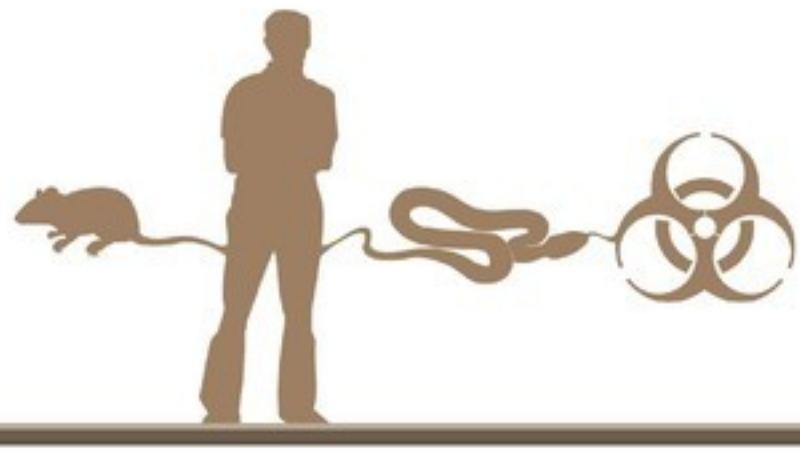
7. Cell Biology and Genetics



XIV Reunião Científica Anual do Instituto Butantan
05 a 07 de Dezembro 2012, São Paulo, SP

7.01 Rabies glycoprotein expression using pseudoparticles virus

Bernardino TC, Rezende AG, Suarez SFP, Paschoal JFB, Pereira CA, Astray RM, Lemos MAN Jorge SAC
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Introduction: The rabies is caused by a virus of genus Lyssavirus, family Rhabdoviridae. This virus is able to infect a lot of species of mammals. The rabies virus glycoprotein (RVGP) is a transmembrane protein that mediates the adhesion to cell receptors and the virus entry in the host cell. The RVGP has been recognized as an antigen able to induce immune response against rabies virus. **Objectives:** The aims this study is establish the expression system of RVGP, using viral pseudoparticles (ppHCV). This ppHCV contains the E1 and E2 proteins of Hepatits C virus, the GAG and POL proteins of murine leukemia virus (MLV) and carries the RNA coding rabies virus glycoprotein (RVGP). **Methods:** We constructed 3 vectors of interest (pCMVGag/Pol, pCMVE1E2 e pCMVRVGP), these were transfected in HEK 293T cells, 48 h after the transfection the supernatant was collected to obtain the recombinant pseudoparticles (ppHCV-RVGP). These ppHCV-RVGP were used to infect Huh 7.0 cells, 48h after the infection, the cell were lysed to measure the RVGP produced by ELISA. **Results and Discussion:** The ppHCV-RVGP presence was confirmed by PCR conventional and the quantification those pseudoparticles were done through quantitative PCR, we observed that pseudoparticles were generated correctly, carrying the mRNA-RGVP. The RVGP was found in infected cells.

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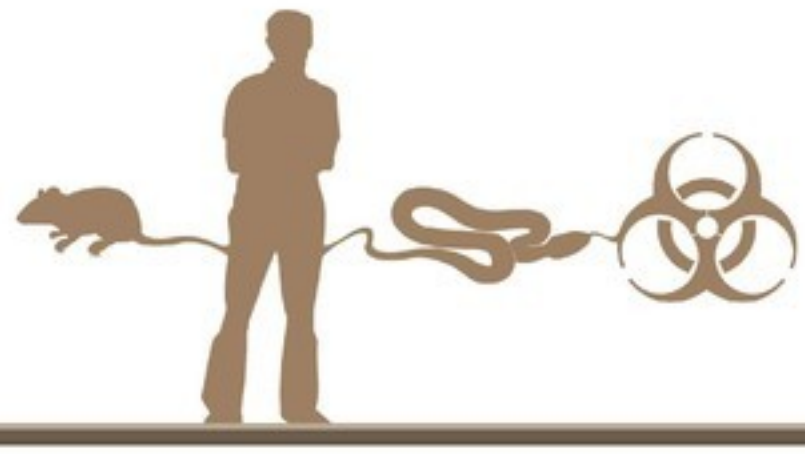

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05 a 07 de Dezembro 2012, São Paulo, SP

7.02 DNA replication analysis of a 347kb fragment from *T. brucei* chromosome 1

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Introduction: In order to duplicate the whole genome in a short space of time during S phase of the cell cycle, many start points of DNA replication (the origins of replication) are needed. In human cells is known that there are about 30 to 50 thousands origins of replication activated at S phase. **Objectives:** In *Trypanosoma brucei* little is known about DNA replication and we are trying to understand its replication pattern. **Methods:** Using the SMARD (Single Molecule Analysis of Replicated DNA) technique we were able to analyze the DNA replication pattern of a 347 kb fragment from chromosome 1 of *T. brucei* that is 1 Mb in length. **Results and Discussion:** We found that the 347 kb fragment from chromosome 1 can be replicated by at least three different origins of replication (named Ori 1, Ori 2 e Ori 3); two of them outside and one origin of replication inside this analyzed fragment. Ori 1 is found inside the 347 kb fragment from chromosome 1 while Ori 2 is downstream and Ori 3 upstream to Ori 1. These three origins are not always activated at S phase and we could observe different combinations of activation of these three origins in order to replicate this fragment. Also based on the molecules analyzed we could suggest that the replication speed rate is about 5 kb/min. These results are just the beginning to understand the replication profile of chromosome 1. However we already can conclude that the *T. brucei* DNA replication is similar to human cells where there are many origins, but not all of them are activated at S phase. And the speed rate replication in *T. brucei* (5kb/min) is similar to mammalian cells (2-3 kb/min).

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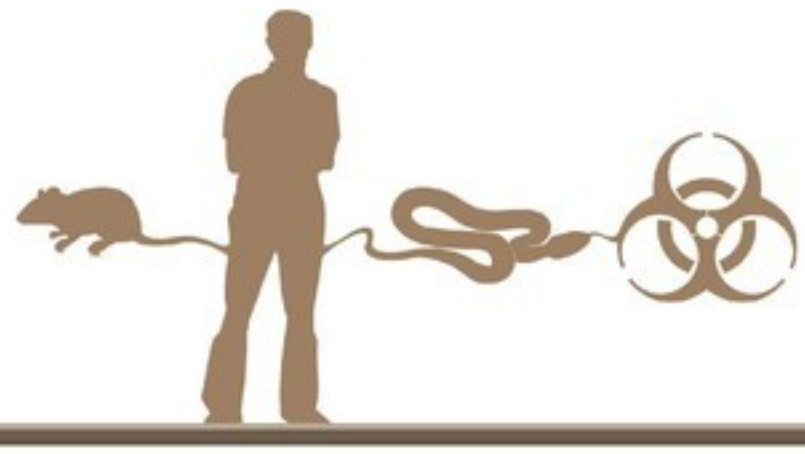

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7.03 Early brain development harbouring pluripotent cells niches

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Introduction: The process of early developmental tissue and morphogenesis is orchestrated by pluripotent and germinative primordial cells. This cells are native of niches until pre- implanted embryo (7, 5 d.d.). Pluripotent/germ cell niche provide expression of specific transcription factor, such as Oct3/4, Nanog, Sox2, responsible for self-renewal capacity and pluripotency. Moreover, primordial germ cells present in epiblast layer migrated during development into all tissues and contribute to ontogenesis. Believes that these cells harboring in adult tissues and contribute to adult niche formation. However, the brain, with its complexity structure and home diversity of cell types, was considered an exception by long time. Recent studies to changed this evidence through discovery of adult neural stem cell niches (NSCn) into subventricular zone (SVZ). Subventricular zone is located the walls of the lateral ventricles, contains a subpopulation of cells with astroglial properties as NSCs, giving rise to intermediate progenitors. **Objectives:** We aimed to identify pluripotent and germ cells in the brain during development of embryos/fetuses of mice of strain 129. In addition, adult brains were used in a comparative way. **Methods:** For this propose we used pluripotency antibodies such as Oct4, NANOG, SSEA3 and primordial germ cell marker (fragilis). Furthermore NSC antibodies such as nestin, glial fibrillary acidic protein (GFAP) and vimentin were evaluated. **Results and Discussion:** In early brain development (anterior neuromeres) had cells imunopositive to Oct4, SSEA-3 and fragilis. While in the fetus with 12 and 15 days of development few cells were imunoreative to factor transcription Oct4 in brain vesicles (prosencephalon, mesencephalon, robencephalon) and SVZ respectively. In contrast to more advantages fetal stages (F18 .d.d) and adult not have cell imunopositive to pluripotent and germinative primordial cells markers. Additionally SVZ of fetal period (fetus with 15 and 18 days of development) had the pattern of adult SVZ such as nestin, vimentin and protein fibrilar glial. These results can contribute with information about the nature of adult NSCs and microenvironment that surrounds them, and the complex process of neurogenesis.

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7.04 Effect of a Phospholipase A_2 isolated from the venom of *Crotalus durissus ruruima* in macrophage function

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Introduction: The venom of *Crotalus durissus ruruima*, a subspecies found at cerrado areas of the Roraima state, presents coagulant and myotoxic activities and contains high levels of crotoxin. From this venom, was isolated a FLA_2 (CB*r*) with high degree of identity with the *Crotalus durissus terrificus* FLA_2, which has been shown to activate macrophage functions, including formation of lipid bodies (LBs). These lipid rich organelles are relevant for lipid metabolism, synthesis and release of inflammatory mediators. Moreover, formation of LBs depends on the scaffold protein perilipin 2 (PLIN2), a member of the PAT family. However, the effects of the CB*r* in macrophages are unknown. **Objectives:** To investigate the effects of CB*r* on isolated macrophages, evaluating: i) LB formation, ii) cytosolic distribution of PLIN2 and iii) protein expression of ciclooxygenases -1 (COX-1) and -2 (COX-2). **Methods:** Thioglycolate-elicited macrophages from male Swiss mice (BI-Ethical Committee 896/12) were incubated with RPMI (control) or non-cytotoxic concentrations of CB*r* for selected periods of time (1 - 24 h). LB formation was quantified by staining with osmium tetroxide (1%) followed by phase contrast microscopy analysis. PLIN2 distribution was analyzed by immunofluorescence assay and COX-1 and -2 protein expression by Western blotting. **Results and Discussion:** Incubation of macrophages with CB*r* at concentrations of 3.25 to 13 µg/mL, but not 1.6 µg/mL, caused a marked increase in LB numbers from 1 up to 24 h, with maximum observed at 3 h with 6.5 µg/mL. Moreover, CB*r* (6.5 µg/mL) increased PLIN2 cytosolic pools, co-localized to LBs at 3h incubation. In contrast, CB*r* neither induced COX-2 protein expression nor affected COX-1 constitutive expression. These data indicate that CB*r* is able to induce LB formation and recruitment of PLIN2 in macrophages. Recruitment of PLIN2 may be relevant to CB*r*-induced LB formation. Similarly to that observed the PLA_2 from *C.d. terrificus* venom, CB*r* does not induce expression of ciclooxygenases.

