7. Cellular Biology and Genetics

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7.01 Circulation of influenza A subtype H3 virus in migrating and wild birds from the Atlantic rainforest in Brazil

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Introduction: Avian influenza A vírus belongs to the family Orthomyxoviridae. There are sixteen subtypes of hemagglutinins and nine subtypes of neuraminidase. The wild and migrating birds may be involved in the H16 subtypes of the virus hemagglutinin and its maintenance and transmission in the interspecies cycle in nature. Several low pathogenic forms of influenza A vírus isolated from birds are transformed into high pathogenic forms and have caused outbreaks and epidemics among both humans and poultry. The samples from species were obtained from reserves and experimental field stations located in São Paulo State - Brazil, during the years 1997 and 1998. **Objectives:** This study aimed to subtype the positive samples using RT-PCR and PCR for rapid detection and identification of the pathogenic subtype. **Methods:** RT-PCR (primer 12U) and PCR (specifc primer, H3) were used. **Results and Discussion:** Of the the 12 samples analyzed, seven showed positivity for subtype H_3 (Samples: 6712, 6715, 6744, 6781, 6782, 6784 and 6841). Among these, 5 samples showed positivity for influenza A, but they were not identified as H_3. Sequencing of the hemagglutinin gene is underway, and it would be worthwhile to compare the sequences of pathogenic potential in these positive samples.

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7.02 Study of endogenous regulatory mechanisms in acute systemic inflammation after intestinal ischemia and reperfusion in mice

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Introduction: Ischemia induces a hypoxic situation starting a sequence of events that are amplified after reperfusion, leading to severe organ injury and dysfunction by the production of reactive oxygen species, leukocyte infiltrate and protein extravasation. Inflammation has been considered the most important cause of injury in organs subjected to ischemia and reperfusion (I/R). Lung is an important target for systemic inflammatory response associated with intestinal I/R. The mechanisms that trigger inflammation after ischemia have been studied in several experimental models. **Objectives:** The aim of this study was to evaluate the endogenous mechanisms responsible for the regulation of systemic inflammation after intestinal ischemia and reperfusion in two lines of mice genetically selected for maximal (AIRmax) or minimal (AIRmin) local acute inflammatory response to a nonimmunogenic substance. **Methods:** Intestinal ischemia was induced by clamping the mesenteric artery for 45 min, and the following parameters were studied at 0, 1, 4 and 24 h after reperfusion. Lung injury was evaluated by MPO activity in lysates and cellular infiltration in the lung parenchyma. Hypoxia and inflammatory cytokine mRNA expression were evaluated in the lungs by real-time PCR, and the protein content in lung lysates was analyzed by 2D-gel eletrophoresis. **Results and Discussion:** We observed in AIRmax ischemic lungs a progressive neutrophilia starting after ischemia with a peak at 4 h ($2.7 \pm 0.3 \times 10^6$ cells/ml). On the other hand, in AIRmin mice the neutrophil content was similar to that of control groups ($1.2 \pm 0.2 \times 10^6$ cells/ml). Corroborating these results, MPO activity was higher in AIRmax ischemic lungs with absorbance (A_{450nm}) of 0.62 ± 0.038 compared to AIRmin ischemic lungs (0.308 ± 0.04). I/R induced upregulation of *hypoxia inducible factor (Hif-1α)* and of *von Hippel Lindau (Vhl)* genes, and of the inflammatory cytokines *Il-1b* and *Il-6* in AIRmax lungs. Conversely, in AIRmin mice these genes were not modulated by I/R. In line with gene expression, the profile of proteins in the lungs showed quantitative and qualitative differences between the two strains. These interline differences observed after intestinal I/R are in agreement with the selected phenotypes of AIRmax and AIRmin mice. The results demonstrate a positive correlation between the inflammatory ability of these strains and the expression in the lungs of genes involved in hypoxia and inflammation in the intestinal ischemia/reperfusion experimental model.

