5: Cellular Biology and Genetic

5.01 Effects of a Kunitz-type inhibitor (Amblyomin-X) on cell cultures of human renal carcinoma

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Introduction: The incidence of renal carcinoma (RCC) has been detected more frequently nowadays, which now represents 50% of newly diagnosed cases. Approximately 30% of patients with RCC have metastatic disease. Being a disease that
highly resistant to chemotherapy and radiotherapy, surgical treatment in early stages, where it is organ-confined, is common. However, after nephrectomy, 60% of patients develop metastasis within 10 years. Amblyomin-X is a recombinant protein produced from *Amblyomma cajennense* cDNA library. This protein is able to inhibit the coagulation factor Xa and also induce apoptosis in different tumor cell lines. **Objectives:** To evaluate the response of renal tumor cells (Caki-1 and Renca) treated with Amblyomin-X and to analyze the presence of inflammation molecules in the microenvironment of treated cells. **Methods:** The morphological characteristics and death of the cells (Renca and Caki-1) were determined by optical microscopy and by MTT assay. DNA content and the phases of the cell cycle were analyzed by flow cytometry (guava system GE), and the levels of interleukin-6 determined by ELISA. **Results and Discussion:** Amblyomin-X induced cytotoxicity in both analyzed cell lines, causing morphological changes. The responses were found to be dose- and time-dependent. Decreases in all stages of the cell cycle and levels of IL-6 were observed after 24 h of treatment. All together, our results suggest that Amblyomin-X exerts a strong cytotoxic effect in renal cells lines.

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5.02 Genetic polymorphism of IL-3Rα confers differential proliferation and greater differentiation to neutrophils in bone marrow cells

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Introduction: Hemopoiesis is a dynamic and gradual process on cell development that occurs by the sequential differentiation of hematopoietic stem cells (HSCs) into multipotential progenitors and terminally differentiated blood cells under the action of hematopoietic cytokines and transcription factors. Mice genetically selected for high (AIRmax) or low (AIRmin) acute inflammatory response show interline differences in the number of infiltrated cells in inflammatory exudate produced by s.c. injection of polyacrylamide beads (biogel). **Objectives:** We analyzed the proliferative capacity of bone marrow cells and neutrophil differentiation in AIRmax and AIRmin mice. **Methods:** To evaluate the myeloid cells proliferation, we used a five-day suspension culture method with IL-3+SCF combined with all-trans retinoic acid (ATRA) as *in vitro* stimulus. **Results and Discussion:** AIRmax mice showed a higher response to synergic SCF+IL-3+ATRA action ($6.31\pm1.63 \times 10^5$ cells/ml) with accelerated neutrophil differentiation ($5.9\pm0.1 \times 10^4$ $CD38^-/GR1^+$ cells) compared to AIRmin mice ($2.4\pm0.1 \times 10^4$ $CD38^-/GR1^+$ cells). The study of genetic polymorphism at the IL-3Rα locus showed that the frequency of the allele that codes for a normal protein was 100% in AIRmax mice. On the other hand, AIRmin mice showed preferential fixation of the alelle related to abnormal protein, present in some inbred strains of mice such as A/J, AKR, A.TH or A.TL which were found to be hyporesponsive to IL-3. The identification of functional polymorphism in the IL-3Rα gene between AIRmax and AIRmin mouse lines, which differ largely in the degree of cell differentiation and in inflammatory response, indicates that this gene is an important regulator of the high and low inflammatory reactivity in both AIRmax and AIRmin mouse lines.

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5.03 Canine amnion-derived stem cells causes tumor in mice

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Introduction: Fetal stem cells are isolated from tissues normally discarded at birth. They are attractive for clinical applications because their use avoids ethical concerns that plague the isolation embryonic stem cells. The amniotic membrane (AM), or amnion, delineates the gestational sac, a highly resilient, transparent, fluid-filled cavity that encompasses a developing fetus during gestation. The amnion is an avascular structure consisting of three discrete layers: an inner epithelial layer, an interposing, acellular basement membrane, and an outer layer of mesodermal cells. A mix of multipotent cell populations, including amniotic epithelial and amniotic mesenchymal cells known as amnion-derived stem cells (ADSC), can be obtained from the amnion (Miki et al., 2005). **Objectives:** The goal of present work was the isolation and characterization of ADSC from canine fetuses (C-ADSC). **Methods:** AM was obtained from a dog fetus at 35 days of gestation. The cells were isolated from the amnion using a tissue explant methodology and cultured according to Marcus *et al.* (2008). Differentiation of ADSC towards mesodermal lineage was performed following routine protocols. Analysis of C-ADSC morphology was performed by transmission electron microscopy (TEM). Before injection into mice, C-ADSC were transduced with retrovirus vectors carrying reporting genes LacZ in order to facilitate cell tracking after implantation. A total of $1x10^6$ cells of normal and Lac Z - cells were injected into right limb of each nude mice (n=8) and Swiss mice (n=2) of both strains. Histological analyses were then performed. **Results and Discussion** C-ADSC showed high proliferative rate after isolation and displayed both embryonic stem (ES) cell-like and epithelial-like cell phenotypes. They were positive with both anti-vimentin and anti-nestin antibodies, suggesting that isolated mixed C-ADSC population was composed of both amniotic epithelial and mesenchymal stem cells. TEM analysis showed the cells, which have ES cells – like morphology, with a large nucleus and with cytoplasm poor in organelles. The cells demonstrated tight contact and gap junction formation. Tumor formation was observed in the right limb of all animals one month after cell implantation. Histological analysis confirms the formation of teratocarcinomas composed of undifferentiated and differentiated cells. Our data suggest that mixed C-ADSC population was composed mainly of cells of the epithelial phenotype, which were able to produce teratocarcinomas in mice. Although culture conditions could promote the isolation of cells with teratogenic potential, caution is needed with respect to fetal stem cell use in the cell therapy. On the other hand, C-ADSC could be an interesting model for cancer research.

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5.04 Analysis of the signaling pathways involved in vasoconstriction induced by angiotensin II (AngII) in the snake *Bothops jararaca*

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Introduction: The renin-angiotensin system produces its effects through the interaction of AngII with the classical angiotensin receptors, AT_1 and AT_2. In some vertebrates, such as the Brazilian snake *Bothrops jararaca* (Bj), AngII interacts with an atypical AT receptor. The majority of AngII responses are due to activation of the AT_1 receptor that is coupled to several intracellular signaling pathways, such as phospholipases C, D and A_2, adenylylcyclase and tyrosine kinases. We have demonstrated that the phospholipase C (PLC)/IP_3 and adenylylcyclase/AMPc pathways in cardiac tissue, and L-type calcium channel in the vascular tissue of Bj, are not involved in AngII response in this animal. **Objectives**: The aim of this study was to evaluate the role of calcium and kinase as second messengers in the AngII response in Bj. **Methods:** A functional assay was used to obtain cumulative AngII (10^{-10}-10^{-6}M) curve with isolated aorta rings, in the absence and the presence of specific inhibitors to examine: 1) the activation of PLC pathway, using U73122 and GF109203X, a PLC and a PKC inhibitor, respectively; 2) the participation of tyrosine kinase and phosphatase, using genistein and sodium orthovanadate; and 3) the role of extra and intracellular Ca^{+2} by removal of external calcium from the medium, or pre-treatment of the tissue with either cyclopiazonic acid (CPA), an inhibitor of the Ca^{+2}-ATPase from sarcoplasmic reticulum, or with caffeine (agonist of the ryanodine receptor in the sarcoplasmic reticulum), or with SK&F96365 (a store-operated calcium receptor inhibitor). **Results and Discussion:** U73122 (10^{-5}M, n=7), genistein (1-30x10^{-6}M, n=8) and sodium orthovanadate (10^{-4}M, n=6) failed to modify AngII response, while GF109203X (10^{-5}M, n=6) reduced Ang II maximum response by 52%. These results indicate that phospholipase C, tyrosine kinase and phosphatase are not involved in AngII response of Bj aorta, but PKC activation is involved. Activation of PKC seems to occur through a phospholipase C-independent pathway. There was an 82% reduction of the Ang II response in calcium-free medium, and a recovery after restoring the normal calcium concentration in the medium. In addition, SK&F96365 (3.10^{-5}M, n=6) caused a 68% reduction in AngII response. All together, these data support the participation of external calcium and store-operated calcium receptor in the Ang II response. CPA (1-30x10^{-6}M, n=7) failed to modify AngII response, but caffeine (10^{-3}M, n=4) reduced the Ang II maximal response by 92%. Therefore, a possible role of the sarcoplasmic reticulum as an intracellular source of calcium for AngII response cannot be completely discarded. Taken together, our results suggest the participation of PKC and calcium as signal transducers of snake AT receptor activation by AngII, in the aorta of Bj.