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7.05 Immature human dental pulp stem cells in the treatment of atherosclerosis in induced animal model

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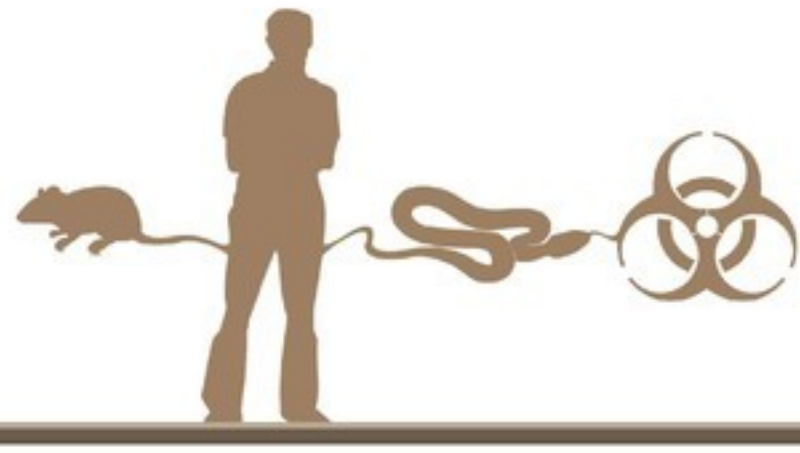
Introduction: Mesenchymal stem cells (MSCs) have anti-inflammatory and immunosuppressive effect from tissue autocrine and paracrine stimulus, promising to be used in allotransplantation for the treatment of various inflammatory diseases. Furthermore, MSC have multiple therapeutic properties such as trophic activity, apoptosis inhibition, scarring suppression and angiogenesis stimulation. Equivalent to MSCs, Human Immature Dental Pulp Stem Cells (hIDPSC) were successful and intensively used into multiple essays for the treatment of muscular dystrophy, induced osteonecrosis of femoral head and corneal reconstruction. These cells present fibroblast-like morphology, retain characteristics of adult MSCs and pluripotent markers such as Oct3/4, Nanog, SSEA-3/4. In contrast, teratoma's formation has never been observed for hIDPSC. **Objectives:** Based on the therapeutic potential of hIDPSC, this project aims to analyze the anti-inflammatory effect of these cells into atherosclerosis-induced model of lagomorph. The purpose of cell transplantation is the regression of atherosclerotic lesions in the aortic arch, and may thus be an alternative for the treatment of human arteriosclerosis. **Methods:** The number of eight New Zealand rabbits had the atherosclerosis induced from high-cholesterol diet at 1% for 60 days. From the 30th day of experiment, the animals were divided into two groups: the first with four cell transplants of hIDPSC (weekly group), and the other with two transplants of hIDPSC (fortnightly group). Each experimental group consisted of three therapy animals and one control. In every intervention were transplanted $2x10^6$ hIDPSC diluted in 1ml of saline via endovenous injection in animals of group therapy and 1 ml of saline in the control group. The animals were euthanized on day 60 and their aortic arches were collected for histological and immunohistochemical tests. The presence of hIDPSC into aortic arch was evaluated by immunohistochemica using anti-human nucleus and anti-hIDPSC markes. The intimal thickening was analyzed by histological tests. **Results and Discussion:** After cell transplantation, anti-hIDPSCs and anti-human nucleus antibody were observed in the intimal layer of both groups' vases; and in middle layer of weekly group's aortic arch, showing the grafting of these cells at the site of inflammation. Histologically was observed a reduction of intimal thickening of aortas from the therapy animals of both groups. These results indicate a tropism of hIDPSCs for damaged aortic arch and, thus, we suggest that hIDPSC may be an alternative treatment for atherosclerosis. Still, more studies are needed to elucidate the anti-inflammatory effects of these cells in atherosclerotic lesions.



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7.06 β-defensin-like genes in Brazilian pitvipers

Corrêa PG[1], Machado T[1], Prieto-da-Silva ARB[2], Ferrarezzi H[1], Germano VJ[3], Gennari DPT[3], Oguiura N[1]
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Introduction: β-defensins are antimicrobial peptides (AMP) found in vertebrates. They constitute an important and conserved component of innate immunity with antimicrobial activities. These molecules are also reported in the venoms of sea anemones, snakes and platypus. In *Crotalus durissus terrificus*, it is known two peptides with β-defensin scaffold: crotamine, a small basic myotoxin, and crotasin, a gene expressed abundantly in several rattlesnake tissues, but scarcely in the venom gland. **Objectives:** Know the evolutionary history of β-defensin-like genes in Brazilian pitvipers. **Methods:** Genomic DNA from different species of *Bothrops, Crotalus* and *Lachesis* snakes were used as template. The primers were designed based on the signal peptide (SP) and 3'UTR, conserved sequences from crotamine and crotasin genes. The gene sequences were aligned with CodonCode Aligner3.7.1, the phylogeny analyzed by maximum parsimony using TNT1.1, and the reconciliation of gene and species trees was performed with Mesquite2.75. **Results and Discussion:** We obtained 13 different sequences from ten species of snakes. The genes presented two introns and three exons that code the SP and the mature β-defensin. The size of intron 1 varied greatly (from 477 to 2018 bp) whereas the intron 2 sequence had conserved size (~153 bp). The mature peptides (MP) had the six conserved cysteines of β-defensin family, basic amino acids residues, net charge from +2 to +11, a N-terminal glutamine as crotasin, a conserved glycine at ninth position and a C-terminal lysine. The gene organization was similar to crotamine, crotasin and other genes found in lizards and teleost fishes. The SP and introns sequences were more conserved than MP sequences. The K_a/K_s ratio showed an accelerated evolution on exon 2 that can increase the peptide variability and the animal protection against diverse pathogens. Phylogenetic analysis using complete gene sequences had a better resolution to recover the phylogenetic relationships among the species than only intron or exon sequences. The reconciliation of β-defensin-like gene trees and species trees allowed inferring the events of gene duplications and extinctions. The reconciled tree showed that the β-defensin-like genes had evolved by gene duplication from an ancestral sequence and a duplication event after rattlesnake speciation gave rise to crotamine toxin. The structure of β-defensin-like genes in Brazilian pitvipers is different from that found in mammals and birds, but is similar to that found in another reptile, the green lizard *Anolis carolinensis*. Our results suggest that the gene structure composed of three exons and two introns can be a pattern for the reptile group.

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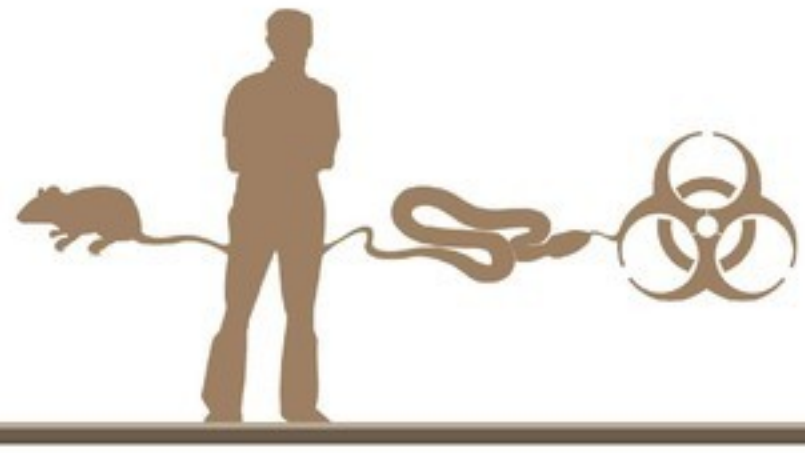

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7.07 Screening of citotoxic effect of crotamine in cancer cells

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Introduction: Crotamine is a major component of rattlesnake (Crotalus durissus terrificus) venom. This molecule is a small non-enzymatic basic polypeptide that once inoculated on host, causes paralysis of the hind limbs and extensive necrosis of muscle cells. On other hands, crotamine at low concentrations, is uptaken by proliferating cells via endocytosis mediated by binding to cell membrane heparan sulfate proteoglycan. In our laboratory was verified that crotamine exhibits citotoxic effect against mouse melanoma cells in vitro besides inhibiting tumor growth in vivo. **Objectives:** The aim of present study was to investigate the mechanism by which crotamine exerts its cytotoxic effect by following its uptake into proliferative human cancer cells mainly breast cancer (MCF-7; MDA MB 231; SKBR3) ,melanoma (SK-Mel-28, Sbcl2, Mel-85) and leukemia cells (Jurkat and GRANTA). **Methods:** To determine the half maximal inhibitory concentration (IC50) the classical MTT test was performed. The effect of crotamine on inducing cell death, on cell cycle and on mitochondria potential was measured through cytometry. Nuclear and cytoplasmatic morphologic changes were accessed by fluorescence microscopy. **Results and Discussion:** The results obtained here, corroborates previous studies conducted in our laboratoy and shown that crotamine exhibits a strong and seletive capacity to inducing cell death targeting mitochondrial pathways in melanoma cell lines since the cytotoxicity to breast cancer and leukemia was more modest. This finding suggests that crotamine can have selective effect in melanoma cells. Given that crotamine may act as a selective cytotoxic agent in melanoma cells, the understanding of its mechanism is still under investigation by our group.