Supported by: CAPES, CNPq

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7.03 Selective cancer cell toxicity of crotamine – a cationic peptide/toxin from the venom of the South American rattlesnake

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Introduction: Crotamine is a toxic peptide of 42 amino acids found in the venom of South American rattlesnakes. Due to the presence of nine lysine residues and three disulfide bonds, crotamine is a very compact and highly positively charged molecule, which shares structural features with some cationic antimicrobial peptides (CAPs). Recently, we reported that crotamine at a non-toxic concentration for normal cells (human fibroblasts, embryonic stem cells and others), was harmful for CHO-K1 tumor cells. **Objectives:** The aim of this study was to evaluate the cytotoxic effect of crotamine on primary invasion of cutaneous melanoma produced by injection of B16-F10 cells into C57Bl/6J mice. **Methods:** Crotamine at 1.0 mM (5 mg/ml) was shown to be cytotoxic to B16-F10 cells *in vitro*, using the MTT assay and Hoechst 33342 and propidium iodide (PI) staining. Fluorescent-labeled crotamine has an intracellular distribution and co-localization with syndecan-1, as visualized by confocal microscopy in cultured B16-F10. The effect of crotamine on the tumor transplantation was studied in two groups each composed of 35 animals: one, receiving cells only (non-treated group) and the other, cells and 0.2 mM (~1mg/mL) crotamine daily (crotamine-treated group), for 21 days. **Results and Discussion:** Drastic delay of tumor implantation and significant reduction of animal death was observed in the crotamine-treated group. Average weight of tumor in non-treated group was 4.60 gm, while in crotamine-treated only 0.27gm was observed in a few animals. According to Kaplan–Meier curves, the crotamine-treated group showed significant survival (n=28) in comparison with the non-treated group (n=7). Our data indicate that crotamine, a mild toxin, is selectively toxic to cancer cells *in vitro* and in *vivo,* with regard to an aggressive and fast-growing type of cancer, which deserves further investigation as a tumor-killing compound.

Supported by: FAPESP, FAPESP/CAT-cepid

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7.04 In utero transplantation of human immature dental pulp in normal dogs

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Introduction: In-utero transplantation of donor cells and tissues in the fetus during gestation is an efficient treatment of several diseases, showing homing in damaged tissues. Immature dental pulp stem cells (IDPSC) were isolated from dental pulp of young patients and well characterized by our group. The fetus is an immunologically privileged environment, which is not susceptible to foreign antigens. **Objectives:** In present study, we aimed at in-utero transplantation of IDPSC into canine fetuses in order to analyze their biodistribution within normal tissues and organs. **Methods:** All experimental procedures were approved by the Ethics Committee and were performed under appropriate anesthesia. Plasmid vector carrying green fluorescent (GFP) gene was introduced into IDPSC. Six million GFP- IDPSC were transplanted following laparotomy and intraperitoneal injection under intra-operative ultrasound control into 5 fetuses at 45 days of gestation. Control animals did not receive the cells. Ultrasound analyses were performed daily. Seven days after cell transplantation, ovarian hysterectomy was performed. Several organs and tissues were collected and fixed or cryopreserved. Ultrafine cryosections were analyzed by confocal microscopy. **Results and Discussion:** The GFP gene was successfully introduced into IDPSC and following selection an expression of protein was observed in 98% of cells. Transplantation procedure was well accepted by the fetuses and the mother. No hemorrhage or intra-abdominal liquid accumulation was observed. The IDPSC, which express GFP protein were detected in several tissues: in thoracic muscle they were localized in blood vessels in tunica externa; in jejunum (gut) they were found in epithelium of the mucosa (cover villi) and in cerebellum they were found in the molecular layer of cerebellar Purkinje cells. Interestingly, IDPSC were detected in placenta, especially in muscle layer (tunica media) of placental artery. The present study showed that in-utero xenotransplantation of hIDPSC was safe. These cells were able to cross the placental barrier and home in on placental vessels. We observed that biodistribution in normal organism is different from that in injured individuals. As it was reported in injured organisms, the cells tend to localize around damaged sites in response to environmental inflammatory factors. In contrast, in normal organism IDPSC traffic throughout all tissues, where they show cell clusters suggesting cell or cells -"founder" event. Our data provide new insight into better understanding of the mechanism of stem cell homing in normal versus damaged tissues.

