12.001 EFFECTS OF CPX ON TRANSFECTION EFFICIENCY AND OVER DIFFERENTIATION IN C2C12.

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Resumo

Title: EFFECTS OF CPX ON TRANSFECTION EFFICIENCY AND OVER DIFFERENTIATION IN C2C12. 1Severino, M., 1Esteca, M. V., 1,2Tamborlin, L., 1,2Luchessi A. D., 1Baptista I. L., 1Faculdade de Ciências Aplicadas, Unicamp, Brasil, 2Instituto de Biociências, Unesp, Brasil.
Introduction: The differentiation process in skeletal muscle cells is highly organized and essential to the regeneration. C2C12 culture cells emerge as reliable model to understand the differentiation process, since those cells have the ability to differentiate in multinucleate myotubes from single myoblasts. These in vitro models are powerful tools for allow genetic manipulation, for example, through the use of plasmids and several transfection reagents. On this study, we used a newly described reagent, ciclopirox (CPX), used to improve transfection efficiency on other cell types, in polyethylenimine (PEI) transfection system.

Objective: The aim of this study was to investigate the effects of ciclopirox over the transfection efficiency and the differentiation process of C2C12 cells. Methodology: C2C12 cells in consolidated culture, was transfected with PEI and a GFP-flag plasmid; other wells also received ciclopirox. To analyze the effect of ciclopirox during the the differentiation,we have used horse serum 2% supplementation for 2 and 4 days. The morphometric parameters were quantifyed by counting cells, through images made by optical microscopy and fluorescence; we also used western blot to quantify where there was higher GFP-flag production, Cyclin (proliferation marker) and Myogenin (differentiation marker).

Fluorescence data was analyzed using T-test (p<0,05).

Results: The percentage of transfected cell was higher in PEI transfection compared to PEI+CPX (PEI 35,5% vs PEI+CPX 21% per mm2 n=3). We also analyzed the transfection efficiency using Western Blot; we observed that CPX reduced the GFP production (~30% of reduction), suggesting that ciclipirox have a stressing effect over C2C12 cells.

We compared the number of cells post transfection, and before differentiation. We observed 102 cells/mm2 in PEI procedure vs 75 cells/mm2 in PEI+CPX, evidencing a reduction of 35%, even starting from 1x105 (n=4). Those data indicate that CPX treatment induced less proliferation compared to PEI transfected cells.

Using Western blot, we detected Flag (GFP), Cyclin and Myogenin proteins during differentiation. We observed that when we applied ciclopirox the Myogenin levels was strong reduced in PEI+CPX (86% vs Control) than PEI transfection (60% vs Control) and control group; the levels of Flag decreased during differentiation (reduction of 40% when compared 2 to 4 days), however in wells that receiving CPX that decrease was slightly higher (30%); the Cyclin levels in PEI+CPX was also reduced compared to PEI (50% of reduction). Our data suggest that in CPX transfection an early differentiation could occur, but without a full progress, as observed in myogenin expression.

Conclusion: Our results showed that in PEI transfection, the protein production was higher compared to PEI+CPX. Moreover, when CPX was applied, it reduced C2C12 proliferation with higher compromised to differentiation, and that could make those cells becoming resistant to the incorporation of the plasmid.

Palavras-chaves: over-expression, c2c12, PEI, CPX, morphology
Introduction: Candida albicans is an opportunistic pathogen, that expressing an unique metacaspase called CaMCA, this enzyme are supposed involved on programmed cell death. Metacaspases are cysteine proteases present in simple eukaryotes, that were identified and were classified based on structural similarity to caspases, but curiously this enzyme is monomeric. The metacaspases presents substrate specificity that contained basic amino acid (Arg or Lys) on P<sub>1</sub> position, and your activity was calcium dependent. Biochemical characterization of the CaMCA activity is important to design new substrates or inhibitor for this protein.

Objective: Obtain recombinant CaMCA metacaspase on E. coli system.

Methodology: pET28a(+) vector (Invitrogen) was chosen to cloning CaMCA. From the synthetic cDNA, amplification was perform with specific primers for CaMCA gene with the HotStar HiFidelity DNA Polymerase (Qiagen). pET28a(+) plasmid and the PCR product were digested with NheI and EcoRI restriction enzymes (Sinapse), these material was binding by T4 ligase (Sinapse). After ligation process CaMCA clone was transformed by thermal shock in E. coli DH5-alpha strain, positive colonies were select by PCR and this material was sequenced by Sanger’s method. Positive colony was chosen and a alkaline lysis was performed to and this material was transformed in an E. coli BL21(DE3) strain and a PCR was make to choose a positive clone. CaMCA was expressed using 0.25 mM of IPTG for 16 hours in 20°C, the material obtained was purified with nickel-sepharose column (GE Lifescience) in different imidazole concentrations (0 - 500 mM).

Results: Amplification of CaMCA gene was availed with a 1% agarose electrophoresis where was observed a unique band around 1360 bp. The material obtained with PCR and a pet28a(+) was digest with NheI and EcoRI restriction enzymes, the digestion material was purified and with t4 ligase we performed the insertion of the DNA into the plasmid. The recombinant DNA was transformed in an E. coli DH5-alpha strain by thermal shock and a positive clones was selected with a kanamycin resistance, in order to confirm a transformation we procedure a PCR with T7 specific primers, a agarose 1% gel confirm the presence of CaMCA gene into pet28a(+) vector. A alkaline lysis was performed in order to obtained a high numbers of copies of the plasmid, these material was used to transformed a E. coli BL21(DE3) strain and to realize the gene sequencing that not presents any mutation. CaMCA was expressed on 20oC using 0.25 mM of IPTG, a SDS-PAGE on 15% acrylamide was used and we could observed a two major protein, one with around 50 kDa that’s corresponding a non-processed form of CaMCA and other with around 40 kDa that correspond a processed form of CaMCA. For purified of CaMCA we used a Ni-sepharose affinity collumn, the protein was shown to be pure after treatment with 300 mM imidazole, the data was confirmed by SDS-PAGE gel.

Conclusion: CaMCA was cloned, expressed and purified successful, our next objectives was testing the activity of this enzyme and procedure some biochemical studies of CaMCA.

Supported by: FAPESP, CNPq and FAEP

Palavras-chaves: Metacaspase, Cloning, Expression, Kinetics, Programmed cell death
12.003 IN VIVO ANALYSIS OF EFFECT BY HYDROGEN PEROXIDE (H2O2) ON Saccharomyces cerevisiae GROWTH

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Resumo

Introduction: The maintenance of life takes place through a process called the cell cycle. Studies have demonstrated the involvement of specific proteases in the regulation of the cell cycle and programmed cell death (PCD). Metacaspases are cysteine proteases, with functional and structural similarity to mammalian caspases, showing marked differences in substrate cleavage specificity and their mechanism of activation. Caspases cleave their substrates after aspartic acid residues, while the metacaspases after arginine or lysine residue. Studies have shown that YCA1 is involved in the regulation and modulation of the cell cycle and on the growth curve of S. cerevisiae. PCD in S. cerevisiae was studied, so far PCD was associated with the accumulation of reactive oxygen species as the regulators of apoptosis in yeast.

Objective: In vivo analyses growth curve on S.cerevisiae with H2O2 in the wild-type strains (BY4742) and knockout strain of YCA1 (Δyca1).