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7.08 The study of potential pathways of proliferation, cell death and maturation of stem cells of yolk sac bovine and its relation to embryonic metabolism

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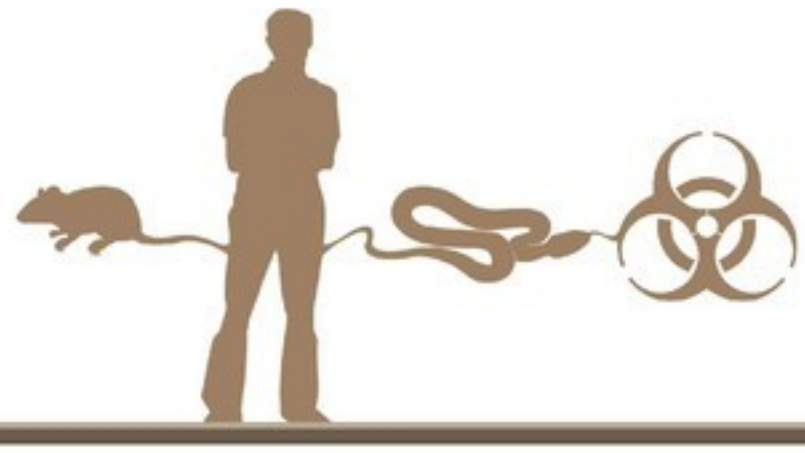
Introduction: It is very difficult to have a clear and homogeneous idea of the embryo metabolism. In fact it may vary from one species to another and also according to the embryonic stage: i.e. before and after genomic activation. The yolk sac (YS) is present in all vertebrate species, and plays important roles in the developing embryo and initial place of origin of stem cells (SC), until the arrival of the placenta. **Objectives:** the study of metabolic potential in YS bovine is related with the normal development of embryonary metabolism. **Methods:** 25 YS of embryos from cattle slaughterhouses in the state of Goiás were studied in the following groups: Group I (23 - 27d), Group II (28 - 32d), Group III (33 - 37d), Group IV (38 - 42d) and Group V (43 - 47d), Group VI (48-52). We used flow cytometry for determination of cell death by expression of Annexin V/PI, Caspase 3, r-TNF, Cytochrome C, electric potential of the mitochondria, synthesis of HSP 47, receptors involved in angiogenesis VEGF-R1 and expression of markers involved in the maturation, differentiation of SC. For experiments of metabolome we used solid state nuclear magnetic resonance (NMR) spectra were acquired on a Bruker Avance 9.4T (400.13 MHz for ^{1}H) spectrometer equipped with a 4 mm triple channel DVT MAS probe, at room temperature, spin at 10 kHz, using spinal64 as decoupling technique. **Results and Discussion:** we finding the important metabolites such as, alanine, myo-inositol, taurine, choline, glycerophosphocholine, cadaverine, glutamate, glutamine, lactate, hydrouracile, creatine, creatinine, aspartate and lysine. These metabolites are related both to the process of proliferation as myo-inositol, and cellular death as choline, both are in a relationship inversely proportional. The only metabolite found in group I is alanine and serving a function of íon transport ammonia until the formation of renal system that is important for survival of the embryo. Adult SC have demonstrated significant levels of primitive characteristic of pluripotency markers (Oct-3/4 e NANOG). Furthermore, YS cells showed significant marking marker CD133, which gene is essentially expressed by the hemangioblast. These characteristics support the hypothesis that YS cells exhibit markers of a broader differentiation potential that should have been lost in stages well before those who have the same profile and maybe this would change if they were subjected to culture conditions, but would not change the fact that the YS is a source of interesting and enigmatic cells, with potential use in various fields of research.

Supported by: CNPq



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7.09 Lipid body formation induced in macrophages by a phospholipase A_2 (CB) from *Crotalus durissus terrificus (C.d.t)* venom and the signaling proteins involved

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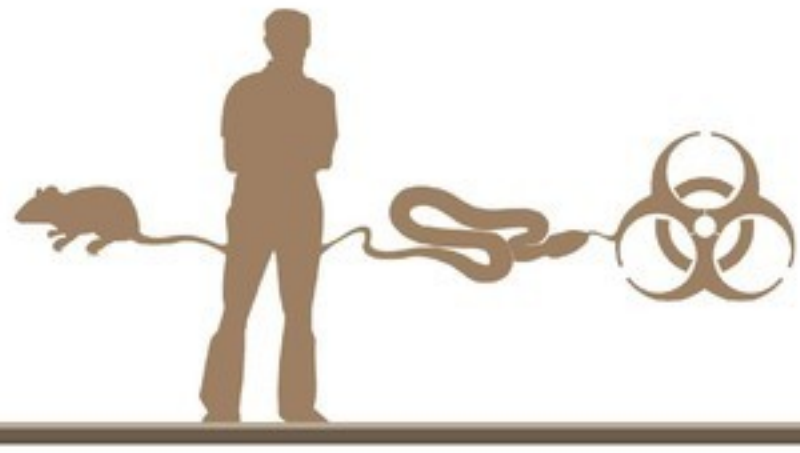
Introduction: CB is a subunit of crotoxin, the main component of *C.d.t* venom. CB is myotoxic, neurotoxic and affects diverse functions of macrophages (MΦs). Upon activation MΦs exhibit an increased number of lipid bodies (LBs), which are relevant organelles for lipid metabolism and synthesis of inflammatory mediators. **Objectives:** To investigate the effects of CB in MΦs, evaluating: *i)* LBs formation and the signaling proteins involved in this effect; *ii)* ultrastructural alterations of MΦs; *iii)* PLIN2 distribution and protein expression *iv)* PGE_2 and PGJ_2 distribution. **Methods:** Murine thioglycolate-elicited MΦs were incubated (1-12h) with either RPMI (control) or non-cytotoxic concentrations of CB (CEUAIB 846/11). LBs formation was evaluated by osmium tetroxide staining and contrast phase microcopy analysis. Ultrastructural modifications were evaluated by transmission electron microscopy, and signaling proteins involved in CB-induced effect by pharmacological interferences. PLIN2 protein expression was determined by W.blotting and distribution of PLIN2, PGE_2 and PGJ_2 by imunofluorescence assay followed by confocal microscopy analysis. **Results and Discussion:** Incubation of MΦs with CB (0.2 to 0.8 μM) significantly increased LBs numbers (1 up to 12 h). Ultrastructural analysis revealed the presence of weakly electrondense LBs with some LBs in association with enlarged RE. Moreover, CB increased PLIN2 recruitment and protein expression at 1, 3 and 12h incubation. Pre-treatment of MΦs with H7 (PKC inhibitor), LY294002 (PI3K inhibitor), FIPI (PLD inhibitor), JNK II (JNK inhibitor) or U0126 (MEK1/2 inhibitor) abolished CB-induced LBs formation. BEL (iFLA_2 inhibitor) reduced, but U73122 (PLC inhibitor), SB202190 ($p38^{MAPK}$ inhibitor), PD98059 (ERK1/2 inhibitor), herbimycin A (PTK inhibitor) or Pyr-2α (cFLA_2 inhibitor) treatments did not affect CB-induced LB formation. Increased PGE_2 and PGJ_2 pools colocalized to LBs were detected in CB-stimulated cells. These data indicate the ability of CB to induce LB formation and both recruitment and protein expression of PLIN2 in MΦs. CB-induced LB biogenesis depends on PLD, PKC, PI3K, MEK1/2, JNK and iFLA_2, but not on PLC, PTK, $P38^{MAPK}$, ERK1/2 nor cFLA_2 pathways. ER may be sites of LBs formation. PGE_2 and PGJ_2 colocalization to LBs suggests LBs as sites of storage and/or synthesis of these prostanoids upon CB stimuli. Due to the modulatory roles of PGE_2 and PGJ_2 in inflammatory processes, LBs may be relevant components of immunomodulation displayed by CB during inflammatory settings.

Supported by: CAPES, CNPq and INCTTOX



7.10 Characterization of angiogenesis´modulated activity by a SVMP - the Bothropasin

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Introduction: Angiogenesis occurs with the degradation of the basal membrane by proteolic enzymes wich are secreted by the endothelial cells involved in the process and their migration and proliferation. In the literature ADAMs(A Disintegrin And a Metaloprotease) have proven modulatory actions in angiogenesis mediated as much by their catalytic activity as by the adhesive properties of their DC domains, and ADAMs hold striking functional and structural similarities to snake venom metaloproteases. These facts provided us with the theoricalto further explore the role(s) of bothropasin (a well-known SVMPs from *Bothrops jararaca)* and of its isolated domainsin modulating endothelial cells migration, proliferation and adhesion as the experimental hallmarks of angiogenesis. **Objectives:** In the present work we show the characterization of bothropasin´sand its domainsactions upon cell proliferation, migration, adhesion and in vivo angiogenesis. **Methods:** Huvec-CS was assayed for mitogenic activity, migration potencial and adhesion to fibronectin, collagen I, collagen IV, laminin and vitronectin, MMPs secretion and integrin activation in the presence of different control and experimental treatments. **Results and Discussion:** Our results shows that in endothelial cells, bothropasin (Bt) is mitogenics and pro-migratory, these actions do not require enzyme´s catalitic activity as proved by the effects of chemical inactivation (EDTA treatment) of the enzyme or by the effects of purified DC domain. We also show that bothropasin acts via commom pathways to the ones triggered by the bFGF and VEGF (Rho-A , MMPs induction, and beta integrins induction); In adhesion to basal membranes components assays a dose-dependent competition to collagen I suggests an action by alpha2beta1 integrin. The "in vivo" assays in chick chorioallantoic membrane (CAM assays).show that embrionic angiogenesis induced by Bt and BtI is significant but accompanied by a hemorrhagic action. The Dc domain, and more specifically the HCR region of bothropasin´s C domain, induces angiogenesis more efficiently without triggering any the hemorrhagic action.