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5.05 Primary cell lines obtained from BPV-related lesions: cytogenetics and immunochemical analysis

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Introduction: The bovine papillomavirus belongs to a DNA oncovirus group, which is characterized as infecting the host epithelium. In cattle, BPV commonly causes only benign skin lesions, but in some cases can lead to neoplasic processes such as enzootic hematuria and upper digestive tract carcinoma. The integration of papillomavirus in host chromatin has not been reported in cattle, but several studies have reported the presence of increased levels of chromosomal aberrations in animals infected by the virus. **Objective:** We present a study of cytogenetic and morphological characterization of cells from primary cultures from skin papilloma, esophagus papilloma and bladder mucosa of animals infected with BPV. **Methods:** For culture establishment, fragments were collected from lesions detected in animals affected by papillomatosis and a sample of skin without visible lesions used as control. The fragments were incubated in DMEM (Cultilab ™), supplemented with 10% fetal bovine serum and maintained at 37 ° C in an atmosphere of 5% of CO ². All animals were tested for the presence of viral DNA in the lesions and in different passages of the culture by PCR using generic and specific primers. In early passages, cytogenetic preparations were performed with hypotonic treatment with 0.075 M KCl solution at 37°C for 30 min and fixed in a 3:1 methanol and acetic acid solution. Slides were stained in 2% Giemsa and examined with a photomicroscope. The cultured cells were also stained with anti-vimentin and anti-pan-cytokeratin for cytological characterization. **Results and Discussion:** All animals showing lesions were confirmed as BPV positive for BPV-1, BPV-2, and some for BPV-4. There was a higher frequency of chromosomal aberrations in affected animals compared to control animals. A greater number of aberrations were visualized in affected animals, particularly those with sequences of BPV4. All cell lines were stained as vimentin positive, which is the intermediate filament that characterizes mesenchymal cells. The same cells were positive for pan-cytokeratin, a marker of cells of epithelial origin, and the most evident expression were found in the esophagus papilloma and bladder mucosa cells. This double labelling feature demonstrated in the cells lines has been already reported in certain types of metastatic cells, characterizing a process called epithelial-mesenchymal transition (EMT): cells show simultaneous cytokeratin and vimentin characteristics. Another point to consider is that cytokeratin genes in cells with malignant growth have been reported with an altered expression, which could indicate the expression of this filament as a possible element for diagnosis in cancer.

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5.06 Identification of suitable loci for discrimination of pathogenic *Leptospira* serovars

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Introduction: Leptospirosis is an infectious disease caused by pathogenic *Leptospira* species. To date, almost 300 serovars have been identified and distributed among 26 different serogroups. Definite serovar identification is performed by the cross agglutinin absorption test (CAAT), employing monoclonal antibodies. However, this technique is considerably expensive. Molecular tools have been described for serovar discrimination, but they have limitations. Multi-locus sequence typing (MLST) is a simple PCR-sequencing approach, which has the ability to discriminate *Leptospira* species. Traditionally, the loci chosen for MLST analyses are housekeeping genes. However, these genes may be highly conserved and, thus, exhibit low discriminatory power. Virulence factor sequences are expected to contain more polymorphic sites. **Objectives:** This study aimed at evaluating the candidate loci *ligB*, *secY*, *rpoB* and *lipL41* (concatenated in this order) in the differentiation of *Leptospira* reference serovars. **Methods:** The sequences were aligned by the ClustalW program and the phylogenetic analyses were performed by the Mega 4.1 software. **Results and Discussion:** This study involved 37 reference strains and employed a 1884-bp locus. The species were resolved into major clusters and the serovars appeared to occupy individual branches within them. Through this analysis, it was possible to obtain separation between serovars Icterohaemorrhagiae strain RGA and Copenhageni strain Fiocruz L1-130. Yet, it was possible to differentiate between strains 56601 and Lai (serovar Lai) and strains M20 and Fiocruz L1-130 (serovar Copenhageni). This suggests that this candidate scheme may be helpful to identify clonal isolates in epidemiological studies. The candidate loci can be amplified from different strains, irrespective of the species to which they belong. Several molecular tools have been described for the characterization of *Leptospira* isolates, but they demonstrated a number of limitations. Contrarily, MLST was demonstrated to be simple, suitable for worldwide application and inexpensive and to generate unambiguous and exchangeable data. The proposal scheme is suitable for discriminating pathogenic serovars of *Leptospira*, per species, as demonstrated in our phylogenetic analyses. Although this approach should, in the future, be extended to a larger number of serovars and clinical isolates, it represents the first step towards serovar molecular discrimination.

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5.07 HPV16 L1L2 gene expressions, protein synthesis and interaction in culture human cells

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Introduction: Of the nearly 200 genotypes identified, at least 15 distinct human papillomavirus (HPV) types are described to be involved in genital, mouth, throat, and skin cancers. Cervical cancer is the second most prevalent cancer in women worldwide, and HPV16 is the most prevalent high-risk HPV type associated with cancer of the cervix. HPV has an 8,000-base pair, circular, double-stranded DNA, containing the E6 and E7 viral oncogenes. L1 is the major and L2 is the minor HPV capsid protein. Together, they compose the small naked icosahedra with a capsid of about 55 nm in diameter, which without viral DNA is known as VLP (virus-like particle). In the worldwide HPV16, VLP L1 has been utilized in prophylactic vaccine development due to their capacity to induce high immunological response, although L2 can induce a low-titer of antibodies to a wide-range of divergent papillomavirus types and species. L2 confer more stability to the VLP and it is also necessary for HPV infection. **Objectives:** We are producing HPV16 L1L2 VLPs to investigate the mechanisms by which virus-cell infection causes cancer. **Methods:** Cultures of the 293T human embryonic kidney (HEK) cell line were transfected with the DNA constructs encoding humanized L1 (L1h) and L2h antigen of HPV16, subcloned into the mammalian expression vectors pUF3L1h and pUF3L2h. Western blotting to control protein expression, immunofluorescence in laser scanning confocal microscopy (LSCM), negative staining and gold immunolabeling for VLPs analyses by transmission electron microscopy (TEM) were used. Pathogen-host cell interaction assays using HPV16 L1L2 VLPs and HEK 293T cells were performed. **Results and Discussion:** Recombinant L1L2 DNA was expressed in HEK 293T cells with high efficiency. At least 85% of cells expressed intracellular L1L2 and VLPs, detected by LSCM and TEM. The HPV16 L1L2 VLPs produced in this study with about 55 nm in diameter interacted with non-transfected HEK 293T cell line, confirmed by LSCM. We are establishing a methodology for an efficient system of recombinant protein expression. The production of HPV16 L1L2 VLPs by transfected HEK 293T cells opens the possibility for new strategies to study HPV-cell interactions and carcinogenesis mechanisms.

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5.08 Renin angiotensin system (RAS) and vascular reactivity in a non-poisonous snake *Oxyrhopus guibei* (Colubridae family)