Methodology: Growth curve was performed from a pre-inoculum contained 10^4 cells/ml cropped in YPD medium at 30°C shaking at 180 rpm. Cell growth was monitored by optical density at 660nm for 72 hours in a spectrophotometer read in interval of 12 hours. Yeast was submitted to three types of growth: with replacement of the culture medium every 12 hours, no exchange of culture medium during the 72 hours and in a presence of different concentrations of H2O2 (0-2 mM).

Results: When cultured in a batch system Δyca1 entered its stationary phase after 70 hours of incubation, while the wild maintained it took about 48 hours, already growing in a continuous stream after 72 hours of incubation both wild strain entered stationary phase, while the knockout strain remained in the exponential phase of growth, but the knockout strain shows an increase in the number of cells. The results show that the metacaspase YCA1 is involved in the regulation of the cell cycle since its inactivation changes the time of the cell cycle, besides acting as a positive regulator of the MCP Wild-type strain when subjected in a presence H2O2, causes apoptosis of S.cerevisiae independent of H2O2 concentration, was demonstrated by higher marking with propidium iodide (PI), on the other hand the knockout strain, presents a decrease of PCD in a presence of low H2O2 concentration, demonstrated by low labeling for PI, once the absence of the metacaspase gene caused a delay in the death process, but in saturating concentrations of H2O2 even the Δyca1 strain shows a cell death rate.

Conclusion: In this study we demonstrated that YCA1 interfered on the growth exponential on S.cerevisiae. We show that the absence of YCA1 metacaspase in different concentrations of H2O2 (0-2 mM) provided a higher resistance to the S.cerevisiae when exposed to H2O2.

Supported by: FAPESP, CNPq and FAEP

Palavras-chaves: Metacaspase, Programmed cell death, Growth curve, Cell cycle

12.004 CLONING, EXPRESSION AND PURIFICATION OF METACASPASE OF Paracoccidioides brasiliensis

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**Resumo**

**Introduction:** Metacaspases (MCAs) are cysteine-peptidases found in lower eukaryotes, such as plants, fungi and protozoa, these enzymes are similar to caspases and are closely correlated with cell death in these organisms. The fungi *Paracoccidioides brasiliensis* (Pb) is the causal agent of paracoccidioidomycosis, a biochemical characterization of the PbMCA activity is important to design new substrates or inhibitor for this protein being an important therapeutic target for the paracoccidioidomycosis.

**Objective:** Clone the PbMCA in heterologous expression in E. coli, expressing and purifying this enzyme.

**Methodology:** pET28a(+) vector (Invitrogen) was chosen to cloning PbMCA. From the synthetic cDNA, amplification was performed with specific primers for PbMCA gene with the HotStar HiFidelity DNA Polymerase (Qiagen). pET28a(+) plasmid and the PCR product were digested with NheI and EcoRI restriction enzymes (Sinapse), these material was binding by T4 ligase (Sinapse). After ligation process PbMCA clone was transformed by thermal shock in *E. coli* DH5-alpha strain, positive colonies was select by PCR and this material was sequenced by Sanger’s method. Positive colony was chosen and a alkaline lysis was performed to and this material was transformed in an *E. coli* BL21(DE3) strain and a PCR was make to choose a positive clone. PbMCA was expressed using 0.25 mM of IPTG for 16 hours in 20°C, the material obtained was purified with nickel-sepharose column (GE Lifescience) in different imidazole concentrations (0 - 500 mM).

**Results:** PbMCA gene amplification was analyzed by 1% agarose electrophoresis and shows a 1427 bp band corresponding at PbMCA. Digestion of the PbMCA and vector (pET28a (+)) was analyzed also with a 1% agarose electrophoresis, and presenting bands around 1400 bp and 5369 bp, respectively. These material was ligated with T4 ligase, the recombinant DNA was introduced into the *E.coli* DH5-alpha strain and confirmed by colony PCR with T7 primers, agarose gel analysis showed the presence of 5 positive colonies, the plasmid was extracted from these colonies and with this material sequencing was performed , through this we realized that cloning was performed without the presence of mutations. Positive colonies were then transfected into the competent bacterium BL21 (DE3) - strain capable of inducing gene expression - and also confirmed by colony PCR, verifying that all colonies were positive. Large-scale expression of PbMCA was analyzed on SDS-PAGE gel, where a band of about 52 kDa was visualized, indicating that the protein was successfully expressed, after expression the PbMCA was purified by column chromatography with nickel, purification was also analyzed by SDS-PAGE where it was found that the protein was left pure with 300 mM imidazole.

**Conclusion:** PbMCA was cloned, expressed and purified successful, our next objectives was testing the activity of this enzyme and procedure some biochemical studies of PbMCA.

**Financial support:** FAPESP, CNPq and FAEP

**Palavras-chaves:** Metacaspase, Cloning, Expression, programmed cell death
MULTIPLICATION AND VIABILITY OF C2C12 CELLS CULTIVATED IN VITRO IN RESPONSE TO ANDROGENIC STIMULATIONS

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Resumo

MULTIPLICATION AND VIABILITY OF C2C12 CELLS CULTIVATED IN VITRO IN RESPONSE TO ANDROGENIC STIMULATIONS

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Introduction: Hyperandrogenism is defined as the state of excessive production and secretion of androgens. The growth, regeneration, maintenance, and adaptation of skeletal muscle tissues are dependent on a number of factors, including serum testosterone (T). Skeletal muscle is one of the most metabolically active tissues and is therefore highly susceptible to hormonal variations, especially in relation to testosterone. The C2C12 cell line is a sub-clone of the murine myoblast cell line; these cells are able to differentiate rapidly, forming contractile myotubes and producing characteristic muscle proteins. Because of their high myogenic potential, C2C12 cells provide an excellent tool for the understanding of specific processes, both in vitro and in vivo.

Objective: To evaluate the effects of different testosterone concentrations on cells counts and viability in C2C12 cell cultures.

Methodology: C2C12 cells were cultured (in triplicates) in Dulbecco's Modified Eagle Medium (DMEM) supplemented with fungizone, streptomycin, penicillin, and 2% of normal equine serum, in 48-well plates (5000 cells / 200 μL medium - day zero). The cultures were kept at 37 °C under a humid atmosphere of 5% CO2 and 95% atmospheric air for up to 10 days, in the absence (control, C) and presence of T (at 10^-10 M and 10^-5 M). The testosterone concentrations used mimicked the normal condition and hyperandrogenemia. The culture medium was exchanged every 48 h. Cell quantification was performed with a Bio-Rad TC20™ cell counter. Cell viability was evaluated by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction method, using absorbance at a wavelength of 570 nm. The data were analyzed using ANOVA and Fisher's test for multiple comparisons. The results are reported as the means ± SD, with p-values<0.05.

Results: The log cell count after 5 days of culture was dose-dependently stimulated by T (C: 4.6538 ± 0.0269; T 10^-10 M: 5.0267 ± 0.0417; T 10^-5 M: 6.4879 ± 0.0484). After 10 days of culture, the cell count decreased for T 10^-10 M (5.1759 ± 0.032), but increased for T 10^-5 M (5.378 ± 0.0104), relative to the control (5.3198 ± 0.0045). The effects of T on cell viability were similar to the effects on cell number. The log cell viability after 5 days of culture was dose-dependently stimulated by T (C: 1.2256 ± 0.029; T 10^-10 M: 1.2587 ± 0.0828; T 10^-5 M: 1.5259 ± 0.1326). After 10 days of culture, the cell viability decreased for
Conclusion: Testosterone stimulated cell multiplication and viability in a dose-dependent manner after 5 days of culture. After 10 days of culture, cell multiplication and viability were restricted by a T concentration of $10^{-10}$ M, while a T concentration of $10^{-5}$ M avoided these inhibitory effects. Thus, the culture of C2C12 cells remained viable for up to 10 days, hence providing a useful in vitro model for studies concerning the effects of T on skeletal muscle cells.