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7.11 Characterization of Alphastatin C – an anti-angiogenic peptides derived from the proteolysis of fibrinogen by Bothropasin

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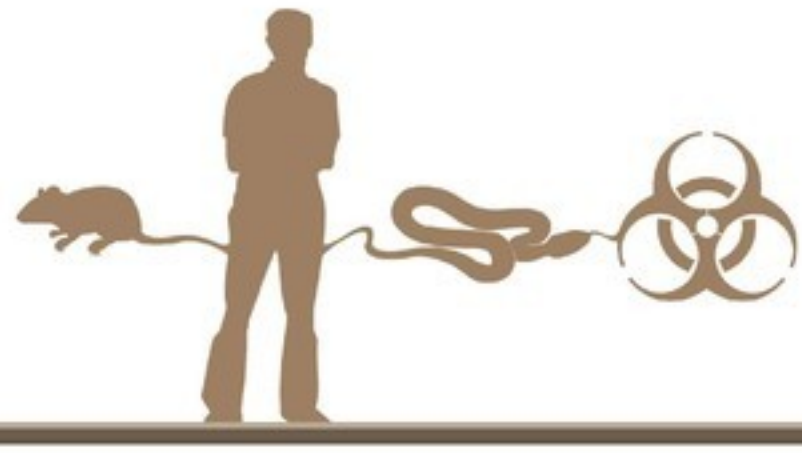
Introduction: Most of the endogenous modulators of angiogenesis described to date are criptical fragments of otherwise structural proteins present in the basal membrane or in the blood plasm that directely contact endothelial cells, *i.e.*Endostatin is derived from Collagen XVIII, Tunstatin is derived fromColagen XVIII, and the Endorepelin is derived from Perlecan. The ADAMs responsible for this kind of physiological processing hold functional and structural similarities to snake venom metaloproteases. These facts provided us with the theoretical basis for the screening of new angiogenesis modulators produced as a byproduct of envenomation, as result of the proteolytic activity of SVMPs upon extracellular membrane and plasma components. **Objectives:** In the present work we show a proof of concept of the new rational approach for angiogenic modulator's characterization proposed in the introduction. We describe the biological activities of the peptidic products of the proteolysis of Fibrinogen by Bothropasin. Our main goal being the identification of peptides potentially active in angiogenesis modulation, we have experimentally segmented this process into three steps: proliferation, migration, and adhesion. We will show modulation of the three events in endothelial and smooth muscular vascular cells. **Methods:** The following cell lines: HUVEC-CS, VSMC were assayed for mitogenic activity, migration potential and adhesion to fibronectin and collagen in the presence of or abscence of Bothrostatin-hidrolysis-Fibrinogen-derived-peptides. As a routine procedure the products of the digestion of Fibrinogen by Bothropasin were precipitated overnight in acetone and quantified using Fluorescamine by INVITROGEN, serial dilutions were employed in biological activity assays as well as HPLC fractions and major peptide components of the active fractions were identified by mass spectrometry, synthetized by solid phase and tested in an additional round of functional assays. **Results and Discussion:** Our results show that the major peptide in the HPLC active fraction is an RGD containing peptide contained in the C-terminus of Fibrinogen alpha chain. This synthetic peptide recapitulates the whole fraction inhibitory activity in a variety of biological assays with different cell lines. e.g. In fibroblasts, HUVEC and smooth muscle vascular cells these peptides inhibit bFGF triggered mitogenesis up to 50%. We will also present quantitative results concerning the activity of these peptides on cell migration as monitored by scratch assays and chemotaxis experiments, and their activity in cell adhesion to specific substrates as laminin, fibronectin and collagen as well as in vivo angiogenic assays in chick chorioallantoic membrane (CAM assays).

Supported by: FAPESP and Bussolegrants



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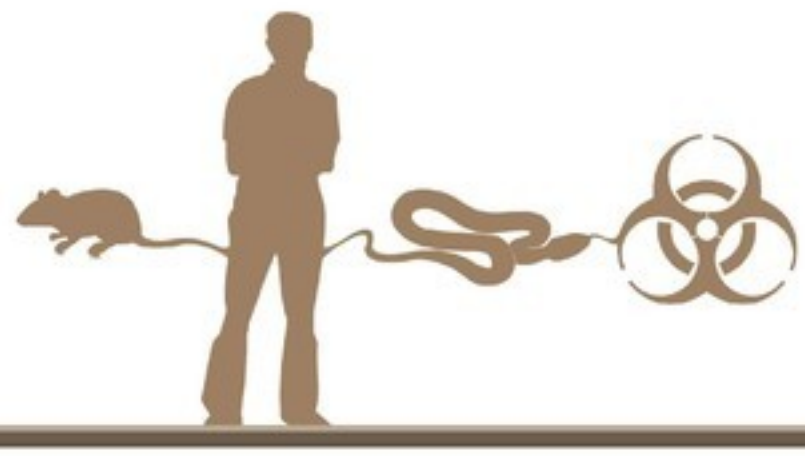
7.12 Intramitochondrial localization of HPV E6 oncoprotein

Kavati EA[1], Canali RA[1], Oliveira HB[1], Marigliani B[1], Sakauchi D[1], Teixeira CFP[2], Armbruster-Moraes E[1,3], Cianciarullo AM[1]
[1]Laboratory of Genetics, Butantan Institute, Brazil; [2]Laboratory of Pharmacology, Butantan Institute, Brazil; [3]Obstetric Clinic of Clinical Hospital, FMUSP, Brazil

Introduction: Every year, approximately 300,000 women die worldwide victims of cervical cancer. The presence of HPV's DNA can be detected in 99.7% of them, being considered its main cause. The oncogenic potential of HPV is based in the capacity of oncoproteins like E6 and E7 to change cellular cycle leading to uncontrolled development, growth, immortality and malignancy. The anti-apoptogenic functions of E6 are known by its interaction with several cellular targets like p53, PDZ proteins, Bak, FAK, TERT and others. Bak is a member of Bcl-2 family proteins found in mitochondrial membrane and when activated allow the formation of pore induced apoptosis. The study of intracellular localization of proteins is important to understand their functions, activities and acting within cellular context. **Objectives:** To study the intramitochondrial localization of the HPV E6 oncoprotein and its possible ligands. **Methods:** HeLa, SiHa and CaSki cells, naturally transformed by HPV, were cultivated and analyzed by immunofluorescence and immunocytochemistry to determine the intracellular localization of E6 protein. Immunofluorescence was also performed with specific mitochondrial stain, MitoTracker®. In addition, mitochondrial isolation using QProteome Mitochondria Isolation Kit for after lysis and co-immunoprecipitation with specific antibody anti-E6, then western blotting and mass spectrometry were performed to determine the ligand to E6 oncoprotein inside the mitochondria. **Results and Discussion:** Due to their cellular targets, E6 has being described as a cytoplasmic protein, but immunofluorescence and immunocytochemistry assays show the presence of E6 co-localized with mitochondria. Thus, mitochondrial isolation was used and its product was lysated and the co-immunoprecipitation with specific antibody anti-E6 showed, the presence of proteins with approximately 55, 28, 21, 17 and 16 kDa, in electrophoresis analysis. They were analyzed by western blotting and recognized by anti-Bak, anti-Bax and anti-E6 specific antibodies. After these proteins were digested by trypsin, mass spectrometry was used to confirm these possible target ligands of E6 oncoprotein. Therefore, the present study demonstrates for the first time the presence of the E6 oncoprotein within mitochondria and their ligands. New studies are being carried out to better understanding and mapping the activity of E6 and their interaction with Bcl-2 proteins in intramitochondrial localization.

Supported by: FAPESP, CAPES, FUNDAP-SES-SP, Butantan Foundation and Butantan Institute



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7.13 Scaling-up of immature dental pulp stem cells

Lizier NF, Kerkis A, Oliveira CF, Madeiro DS, Kerkis I
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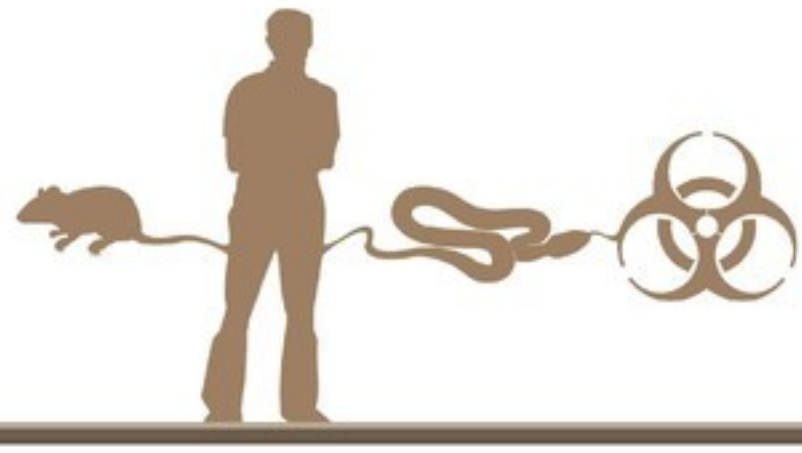
Introduction: Dental pulp (DP) can be extracted from child's primary teeth (deciduous), whose loss occurs spontaneously by about 5 to 12 years. Thus, DP presents an easy accessible and painful source of stem cells without ethical concerns. Substantial quantities of stem cells of an excellent quality and at early (2-5) passages are necessary for clinical use, which currently is a problem for use of adult stem cells. **Objectives:** We aimed to establish a new method based on tissue explant culture and mechanical (non-enzymatic) transfer in order to obtain a long-term culture of DP providing substantial quantities of immature stem cells from dental pulp (IDPSC) without aberrant genetic and biologic changes. **Methods:** We evaluated such characteristics as: morphology (light and electronic microscopy), expression of specific MSC-phenotypes and ES cell proteins and genes (immunofluorescence, FACS and RT-PCR), karyotype, growth rate and differentiation ability of IDPSCs just after DP extraction (early population, EP) and after multiple DP transfer (late population, LP). Some of these parameters were evaluated after cryopreservation and with culturing IDPSCs in three distinct culture media. The used of antibody against BrdU incorporated in DP just after plating and three days after DP cultivation gave insight into the mechanism of IDPSCs generation by explant culture. Additionally, to distinguish SCs in DP, immunohistochemical staining against nestin, vimentin, Oct3/4 and STRO-1 has been performed. **Results and Discussion:** DPs were cultured generating stem cells at least during six months through multiple mechanical transfers into a new culture dish every 3-4 days. No changes, in both EP and LP, were observed in morphology, expression of stem cells markers, chondrogenic and myogenic differentiation potential, even after cryopreservation. Six hours after DP extraction and *in vitro* plating, rare BrdU positive cells were observed in pulp central part. After 72 hours, BrdU positive cells increased in number and were found in DP periphery, thus originating a multicellular population of stem cells of high purity. Multiple stem cell niches were identified in different zones of DP, because abundant expression of nestin, vimentin and Oct3/4 proteins was observed, while STRO-1 protein localization was restricted to perivascular niche. Our data demonstrated that IDPSC can be easily isolated and successfully expanded *in vitro*, while maintain their undifferentiated status preserving their properties even after cryopreservation. Our finding is of importance for the future of stem cell therapies, providing scaling-up of stem cells at early passages with minimum risk of losing their "stemness". These stem cells represent a biotechnological product ready to go throughout stem cell-based therapy market.