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Introduction: Snakes are interesting vertebrates to study cardiovascular function because they have an elongated blood column, and have to adapt its blood circulation to different gravitational influences. RAS is important in cardiovascular control, and its study in Brazilian snakes from Viperidae family points to the presence of a RAS relatively conserved compared with other vertebrates species. The angiotensin converting enzyme (ACE) plays a significant role in RAS by removing two amino acids from the inactive angiotensin I (Ang I), converting this peptide into its active form angiotensin II (Ang II). **Objectives:** The aim of this study was to investigate the presence of a functional RAS in a vascular tissue, aorta, of the snake *Oxyrhopus guibei*, which belongs to a family of non-poisonous snakes, namely the Colubridae. We analyzed the presence of an active ACE and used pharmacological tools to characterize the Ang II receptor. **Methods:** Using an *in vitro* assay with vascular smooth muscle from *Oxyrhopus guibei*, we obtained cumulative concentration-effect curves for AngI and Ang II (10^{-10} - 10^{-6}M) in the absence and presence of an inhibitor of ACE, captopril (10^{-6}M). Cumulative concentration-effect curves for Ang II (10^{-10} - 10^{-6}M) were also constructed in the absence and presence of the non-selective antagonist of the Ang II receptor, [Sar^{1}, Ile^{8}] Ang II (10^{-7}-10^{-5}M), or an agent that reduces disulfide bridges in the receptor structure, dithiothreitol – DTT ($3x10^{-3}$M). **Results and Discussion:** Pre-treatment with the ACE inhibitor, captopril, shifted the Ang I curves to the right (pD_2 6.9 to 5.9, n=6), but was not able to displace the Ang II curves (pD_2 7.1 to 7.1, n=5). Taken together, these results indicate the existence of angiotensin converting enzyme in the vascular tissue of the snake, which is functionally active and responsible for converting Ang I into Ang II. The initial pharmacological characterization of receptor for Ang II in the snake aorta using three different concentrations of the non-selective antagonist [Sar^{1}, Ile^{8}] Ang II (10^{-7}-10^{-5}, n=3) showed a shift to the right in the Ang II curve and a reduction in the maximum effect. These data suggest the presence of an Ang II receptor in this snake, but selective antagonists should be used to identify the subtype of the Ang II receptor. Cumulative concentration-effect curves for Ang II were reduced after DTT treatment (n= 2), suggesting the presence of at least one disulfide bridge functionally important in the Ang II receptor structure. DTT is also reported to produce similar reduction of Ang II response in mammalian species and in two Brazilian snakes from the Viperidae family. These results contribute to the knowledge of RAS in vertebrate species.

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5.09 Identification and evaluation of the bovine papillomavirus (BPV) in blood and peripheral lymphocyte cultures from dairy cows in Pernambuco State, Brazil

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Introduction: Bovine papillomaviruses (BPVs), family *Papillomaviridae*, act as causal agent of warts and bladder and digestive tract cancers, causing significant economic losses to the cattle industry. Ten types of BPVs have been well characterized (BPV-1-10). **Objectives**: The main objective of this study was to evaluate sequences of BPV genomes in blood samples collected on a dairy farm in Pernambuco and in respective peripheral lymphocyte cultures, to determine the presence of BPV 1, 2 or 4 and their simultaneous presence. **Methods**: The polymerase chain reaction (PCR) technique was used for viral diagnosis, using specific primers for types 1, 2 and 4, targeted to genes L1, L2 and E7, respectively. Confirmation of the amplified products was performed with enzymatic digestion and sequencing. **Results and Discussion**: Viral sequences were detected in all the animals, regardless of the apparent presence of papillomas. Types 1 and 2 were detected directly from blood samples and correlated with lymphocyte cultures, and type 4 was not detected in any samples. The positive results were confirmed by enzymatic digestion and sequencing results were compared with published sequences available in *GenBank*. The viral presence in blood corroborates studies that argue BPV dissemination through blood while the detection in culture suggests viral maintenance in this system. The sequencing of some positive samples for BPV-1 suggests the occurrence of a new viral variant. Taken together, the results suggest the role of lymphocytes as sites of viral latency, in addition to the presence of a variant of BPV-1 circulating among the animals studied.

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5.10 Cytogenetics and molecular phylogeny of the genus *Oligoryzomys* (Sigmodontinae, Rodentia) from new Brazilian localities

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Introduction: Pygmy rice rats of *Oligoryzomys* are widespread from Mexico to Tierra del Fuego in a variety of habitats and climates. Recently, 18 species were recognized for the genus, 10 being recorded in Brazil, inhabiting the Atlantic and Amazonian rainforests, Cerrado, Caatinga and Pampa. Some of them are agricultural pests or reservoirs of Hantavirus. They exhibit species-specific karyotypes and the diploid numbers vary from 44 to 70. **Objectives:** The aim of the present study was to characterize karyotypes of several *Oligoryzomys* species and to establish the phylogenetic relationships between them. **Methods:** We used samples of *Oligoryzomys* from 46 localities of 11 Brazilian states: Piauí, Ceará, Bahia, Minas Gerais, Espírito Santo, São Paulo, Paraná, Rio Grande do Sul, Mato Grosso, Goiás, and Tocantins. Cytogenetic data were obtained from bone marrow, spleen and fibroblast culture, and DNA was extracted from liver and muscle. **Results and Discussion:** Conventional and differential staining and fluorescence *in situ* hybridization (FISH) for cytogenetic study evinced *Oligoryzomys nigripes* with 2n=62 and FN=78, 80, 81, 82; *O. flavescens* with 2n=64, 65, 66 and FN=66, 67, 68; *Oligoryzomys microtis* with 2n=64 and FN=64; *Oligoryzomys moojeni* with 2n=70 and FN=74; and *Oligoryzomys fornesi* with 2n=62 and FN=64. FISH with telomeric probes showed exclusively telomeric signals even in rearranged pairs and supernumerary chromosomes. Sequences of 750 bp of the mitochondrial cytochrome-b gene were used for phylogenetic reconstruction. Parsimony and Bayesian analyses recovered the genus as monophyletic and the clades were related to the biomes where animals were trapped: Amazonian, Atlantic forest, Cerrado areas, and a clade composed of samples with 2n=62 from Ceará - from a transitional area between Atlantic and Amazonian rainforests called "Brejos" - and Bahia. The monophyly of *Oligoryzomys* is well corroborated in the literature; however, the relationships within the genus are still unclear. In our data, the Amazonian clade is related to *Oligoryzomys microtis*; the clade of Atlantic forest comprises several localities of Southeast and South Brazil and is composed of representatives of *Oligoryzomys nigripes*. Distinct clades were recovered from different areas of the Cerrado. Besides, we emphasize karyotypes as important marker for this genus and that the recovered clades are related to the biomes that the animals inhabit.

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5.11 Polymorphism in codon 72 of p53 gene in women examined during routine gynecological examination in Ouro Preto, Minas Gerais, Brazil

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Introduction: The p53 gene (17p13.1) has 11 exons: the first is not transcribed. The p53 protein has 393 aminoacids, with four segments with distinct functions. The p53 protein is important in carcinogenesis by acting in the preservation of genomic integrity, the transcriptionally active p21 gene, inducing the synthesis of protein p21. p53 activates the gene GADD-45 (growth arrest DNA damage inducible) which acts in correcting DNA lesions. Protein p53 also activates genes involved in the mechanism of apoptosis and suppresses the action of anti-apoptotic genes. The E6 protein of high risk human papillomavirus is able to bind to p53 leading to its rapid degradation. The codon 72 has different alleles, causing the insertion of different amino acids in this position of the protein: arginine (Arg - GCC) and proline (Pro - CCC), generating the genotypes: Arg / Arg, Arg / Pro and Pro / Pro. **Objectives:** To evaluate the frequency of polymorphism in codon 72 of p53 gene in 348 randomly selected women in the routine gynecological examination for HPV detection. **Materials and Methods:** Analysis was performed in cervical samples obtained from 348 women of Ouro Preto, Minas Gerais. The polymorphism at codon 72 of exon 4 of gene p53 was determined by PCR, with specific primers for each allele. The PCR products were analyzed in a 2% agarose gel by electrophoresis. **Results and Discussion:** The frequencies of genotypes were: Arg / Arg 41% (141), Arg / Pro 48% (168) and Pro / Pro 11% (39). HPV was demonstrated in 15.3% (53) patients; 8.0% (28) showed substantial alterations in cytological examination. Correlating cytological alteration and the genotypic frequencies, it was possible to show: ASC-US: Arg / Arg 21.4% (6), Arg / Pro 21.4% (6), Pro / Pro 7.1% (2). LSIL: Arg / Arg 10.7% (3), Arg / Pro 14.3 (4), Pro / Pro 3.6% (1). ASC-H: Arg / Arg 14.3 (4), Arg / Pro 7.1% (2). The genotype Arg / Arg was not more frequent in samples verified as ASC-US and LSIL, but was more frequent in the samples showing cytologically more severe diagnosis.