Funding: CNPq

Palavras-chaves: cell multiplication, Hyperandrogenism, skeletal muscle, Testosterone, viability in C2C12

12.006 SECOND-DEGREE BURN HEALING IS FAVORED BY THE EXTRACT OF Casearia sylvestris.

Introduction: The complications associated with diabetes are several, mainly regarding the difficulty of repairing burns. Therefore, the study of Casearia sylvestris, an alternative in the repair of lesions associated with diabetes, becomes of great relevance. Objective: Investigate the action of the hydroalcoholic extract from the leaves of C. sylvestris in the repair of second-degree burns in diabetic and non-diabetic rats. Methodology: Study approved in CEUA-UNIARARAS (nº 023/2015). A total of forty non-diabetic and forty diabetes-induced rats (150 mg/kg of intravenous aloxane) were used and those with glycemia above 200 mg/dL, 30 days after induction, were included in the study. The second-degree burns on the skin of the dorsum of the animals were performed with a metal plate (2.0 cm in diameter/120ºC/20 seconds), after anesthesia (ketamine: 1.0 mL/kg and xylazine: 0.2 mL/kg). The experimental groups were: (C) non-diabetic animals treated topically with carbopol gel; (G) non-diabetic treated with an extract of C. sylvestris in carbopol; (DM-C) diabetics treated with carbopol; (DM-G) diabetics treated with C. sylvestris in carbopol. Lesion samples were collected on the 3rd, 7th, 14th and 21st days after the lesion for biochemical analysis: Dosages (µg/g of dry tissue) of hydroxyproline (HO-Pro) and glycosaminoglycans (GAG) for MEC remodeling evaluation and protein expression of TGF-β1, Collagen types I and III by the Western blotting technique (arbitrary units). The quantitative values were analyzed using ANOVA and Tukey's post-test (p<0.05) and expressed as mean±standard error. Results: On the 14th experimental day, it was observed that C. sylvestris treatment favored increased hydroxyproline quantification in the DM-G group (33.40±3.44) in relation to DM-C (18.47±5.71). In relation to glycosaminoglycans quantification, an increase in DM-G (3d:5.03±2.34, 7d:4.07±0.95) was observed in relation to DM-C (3.67±0.28) and G (7d:0.87±0.1). In the Western blotting analysis for TGF-β1 protein expression, in the day 3 was observed an increase in G (11,478.05±3,022.05) in relation to the other groups. On the 7th day, DM-G (23,544.32±2,489.94) was superior to the other groups with a reduction in the later periods. In the determination of collagen type III, on the 3rd and 14th days group G (3d:21,081.86±3,535.98, 14d:20,800.87±1,360.41) was superior to the others and the group DM-G (3d:13,599±2,299) higher than the DM-C group. On day 7, group G (7d:16,589±705) was superior to group C (7d:7,205±52.42). On the 14th and 21st days, there was an increase in group C (14d:12,273 ± 2,001, 21d:10,719±5,191) in relation to the DM-G group. Group G at day 21 (15,127±1,126) was superior to DM-C and DM-G groups. In the analysis of Collagen type I, a gradual increase was observed in groups G and DM-G during the whole experimental period, being...
higher in DM-G on the 14th day (17,143±566) in relation to C and G. On the 21st day G (14,001±1,481) was superior to C and DM-C and DM-G (18,766 ± 1,343) superior to the other groups. **Conclusion:** *C. sylvestris* can be considered an alternative for the healing process of second-degree burns in diabetics, as it favored repair by increasing inflammation in the initial periods with subsequent reduction, also providing reorganization of the extracellular matrix and collagenesis. **Funding:** FHO.

**Palavras-chaves:** Burn, Healing, Herbal Medicine, Diabetes

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**12.007 HISTOLOGICAL CHARACTERIZATION OF PERIPROSTATIC ADIPOSE FROM OBESE MICE AND ITS ROLE ON PROSTATE TISSUE.**

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**Instituição** ¹ UNICAMP - Universidade Estadual de Campinas

**Resumo**

**Introduction:** The lower urinary tract symptoms (LUTS) has a high prevalence worldwide and affect both genders, however, obese men are more likely to develop these symptoms. Obesity promotes an increase of adipose tissue, which is an important source of pro-inflammatory, pro-contractile and pro-proliferative substances. The adipocytes are divided into white and brown adipose tissue. The white adipose tissue (WAT) is composed of large diameter solid lipid droplet, cytoplasm and nucleus. While the brown adipose tissue (BAT) is composed of smaller cells containing small lipid droplets. In a recent study published by our group the prostate weight and epididimal fat of obese mice are significantly greater when compared to lean mice tissues and these animals presented prostate hipercontractility.

**Objective:** Therefore, the aim of this study is to characterize the periprostatic adipose tissue from obese mice.

**Methodology:** All experimental protocols were approved by Ethics Committee for the use of experimental animals (CEUA 4720-1/2-2017). C57BL/6 male mice with 4 weeks old were fed with standard chow (control group, N=5) or high-fat diet (obese group, N=5) for 12 weeks. At week 12, prostate and periprostatic adipose tissue were collect for histopathology and immunohistochemistry to evaluate proliferative (ki-67). Student's t-test analysis was used. P<0.05 was accepted as significant.

**Results:** The histological analysis showed that the adipocyte area from periprostatic adipose is significantly greater in obese than in lean mice. With respect to the histological analysis of prostate sections, in the control group a simple columnar epithelium and a thin layer of smooth muscle was observed. On the other hand, the prostate from obese mice showed epithelial hyperplasia. These results were confirmed with the proliferative index (Ki-67) test, which showed greater values (an increase of 93%; P

**Conclusion:** Since the area from periprostatic adipose tissue is significantly greater in obese than in lean mice, our results suggest that the adipose tissue could release proliferative substances thus contributing to the development of prostatic hyperplasia.

**Founding:** CAPES

**Palavras-chaves:** Prostate, periprostastic adipose tissue, LUTS

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**12.008 Antioxidant and anti-adipogenic activity of Campomanesia adamantium O.Berg (Myrtaceae)**
Resumo

Title: Antioxidant and anti-adipogenic activity of Campomanesia adamantium O.Berg (Myrtaceae) 1Espindola, P.P. T.*, 1Rocha, P. S., 1Campos, J.F., 2Carollo, C.A., 1Santos, E. L., 1de Picoli Souza, K. 2Federal University of Mato Grosso do Sul, Campo Grande/MS, 1Universidade Federal da Grande, UFGD, Dourados/MS.

Introduction: The use of medicinal plants over the centuries and their chemical diversity has stimulated new studies in search of natural compounds that can be used in the prevention and treatment of various diseases, as obesity. Campomanesia adamantium O. Berg., popularly known as “guavira”, is a plant native to Brazil, occurring in the Cerrado, including Mato Grosso do Sul State, and it is used for several purposes. Leaves, fruits and roots of this plant have medicinal properties already described as effect to decrease body mass, hypoglycemic, hypolipidemic and antileukemic.

Objectives: To evaluate the antioxidant activity, cytotoxicity and anti-adipogenic effect of Campomanesia adamantium leaves.