Supported by: FAPESP



7.14 Isolation and characterization of cancer stem cells from patients with Li-Fraumeni syndrome and Li-Fraumeni-*like* syndrome

Mambelli LI[1,2], Achatz MIW[1], Hainaut P[3], Kerkis I[2]
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Introduction: Li-Fraumeni syndrome (LFS), an inherited syndrome associated with germ line mutations in TP53. is characterized by high risk of multiple, early cancers. In Brazil, a variant form of LFS is exceedingly frequent due to a widespread founder TP53 mutation, p.R337H, detected in about 0.3% of the general population in Southern Brazil. This mutation occurs in p53 oligomerization domain and its effect on p53 oligomerization is supposed to be dependent unpon pH conditions. **Objectives:** The aim of the present study was to isolate and to characterize cancer stem cells from patients with Li-Fraumeni Syndrome and Li-Fraumeni-*like* Syndrome. **Methods:** We have isolated and characterized Cancer Stem Cells from tumors of p.R337H carriers. After informed consent, surgical resection fragments were dissociated and brought in culture. Adherent cells and spheroid were derived from different tumor types. **Results and Discussion:** Spheroids derived from a breast cancer (BC) were further analyzed to demonstrate positive immunolabeling for CD44, CD24, Oct4 and Sox2 antibodies. Time-lapse videomicroscopy showed rapid growth, frequent asymmetric division and absence of senescent phenotypes for least 17 passages. At confluence, some cells spontaneously differentiated into adipogenic and muscle–like cells. Undifferentiated cells were further submitted to osteogenic differentiation being positively stained by von Kossa. Colony-forming units (CFU) assay was performed in duplicate using adherent cells. After 15 days in culture, by changing the medium every three or four days, 1000 cells were able to form 449 and 442 colonies, respectively. Floating and adherent spheroids contained self-renewing cells capable of long-term proliferation without losing their primary characteristics. These properties are similar to those of stem cells with intactivated p53 protein. However, treatment of adherent cells with the DNA damaging agent doxorubicin elicited p53 accumulation, p21 induction, cell cycle arrest at low doses of Dox and cell death/apoptosis at higher doses. These results are consistent with the notion that p.R337H may retain a quasi-normal p53 function in response to stress while expressing properties similar to mutant p53 in long-term CSC culture. These p.R337H CSC may provide a very useful model for studying the defects underlying inherited cancer in p.R337H carriers.

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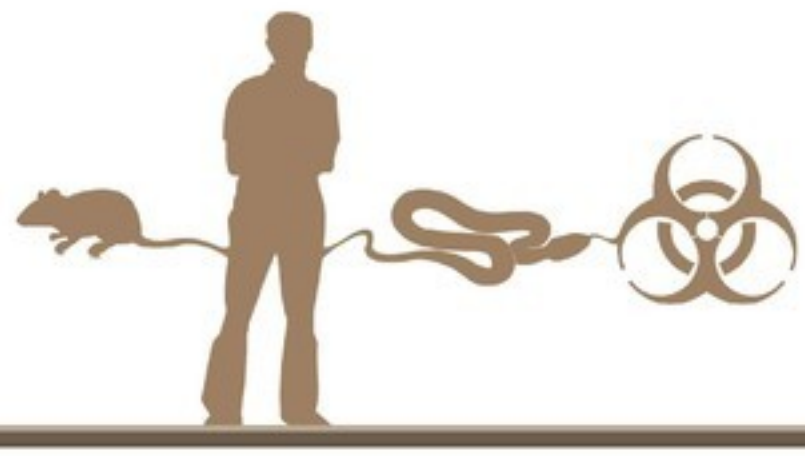

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7.15 Improvement of SL-pre-SSUrRNA molecules detection in *Leishmania (Leishmania) amazonensis* by quantitative RT-PCR

Mayer MG[1], Floeter-Winter LM[2]
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Introduction: SL addition *trans*-splicing is described as a fundamental processing for the generation of mature mRNAs in trypanosomatids. However, recently, we have described SL addition *trans*-splicing of pre-SSU rRNA at the 5'ETS region of four different trypanosomatids: *Trypanosoma cruzi*, *Trypanosoma brucei*, *Chrithidia fasciculata* and *Leishmania (Leishmania) amazonensis*. We have also shown that SL-5'ETS hybrid molecules are present in low amounts (10^3-10^4 molecules/ 5μg total RNA) in *Leishmania (Leishmania) amazonensis* promastigote cells. This low number of processed molecules can be raised up by treatment of promastigotes with 5-FU (5-fluorouracil), an inhibitor of the rRNA exosome decay pathway, suggesting that these hybrid molecules take part in 5'ETS pre-rRNA spacer region degradation. **Objectives:** Improve detection of SL-5'ETS hybrid molecules by quantitative RT-PCR in 5-FU exosome inhibition assays. **Methods:** *Leishmania (Leishmania) amazonensis* were treated with increasing doses of 5-FU (0, 4, 16, 32 and 64 μM). Total RNA were extracted by the Trizol method and depleted of DNA by DNase RNase-free treatment. RNA (5μg) was reverse transcribed using specific primer, and 1/20 of the reaction was then used in qRT-PCR. We used the SYBR Detection System (Appied Biosystems, USA) according to manufacturer's instructions in a 50μl final reaction volume and 7300 System (Appied Biosystems, USA). We tried a three-step PCR approach instead of a two-step approach used in initial experiments: The reactions were initially maintained at 50°C and then held at 94°C for 10 min. A total of 50 cycles were then performed, consisting of a denaturation step at 94°C for 30s, an annealing step at 50° for 30s, and an extension step at 72°C for 30s. Known amounts of the cloned DNA products were diluted to produce a standard curve. Statistical significance was determined using Student's *t* test. **Results and Discussion:** Although three-step protocol is a longer procedure than the two-step protocol, our results show that three-step protocol was more accurate than the two-step protocol, and should be the choice method for the detection of low amount SL-5'ETS hybrid molecules. These results also certify the previous ones which suggest that SL-5'ETS molecules participate in the exosome degradation pathway.

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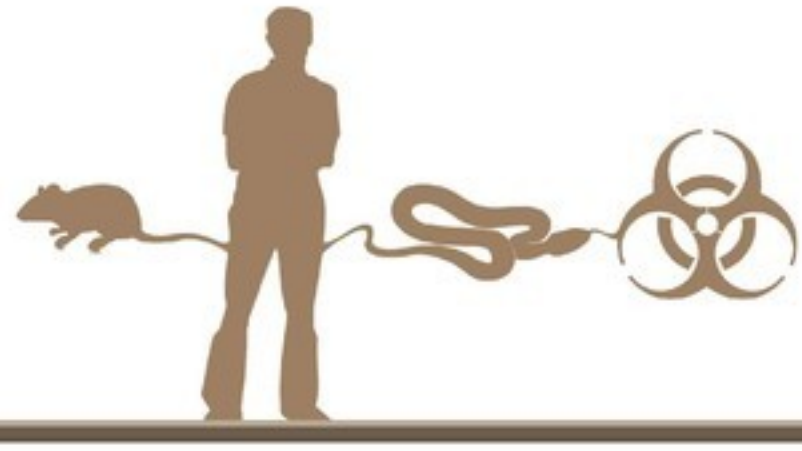
7.16 Phylogenetic classification of a new putative bovine Papillomavirus type associated with skin lesions in cattle

Melo TC[1,2], Mazzuchelli-de-Souza J[1,3], Diniz N [1,3], Araldi RP[1], Assaf SLMR [1], Sakata ST[1], Ruiz RM[1], Carvalho RF[1], Beçak W[1]; Stocco RC[1,3]
[1]Laboratório de Genética, Instituto Butantan; [2]Programa de Pós-Graduação em Biologia Estrutural e Funcional; UNIFESP; [3]Programa de Pós-Graduação Interunidades em Biotecnologia, USP

Introduction: Bovine papillomaviruses are recognized as causal agents of benign and malignant tumors in cattle. BPVs have a double-stranded circular DNA genome from 8Kb, its open reading frames is divided in two regions: early and late. 12 types of BPV were identified, classified in the genera - *Delta, -Xi,* and *-Epsilon papillomavirus,* with the exception of BPV-7, classified apart. **Objectives:** Determine the phylogenetic position of the BPV/JN, detected in the State of São Paulo, Brazil, and to evaluate the clinical and histological characteristics of the animals infected by this new putative type. **Methods**: *Animal selection:* four Simmental cows, with cutaneous papillomatosis were selected. Warts were collected in buffer for DNA extraction. *Histopathologica:* Papilloma samples were fixed in 10% formalin, embedded in paraffin, sliced into 5um sections and stained with hematoxylin and eosin. *DNA extraction and PCR*: Collected papilloma samples was subjected to DNA extraction, using *mini Kit Tissue Protocol* – Qiamp DNA. *Molecular identification:* Was made reaction of PCR using MasterMix (Promega) and degenerate primers FAP59/FAP64. *Cloning and Sequencing*: The PCR products were purified for sequencing and analyzed in gel electrophoresis stained with GelRed™. DNA the amplification products were cloned PCR 4.0 TOPO TA plasmid. Recombinant plasmids were used to transform competent DH5 α E. coli cells and the plasmids were subsequently recovered for sequencing reactions: three independent sequencing reactions were made for each cloned PCR fragment in an ABI377 PRISM Genetic Analyzer™. The sequences ware assembled using the BioEdit software. Assembled sequences were aligned using ClustalX 1.83. Homologies analyses were performed with BLAST in the GenBank database. *Phylogenetic Analysis:* Nucleotide and amino acid sequences from other BPV types were retrieved from the GenBank for comparison with the sequences obtained here. Alignments were performed using the MEGA 5.0 software. Phylogenetic analysis comparing other PV nucleotide sequences was performed and neighbour-joining trees were drawn using TreeView (1.6.6). **Results and Discussion**: Analysis of the complete L1 ORF sequence had shown that BPV BPV/JN (JQ280500.1) was most closely (72%) related to BPV-1 and BPV-2 (72% of genetic similarity). Macroscopic and histological data also indicated that the collected lesions have fibropapilloma characteristics.t These data suggest that the BPV BPV/JN can be classified as a new *Deltapapillomavirus*.