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5.12 Bovine papillomatosis in dairy herd: preliminary evaluation in Ouro Preto, Minas Gerais, Brazil

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Introduction: Bovine papillomavirus (BPV) is a double-stranded DNA virus that induces lesions (such as warts) in the epithelium. These lesions can develop into tumors when exposed to certain co-factors. This results in massive economic losses. Bovine papillomatosis is frequent in several regions of Brazil, with dramatic impairment to cattle breeding, but without systematic evaluations of its occurrence. **Objectives**: We investigated a dairy herd in Ouro Preto – Minas Gerais, collecting lesion fragments for detection and identification of papillomavirus types. **Methods:** Lesion fragments and peripheral blood samples were collected from affected cattle. Morphological alterations in infected tissues have been analyzed by anatomopathologic studies performed in wart fragments. DNA was extracted with a Tissue kit (Qiagen), in accordance with the manufacturer´s instructions. All samples were investigated concerning the presence of papillomavirus genome by PCR techniques, using specific and generic primers. In all positive samples, enzymatic digestion was performed for confirmation of the virus type and analyzed by electrophoresis in a 2% agarose gel stained with GelRed[tm]. **Results and Discussion:** All the samples using primers BPV-1 and FAP were found to be positive concerning the presence of papillomavirus genome. The positive products of PCR for FAP primers were confirmed by enzymatic digestion. The BPV positive products were confirmed by enzymatic digestion and sequencing. The identification of herds showing one specific BPV type is important in considering an evaluation of patterns of virus transmission and final vaccine procedures.

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5.13 Differential display as a cost-effective alternative for the study of eukaryotic transcriptomes

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Introduction: Systemic characterization of altered gene expression has been shown to provide useful data for molecular phenotyping and classification of malignant tumors as well as for identification of potential tumor-specific therapeutic targets. For these reasons, techniques capable of detecting differentially expressed genes across transcriptomes are now considered standard investigation methods for oncology. Adrenocortical carcinoma is a rare and aggressive malignant disease for which no effective treatment, besides surgery, is available. Only a few published studies have targeted the expression profile of adrenocortical tumor tissues. Therefore, we aimed to study the transcriptomes of adrenocortical tumor cells and tissue with the use of differential display (DD) - a strategy of transcriptome characterization that requires minute amount of starting RNA and standard equipment used in molecular biology. **Objectives:** To detect relevant differences in gene expression between tumor and normal adrenocortical tissues. **Methods:** RNA from an adrenocortical carcinoma cell line and from specimens of an adrenocortical carcinoma tumor were compared to a commercial pool of normal adrenal RNA using DD, which was based on: 1) systematic radiolabeled amplification of the mRNAs 3' termini; 2) high resolution polyacrylamide gel electrophoresis followed by exposure to X-ray films; and 3) retrieval of fragments of interest for cloning, sequencing and identification. The transcription patterns of normal tissue and adrenal cortex carcinoma were compared side-by-side, using DD: those bands with difference in intensity (reflecting differential expression) were candidates for analysis. In order to reliably identify differentially expressed transcripts we adopted optical density-based criteria to select bands of interest. **Results and Discussion:** Transcripts differentially accumulated in adrenocortical tumor cells were detected and represented virtually all chromosomes. A significant portion of these transcripts represent intro-derived sequences. Differential expression of most of these transcripts has not been reported for adrenocortical tumors. Optical density-based criteria and other alternative procedures introduced to some steps of DD reduced significantly some major drawbacks of this technique. Several aspects discourage the use of DD, such as difficulty in visual comparison of hundreds of bands/gel lane and multiple clone analysis for each fragment. Besides, it is generally considered to be a labor-intensive and time-consuming technique. Other negative points are heterogeneity of gel background and low half-life of radiolabeled nucleotide. But, important advantages of DD over other techniques (e.g., microarray) should be taken into account: no requirement of previous knowledge in mRNA sequences, low RNA quantity demand, sensitivity to abundant and rare transcripts and technical simplicity and accessibility. The technical improvements introduced in our work and other considerations may render DD more atractive as a method to compare eukaryotic expression patterns.

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5.14 Interaction of HPV16 L1L2 VLP with human amniotic fluid $CD34^+/CD117^+$ stem cells

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Introduction: The naked icosahedral capsid of HPV (human papillomavirus) is composed of 2 structural proteins called L1 and L2, which contain a double-stranded, circular DNA genome. VLPs (virus-like particles) are capsid without DNA, used as vaccines and for host-pathogen interaction studies. The infection of HPV occurs preferentially in epithelial cells; however, the presence of virus in tissue fluids is of interest from a clinical and basic research viewpoint. HPV16/18 DNA was detected in the amniotic fluid in pregnant women with cervical diseases related to HPV. The clinical meaning of HPV presence in the amniotic fluid before birth raises a great discussion around the possibility of newborn contamination. Prenatal HPV transmission was suggested when it was confirmed that surgical delivery did not protect children from mother-fetal transmission. It is important to confirm if newborns had a previous contact with HPV DNA sequences, before birth. For this, some host-pathogen interaction assays have been performed. **Objective:** This study investigated the possibility of HPV16 L1L2 VLPs interaction with cells from human amniotic fluid. **Methods:** Isolation and characterization of the cell types present in human amniotic fluid in different gestational stages were performed using cell culture. Cell samples were obtained from amniotic fluid by transabdominal amniocentesis from women clinically indicated for this procedure. Interaction assays using HPV16 L1L2 VLPs in immunofluorescence methods were analyzed by laser scanning confocal microscopy (LSCM). Anti-CD34 and anti-CD117 antibodies were used recognize stem cell markers, along with anti-transferrin receptor (anti-CD71). **Results and Discussion:** It was possible to identify fibroblasts and hematopoietic precursor stem cells in the cultures. The $CD34^+/CD117^+$ stem cells interacted with the HPV16 L1L2 VLPs by the internalization through CD71 receptor, confirmed by LSCM. The possibility of HPV16 infecting stem cells from hematopoietic precursor lineage can support the hematological route as a possibility of infection, which not considered in public health nowadays, mainly in the mother-fetal transmission hypothesis of HPV DNA sequences. In addition, these findings stimulate thought about the potential application of these cells for fetal therapy and tissue engineering.

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5.15 Partial sequencing analysis of the genome of influenza A samples of subtypes H3 and H8 isolated from wild migrating birds in São Paulo State, Brazil

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Introduction: Avian influenza virus belongs to the family *Orthomyxoviridae.* In the last years, highly virulent avian influenza subtypes, H5, H7, H9 and H10 have caused outbreaks and epidemics in poultry and fatal infections in humans. The wild and migrating birds may be participating in the maintenance and interspecies transmission of the sixteen subtypes of hemagglutinin. **Objectives:** To sequence the NS1 and M1 region from two influenza A viruses isolated from wild and migrating birds and comparison to sequences of all subtypes of influenza A available from public databases in order to determine the homology between these isolates. **Methods:** The samples from species *Sporophila caerulescens* (LE 6744) and *Elaenia mesoleuca* (LE 6712) were collected in reserves and experimental field stations located in São Paulo State - Brazil, during the years 1997 and 1998. The two viral types isolated from the samples (LE 6744 and LE 6712) were identified by the hemagglutination inhibition test (HI) using the 21 antibody patterns anti-influenza A type and one for the influenza type B. Other techniques used were transmission electron microscopy (TEM) and RT-PCR**. Results and Discussion:** The HI test demonstrated that the *Elaenia mesoleuca* sample showed an antigenic relationship with 80 HAIU to A/Turkey/Ont./6118/68 (H8N4) and the *Sporophila caerulescens* sample reacted with 160 HAIU to A/Hong Kong/1/68 (H3N2), A/Equine/Miami/63 (H3N8), and A/Duck/Ukraine/63 (H3N8) antiserum. TEM revealed structures of viral particles measuring 40 to 120 nm. RT-PCR detected the specific site for influenza A virus gene: NS1 oligonucleotides amplified a 189-bp fragment and primers for a fragment of M gene amplified a 340-bp fragment. The sequencing analyses of these two isolates revealed a high homology between these two strains or NS and M genes. Sequencing of the hemaglutinin genes is under way, and it is important to confirm the subtypes and to analyze the pathogenic potential of the samples.