7.05 On the way to the clinic: successful corneal reconstruction by human immature dental pulp stem cells

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Introduction: Total limbal stem cells deficiency (LSCD) is caused by a variety of conditions, such as genetic disorders, chemical and thermal injury, inflammatory diseases, and others. It often results in persistent corneal epithelial defect or abnormal reepithelialization by conjunctival epithelial cells, leading to a significant loss of visual acuity. Searching for an alternative that could be potentially used in corneal reconstruction, we turned our attention to a population of stem cells isolated by our group, which are called human immature dental pulp stem cells (hIDPSC). These cells were shown to express mesenchymal and embryonic stem cell markers. Recently, we demonstrated that hIDPSC express a set of specific markers of limbal stem cells (LSC), such as integrin β-1, vimentin, p63, ABCG2, connexin 43 and K12, *in vitro*. **Objective:** To provide a new source of limbal epithelial stem cells, we aimed to determine the outcome of hIDPSC transplantation for ocular surface reconstruction in an animal model of LSCD after chemical injury. **Methods:** LSCD was induced by the application of 0.5 M NaOH to the right eye of rabbits for 25 s (mild chemical burn [MCB]) and for 45 s (severe chemical burn [SCB]). After 1 month, a superficial keratectomy was performed to remove the fibrovascular pannus that covered the animals' burned corneas. A tissue-engineered hIDPSC sheet was transplanted onto the corneal bed and then covered with deepithelialized human amniotic membrane (AM). In the respective control groups, the denuded cornea was covered with AM only. After 3 months, a detailed analysis of the rabbit eyes was performed with regard to clinical appearance, histology, electron microscopy, and immunohistochemistry. **Results and Discussion:** Corneal transparency of the rabbit eyes that underwent hIDPSC transplantation was improved throughout the follow-up, while the control corneas developed total conjunctivalization and opacification. Rabbits from the MCB group showed clearer corneas with less neovascularization. The clinical data were confirmed by histologic analysis that showed healthy uniform corneal epithelium, especially in the MCB group. We also showed that in the SCB group reconstruction of the stromal layer was observed, sugesting the next surgery would lead to complete corneal reconstruction. The corneal tissue showed expression of human cornea-specific proteins, therefore corneal tissue formed after transplantation was of hIDPSC origin. In the control corneas, none of these human antigens were detected. Overall, these data showed that transplantation of a tissue-engineered hIDPSC sheet was successful for the reconstruction of corneal epithelium in an animal model of LSCD. Currently, CONEP permission was obtained by our group for transition of this stem cell technology from pre-clinical to clinical applications of hIDPSC in corneal injuries in humans. It is noteworthy that corneal reconstruction using stem cells is also supported by ISSCR among a very small number of therapies which are considered safe.

Supported by: FAPESP

7.06 Epistatic relationship between cyclin D1 and Ki-Ras in Y-1 malignant cell line

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Introduction: Arginine vasopressin (AVP) and FGF2 (fibroblast growth factor 2) are two proteins whose pro-survivor and mitogenic effects are well established in several cell lines. However, our group and others have demonstrated that these factors can also act, selectively in malignant cells, inhibiting cell proliferation. Using a ras-driven mouse malignant cell model (Y-1), we showed that this cytotoxic effect of FGF2 depends on high levels of Ras-GTP protein. On the other hand, the cytotoxic effect of AVP depends on the maintenance of high levels of Ras-GTP protein but also involves the inhibition of cyclin D1. We also know that in these cells cyclin D1 does not play its ordinary role in promoting the transition G0 → G1 → S. This function is restricted to cyclin E. These results suggest that cyclin D1 plays a distinct role other than its classical function, in ras-dependent malignant cells. Our hypothesis is that cyclin D1 is a co-oncogene of Ki-ras, ensuring cell survival against the Ras-oncoprotein stress. In addition, we assume that the Ki-ras gene modulates the function of cyclin D1 gene in an epistatic manner. This hypothesis is supported by the phenotype of the sub-lines resistant to FGF2, which are also resistant to AVP. **Objectives:** We aimed to test the hypothesis of epistatic relationship between cyclin D1 and Ki-ras genes through selection of clones resistant to AVP from the parental line Y-1, and analysis of their phenotype concerning the expression of cyclin D1, level of ras-GTP protein and vulnerability to both AVP and FGF2 cytotoxic effects. **Methods:** The parental line Y-1 was submitted to AVP for selection of resistant colonies. Eleven clones survived in culture and were analyzed for sensitivity to AVP and FGF2 by growth curves and clonogenic assays. The expression of cyclin D1 and Ras-GTP were analyzed by PCR and Western blotting. In cytogenetic characterization of the clones, we used conventional Giemsa staining and G, C and Ag-NOR banding. **Results and Discussion:** On the Y-1 cell line, 2 marker chromosomes, HSR-I and HSR-II, carry the amplification of the Ki-ras gene. The cytogenetic study of the clones resistant to FGF2 revealed that they lost the chromosome HSR-I. HSR-II cannot be lost because it is the only one that transcribes rRNA. Three clones resistant to AVP were analyzed after the selection with AVP. They showed resistance to AVP, as demonstrated by growth curves and clonogenic assays. We also analyzed the effect of FGF-2 on these three clones and found that they maintained sensitivity to this factor, which is consistent with our hypothesis. We are now analyzing the expression of cyclin D1, cyclin E1, p27, and Ras protein.