Methods: Aqueous extract of the leaves of C. adamantium (EAFCa) was prepared by solvent accelerated extraction (DIONEX35). The antioxidant potential of EAFCa was investigated by the DPPH capture method, its cytotoxicity in 3T3F442a pre-adipocytes and its effects on the differentiation of adipocytes by accumulation of lipids in cell culture of 3T3F442a pre-adipocytes with oil red staining. The data were submitted to one-way analysis of variance (ANOVA) followed by Student Newman Keuls test using the Prism 5 GraphPad Software. The results were considered significant when $p<0.05$.

Results: The concentration required to inhibit 50% free radicals (IC$_{50}$) in the DPPH assay of the EAFCA was 11.6 ± 1.5 μg/mL, compared to BHT 28.1 ± 6.3 μg/mL and ascorbic acid 2.1 ± 0.6 μg/mL, synthetic and natural control antioxidants, respectively. EAFCa did not induce death of 3T3F442a pre-adipocytes and its effects on the differentiation of adipocytes by accumulation of lipids in cell culture of 3T3F442a pre-adipocytes with oil red staining. The data were submitted to one-way analysis of variance (ANOVA) followed by Student Newman Keuls test using the Prism 5 GraphPad Software. The results were considered significant when $p<0.05$.

Conclusion: In summary, the preliminary results indicate the antioxidant and anti-adipogenic potential of EAFCa without presenting effects of cytotoxicity.

Funding: UFGD, FUNDECT and CNPq.

Palavras-chaves: antioxidants, cytotoxicity, differentiation, leaves, prevention
Resumo

Introduction: Yellow fever (YF) is an infectious, non-contagious disease caused by an RNA virus of the Flaviviridae family, which is transmitted to humans by the bite of hematophagous mosquitoes. It causes severe disease involving especially the liver, with lesions characterized by midzonal apoptosis. The mechanism by which liver cells undergo apoptosis in response to YF infection remains unclear.

Objective: To provide further information on the mechanism of apoptosis in YF infected hepatic cells we investigated whether the virus causes endoplasmic reticulum (ER) stress and intracellular Ca\(^{2+}\) signaling disturbs.

Methodology: Explants and biopsies from patients diagnosed with hepatitis (Certificate of Presentation for Ethical Consideration: 77877417.9.0000.5125) caused by YF infection were analyzed by hematoxylin and eosin staining and immunohistochemistry. HepG2 cells were infected with the attenuated YF strain (17DD) and isolated YF (RB) to evaluate the viral cytotoxicity by violet crystal and by rezasurin metabolism. Proteins involved in Ca\(^{2+}\) signaling and ER stress were assessed by immunofluorescence and immunoblot. Mitochondrial Ca\(^{2+}\) signaling, with Rhod-2/AM, was monitored in individual cells by time lapse confocal microscopy after adenosine triphosphate (ATP) or tumor necrosis factor (TNF-a) stimulation. The results were expressed as means and standard errors of the mean, and tested by one-way analysis of variance followed by Tukey posttests, where p

Results: Histological analysis of liver samples from YF patients showed a range from mild to severe hepatic damage, where Councilman bodies, macro and microvesicular steatosis, and lytic necrosis were found within the midzonal region of the lobule in mild cases, or in the whole organ in the fulminant hepatitis cases. Immunohistochemistry staining showed that several hepatocytes were positive to flavivirus antigen and to apoptosis (ssDNA). Moreover, the 1,4,5-trisphosphate receptor subtype 3 (InsP3R-3), an isoform that is absent under normal conditions in hepatocytes, was upregulated in YF patient samples. In vitro experiments using the hepatic cell line HepG2 showed that YF infection is cytotoxic to this cells, wherein the cell death and cytotoxicity were more pronounced after RB infection compared to 17DD strain (61.0 and 31.4% respectively, p\(^{2+}\) signaling was increased in the cells infected with YF compared to control. The amplitude and duration of mitochondrial calcium signaling were significantly increased in RB infected cells compared to control after ATP and TNF-a stimuli (2.2x and 1.7x respectively, p

Conclusion: Based on these findings, we conclude that the disturb in the intracellular Ca\(^{2+}\) signaling machinery and the activation of ER stress cascade play an important role in the genesis of hepatic lesions in severe yellow fever, by inducing a massive hepatocyte apoptosis.

Funding: Liver Center at UFMG, CNPq and FAPEMIG.

Palavras-chaves: Ca2+ signaling, Yellow fever, apoptosis, ER stress, Inositol trisphosphate receptor
12.010 CRITICAL ROLE OF PARKIN DURING THE SKELETAL MUSCLE REGENERATION

Marcos Esteca Marcos Esteca

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Resumo

Title: CRITICAL ROLE OF PARKIN DURING THE SKELETAL MUSCLE REGENERATION. 1Esteca, M. V., 1Severino, M., 2Silvestre, J. G. O., 3Gustafsson. A., 2Moriscot, A. S, 1Baptista, I. L., 1Faculdade de Ciências Aplicadas, Unicamp, Brasil, 2Instituto de Ciências Biomédicas, Departamento de Anatomia, Universidade de São Paulo, Brasil, 3Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, United States.

Introduction: The regenerative process of skeletal muscle is a process highly ordered. Several studies have suggested that proteolytic pathways such as ubiquitin-proteasome and autophagic are necessary on both phases, inflammatory (1-3 days post injury) and regenerative (3-21 days post injury). One critical step on differentiation of myogenic precursor cells, during the regeneration, is a metabolic shift from glycolytic to oxidative. At this point, we suggest that Parkin E3 ligase, required for mitochondrial turnover, may influence the process of muscle regeneration.

Objective: The aim of this study was to investigate whether Parkin is important for mitochondrial turnover of myogenic precursor cells, influencing the differentiation process during skeletal muscle regeneration.

Methodology: 10 wild-type animals (WT) and 10 knockout animals for Parkin (Parkin−/−) were used for the experiments. The injury was induced by cardiotoxin (CTX1 10μM) inoculated in the tibialis anterior muscle and the sacrifice of the groups was performed 3 days after injury (CTX3D) and 10 days after injury (CTX10D). The contralateral paw was used as control group (CTRL). All procedures were approved by CEUA (46871/2017). Data were compared between groups using One-Way ANOVA and Post-hoc Tukey test (p<0.05) and for two groups the T-test was used (p<0.05).

Results: Parkin−/− animals had a mean area 24.5% lower than CSA compared to WT animals at 10 days after injury (WT 1180μm² ± 695 vs Parkin−/− 897 ± 641μm² n=4). We found a large increase of Parkin protein levels in the CTX3D group compared to the CTRL, which remained elevated until 10 days after the injury (n=3). In cell location, we detected Parkin co-localized with VDAC2 on 94.5% (±2.5) of myogenic precursor cells in the CTX3D group and 71.5% (±9.5 n=2) in the CTX10D group. We observed an important drop on protein content of VDAC2 in the WT and Parkin−/− animals, 3 days after injury compared to its related CTRL (n=3). At 10 days after the injury the levels of VDAC2 increased near the basal level in WT animals, but not in Parkin−/− animals (n=3). In the WT animals a large increase in LC3-I occurs 3 days after lesion and remained elevated until 10 days post injury (n=3); we also observed a gradual increase in LC3-II on both, 3 and 10 days after injury (n=3). In Parkin−/− animals, LC3-I and LC3-II only increased 10 days after injury (n=3). Using immunofluorescence, Parkin−/− animals showed a higher number of LC3/VDAC2 positive cells at 10 days post-injury compared to WT animals (WT 7.6%± 0.9 vs Parkin−/− 24.5% ±1.3, n=2). We also analyzed the p62 protein content and that protein was downregulated 3 days post injury and remained below baseline until 10 days after injury on both, WT mice as Parkin−/− animals (n=3).