5.16 Lipid body formation induced by a snake venom phospholipase A_2 (PLA_2) in macrophages and the signaling pathways involved

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Introduction: Lipid bodies are cytosolic inclusions present in most eukaryotic cells, containing neutral lipids surrounded by a single phospholipid membrane and specific proteins, such as the protein related to differentiation of adipocytes (ADRP). These inclusions compartmentalize signaling proteins such as MAPK, PKC, PI3K, enzymes responsible for synthesis of eicosanoids and ADRP. Increased LB formation occurs in activated inflammatory cells and has been associated with inflammatory diseases such as atherosclerosis, obesity and diabetes. Some of them show high levels of circulating secretory PLA_2 ($sPLA_2$). MT-III, a $sPLA_2$ isolated from *Bothrops asper* snake venom, induces local inflammation and increases the activity of macrophages (MΦs), which are central cells in inflammation. **Objectives:** To evaluate the ability of MT-III to induce LB formation in MΦs and the pathways involved, analyzing: a) the number of LBs formed, b) intracellular distribution and protein expression of ADRP and c) involvement of major signaling proteins. **Methods:** Thioglycolate-elicited MΦs from Swiss mice were incubated with MT-III (6.3 μg/mL) or RMI (control) for 1 h. LB formation was assessed by both staining with osmium tetroxide (1%) followed by counting under phase contrast microscopy and electronic microscopy after conventional procedures. Cell ADRP distribution and expression were evaluated by immunofluorescence assay and Western blotting, respectively. Participation of signaling proteins was evaluated by treatment of cells with specific inhibitors before stimulation with MT-III. **Results and Discussion:** Incubation of MΦs with MT-III resulted in increased numbers of LB (4±0.18 LBs/cell; control: 1.2±0.11). The ultrastructural analysis showed both light and strongly osmiophilic LBs in MT-III-stimulated MΦs with some LBs in close association with endoplasmic reticulum (RE). Enlargement of both RE and Golgi cisterns were also observed. In addition, MT-III upregulated ADRP expression (369%), a marker of LB formation, at 6 h after incubation and increased the intensity of fluorescent ADRP in the cytoplasm. Pretreatment of cells with either LY294002 (1 μM) or Wortmannin (5 nM), PI3K inhibitors, reduced MT-III-induced LB formation by 58%. SB202190 (p38MAPK inhibitor, 5 μM) or $AACOCF_3$ (20 μM) or Bel (2 μM), intracellular phospholipases inhibitors ($cPLA_2$ and $iPLA_2$, respectively) or PD98059 (ERK 1/2 inhibitor, 25 μM) or H7 (PKC inhibitor, 20 μM), reduced MT-III-induced LB formation by 51, 59, 50, 45 and 67%, respectively. However, herbimycin (PTK inhibitor, 10 μM), indomethacin or etoricoxib (cyclooxygenase inhibitors, 1 μM), zyleuton (5-lipooxygenase inhibitor, 1 μM) did not alter MT-III-induced effect. MT-III is able to induce the formation of LBs in macrophages. This effect is dependent on PI3K, p38MAPK, $cPLA_2$, $iPLA_2$, ERK1/2 and PKC but not on PTK nor metabolites from cyclooxygenases-1 and -2 or 5-lipooxygenase. Moreover, MT-III is able to recruit ADRP and up-regulate its expression. These events together with activation of RE and Golgi complex may be important in LB formation induced by MT-III. These data give new insights into the role of secretory PLA_2 in inflammatory diseases involving lipid body formation.

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5.17 Dental pulp stem cells as a source for neural regeneration

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Introduction: Dental pulp contains different populations of stem/progenitor cells that reside within the perivascular niche and originate from migrating neural crest cells. In the past years, studies have demonstrated the self-renewal capacity, clonogenic efficiency and multi-lineage differentiation potential of human dental pulp stem cells (DPSC). However, only recently was it demonstrated that these cells have the ability to differentiate towards functional neurons after chemical induction of differentiation (Arthur *et al.*, 2008). Previously, we showed that immature DPSC were able to undergo neuronal differention spontaneously (Kerkis *et al.,* 2006). **Objectives:** The goal of our study was to evaluate the capacity of human adult DPSC and immature DPSC to undergo spontaneous differentiation into different neural cells *in vitro*. **Methods:** Human adult and immature DPSC were characterized and maintained as previously described (Kerkis *et al.*, 2006). Differentiation towards neural cells was performed under culture conditions developed for neuronal cells in the absence of known growth factors, such as: epidermal growth factor (EGF), basic fibroblast growth factor (bFGF) and retinoic acid (RA). Anti-human antibodies: mouse anti-beta-III-tubulin, goat anti-nestin and rabbit anti-glial fibrillary acidic protein (GFAP) and others were used after cell fixation in 4% paraformaldehyde. The expression of cell specific proteins was analyzed under confocal microscopy. Morphological studies were performed using hematoxylin/eosin, as well as neutral red staining and analyzed by light microscopy. Functional tests are being carried out. **Results and Discussion:** Human adult DPSC and immature DPSC show rapid proliferation and expansion *in vitro*. They can be maintained for a long period in culture, which indicates their self-renewal potential. These cells expressed mesenchymal stem cells markers, as well as reacting positively with human embryonic stem cell markers. Undifferentiated immature DPSC cultured in basal medium already expressed neural progenitor markers, such as nestin and GFAP. These cells were able to respond to culture conditions usually used for neuronal cells, even without the use of chemical inducers, showing acquired neural cell-like morphology after eleven days of culture. The decrease in the expression of nestin and GFAP proteins was demonstrated during the process of neuronal differentiation. At the same time, the cells showed increasing expression of immature neural proteins. We observed that the cell populations that undergo neural differentiation showed terminally differentiated neuronal cell types and at the same time showed neurosphere formation. Terminally differentiated neural cells survive during a long period in culture. We showed that undifferentiated human adult and immature DPSC are already committed to originating neuron- and glial-like cells. The suggested model mimics neural stem cells growing and differentiating *in vitro* and can be possibly used to analyze the various stages of neural cell development. Dental pulp is a readily accessible source of stem cells which have a potential use in cell therapy to treat neurological disease.

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5.18 Characterization of multipotent equine adipose tissue-derived progenitor cells. Clinical case reports of allogeneic cell-therapy in horses

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Introduction: In horses, stem cell therapies are a promising tool for the treatment of many injuries, which are common consequences of athletic endeavor, resulting in high morbidity and often compromising performance. We reported the isolation and characterization of equine adipose tissue-derived progenitor cells (eAT-PC) before and after crypreservation (banking of eAT-PC). The aim of this study was further characterization of eAT-PC differentiation potential and application of allogenic eAT-PC for the treatment of tendonitis in horses. **Methods:** eAT-PC was maintained under conditions previously described. Differentiation towards muscle and neuronal cells was performed following routine protocols. Mouse anti-human antibodies, anti-myosin, anti-α-actinin, anti-MyoD1, anti-beta-tubulin-III, as well as rabbit anti-human anti-nestin and anti-glial fibrillary acidic protein (GFAP) were used. Twelve animals with tendonitis received 10^7 of eAT-PC into the injured tissue under local anesthetic and ultrasonographic control. After one month, ultrasonographic control was performed again. Since our study was based on clinical cases, the animals were heterogenous for age, weight and sex, but all of them were athletic horses. All procedures were approved by horse owners under signature of a veterinary service contract. **Results and Discussion:** After the induction of myogenic differentiation, the cells showed first signs of morphological changes similar to muscle cells, at day 10. Myosin, α-actinin and MyoD1 antibodies showed positive immunostaining with the cells confirming muscle cell differentiation. Prior differentiation into neuronal lineages, eAT-PC already showed strong nestin positive immunolabelling. Neuronal differentiation was evidenced by outgrowth formation and nucleous dislocation. Neuron-like cells derived from eAT-PC reacted positively with such markers as beta-III-tubulin and GFAP. Functional test are being carried out. One month after eAT-PC application into the lesion, the formation of healthy tissue has been observed. All treated horses showed a functional recovery and were able to return to their normal activity, without lesion recurrence. Extending our previous findings, we showed that eAT-PC were able to produce smooth and skeletal muscles and neuron-like cells. Their application in horses provided functional recovery of damaged tendons, and treated animals were capable to return to their normal activity. Our findings classify eAT-PC isolated and cultured *in vitro,* as a promising tool for cell therapy, which maintain their potential even after cryopreservation. Further studies are needed in order to understand the mechanism of their action on the recovery of damaged tissues.