Supported by: CNPq, Fundação Butantan, CAPES



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7.17 Intracelular peptides analysis in cells expressing the immune proteasome: possible correlations to cell signaling

Monte ER[1], Castro LM[1], Russo LC[1], Gozzo FC [2], Rioli V[3], Ferro ES[1]
[1]Departamento de Biologia Celular e do Desenvolvimento, Instituto de Ciências Biomédicas, Universidade de São Paulo, SP, Brasil, [2]Laboratório Dalton de Espectrometria de Massas, Instituto de Química, Unicamp, SP, Brasil e [3]Laboratório Especial de Toxinologia Aplicada, Instituto Butantan; São Paulo, SP, Brasil.

Introduction: Proteasomes are multisubunit multicatalytic proteases that are responsible for the majority of nonlysossomal protein degradation within eukaryotic cells. The 20S catalytic core is composed of 28 subunits assembled in four stacked seven-membered rings. The outer rings contain seven different non-catalytic α-type subunits, and the inner rings contain seven different β-type subunits, three of which are catalytic (beta1, beta2 and beta5). Under conditions of stress or immune response, these three subunits may be replaced by β1i, beta2i and/or beta5i to form the immune proteasome, which has different catalytic specificities of the constitutive proteasome. The inducible subunits appear to be responsible for altered peptidase specificities in IFN-y-treated cells. Moreover, it is known that this modification provides a better antigen presentation via MHC class I. **Objectives:** Our aim in this work is to characterize the intracellular peptide content after immune proteasome induction. **Methods:** HeLa cells were grown under standard culture conditions until 60% confluence and subsequently incubated with (200U) or without INF-y for 48 hours. After confirmation of the immune proteasome expression by Western Blotting using specific antibodies against subunits beta5 and beta5i, peptides extracted from control and experimental group were quantified using fluorescamine and then labeled with light isotopes (D0-TMAB) or heavy isotopes (D3-TMAB). The semi-quantitative analysis and identification of peptide sequences were performed mass spectrometer followed up by searches in the NCBI database using the MASCOT software. **Results and Discussion:** A total of 85 peptides were identified, all derived from intracellular proteins (nuclear, mitochondrial and cytosolic). Only one of these peptides appear significantly increased in HeLa cells stimulated with IFN-y compared with controls, suggesting that the immune proteasome induction only changes specific intracellular peptides.

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7.18 Immature dental pulp stem cells cultivated on deepithelialized and epithelialized human amniotic membrane in different media

Monteiro BG[1,2], Gomes JAP[2], Kerkis I[1]
[1]Laboratório de Genética – Instituto Butantan; [2]Centro Avançado de Superfície Ocular, Departamento de Oftalmologia da Universidade Federal de São Paulo

Introduction: Searching for an alternative stem cell source that could be potentially used in corneal reconstruction, we turned our attention to a population of stem cells isolated by Kerkis' group from deciduous teeth, which were named human immature dental pulp stem cells (hIDPSC). These cells exhibit all characteristics of multipotent adult stem cells, expressing mesenchymal stem cell, and several human embryonic stem (ES) cell markers (Oct ¾ and Nanog). We also verified that these cells expressed markers of limbal stem cells such as ABCG2, ß1-integrin, vimentin, p63, connexin 43, but were negative for the corneal cell marker keratin 3/12 when cultured in vitro. Based in these date we evaluated the better culture medium to induce hIDPSC into corneal epithelium cells. **Objectives:** Evaluate the capacity of differentiation of human Immature Dental Pulp Stem Cells into corneal epithelium in different media and onto epithelialized and deepithelialized amniotic membrane. **Methods:** The amniotic membranes (AM) were provided by Obstetrics Department of Federal University of São Paulo, and were previously prepared. The culture of human Immature Dental Pulp Stem Cells (hIDPSC) were divided in two groups, that represent deepithelialized and epithelialized AM (AMD and AME, respectively), and subdivided into four groups in accordance to the culture media (Shem, KSFM, Knockout, DMEM and Epilife). After seven days of culture, the samples were fixed and prepared for immunocitochemistry analysis. The differentiation was comproved using K3/12 antibody. Antibodies against ABCG2, connexin 43 and p63 were also tested. The control groups were cultivated with medium that didn't induce differentiation in both groups of AM. Histological and eletron microscopy (EM) analysis were performed. **Results and Discussion:** Immunocitochemistry analysis for differentiation showed positive reaction for K3/12 in the group of AMD with SHEM and Knockout DMEM media. Epilife and KSFM didn't reacted. Control group of AMD presented positive reaction for ABCG2, p63 and connexin 43, which indicates that those cells remain undifferentiated, and negative reaction for K3/12. AME did not present reaction for any antibodies. In the histological and EM analysis of AMD with SHEM and Knockout DMEM media, we could observe similarities with corneal epithelium. These similarities couldn't be observed in Epilife and KSFM media in AME and control groups. AME group presented cells like trophoblasts. These data prove that hIDPSC onto the AME differentiated into another type of cell. Our results showed that hIDPSC onto AMD with Shem and Knockout media are able to differentiate into corneal epithelium cells. And AME are not indicated to be used as support for hIDPSC.

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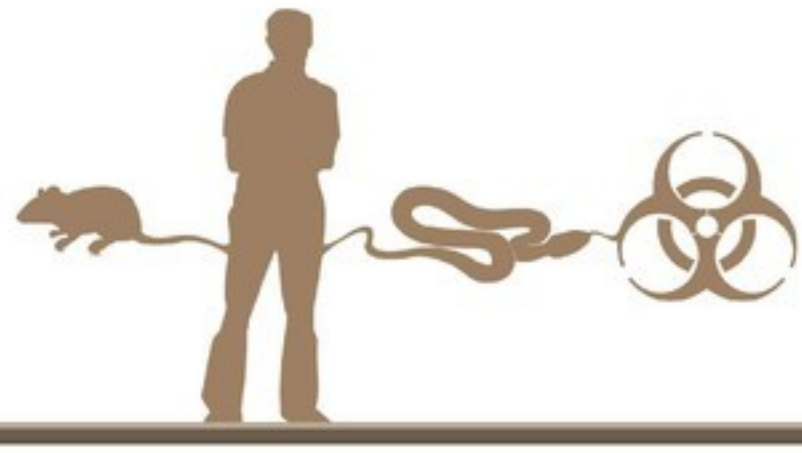

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7.19 Embryonic cells of *Amblyomma cajennense* (Acari: Ixodidae) as a substrate for growth and isolation of pathogens

Cirelli-Moraes A[3], Mendonça RZ[1], Marcili A[2], Nieri-Bastos FA[2], Carneiro SM[4]; Badari JC[1], Barros-Battesti DM[3]
[1]Laboratório de Parasitologia, [3]Laboratório Especial de Coleções Zoológicas, [4]Laboratório de Biologia Celular, Instituto Butantan, São Paulo-SP, Brazil; [2] Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo-SP, Brazil

Introduction: After the mosquitoes, ticks are the main vectors of pathogens. These positions were reversed when we analyze the devastating impacts caused by them. Obligate ectoparasites and hematophagous, ticks provide relevant economic loss and damages to human and animal health, transmiting viruses, bacteria and protozoa. Many micro-organisms do not grow on artificial medium. Embryonic cell cultures of ticks have been established with the purpose of growth and isolation of these pathogens. In Brazil, the obtaining of embryonic cells of ticks is recent, and few species have been studied. Primary cultures of *Amblyomma cajennense* (Acari: Ixodidae) from egg masses of different ages have been routinely obtained in the Laboratory of Parasitology of the Butantan Institute. **Objectives:** The goal of this work is demonstrate that cell cultures of *A. cajennense* could be a good substrate for the isolation and growth of pathogens. **Methods:** Cultures already established, are being kept in the L-15 B medium with sugars and amino acids, supplemented with 10% of FSB, and incubated at 32°C, with weekly medium changes. When the cultures formed a confluent monolayer, they were subcultured and some of them were frozen. Such cells have been tested for the growth of different pathogens such as bacteria (*Rickettsia parkeri* and *Rickettsia belli*) and protozoa (*Leishmania infantum chagasi* and *Trypanosoma theileri*). **Results and Discussion:** There was proliferation or maintenance of the pathogens tested, demonstrating that the cell cultures obtained from *A. cajennense* are potential substrates for growth and isolation of these micro-organisms, as well as for studies of host-parasite interaction. Consequently, it is a tool in the development of vaccine candidates and bioacaricides, that will improve both the control not only of the ticks as well as the pathogens transmitted by these vectors.