Conclusion: At this point, our results point out that Parkin presence is important to mitochondrial turnover during the myogenic differentiation, and a deficiency in Parkin expression can lead to a weakened autophagic flow and a consequent loss of regenerative capacity of skeletal muscle.

Palavras-chaves: Parkin, Eskeletal muscle, Regeneration, Mitophagy, Stem cell
12.011 Prostatic Smooth Muscle Cells: Environment-Induced Phenotypical Switch

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**Resumo**

Introduction: The prostatic smooth muscle cells (SMCs) are surrounded by an external (basal) membrane and stromal type I collagen. The prostate is highly dependent on androgens. After the first hours of castration the endothelial cells suffer apoptosis and the resulting vascular permeability leads to the formation of a fibrin network. This also results in exposure of SMCs to serum-derived cytokines, such as TGFβ3 and TGFα, which are migratory stimuli for some cell types after tissue injury. The external stimuli produced by extracellular matrix (ECM) are translated through transmembrane receptors, the integrins, which bind specific ECM proteins. The integrin cytoplasmic domain interacts with several proteins in a complex called focal adhesion. One of these proteins is focal adhesion kinase (FAK), which is a 125-KDa protein-tyrosine kinase and acts as an integrator of the ECM and the cytoskeleton.

**Objective:** To identify how different (ECM) substrates and cytokines (TGFβ3 and TGFα) affect prostatic SMC phenotype and FAK content/distribution.

**Methodology:** SMCs were obtained by culturing ventral prostate explants from male Wistar rats (Protocol: CEUA 4132-1), in DMEM supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, 10 nM dihydrotestosterone, and 5 µg/mL insulin. Subsequently, SMCs in the fourth-to-seventh passages were seeded on type I collagen, fibrin and laminin-rich (Matrigel) extracellular matrix 2D-substrates. After 48h in culture, cells were examined by immunofluorescence and Western blotting, using antibodies against α-SMA and total FAK, and by transmission electron microscopy. A wounding healing assay was used to study the effect of TGFβ3 and TGFα in the SMC migration.

**Results:** The morphology of the cells after 48h of culture on the substrates and FAK distribution was remarkably different. Western blotting and fluorescence techniques did not show changes in α-SMA or FAK levels. SMCs adhered very quickly to type I collagen. Their cytoskeleton showed several actin bundles and focal adhesions. When seeded on a fibrin matrix, the cells were very active, showing numerous processes, and promoted degradation of the fibrin matrix. It was observed that the cells on laminin-rich substrate had several processes, and formed cell clusters in the first hours of culture. Actin filaments in fibrin and laminin-rich substrate did not organize in thick bundles. The scrapping/wounding showed that TGFα, but not TGFβ3, stimulated cell migration.

**Conclusion:** These observations suggest that ECM components and cytokines regulate behavior, morphology and cytoskeleton, confirming the remarkable phenotypical plasticity of differentiated SMCs.

**Financial Support:** FAPESP (Grant No. 2009/16150-6 and 2015/03235-4).

**Palavras-chaves:** Cytokines, Extracellular Matrix, Focal Adhesion Quinase, Prostate, Smooth Muscle Cell

12.012 Toxoplasma gondii affects neurogenesis and neuritogenesis in Neuro2a cells and reduces migration and proliferation in mouse neurospheres.

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**Resumo**

**Abstract**

Toxoplasma gondii affects neurogenesis and neuritogenesis in Neuro2a cells and reduces migration and proliferation in mouse neurospheres.
INTRODUCTION: Toxoplasmosis is a worldwide spread zoonosis caused by *Toxoplasma gondii*, an obligate intracellular protozoan that affects all warm-blooded animals, including humans. The ability to transmigrate through the placental barrier and to replicate in different fetal tissues, without being affected by the immune system, makes *T. gondii* infection an important cause of prenatal complications. Vertical transmission may interfere with the fetal development, leading to abortions or several malformations, such as neurological defects, blindness and microcephaly. The parasite has a tropism for the Central Nervous System where tissue cysts are found within neurons. However, little is known about the impact of infection on the differentiation of neuronal cells that may lead to alterations in cortical neurogenesis.

OBJECTIVES: The aim of this project is to study the mechanisms involved in the pathogenesis of toxoplasmosis, focusing on neurogenesis and neuritogenesis.

METHODS: Initially we used Neuro2a cells (N2a), a neuroblastoma cell line that have the potential to differentiate into neurons upon serum withdrawal. N2a were infected with tachyzoites of the ME-49 (type II) strain for 1 day in the presence of Fetal Bovine Serum (FBS, 10%) and were induced to differentiate with DMEM containing Bovine Serum Albumin (BSA, 0.1%). As a second approach, neurospheres composed of neural progenitor cells, isolated from Swiss Webster mice (CEUA-IOC: L-048/2015), were also used. The proliferation of floating neurospheres’ was analyzed after 2 (N=6 neurospheres control and infected) and 3 days post infection (N=13 control, N=8 infected). For differentiation assays, neurospheres were plated in glass coverslips coated with fibronectin and Poly-L-lysine, in differentiation medium (DMEM-F12 containing N2, B27 with retinoic acid and Glutamax). The migrated area was calculated after 48 and 120 hours (N=3 and N=1, respectively), and the immunoreactivity of different markers was analyzed by immunostaining.

RESULTS: The infection of N2a cells with *T. gondii* significantly decreased the rate of Neurofilament-positive neuroblasts (3.833 ± 0.8122% of uninfected and 1.181 ± 0.4633% of infected, N=3, Student’s t test) and the length of neurites (4.847 ± 1.029% of uninfected and 1.862 ± 0.8936% of infected, N=3, Student’s t test). We observed that *T. gondii* infection significantly reduces the cumulative rate of migration by 20 (p<0.01) and 40% (p<0.001) at 24 and 48 hours, respectively (2-Way ANOVA with Bonferroni post-test). Our preliminary data indicate that infected neurospheres showed decreased numbers of GFAP (intermediate filament, marker of glial cells) and NeuN (nuclear marker of neurons)-positive cells per perimeter (*GFAP*: 7.684 ± 0.7621% N=8 control versus 5.478 ± 1.394% N=5 infected; *NeuN*: 1.000 ± 0.2224% N=4 control versus 0.6140 ± 0.08778% N=5 infected). Furthermore *T. gondii*-infected floating neurospheres showed a reduced number of proliferating cells after 2 and 3 days post infection as shown by Ki67 immunostaining.

CONCLUSION: We conclude with the results found until this moment that *T. gondii* affects neuronal differentiation and neurite outgrowth, with reduction in progenitor cells proliferation and migration. This data will contribute with the understanding of the mechanisms by which this parasite affects cortical development in mice.

FUNDING: This work was supported by Fundação Oswaldo Cruz, CNPq (Edital Universal 2014 and PAPES VII) and FAPERJ.