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5.19 Increased level of chromosome aberrations in bovineS (*Bos taurus)* infected with BPV-1 and 2

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Introduction: Bovine papillomavirus (BPV) is represented by ten virus subtypes, epitheliotropic or mucosotropic, which are transmitted through contaminated blood and ephitelial contact. This virus has been related to chromosomal instabilities. **Objective:** The purpose of this study was to evaluate the levels of chromosome aberrations in samples of peripheral blood collected from cattle (*Bos taurus taurus*) to determine the presence of sequences of BPV by polymerase chain reaction (PCR). **Methods:** Sixty-one blood samples were collected from cattle showing papillomatosis (symptomatic animals) and without clinical signals (asymptomatic animals). Short-term lymphocyte cultures were performed for cytogenetic studies. Cytogenetic analyze was performed in a blind test. **Results and Discussion:** The results showed that 28 animals were not infected by BPV (Control group), 33 animals were infected by BPV types 1 and/or 2. The BPV-infected group included animals with papillomatosis and animals without detected lesions. Seventeen females (with papillomatosis) exhibited 42.71% of cells with chromosome aberrations; 16 animals (without papillomatosis) displayed 40.19%. A total of 2203 cells were analyzed: 918 showed one or more chromosomal aberrations. The chromosomal aberration rate in symptomatic and in asymptomatic animals was respectively 42.7± 7.8 and 40.2 ± 11, compared with an aberration rate of control group of 4±2. The Kruskal-Wallis test followed by the Mann-Whitney test was used for statistical analysis ($P < 0.0001$). Significant differences were not observed between infected subgroups ($P = 0.62$). The identified chromosomal aberration types were: centric association (CA); acentric fragment (AF); telomeric association (TA); telomeric association by a single chromatid (TAcr); chromatid breaks (CtB); chromosomic breaks (CmB); gaps; aneuploidy; polyploidy; addition or loss of chromosomal segment (add or del) and early chromatid separation (EcrS). The possibility of distinguishing infected and non-infected animals by levels of chromosome aberrations establishes evidence of the virus interaction with host chromatin.

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5.20 HPV detection and typing of women seen for routine evaluation in Health Department, Ouro Preto, Minas Gerais, Brazil

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Introduction: The human papillomavirus (HPV) is widely distributed in the world and virus persistent infection is recognized as an important cause, for the development of cervical cancer. Primary lesions detected by cytological examination can progress or regress spontaneously. The challenge in cervical cancer screening is to detect the risk of progression to cancer. Besides the presence of the virus, the main determinants of cervical cancer clinical progression include the involvement of high-risk viral types, viral load, integration of viral DNA in the host chromosome and interaction with different co-factors. **Objective:** We investigated the types of HPV in women of the city of Ouro Preto, MG, relating to HPV cytological alterations and the cervical cancer development. **Methods:** Patients received at the City Health Department for routine gynecological were submitted to anamnesis for assessing socio-demographic characteristics and family, sexual and reproductive history. Cervical samples were collected for cytological examination and molecular analysis (HPV). Detection and typing were done by polymerase chain reaction with primers My09 (5'-CGT CCA / AAA C / G GGA A / TAC TGA TC-3 ') and My11 (5'-GCA / CAG GGA C / AAC CAT T / T AAT GG-3 '), RFLP and sequencing. **Results and Discussion:** We evaluated 461 patients, mean age 38 years, where the majority were from the urban area (67.4%), married (46%), and with only 1 sexual partner (49.5%). Eighty-one (17.5%) presented with HPV infection, and 50 women (11%) had cellular changes in the Papanicolaou test: 28 women had cellular atypia of undetermined significance may be not neoplastic (ASC U.S.), 8 showed no cellular atypia discarding injury of high degree (Asch), 1 had non-neoplastic glandular cells (AGS-NOS) and 11 had squamous intra-epithelial lesion of low-grade (LSIL). A total of 65 had viral type identified by the method PCR / RFLP, where 60% had infections with oncogenic HPV high risk, 34% low risk HPV, 6% risk oncogenic HPV indefinite and 17% multiple viral infections. The data show the presence of different viral types in the city of Ouro Preto in women with or without various changes and cytological studies, indicating that prevalence studies should be developed previous to vaccination approaches, for the survey and understanding of the determinants of the development and progression of cervical cancer.

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5.21 Expression of extracellular matrix proteins (ECM) and matrix metalloproteinases (MMP) in human dental pulp stem cells

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Introduction: Adult stem cells can be isolated from different tissues including the human dental pulp, a structure originating from the dental papillae. These cells are important in a series of studies, such as the analysis of some odontogenic tumors. **Objective:** The aim of this study was to infer the histogenesis of odontogenic myxoma (OM), a benign odontogenic neoplasia, analyzing the extracellular matrix proteins (ECM) and matrix metalloproteinases (MMP) expressed in human dental pulp stem cells. **Methods:** Three different immature dental pulp stem cell cultures (IDPSC) (DL-1, DL-2 and DL-4) were used. The proteins searched were those routinely used to characterize the OM: vimentin, type I collagen, fibronectin, tenascin and hyaluronic acid (HA). MMPs frequently highly expressed in invasive tumors (MMP-1, MMP-2 and MMP-9) were also analyzed. Immunofluorescence and enzymatic assays were performed to determine the presence of these proteins inside the cells and in the conditioned media, respectively. **Results and Discussion:** All cell lines expressed vimentin, but none of them expressed HA, a protein frequently involved in cell migration and proliferation. The DL-1 line expressed all the other ECM proteins, and the expression of type I collagen was not observed in DL-2. Fibronectin and tenascin were not observed in DL-4. All cell lines expressed all the MMPs, but the release of MMP-2 in the conditioned media was significantly higher than with others. Based on the conditions of this study, it is possible to conclude that expression of ECM proteins and MMPs in IDPSCs were similar to those found in OMs. The lack of HA expression in these cells, a protein which characterizes the tumor, needs more investigation.

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5.22 Effects of ovariectomy and 17β-estradiol replacement on ERK1/2 activation in rat hippocampus

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Introduction: 17β-estradiol plays a potent neurotrophic and neuroprotective role in brain (reviewed in Brann et al., Steroids 72:381, 2007). The mechanisms underlying estrogen neuroprotection are not fully understood; however, several candidate targets have been identified, for example, members of the Bcl-2 family (Nilsen and Brinton, Proc. Nat. Acad. Sci. USA, 100: 2842, 2003). Recent studies from our laboratory have shown that 17β-estradiol may help maintain long-term neuronal viability in the hippocampus by regulating the expression of Bcl-2 family members if initiated immediately after ovariectomy (Sayuri et al., Anais da XXIII Reunião Anual da FeSBE, pp. 89, 2008). 17β-estradiol has also been shown to activate extracellular signal-regulated kinase (ERK) which mediates neuroprotection in the hippocampal CA1 after global ischemia (Jover-Mengual et al., Endocrinology 148:1131, 2007). Whether ERK signaling cascade is involved in estrogen-induced expression of Bcl-2 after ovariectomy remains to be explored. **Objective:** The aim of the present study was to determine the effects of ovariectomy and 17β-estradiol replacement on ERK1/2 activation in adult rat hippocampus. **Methods:** The experimental procedures were approved by the Research Ethics Committee of Instituto Butantan (number 569/09). Hippocampi were obtained from rats in proestrus (control), rats ovariectomized for 15 days (C15) and 21 days (C21), rats ovariectomized for 15 days and then treated with 17β-estradiol benzoate for 7 days (10 μg/rat, s.c., every other day) (E7) and rats ovariectomized and immediately treated with 17β-estradiol benzoate for 21 days (10 μg/rat, s.c., every other day) (E21). Western blotting for detection of ERK1/2 and phospho-ERK1/2 was performed as previously described (Lucas et al., Biol Reprod., 78: 101, 2008). **Results and Discussion:** Ovariectomy for 15 and 21 days (C15 and C21) did not have any effect on ERK1/2 phosphorylation compared to values obtained from control animals. Similar results were obtained with 17β-estradiol replacement limited to the last week of hormonal deprivation (E7). On the other hand, 17β-estradiol replacement throughout the post-ovariectomy period (E21) induced a rapid increase in the phosphorylation state of ERK1/2 (pERK1, 199.56 ± 14.81, n=3; pERK2, 139.03 ± 19.27, n=3) ($P<0.05$) compared to values obtained from control (100%) or ovariectomized rats (pERK1: 128.59 ± 21.19%, n=3; pERK2: 105.93 ± 5.93:, n=3 and pERK1: 104.38 ± 4.38%, n=3; pERK2: 106.76 ± 4.35%, n=3, respectively, C15 and C21). These results suggest that 17β-estradiol is involved in the regulation of ERK1/2 phosphorylation in rat hippocampus if initiated immediately after ovariectomy. Neuroprotection by estrogen could be mediated in part by expression of Bcl-2 through ERK1/2 phosphorylation. Further experimental approaches will be important to clarify these events.