Supported by CNPq, FAPESP

7.07 Non-hematopoietic progenitor/stem cells derived from yolk sac, liver and bone marrow of canine fetus

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Introduction: Due to the functional role of nonhematopoietic/progenitor stem (nHPS) cells during fetal development in supporting hematopoiesis, these cells co-localize with transient hematopoietic stem cell niches (tHSCN) such as yolk sac (YS), liver (LV) and bone marrow (BM). These nHPS are also responsible for rapid growth and amplification of hematopoietic stem cells and later for formation of adult permanent hematopoietic stem cell niches (pHSCN). These multiple functions of nHPS cells, which localize in different organs, reflect on nHPS cell properties after *in vitro* isolation. It seems that nHPS cells can be isolated only from functional tHSCN during very time-restricted developmental window. Thus, successful isolation of nHPS cells directly depends on correct definition of fetal age. This is the first study aimed at the isolation of nHPS cells in dogs. **Objectives:** We isolated and characterized nHPS cells from canine fetal tissues (YS, LV and BM) **Methods:** The fetuses at different ages were used in order to establish the best fetal age suitable for the isolation of nHPS cells. Crow-rump technique was used for age estimation. The nHPS cells were obtained from the culture of tissue-explants. **Results and Discussion:** All nHPS cells showed fibroblast-like morphology. Ultraestructural analyses showed that populations of BM- and YS-nHPS cells were composed of cells with embryonic- and mesenchymal-like morphology. The LV-nHPS cells, in turn, were of the mesenchymal- and epithelial-like type. CFU-F assay was more efficient in LV-nHPS cells. All nHPS cell expressed vimentin, nestin and CD44 proteins. Expression of cytokeratin-18 was observed in BM- and LV-nHPS cells, while VE-cadherin was expressed only in YS-Nhps cells. The BM-, LV- and YS-nHPS cells expressed pluripotent stem cells markers, such as Oct3/4 and Sox2. Karyotype analysis revealed a normal diploid chromosome set (2n=78) in all cell cultures. The nHPS cells from YS and BM were able to undergo osteogenic and chondrogenic differentiation, while LV-nHPS cells showed osteogenic commitment only. None of nHPS cells were able to differentiate into adipocytes. None of the nHPS cells showed teratoma formation after injection into muscle of nude mice. The efficient isolation of nHPS cells was delineated during fetal development in dogs. We showed that morphologically more homogeneous population of nHPS cells can be isolated from BM, LV and YS between days: 50 -60, 30-45 and 25- 30 of canine pregnancy, respectively. Overall, we isolated heterogeneous populations of BM-, LV- and YS-nHPS that displayed distinct plasticity, when compared to NHPS cells isolated from adult tissue.

Supported by: FAPESP

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7.08 Isolation of embryonic-like stem cells from fetal brain without feeder layer