Palavras-chaves: Congenital toxoplasmosis, Cellular differentiation, Neuritogenesis, Neurogenesis, Toxoplasma gondii
Background: The antimicrobial peptides (PAMs) Gomesin, Protegrin, Tachyplesin and Poliphemusin have demonstrated potent anti-tumor activity. These peptides exhibit cytotoxicity against tumors by various cellular mechanisms. However, how the PAMs diffuse across cells and the participation of glycosaminoglycans (GAGs) in this process remain unclear. Aim: Investigate the internalization of PAMs and the participation of GAGs on internalization. Methods: Intrinsic tryptophan fluorescence was monitored to determine the Kd of GAG with PAMs by spectrofluorimetry. Structural modifications were performed on heparin to verify the participation of GAGs and the citotoxic ability of PAMs was evaluated by MTT assay, in addition, endocytosis inhibitors were also used. Modifications in the amount and sulfation of the GAGs were performed in cultures of HeLa cells by addition of sodium chlorate and xylopyranoside, respectively. The quantification of the entrance and internal location of the PAMs was performed by confocal microscopy and flow cytometry. Results: High affinity of PAMs, around micromolar concentration, was observed for the GAGs tested (heparan sulfate, chondroitin sulfate, hyaluronic acid and modified heparins). The presence of heparin and xylopyranosidase reduced the cytotoxic effect of PAMs on HeLa cells. Desulfation of GAGs did not alter the cytotoxic effect of PAMs. Endocytosis structures such as clathrin, caveole and lysosome were labeled, but no co-localization with PAMs were observed. E-64 and Chloroquine potentize the effect of PAMs. On the other hand, dynasore (protegrin) and cytochalasin-D (Tachyplesin and Polyphemusin) inhibited the cell death of PAMs. Conclusion. All GAGs are able to bind to PAMs with high affinity and may be involved in the entry of peptides into the tumor cell, regardless of the charge. Furthermore, no co-localization of PAMs with clathrin, caveola and lysosome was observed, but was inhibited by cytochalasin-D and dynasore, suggesting that PAMs could pass through by macropinocitosis/endocytosis dependent of RhoA/Rac across cell membrane.

Palavras-chaves: antimicrobial peptides (PAMs), glycosaminoglycans (GAGs), cell membrane

12.014 ANTIMITURAL ACTION OF PORPHYRIN TMPYP UNDER IRRADIATION WITH WHITE LIGHT

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Resumo

Introduction: The porphyrins and their derivatives have been, over the years, the target of various studies in different areas, giving raise to several applications. The porphyrins are polycarboxylated tetra pyrrole macrocycles that may contain a metal core; the antitumoral action has been associated with the fact that when photosensitized they generate singlet oxygen and DNA damages. Therefore, the porphyrins are toxic to tumor cells. This phototoxicity is derived from the photophysical and photochemical characteristics. The porphyrins relevance is due to their high affinity for tumor tissues and relatively low toxicity.

Objective: Determine the antitumoral action of porphyrin TMPyP in epidermoid carcinoma of the mouth cells when radiated with white light.

Metodology: The porphyrin TMPyP 5,10,15,20-Tetrakis (1-methyl-4-pyridinium) porphyrin tetra (p-toluenesulfonate) was acquired from Sigma-Aldrich and was tested against rabbit aortic smooth muscle cells (MLAC) and epidermoid carcinoma of the mouth cells (ATCC SCC9). The cells were cultured and incubated in a controlled environment of 37ºC and 5% CO2. After cell confluence between 80 and 90%, two 96-well test plates were assembled for each cell type with 100 μL of suspension containing 1.0 x 105 cells/mL per well. The plates were maintained for 24 hours in incubation at the same temperature and atmosphere conditions as mentioned above. After cell growth on the test plates, increasing concentrations of porphyrin were added in the dark. All test were performed in triplicate. One of the plates from each cell was placed directly in the incubator, without light irradiation. Another plate was irradiated with direct white light for 10 minutes at a
distance of 10 centimeters. All plaques were analyzed by optical microscopy every 24 hours, and the cell viability was tested by MTT - [4,5-dimethylthiazol-2-yl] -2,5-diphenyl tetrazolium bromide after 40 hours of incubation. The absorbance values obtained were converted into a percentage of viable cells, considering as live control (100% viability) the sample containing only the cells, without addition of any additional compounds. All data were tabulated using Prism software (GraphPad Software Inc., San Diego, CA, USA).

**Results:** There was statistically significant differences in the percentage of cell viability for the post-irradiation samples with white light compared to the dark for both cells. A dose-dependent effect was observed for both cells, which became more evident in the plates irradiated with white light. The cytotoxic effect exerted by porphyrin TMPyP4 was greater in tumor cells than in normal cells both in the presence of light and in the dark.

**Conclusion:** The results show the cytotoxic effect of porphyrin TMPyP4 on normal rabbit aortic cell and in epidermoid carcinoma of the mouth cells. This effect was increased by the irradiation with white light. The results are promising and more studies are needed.

**Funding:** CNPq e Faep.

**Palavras-chaves:** Cytotoxicity, Mouth carcinoma, Porphyrins

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**12.015 TOXOPLASMA GONDII INFECTION IMPAIRS MYOGENESIS IN VITRO**

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**Resumo**

**INTRODUCTION:** Toxoplasma gondii is an intracellular parasite capable of crossing the transplacental barrier and infecting fetal tissues, causing congenital toxoplasmosis. Previous studies of our group showed development defects in a murine model of congenital toxoplasmosis. The parasite has a strong tropism for the skeletal muscle cells, in which it forms tissue cysts. In experimentally infected pregnant mice, the skeletal muscle showed atrophy and myositis. The mechanisms by which the infection affects the development of the skeletal muscle has not been addressed until this moment.

**OBJECTIVE:** Investigate the molecular mechanisms that could interfere in the myogenic programesis during *T. gondii* infection.

**METHODOLOGY:** The mouse myoblast C2C12 cell line was utilized, due to its ability to differentiate *in vitro*. Myoblasts were infected with ME49 strain of *T. gondii* 24 hours after plating. 24 h after infection proliferation medium was replaced for differentiation medium, as well documented in the literature. At 24 or 120 hours after induction, cells were processed to immunofluorescence or qRT-PCR. In addition, at the same points the conditioned medium was collected to for cytokines/chemokines analysis profiling using Cytokine Bead Array or ELISA for TGF-beta1. Gelatinase activity of Matrix metallopeptidases (MMPs) was determined by zymography.

**RESULTS:** The infection can drastically reduce the differentiation and fusion indexes, as well as the number of mature myotubes and the diameter of the such myotubes. The expression of myosin heavy chain, a marker of terminal myogenesis was reduced 3,5-fold in infected cells (n=3). Expression of myogenic regulatory factors (MRFs) was also altered by infection. Myf5 and Mrf4 had increased expression and decreased myogenin (MyoG) expression after 120 h of induction in infected cells. While in uninfected cultures MyoG was upregulated 6-fold with 24 h of induction of myogenesis, in infected dishes this increase was only 2-fold (One-Way ANOVA with Bonferroni post-test, n=4). To assess changes in the proliferation rate we performed immunostaining of for Ki67. After 120 h of cultivation in differentiation medium, infected cells showed a significant increase of Ki67-positive cells immunostaining (p<0.05, One-way ANOVA with Bonferroni post-test, n=4). CBA revealed that IL-6 and MCP-1 was were highly increased in *T. gondii* infected cultures (n=2). TGF-β secretion was decreased in infected cells after 120 h (n=2). Gelatinase zymography indicated that MMP-3 is activated in infected cultures (n=2). In order to investigate damages to upstream steps of the myogenic program induced by the infection, we performed immunostainings for β-catenin and looked for nuclear localization of such protein. We found that after 10 hours of
induction with differentiation medium, infected cultures showed decreased fluorescence intensity (p<0.05, One-Way ANOVA, n=3) and decreases rates of nuclear staining.