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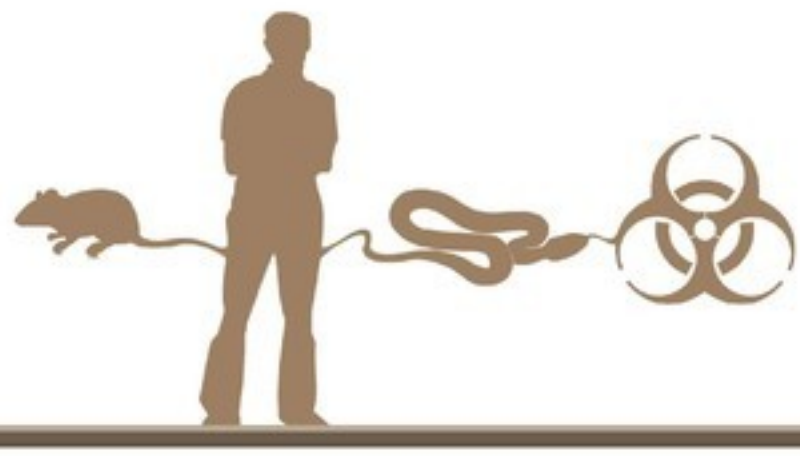

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7.20 Expression of early retinal marker in three-dimensional culture of immature dental pulp stem cells

de Morais BP[1], Mambelli LI[1], Lizier NF[1], Monteiro BG[1] , Gomes JAP[2], Kerkis I[1]
[1]Genetics Laboratory, Butantan Institute; [2]Department of Ophthalmology, UNIFESP - Sao Paulo, SP, Brasil

Introduction: Retina is the light-sensitive eye tissue which converts captured energy in image by specialized neuronal cells. If these cells are damaged, the vision is permanently lost, once they are unable to regenerate. Treatments aim only to decrease retinal damages instead of promoting an effective vision recovery. Stem cells (SC) are a promissive source to substitute injured tissue. Immature dental pulp stem cells (IDPSC) have characteristics of pluripotent SC and are able to acquire properties of almost all cell types. **Objectives:** We aim to evaluate the potential of IDPSC to develop retinal spheres, which can represent a new source for treatment of retinal degenerative diseases. **Methods:** Undifferentiated IDPSC, previously established by us, were analyzed by immunocytofluorescence to evaluate CD73 expression, an early photoreceptor marker. We also analyzed the expression of specialized retinal neurons antibodies: Rhodopsin, Calbindin, Crx, Nrl, Pax-6 and Chx-10. Further, these cells were submitted to progenitor neural differentiation. The capacity of IDPSC to differentiate towards retinal spheres similar structures was evaluated by the expression of Nestin, β-III-tubulin and Pax-6 antibodies. Once these structures were formed, retinal progenitors differentiation was initiated. **Results and Discussion:** Undifferentiated IDPSC reacted positively to CD73 and were negative to specialized retinal neurons antibodies. We also observed the positive reaction of anti-nestin and anti-β-III-tubulin in retinal spheres, indicating that these structures have previous commitment with neural lineage. Anti-Pax-6, a marker of progenitor retina cells, was also positive in retinal spheres. Nevertheless, it is still required some factors that can facilitate the induction of mature retinal characteristics. We demonstrated that IDPSC present the potential of developing retinal spheres structures, with neural and retinal properties *in vitro,* which can be maintained for a long-term in culture. Our data demonstrated that IDPSC can be an alternative source to regenerate damaged retinal tissues. Further studies are needed in order to elucidate respective roles of retinal tissue formation.

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7.21 Endoplasmic reticulum stress in apoptotic signaling of the novel tick recombinant molecule in renal cell carcinoma

Morais KLP[1,2], Souza JG[1,2], Simons SM[1], Berra CM[1], Sato RF[3], Chammas R[3], Chudzinski-Tavassi AM[1]

[1]Laboratório de Bioquímica e Biofísica, Instituto Butantan, SP, Brazil; [2]Departamento de Bioquímica, Universidade Federal de São Paulo, Brazil; [3]Departamento de Radiologia e Oncologia, Faculdade de Medicina da Universidade de São Paulo, Brazil

Introduction: A cDNA library of the *A. cajennense* tick salivary glands was constructed and used to identify a gene encoding a Kunitz-type protease inhibitor. A recombinant protein, named Amblyomin-X was over expressed in *E. coli*. The expressed protein is able to promotes apoptosis in murine renal adenocarcinoma (RENCA), decreased proteasomal activity and increased pool of poly-ubiquitinylated proteins in some tumor cell lines, suggesting an endoplasmic reticulum (ER)-stress. However, the mechanistic effects of this protein are still unclear. **Objectives:** To investigate the involvement of ER-stress in tumor cells treated or not with Amblyomin-X. **Methods:** To evaluate two markers of the ER-stress, GRP78 and GADD153, the genic expression was perfomed by ABI 7500 Real Time PCR System (Life Technologies) using forward and reverse specific genes primers and protein expression through analysis for Western Blot with specific monoclonal antibodies. **Results and Discussion:** RENCA cells treated with Amblyomin-X showed a modest effect in genes related to ER-stress, but significant differences in protein expression was observed. The results suggest that ER-stress is involved in this process pro-apoptotic of the Amblyomin-X.

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7.22 Hematopoiesis in *Oxyrhopus guibei* (Ophidia: Dipsadidae) snakes: Morphological and cytochemical characterization

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Introduction: Hematopoiesis in snakes begins early during embryogenesis and the process changes through fetal life. The first erytropoietic activity is extraembryonic from mesoderm cells of the yolk sac and during the embryonic development it becomes intraembryonic. In newborn and adult snakes, the main site of hematopoiesis occurs in the bone marrow. **Objectives**: The aim of this study was to characterize different stages of blood cells maturation of *Oxyrhopus guibei* snakes, based on microscopic studies including cytochemical stains and ultrastructural features. **Methods:** Fragments of vertebrae of newborn and adult snakes (n= 11) were collected to obtain bone marrow that was fixed in Bouin or formol calcium and processed routinely for histology. Tissue sections, *imprint* of bone marrow and blood smears were stained with Rosenfeld, hematoxylin and eosin or methylene blue. The cytochemical reactions performed were periodic acid-Schiff (PAS), toluidine blue (TB), sudan black B (SBB), benzidine peroxidase (BP) and acid phosphatase (AP). For transmission electron microscopy (TEM), bone marrow was fixed in paraformaldehyde 4% + glutaraldehyde 2% in Tyrode buffer, postfixed in 1% osmium tetroxide and embedded in Epon 812 resin. **Results and Discussion:** Most of progenitors of blood cells were identified in the active hematopoietic focus in bone marrow of vertebrae and ribs. The azurophilic and lymphocytic series were morphologically similar to those of other reptiles. Granulocytic lineage was classified as myeloblast, promyelocyte, myelocyte and mature granulocytes. Promyelocyte can be differentiated into basophils, with large, round and electron dense granules or heterophils, with fusiform granules with varied size in the TEM analysis. TB and PAS were positive in the immature and mature basophils granules. On the other hand, heterophils and azurophils showed strong positive reaction for lipids staining of SBB and BP. AP was found on azurophils in various stages of maturation. The different stages of erythrocytes were classified as: proerythroblast, basophilic erythroblast, polychromatic erythroblast, pro-erythrocyte and mature erythrocytes. Thrombocytics cells showed PAS positive and characteristics of mature and immature cell were defined using TEM identifying the dense bodies, alpha granules and open canalicular system. The rib or vertebral bone marrow is an important hematopoietic site in the newborn and adult *O. guibei* snakes and the morphologic, cytochemical and ultrastructural characteristics are useful to identify and characterize different stages of maturation of blood cells.

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7.23 The interaction among Glyceraldehyde 3-phosphate dehydrogenase protein and telomeric DNA is controlled by the steady-state internal redox (NAD^+/NADH) in *Trypanosoma cruzi*

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Introduction: Several studies show that the intracellular redox state is fundamentally important in maintaining the cellular metabolism and function. Among these redox balances one of the most important is the redox pairs NAD^+/NADH, which regulates the function of many molecules. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is a multifunctional protein with defined functions in numerous subcellular processes. Among these functions it should be stressed the protection of chromosomes ends in mammalian cells against rapid degradation through its ability to bind telomeric DNA. **Objectives:** In this study, we demonstrated that recombinant GAPDH from *Trypanosoma cruzi* (rTcGAPDH) is able to interact with telomeric DNA and we have showed that rTcGAPDH binds directly a single-stranded oligonucleotide bearing at least one telomeric repeat. **Methods:** Were used gel-shift assays, ChIP assay, and quantitation of NAD^+/NADH. **Results and Discussion:** We could demonstrate that GAPDH is in fact bound to telomeric DNA *in vivo* in the proliferative epimastigote cells. Interestingly, [NADH] is higher than [NAD^+] in these cells, but exogenous NAD^+ was able to block GAPDH-telomere interaction. Corroborating with this hypothesis that NAD^+/NADH balance determines GAPDH-telomere interaction, we verified that the non-proliferative/infective trypomastigote present higher intracellular concentration of NADH compared to NAD^+ and in these cells GAPDH is not able to bind telomeric DNA. Moreover, exogenous NADH is able to rescue GAPDH-telomere interaction in this stage. Our data show the importace of NAD^+/NADH balance in the interaction of telomeric DNA/GAPDH in trypanosomes, strongly suggesting that the protection of parasite chromosomes ends might be regulated by the metabolic state of the cells.