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5.23 Preliminary assessment of the use of recombinant protein l2 of bovine papillomavirus

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Introduction: Papillomatosis is an infectious disease characterized by verrucous lesions, in the skin or mucosa, which affects several species of mammals, including man. In cattle, this disease is responsible for dramatic economic losses, as in reduction in milk production and weight loss. The tumors are usually benign and often regress spontaneously. However, the lesions can process to cancer. Bovine papillomavirus types -2 and -4 (BPV-2 and BPV-4) are associated with cancers of the bladder and upper digestive tract. **Materials and Methods:** The cloning of the L2 gene and expression in a bacterial system is a viable approach for the production of immunological inputs, such as diagnostic tests or vaccines. The N-terminal portions of the protein L2 (BPV-2 / BPV-4), previously cloned, were purified by affinity chromatography system for glutathione. The protein purification resulted in 5.0 mg / mL of the protein L2-BPV-2 and 7.5 mg / mL of the protein L2-BPV-4 which were used for inoculation of calves, two doses of 1.0 mg each protein were mixed 1:1 with aluminum hydroxide. The proteins were subjected to electrophoresis in denaturing polyacrylamide gel, transferred to nitrocellulose membrane and subjected to immunodetection using the pre-and post-immune serum. The levels of antibodies were analyzed in an ELISA reader, using microplates prepared with 1.0 g of each protein. **Results and Discussion:** The immune response obtained by vaccination of calves was satisfactory, indicating the feasibility of this approach for the implementation of a vaccine-BPV in the national herd. Further analyses of specific antibodies directed against the protein L2 of bovine papillomavirus types -2 and -4 are being improved.

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5.24 Secretion cycle of primary duct after milking in the snake *Bothrops jararaca*

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Introduction: Venom gland apparatus of Viperidae snake has four distinct parts: main venom gland, primary duct, accessory gland and secondary gland connected to the fang canal. The main venom gland is well studied. This gland has a central lumen where venom is stored and the secretory cells are stimulated for a new cycle of venom synthesis after emptying the lumen either by manual extraction or biting. The venom production cycle lasts around 30 – 50 days, peaking at 4 days after milking. There are few studies about the primary duct, and we have shown that the primary duct of *Bothrops jararaca* is folded and has a pseudostratified epithelium composed of secretory cells with negative reactivity for PAS and alcian blue, horizontal cells and mitochondria-rich cells. **Objective:** The aim of this study was to determine the secretion of secretory cells of primary duct of *Bothrops jararaca* snake during the venom production cycle. **Methods:** Female *Bothrops jararaca* snakes were anesthetized with pentobarbital sodium (30 mg/kg) and the primary duct from unmilked, 1 hour milked (n=3 each), 1 (n=1), 4, 7 (n=3 each) and 15 (n=2) days milked snakes were excised after decapitation. The primary ducts were fixed and embedded in Epon resin. Histological sections with thickness of about 1 μm were stained by Toluidine Blue. Animal care and procedures used were in accordance with the guidelines of the Animal Ethics Committee of Instituto Butantan (374/2007), and by the Brazilian Institute of Environment (IBAMA, License 01/2009). **Results and Discussion:** In unmilked snakes, the secretory cells of primary duct showed a large number of vesicles with different electrodensities and a lumen full of secretion. The presence of the secretion inside the lumen began to decrease 1 day after milking and was almost empty 4 days after milking. Afterward, the lumen started to be replenished and 15 days after milking was not completely full. Just after milking, the number of vesicle decreased. Large number of vesicles can be detected only 4 days after milking and persists until 15 days after milking. Therefore, exocytosis occurs just after milking, but the replenishment of the lumen starts only 4 days after milking. Our data show for the first time the secretion of the primary duct of *Bothrops jararaca* snake after milking. These results suggest a cycle of secretion similar to that found in the main venom gland.

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5.25 Isolation, culture and characterization of stem cells derived from feline adipose tissue and their multilineage differentiation potential

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Introduction: Mesenchymal stem cells (MSCs) are a promising tool for tissue regeneration, due to their particular characteristics of proliferation and differentiation into lineage-specific tissues such as bone, cartilage, fat, tendon, and muscle. In addition to the differentiation into their natural derivatives, multipotent MSCs have the potential to differentiate into other types of tissue-forming cells such as hepatic, renal, cardiac, and neural cells. Several publications have shown that stem cells reintroduced in the organism were capable to restore a tissue and its function. Although stem cells have been isolated from rodent and human tissues as dental pulp, muscle, skin and fat, very few data exist about stem cell isolation from nonrodent animals, such as dogs and cats. Adipose tissue is an attractive source of MSCs due to its abundance and ease of access with minimal donor site morbidity. **Objectives:** The aim of this current study was to provide evidence that feline adipose tissue–derived mesenchymal stem cells (fAD-MSC) can be isolated and characterized. Regarding their potential of proliferation and differentiation we directed our study to their use in cell therapy in pets. It is the essential first step toward their use in domestic cat diseases. **Methods:** First, fAD-MSCs were isolated using an explants method followed by freezing in liquid nitrogen until use. After thawing, cells were analyzed for proliferative potential, and subsequently their differentiation capacity in osteogenic, adipogenic, and chondrogenic inductive media. Differentiation was assessed by morphological criteria and immunohistochemistry. **Results and Discussion:** The cells showed similar fibroblast-like cell morphology *in vitro* before and after cryopreservation. After the induction of osteogenic differentiation, *von Kossa* staining revealed the formation of calcified extracellular matrix, and immunocytochemistry studies revealed positive immunostaining for osteocalcin (LF-32 and LF-126), as well as bone sialoprotein, confirming the osteogenic potential of these cells. The adipogenic differentiation was confirmed by oil red O staining, which revealed significant lipid droplet accumulation. Chondrogenic differentiation was observed after induced pellet culture and evidenced by histological (toluidine blue) and immunohystochemistry studies using specific antibodies, such as aggrecan and collagen type II. Under the influence of the 3 different media, fAD-MSCs were capable of advancing into all 3 differentiation pathways. In conclusion, the stem cells isolated from feline adipose tissue show characteristics similar to those of human mesenchymal stem cells. They are a suitable source of multipotent stem cells for future tissue engineering strategies and cell-based therapies.

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5.26 New system for gene therapy delivery: crotamine kinetic in cell cycle and penetration mechanisms

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Introduction: Cell penetrating peptides (CPPs) hold great potential as delivery vectors, *in vitro* and *in vivo,* for use in research and medicine. The current use of these CPPs is limited due to a lack of cell specificity in CPPs-mediated cargo delivery, as well as insufficient knowledge of their uptake mechanism. Previously, we isolated and characterized crotamine, a rattlesnake venom compound able to penetrate into the cytoplasm and the nuclei. The interaction of crotamine with centrioles and chromosomes during cell proliferation and division suggests that its uptake depends on the cell cycle. Previously, our group demonstrated that crotamine penetrates into cells via endocytosis and interaction with heparan sulfate. **Objectives:** The aims of the present study were to determine: a) the crotamine effect in cell proliferation; b) its uptake in different cell cycle phases; c) the crotamine interaction with different syndecans (1 to 4); and d) clathrin and/or caveolin-mediated endocytosis involved in crotamine uptake. **Methods:** For this study, we performed MTT assay, immunofluorescence and flow cytometry using B16-F10, CHO-K1 and mouse peritoneal cells. Crotamine was conjugated with fluorophores FITC or Cy3 for the visualization of this CPP into the cell. For the proliferation assay, the crotamine concentration was 0.01-10 μM. To analyze crotamine kinetics into cell cycle, we synchronized the cells in G0/G1 phases and the uptake was determined every four hours, for 28 h. To verify the colocalization of crotamine with syndecans and the aforementioned proteins related to endocytosis, the following antibodies were used: anti-syndecans 1-4, anti-clathrin and caveolin. **Results and Discussion:** We observed that at a concentration of up to 1 μM, crotamine induced higher cell proliferation, up to 30% in comparison with proliferation in control culture. After cell synchronization, we analyzed crotamine fluorescence in different cell cycle phases. This CPP did not reveal any difference in treated cultures, suggesting that crotamine uptake by cells occurred independent of cell cycle phase. Crotamine did not show specificity for any syndecans tested, the colocalization of these molecules was observed on the plasma membrane and in cytoplasmic vesicles. We also observed crotamine colocalization associated with clathrin and caveolin. Our data indicates that crotamine: a) at a low concentration increased cell proliferation; b) uptake did not depend on phase of cell cycle; c) interacted with four syndecans types; and d) can be internalized concomitantly by different mecanisms: clathrin and caveolin endocytosis pathways. These data show that crotamine behaves like other CPPs such as Tat and Antp.