CONCLUSION: T. gondii induces a severe disarray of the skeletal muscle cell culture, with the increased secretion of IL-6, MCP-1 and MMP-3 and decreased secretion of TGF-beta. This was concomitant with deregulation of the expression of MRFs, thus leading the culture to remain in a proliferative state and unresponsive to myogenic initiation, including the activation of the Wnt-b-catenin pathway.

FUNDING: Fundação Oswaldo Cruz, CNPq (Edital Universal 2014 and PAPES VII) and FAPERJ.

Palavras-chaves: C2C12, Myogenesis, Toxoplasma gondii

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12.016 WOUND HEALING TREATED WITH Azadirachta indica IN WISTAR DIABETIC AND NON-DIABETIC RATS

Autoren

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Resumo

Introduction: Complications associated with the healing in diabetes become essential the investigation of alternative methods that helped the tissue restoration. Herbal medicines such as Azadirachta indica (popular name "Neem"), can be potential therapeutic alternatives for wounds associated with diabetes.

Objective: To evaluate the healing of wounds treated with A. indica extract in diabetic and non-diabetic rats by histomorphometric analysis (quantification of inflammatory infiltrate, angiogenesis, fibroplasia, and collagenases) and analysis of wounds reepithelialization.

Methodology: Study approved by CEUA-UNIARARAS (nº 025/2017). A total of 60 non-diabetic rats and 60 diabetes-induced rats (45 mg/kg aloxane, intravenous) were used, which presented glycemia above 200 mg/dL after 7 days post-induction. After ketamine (3.0 mg/kg) and xylazine (1.0 mg/kg) anesthesia, the lesions were performed with histological punch (15 mm diameter). Then, the animals were divided into: S- non-diabetic treated with carbopol gel; N- non-diabetic treated with the A. indica extract on carbopol gel; DM-S- diabetics treated with carbopol gel; DM-N- diabetics treated with the extract of A. indica. The animals were euthanized on the 2nd, 7th and 14th days post-injury. The lesions were photographed on the initial day (0d) and on the segment days (2d, 7d, and 14d) for analysis of wound healing rate (WHR = initial area - final area/initial area). Histological (HE and Gomori trichrome staining) was performed for histomorphometric analysis (quantification of inflammatory infiltrate, fibroblasts, blood vessels and collagen) calculated by ImageJ. The results (mean±standard error of medium) were analyzed by ANOVA Two-Way and Tukey post-test (p<0.05).

Results: The DM-S group (332.9 ± 38.54) on 2nd day presented superior inflammatory infiltrate than S (198.5 ± 6.3) and N (152.3 ± 12.74); on the 7th Day it was higher than N (106.50 ± 18.18) and on 14th day the groups S (229.4 ± 25.04), DM-S (330.2 ± 29.4) and DM-N ( 214.1 ± 40.5) were higher than N (80.2 ± 11.9). As for angiogenesis, the groups S (31.2 ± 2.7) and DM-N (30.5 ± 2.0) on 2nd day were
higher than \( N (17.7 \pm 0.7) \). Regarding fibroplasia, \( S \ (291.5 \pm 14.8) \), DM-S \( (295.0 \pm 33.9) \) and DM-N \( (296.8 \pm 21.8) \) groups on 7th day were superior to \( N \ (146, 1 \pm 24.0) \). Collagenase was higher in group \( N \ (53.5 \pm 4.3) \) than \( S \ (32.4 \pm 7.1) \) and DM-S \( (33.8 \pm 4.1) \) on 2nd day. The groups \( S \ (0.9 \pm 0.03) \) and \( N \ (0.8 \pm 0.06) \) showed superior reepithelialization of the wounds than DM-N \( (0.61 \pm 0.03) \) on the 14th day.

**Conclusion:** The extract of *A. indica* favored the wound healing in non-diabetic animals. However, when it was used in animals induced to diabetes, the wound healing was not favorable.

**Funding:** HERMINIO OMETTO FOUNDATION

**Palavras-chaves:** Diabetic, Herbal medicines, Wound Healing

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**12.017 PROTEOMIC ANALYSIS APPLIED TO THE STUDY OF LIPID BODIES: ASSOCIATION WITH CALCINEURIN IN THE FUNGUS Paracoccidioides brasiliensis**

**Autores**

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**Resumo**

Indroduction: *Paracoccidioides brasiliensis* is one of the species of a fungus that causes Paracoccidioidomycosis (PCM), a systemic mycosis found in Brazil. Calcineurin, a \( \text{Ca}^{2+} \)-calmodulin-dependent phosphatase, regulates processes related to cell dimorphism and proliferation in *P. brasiliensis*, while apparently also regulates lipid metabolism. Yeasts cells treated with cyclosporin A (CsA), a calcineurin inhibitor, present a larger lipid bodies when compared to non-treate yeasts.

Objective: In order to investigate the role of calcineurin in lipid metabolism, the present study evaluated, by mass spectrometry, the protein profile of yeasts of *P. brasiliensis* grown at different concentrations of glucose (0.2 and 2%) in the absence and presence of the calcineurin inhibitor.

Methodology: Peptides were separated by a nanoflow liquid chromatography performed on an Easy nanoLC system coupled to an LTQ-Orbitrap Velos mass spectrometer. Spray voltage was 2.1 kV a 200 °C and the equipment was operated in Data Dependent Acquisition mode - DDA (m/z from 300 to 1650) followed by MS/MS acquisition using Collisional Induced Dissociation (CID) of the top 15 intense ion from the MS scan. MS spectra were acquired in the Orbitrap analyzer at 60,000 (400 m/z). Raw data were analysed with MaxQuant software (version 1.5.3.17). FDR was 1%. The target-decoy strategy (ELIAS; GYGI, 2007) was used to analyse the target database, limited to Fungi taxa from UniProt. Label Free Quantitation (LFQ) was used. Protein intensities were log2-transformed and normalized using the R scripting and statistical environment. Proteins with a P-value ≤0.05 and log2(fold change)\(> 1\) and\(<-1\) were considered to be differentially expressed. When no annotation was found, a BLASTP search was performed using the Blast2GO software. Protein network analysis was carried out using KEGG pathway. Samples were from three independent biological replicas.

Results: Our results indicate that yeasts grown at 2% of glucose have a metabolic profile directed to an active metabolism, with oxidative phosphorylation, proteins fatty acids synthesis and oxidation. In contrast, yeasts under 2% glucose plus 1 microg/mL CsA, presented a catabolic profile of the protein metabolism, an active
gluconeogenesis pathway and glyoxylate cycle, a mal-functioning mitochondria, a putative inability to access lipids for beta oxidation, which may be at least partially impaired.

Conclusion: Our hypothesis is that calcineurin may be involved in the consumption / degradation of lipid bodies of *P. brasiliensis*. The proteomic profile found in yeast treated with CsA may reveal a mechanism of metabolic modulation by calcineurin not yet described, which may allow the identification of one or more key enzymes for cellular integrity and, consequently, a potential therapeutic target for this and others pathogenic fungi. However, further research is needed since CsA also inhibits cyclophilins.