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7.24 The involvement of RPA complex and Orc1/Cdc6 protein in the replication of chromosomal ends in trypanosomes

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Introduction: *Trypanosoma cruzi* is the etiologic agent of Chaga´s disease, the second highest illness burden among neglected tropical diseases. Nowadays, Chagas disease still causes 40,000 new infections per year. Thus, improving the knowledge about the molecular biology of this parasite may facilitate the discovery of new therapies and the development of antiparasitic drugs. Our group study the pre-replication and replication machinery in trypanosomes and in this work we propose that some of these components play a role in telomere complex maintenance in trypanosomes. Telomeres are formed by the interaction of DNA with protein complexes which are responsible for maintaining these terminals. The ORC complex is part of the pre-replication machinery and during replication is found along the chromosome, including the telomeric ends. Replication Protein A (RPA) comprises a trimeric complex formed by three subunits, that performs, alone or together with other proteins, various vital functions in DNA metabolism, being a fundamental player during replication and also in telomere maintenance. In trypanosomes, such as *Leishmania*, only subunit 1 of the RPA is found associated to telomeric DNA. In trypanosomes Orc1/Cdc6 is a member of the pre-replication machinery but the role played by the trypanosome RPA in this context has not yet been characterized. **Objectives:** This study aims to characterize the *T. cruzi* RPA, as well as analyze the interaction of this protein and Orc1/Cdc6 with parasite telomeres. **Methods:** After cloning and expression of recombinant RPA and Orc1/Cdc6 proteins we perform Circular Dichroism and electrophoresis mobility shift assays (EMSA) to study interaction of these molecules with telomeric DNA. **Results and Discussion:** We have already cloned, expressed and purified recombinant RPA and Orc1/Cdc6. RPA is an insoluble protein and needed heparin as a chaperone for its perfect refolding, analyzed by Circular Dichroism. We have observed using this technique that RPA folding change subtly with the addition of telomeric oligos. Through EMSA assays we show that RPA interacts with telomeric G strand. These data strong evidence that RPA associates with telomeric DNA in *T. cruzi* and therefore might be involved with DNA replication and protection of chromosome ends. Finally, once involved in such important functions, RPA is a possible target for drug design against Chagas' Diseases.

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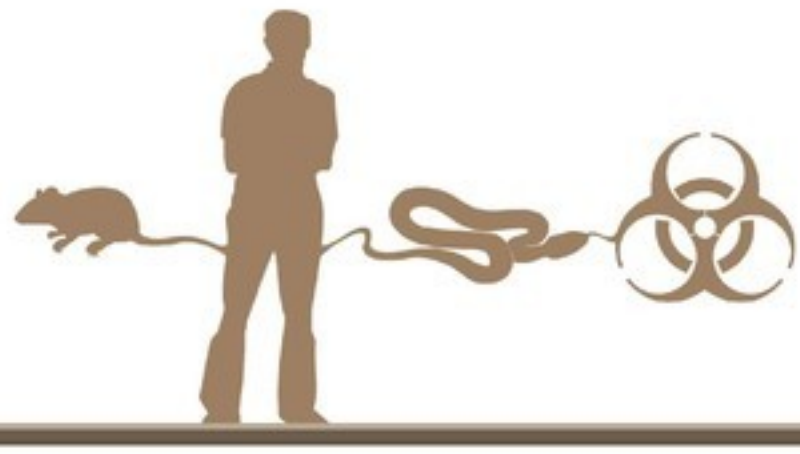

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7.25 Obtention and characterization of mesenchymal stem cells from equine synovial membrane

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Introduction: Equine mesenchymal stem cells (MSCs) have been reported to be present in bone marrow, adipose tissues, dermis, muscles and peripheral blood. It has the potential to differentiate along different lineages including those forming bone, cartilage, fat, muscle and neuron. This differentiation potential makes MSCs excellent candidates for cell-based tissue engineering. MSC are extremely promising as a treatment of diseases such as nerve damage, arthritis, ligament ruptures and tendon injuries. Human multipotent MSCs were isolated from the knee joints. **Objectives:** The aim of this study was to obtain and characterize equine synovial membrane cells and analyze its therapeutic potential. **Methods:** In this study, synovium samples were collected from arthroscopic procedures at the Department of Equine Surgery, Veterinary Hospital, FMVZ-USP, in São Paulo. The fragments were submitted to cell culture. Growth curves were made to find the ideal concentration of cell growth and their kinetic behavior. **Results and Discussion:** The different cell densities used in this study showed that 10^4 cells have an optimal density to *in vitro* cell growth. The cell proliferative capacity was observed until 10^{th} passage. Flow cytometry analysis showed the expression of embryonic stem cells markers: OCT3/4 (49.0% ±7.4), NANOG (46.8% ±7.8), TRA-1-81 (31.8% ±2.6); hematopoietic stem cells markers: CD45RO (24.4% ±7.3), CD117 (28.9% ±5.8), CD 133 (31.2% ±9.9), VEGF-R1 (30.6% ±6.8); and mesenchymal/hematopoietic stem cell markers: CD 105 (43.3% ±4.5), CD90 (45.0% ±7.6), CD34 (38.4% ±1.9). Our study demonstrates that equine multipotent undifferentiated MSCs can be isolated from synovial membrane. These cells have the ability to proliferate extensively in culture. Synovial MSCs are promising candidates for therapeutic strategies.

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7.26 Analysis of the importance of the ATPase activity and the ATP binding Orc/Cdc6 Trypanosomes to the stability of the complex pre DNA replication

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Introduction: The process of chromosomal replication starts with the formation of the complex pre-replication, DNA regions known as origins of replication. In yeast, the pre-replication machinery (pre-RC) is composed of the ORC complex (Orc 1-6), Cdc6, Cdt1 and MCM complex (Mcm 2-7) that has activity helicase. The tripanossomes doesn't have in its pre-RC Cdc6 and Cdt1, but only a protein homologous to Cdc6 and Orc called Orc1/Cdc6 by our group, which interacts with the MCM licensing origins of replication. In yeast cells, the binding of ATP to ORC is a essencial for its interection to origins of replication. And the ATP hydrolysis by Cdc6 and ORC ensure the specific interaction between DNA and the pre-RC as the stability of MCM complex. The primary sequence of Orc1/Cdc6 *T. cruzi* and *T.brucei* presents a site of interaction to ATP / GTP, and also the sensor regions 1 and 2 that are essential for ATPase activity.Our laboratory has demonstrated that these recombinant proteins bind and hydrolyze ATP "in vitro" and that the ATPase activity increases in the presence of nonspecific DNA. **Objectives:** In this sense, the objective of this study is to evaluate the importance of ATP binding and hydrolysis to the formation and stability of pre-replication machinery in the genome of trypanosomes. **Methods:** Even so, we generated recombinant proteins of Orc1/Cdc6 *T. cruzi* and *T. brucei* mutated at regions sensor 1 or 2 regions which are unable to hydrolyze ATP and mutated in the linker region of ATP. These genes (*T. cruzi* and *T. brucei*) were cloned into vectors for transfection into both parasites. The mutated proteins contain a tag (flag) that allow discrimination of the endogenous protein and mutated. The protein-DNA interaction will be analyzed by extract differential. **Results and Discussion:** A mutation in the sensor 2 region of *T.brucei* drastically reduced ATPase activity compared Orc1/Cdc6 with Wild type. It will analyze the importance of ATP binding and hydrolysis in parasites transfected with Orc1/Cdc6 mutated proteins.

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7.27 Evaluation of potential of crude saliva tick to reduces mitochondrial potential (Δψm) triggering apoptosis in melanoma cells

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Introduction: Ticks are blood-feeding arthropods that secrete a complex mixture of bioactive components through their saliva to maintain the fluidity of blood during its feeding. Molecules from the saliva of hematophagous animals are useful tolls to understand many pathophysiological processes (anti-haemostatic, cell death, etc). Saliva tick possesses proteins toxins having numerous biological activities. In this study the crude saliva obtained from the *Amblyomma cajennense* tick is shown to contain biological effects such as trigger apoptosis and decrease the potential of mitochondria of murine (B16F10) and human (Mel-85, Sk Mel-28, Sbcl2) melanoma cells. **Objectives:** In this work was evaluate the potential of crude saliva from *Amblyomma cajennense* tick to alter the mitochondrial (Δψm) potential of melanoma cells and thus, leading the cells to apoptosis. **Methods:** The saliva was collected according to the method described by Kaufman and keeping at -80° C. Changes in morphologic profile, was evaluated through the fluorescence microscopy. To evaluate the mitochondrial potential the cells were harvested and dyed with TMRE and flow cytometry was performed. **Results and Discussion:** The treatment with crude saliva induced alterations in cells morphology consistent with apoptosis and beyond this, reduced the TMRE signal fluorescence, analyzed thought of 3D dot plot, indicating that the decreased of Δψm changes in response to the treatment.

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7.28 Heterogenous multipotent stem cells isolated from porcine adipose tissue

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Introduction: Adipose tissue (AT) is an attractive source of MSCs because of its abundance and ease of access with minimal donor site morbidity. However, the stromal vascular fraction (SVF) of AT is composed by heterogeneous cell population comprising a subset of stem cells (or different subsets of stem cells) and more differentiated (progenitor) cells. Thus is critical to for further stem cells therapy the standardization of immunophenotype of SC isolated in vitro. Porcine are used in biomedical research and are the preferred species for physiology and disease studies. In this context investigation of SC in pig is essential for progresses SC research and to future applications in humans. **Objectives:** Isolation and characterization of porcine adipose multipotent stem cells (pAMSC). **Methods**: In order to isolation of pAMSC were use clinically healthy adult pigs. The AT from pigs was collected from subcutaneous fat depots using undergoing experimental surgeries unrelated to this study. The adipose tissue sample was digested for 30 minutes at 37C with collagenase type I. Following the cells obtained trough digestion were plated in flasks. The pAMSC were cultivated in DMEM-HG and 15% SFB-hyclone. These cells were characterized using following SC antibodies: Oct3/4, nanog, Sox2 CD44, CD146, nestin, vimentin, and cytokeratin-8. Also differentiation assays were performed. **Results and Discussion**: After 48 hours of cultivation first fibroblast- like and epithelial like colonies appeared in AD cells culture. However after four passages remained fibroblast-like cells. Fibroblast-like and epithelial-like cells react positively and uniformly with intermediary filaments vimentin and nestin. The majority of cells epithelial cells colony was positive to cytokeratin-18 and CD44 antibodies. While few fibroblast-like cells react positively to CD44 and cytokeratin-18 antibodies. A few Oct3/4 positive cells were found, presenting perinuclear localization. In contrast fibroblast-like and epithelial-like cells were negative to pluripotent markers such as nanog and Sox2. The cell populations showed successfull osteogenic, chondrogenic and adipogenic differentiation. In addition pMASC did not present teratomas formation 40 days after injection into muscle of nude mice. Porcine multipotent adult stem cells are heterogeneous population and express proteins of mesodermal and ectodermal commitment. These heterogeneous cells showed ability to mesodermal differentiation similar to mesenchymal stem cells from adult bone adipose.

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