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5.27 Expression of laminin-5 in actinic cheilitis and human lip squamous cell carcinoma

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Introduction: The actinic cheilitis is the initial and incipient stage of lip squamous cell carcinoma, resulting from excessive and longterm exposure of the components to_ultraviolet solar radiation. Laminin-5 plays important role in the development and in the invasion of squamous cell carcinoma. **Objectives:** To analyze and evaluate through immunohistochemical techniques the expression and distribution of laminin-5 in actinic cheilitis and in lip squamous cell carcinomas. **Methods:** Paraffin blocks of actinic cheilitis, superficially invasive squamous cell carcinoma and invasive squamous cell carcinoma, from Hospital das Clínicas da Faculdade de Medicina da USP, were sectioned. Immunohistochemical reactions to laminin-5 gamma-2 chain (Clone: 4G1 - Dako) were carried out, and the slides were examined with a light microscope. **Results and Discussion:** The majority of actinic cheilitis cases showed no cytoplasmic staining for laminin-5 gamma 2 chain. All cases of superficially invasive carcinoma and invasive carcinoma showed laminin 5 gamma 2 chain positivity located in the extracellular matrix and in the peripheral cells of tumor invasive front, but the expression was not homogeneous. No cancerous tissue close to invasive areas showed cytoplasmic expression in the epithelial basal layer. Thus, it is possible to conclude that expression of laminin-5 gamma 2 chain is closely related to the degree of tumor epithelium dysplasia.

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5.28 Characterization of *Bothrops jararaca* genomic sequences encoding toxins with antimetastatic potential

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Introduction: Jararhagin and bothropasin are metalloproteinases present in *B. jararaca*'s venom that have the potential to be used as tools for inhibition of tumor metastasis. The cDNA sequence identity of these toxins shows 65 % identity in the pro-domain region; 74 % in the catalytic domain; 93 % in disintegrin-like domain; and 100 % in the cysteine-rich region. The published cDNAs have been obtained from mRNA of *pools* of specimens. **Objective:** This work aimed at the characterization of the genes encoding those toxins through the identification of exons and introns in the genomic DNA from a single specimen. **Methods:** Genomic DNA was extracted from the blood by current procedures and PCR *primers* were designed according to the published cDNAs for both toxins. The amplification products were cloned using the *pGEM T Easy Vector* and DH5α *E.coli* according to current procedures. The clones were sequenced using the *Big Dye Terminator Kit* and *ABI Prisma 3100 (Applied Biosystems)*. **Results and Discussion:** The total length of the jararhagin gene is about 7402 bp and that of bothropasin is 7849 bp. Twelve exons and 8 introns have already been identified and may be accessed in the GenBank database. Approximately 886 bp only are still missing to finish the whole sequence of each toxin gene. Both toxin genes had the same numbers of exons and introns, but there were several differences between the sequences of exons and introns, and also in the sizes of introns. These findings constitute the first report in the literature concerning the determination of exon and intron boundaries for jararhagin and bothropasin. The data indicate that they are encoded by two different genes that did not undergo accelerated evolution following gene duplication, since the main differences between them are found in intronic sequences. Clones containing sequences of interest, especially those encoding the disintegrin domain, may be used in the future to express protein domains particularly involved with the inhibition of tumor cell growth *in vitro* and *in vivo*.

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5.29 Expression of hypoxia regulatory genes during lung inflammation produced by intestinal ischemia-reperfusion in AIRmax and AIRmin mice

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Introduction: Oxygen homeostasis is essential for survival and physiologic development of organisms. Lack of oxygen to tissue is a common underlying factor in morbidity and mortality for numerous serious medical conditions. Myeloid cells exert their functions in specialized areas of hypoxia. The group of proteins responsible for adaptation of myeloid cells in hypoxia includes the products of the *Hif-1α* gene. Hypoxia-inducible factor is a transcription factor which responds to changes in oxygen and induces the transcription of the genes such as *Vhl*, *Vegf* and pro or anti-inflammatory cytokine-encoding genes. **Objectives:** The aim of this study was to characterize the cellular and molecular mechanisms operating in hypoxia state induced by intestinal I/R on lung inflammatory reaction in two lines of mice genetically selected for maximal (AIRmax) or minimal (AIRmin) acute inflammatory response. **Methods:** Lung injury was evaluated by MPO activity, cellular infiltration in the lung parenchyma, CD11b and CD62L expression on BM, blood and lung cells and hypoxia gene expressions by real-time PCR. **Results and Discussion:** We observed an intense neutrophily in the AIRmax lung ($2.6\pm0.3x10^6$cell/ml) and a low infiltrate in AIRmin mice with equivalent levels compared to the control groups ($0.8\pm0.2x10^6$cell/ml). Adhesion molecules analyzed showed higher expression of CD62L in the AIRmax I/R lung than AIRmin I/R or control groups. A high expression of *Hif-1α, Vhl, Il-1β* and *Il-6* was observed in AIRmax I/R mice. Conversely, AIRmin I/R mice showed low expression of these genes. AIRmax mice display a higher acute inflammatory reaction after I/R than do AIRmin mice, characterized by massive neutrophil infiltration. High levels of *Hif-1α, Vhl, Il-1β and Il-6* were determinant for high inflammatory response in AIRmax mice. This interline difference is in accordance with selection phenotypes indicating these lines as a model for the study of *Hif* regulation in the inflammatory response after I/R.

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5.30 Yolk sac and bone marrow progenitors can be isolated from canine fetus

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Introduction: The fetus is a source of non-embryonic stem cells (SC), and these cells are easy to obtain and to expand undifferentiated, while showing high differentiation abilities. The dog model mimics important aspects of human anatomy, physiology and pathology producing pre-clinical safety results after xenotransplantation of stem cells. On the other hand, the pet is a growing market of stem cells application. **Objective:** In our present study we aimed at the isolation and characterization of yolk sac (YS) and bone marrow (BM) progenitor cells from canine fetus. **Methods:** All experimental procedures were approved by the Ethics Committee of the School of Veterinary Medicine and Animal Science of São Paulo University (No. 931/2006). Canine fetuses at 30 and 55 days gestation were obtained through an ovarian hysterectomy and under general anesthesia. The explants of YS tissues and BM cells flushed from femur bone were cultured in α-MEM + 15% fetal bovine serum. Morphology of the cells was evaluated with an inverted microscope and by transmission electron microscopy (TEM). Antibodies: goat anti-Oct3/4; mouse anti-vimentin; mouse anti-VE-cadherin; rabbit anti-nestin; mouse anti-cytokeratin. Expression of anti-CD44 antibody was analyzed by flow cytometry. Osteogenic, adipogenic, neuronal differentiation assays as well as karyotype analysis were performed according to routine protocols. **Results and Discussion:** Two days after cultivation, first fibroblast-like colonies appeared in BM cell culture as well as outgrowth of fibroblastic cells around YS explants. TEM analysis demonstrates that the cells from both sources showed two cell populations with a high nuclear-to-cytoplasmic ratio and fibroblast-like morphology. Both, YS and BM fibroblast-like cells reacted positively and uniformly with vimentin antibody, and the majority of cells were positive to nestin antibody. In addition, cells isolated from BM showed positive immunostaining with CD44 (~ 96.6 %) and cytokeratin. Several fibroblast-like cells isolated from YS were also positive for Oct 3/4 and VEGF antibodies. Both cell populations showed successful osteogenic and chondrogenic differentiation. In addition, BM-derived fibroblast-like cells were able to produce neuron-like cells. Karyotype analysis performed at passages 6 and 7 revealed a normal diploid chromosome number (2n=78). Our data suggest that undifferentiated cells can be isolated from BM and YS and maintained in culture during successive passages, showing a normal karyotype. TEM analysis indicates that BM and YS undifferentiated cells are composed of two different cell populations: i. cells show characteristics similar to very small embryonic-like (VSEL) stem cells and ii. cells show characteristics of bone marrow mesenchymal stem cells (MSC), both described previously for human BM – MSC. Unexpectedly, antigen expression profile BM of progenitor cells and differentiation capacity suggest their ectodermal commitment.