Funding: CAPES

**Palavras-chaves:** Calcineurin, Lipid bodies, Metabolism, Paracoccidioides brasiliensis, Proteomic

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**12.018 PROSTATIC EPITHELIAL CELL-INDUCED MACROPHAGE DIFFERENTIATION.**

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**Resumo**

**Title:** PROSTATIC EPITHELIAL CELL-INDUCED MACROPHAGE DIFFERENTIATION. Aline Siqueira Berti1 and Hernandes F Carvalho1 1Department of Structural and Functional Biology, State University of Campinas, Campinas/SP, Brazil.

**Introduction:** Androgens are important for prostate gland development and homeostasis. Androgen deprivation leads to the influx of immune cells, in particular, of different populations of macrophages. Macrophages have a prominent role in development, tissue homeostasis and resolution of the inflammatory process. We have shown that one subpopulation of macrophages control tissue inflammation after castration (Prostate 2018, 78: 95).

**Objective:** The objective of this work was to analyze if isolated prostatic epithelial cells can induce macrophages differentiation into the M1 and M2 phenotypes.

**Methodology:** The Raw 264,7 macrophage cell line was cultured in complete medium, supplemented with 50% of LNCAP conditioned medium either in the presence or absence of R1881 examined by time lapse microscopy for 24h. RWPE-1 cell line and LNCAP cell line were cultured for 15 days in matrigel of spheroid formation. After this period, the spheroids were cocultured with Raw 264,7 and observed for 24h. After 24h, both cultures were immunostained for iNOS. Macrophages were also examined for the expression of ZNF 142. Mitochondria were stained with Mitotracker Red.

**Results:** We have observed that androgens induced the expression of iNOS by macrophages cultured in either 2D or 3D. Macrophage motility in 2D cultures also increased in the presence of LNCaP conditioned medium. In the presence of androgens there was an increased of arginase expression and in the number of mitochondria. There was no change in the amount of ZNF 142 in macrophages in the presence and absence of androgenic stimulation.

**Conclusions:** These observations suggest that prostatic epithelial cells cultured in the absence of androgenic stimulus lead to the production of soluble factors that influence macrophage differentiation and motility.

**Funding:** CNPQ

**Palavras-chaves:** Macrophage, Culture, Prostatic, Epithelial, Differentiation
12.019 AG73, a laminin-derived peptide improves adhesion, migration and differentiation of myoblasts

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Resumo

Duchenne Muscular Dystrophy (DMD) affects 1 in 3600 to 6000 male births and is an X-linked recessive disorder. It is caused by mutations in the dystrophin gene, leading to lack of expression of dystrophin, a protein that stabilizes the sarcolemma during muscle contraction. There is a progressive muscle weakness and wasting in DMD, resulting in loss of motor function, cardiorespiratory complications and early death around the 20s. Myoblast transplantation is an important cell therapy in DMD, in which the myoblasts are able to fuse in dystrophic muscle, improving the muscular strength and dystrophin expression. However, the injections of these cells present some problems: high cell death, low proliferation, impaired migration and receptor's immune response that decrease the efficacy of the therapy. Laminin-111(LAM-111) was proposed as an effective protein-based therapy for DMD, as it prevents the pathology in mdx mice, a murine model for the disease. Furthermore, myoblast transplantation with co-injection of laminin-111 improves the therapy efficacy in mdx mice. Partial proteolysis of matrix extracellular proteins, as laminin-111, produces peptides called matrikines and many of them are able to modulate cell activity. AG73, SIKVAV and C16 are peptides derived from laminin-111 that has diverse biological functions in vivo and in vitro assays. In this study, we evaluated the effect of LAM-111-derived peptides on myoblast's adhesion, migration, differentiation and proliferation. To investigate cell adhesion, C2C12 cell line was incubated in a plate with 1μg of LAM-111 or its derived peptides with BSA blocking. The peptide AG73 was able to promote the myoblast adhesion as LAM-111. Next, we evaluated the myoblast migration using Transwell assay, the LAM-111 or peptides were incubated 1, 50 or 100 μg in the bottom chamber. On the upper chamber, we incubated C2C12 with serum free medium. After 16 hours, we counted the cells in the lower part of membrane. The peptide AG73 and LAM-111 improved myoblast migration as the positive control with serum. To evaluate the myogenic differentiation, we seeded C2C12 cell line in a plate coated with 10μg LAM-111 or peptides and we induced differentiation until the third day. Interestingly, the peptide AG73 enhanced the miotube's diameter but not the other conditions. Next, we cultivate the myoblasts in a plate coated with 1 or 10μg of LAM-111 or peptides for evaluation of proliferation after 24 and 48 hours. We didn't observe any differences between the groups. In conclusion, the AG73 peptide improved adhesion, migration, differentiation and proliferation of C2C12 myoblast. Thus, this peptide may contribute on efficacy of cell therapy in DMD.

Palavras-chaves: Duchenne, matrikines, treatment, muscle, myoblast

12.020 POTENTIAL USE OF EXTRACELLULAR CYTOCHROME c AS A SPECIFIC AND EARLY MARKER OF CELL DEATH IN ANTITUMOR THERAPY

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Resumo

Introduction: Cytochrome c is a highly preserved intramitochondrial hemeprotein, allocated on the outer face of the internal mitochondrial membrane, involved in the transport of electrons coupled to the generation of ATP. When released in the middle intracellular triggers the activation of caspases, which culminates in the process of cell death by apoptosis. It was recently discovered that cytochrome c can by an unfamiliar mechanism transports from intra to extracellular during the cellular death process. The levels of this protein are measurable in human plasma and studies show that patients with neoplasms have high levels of this protein in the blood, especially after being subjected to chemotherapy treatment. We proposed to analyze the relation in the extracellular medium of cytochrome c and lactate-dehydrogenase, traditional marker of cellular lesion.

Objective: Quantify cytochrome c and lactate-dehydrogenase extracellular post induction of death cell in vitro.

Methodology: Cells culture of RbAOSMC (rabbit aortic smooth muscle) and K562 (chronic myeloid leukemia) were exposed to the chemotherapeutic agents Actinomycin D and Doxorubicin for 48 hours. After 48 hours, the culture supernatant was removed. The first cell fraction was subjected to viability assay by reduction of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium-bromide), the cell fraction was subjected to viability assay by reduction of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium-bromide). For the quantification of cytochrome c extracellular and lactate dehydrogenase obtained from the culture supernatant, Elisa sandwich methods and the enzymatic method, respectively, were used. Analysis of the mechanism of death was performed using the annexin V-FITC and propidium iodide markers by flow cytometry.

Results: In both cellular lines, actinomycin d promoted the release of lactate dehydrogenase and cytochrome c to the extracellular medium, and the concentration of lactate dehydrogenase was always inversely proportional to cell viability. For doxorubicin, both cellular lines had quantifiable lactate dehydrogenase, but only for tumor cells (k562) cytochrome c was quantifiable. Both doxorubicin and actinomycin D potentially induced cell death by apoptosis.

Conclusion: The results showed important differences regarding the levels of cytotoxicity induced by different chemotherapeutic drugs. The different patterns of release cytochrome c and lactate dehydrogenase suggest that employment of lactate dehydrogenase as a marker of cellular injury should be used with caution, because they are not necessarily linked proportionally to the level of cellular damage resulting from the action of chemotherapy. The extracellular cytochrome c has proved a marker earlier and more specific to the process of death of tumor cells than lactate dehydrogenase.

Funding: CNPq and FAEP UMC.

Palavras-chaves: EXTRACELLULAR CYTOCHROME c, apoptosis, neoplasms