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Introduction: Currently, stem cell research has grown, especially regarding the search for new sources of stem cells. Embryonic stem cells are isolated from the inner mass of the blastocyst. These cells are an important model of mammalian development in vitro and are the focus of a great deal of research for their use in cell therapy. Recently, it was demonstrated that pluripotent stem cells can be isolated from the epiblast. Consequently the epiblast will differentiate very early into primordial germ cells (PGC). During embryo development, PGCs migrate into the aorta-gonad-mesonephros region (AGM), which is responsible for waves of hematopoiesis. This region derives hematopoietic stem cells (HSC) and together with the PGC to promote the colonization of the fetal liver, thymus, spleen and bone marrow (BM). These results are new and there is much to be studied on the characteristics and properties of populations of stem cells from embryo and fetal tissues, mainly in fetal brain. **Objectives:** We aimed at the identification and isolation of embryonic-like stem cells from fetal brain. **Methods:** The canine embryo was used in order to isolate embryonic-like cells. The crow-rump technique was used for age estimation. The nHPS cells were obtained from the culture of tissue-explants in cell culture medium composed of DMEM-F12 supplemented with 15% fetal bovine serum, 1% non-essential amnino acids, 1% streptomycin/penicillin and 1% glutamine. **Results and Discussion:** After 48 h, we observed in the fetal brain explants, as expected, the formation of cells with typical morphology of stem cells from the nervous system, such as neurospheres, rosettes and neurons. At the same time other colonies, which displayed embryonic-like stem cell morphology were found in the culture. These colonies remained undifferentiated for 5 days. After splitting, these colonies were then able to grow on both a feeder layer of mouse embryonic fibroblasts and matrigel, while maintaining an undifferentiated state and typical ES cell-like morphology, without LIF. This finding is innovative because it suggests that ES-like pluripotent cells can be isolated at the advanced stages of the development from the fetus. It opens a discussion about maintaining primordial ES cell niches not only in early embryos (blastocysts), but also in some other tissues, such as brain and testis, during fetal and adult development. The function and developmental sense of this phenomenon needs to be clarified.

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7.09 Search for therapeutic strategies in cells transformed by human papillomavirus

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Introduction: Cervical carcinoma expresses E6 and E7 oncoproteins from high risk human papillomavirus (HPV). A more significant role for malignant transformation can be assigned to the *E6* and *E7* oncogenes and their respective proteins, which neutralize cellular tumor suppressor function, through degradation of p53 and pRB (retinoblastoma protein), respectively, contributing to tumorgenesis. The E6 oncoprotein encoded by the virus is multifunctional, demonstrating several cellular targets; however, it is not clear yet if all of these activities are related to tumor malignancy. The E7 oncoprotein shows isoforms which act in different ways. **Objectives:** The aim of this study was to evaluate the distribution of viral oncoproteins E6 and E7, mitochondria, transferrin receptor (TfR), transferrin (Tf), ferritin (Fe), clathrin, cytochrome c, porin and F_0F_1-ATPase, in human cells transformed and non-transformed by HPV, to search for strategies against carcinogenesis. **Methods:** HPV-negative cell lines were transfected with pLXSN vectors, containing complete E6 and E7 genes sequence, used as positive controls. Cells were analyzed through immunofluorescence assays, cellular fractionation for mitochondrial isolation in HPV-positive and HPV-negative cells, for ultrastructural immunocytochemistry and Western blotting. **Results and Discussion:** The antigens E6 and E7 oncoproteins were well recognized by the antibodies in HPV-transformed cells and in pLXSN vector transfected cells. TfR were detected in abundance at the plasma membrane of cells, as well as Fe being labeled in the cytoplasm, nucleus and mitochondria and cytochrome c, porin and F_0F_1-ATPase preferentially in the mitochondria. The great amount of iron suggests a participation of this element in the HPV cell transformation, maintaining mitochondrial cytochrome c levels. Co-localizations of E6 in isolated mitochondria were detected in HPV-transfected and HPV-transformed cells, by ultrastructural immunocytochemistry. In conclusion, these findings point to the fact that E6 acts as an anti-apoptogenic factor, and all anti-apoptogenic factors detected are being evaluated as potential prophylactic and therapeutic strategies in the development of vaccines against cervical and anogenital cancers.

Supported by: FAPESP, CAPES, Fundação Butantan, Instituto Butantan

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7.10 Erythropoiesis in the spleen of the snake *Bothrops jararaca* (Viperidae, Crotalinae)

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Introduction: The spleen changes over vertebrate evolution both structurally and functionally.In most adult fish and amphibians, the hemopoietic function of the spleen is remarkable, while in Amniota immunological function is predominant, and the bone marrow is most responsible organ for blood supply. Moreover, in mammals and birds, on ontogeny the spleen has an important role in hematopoiesis. In severe cases of anemia, even in mammals, it can produce red blood cells. These functions appear in some groups of reptiles, but most studies focus on morphological or immunological analysis, and few studies have reported on hemopoietic activity. **Objectives:** The aim of this study was to obtain detailed information on the structure of the spleen, mainly regarding the presence of erythropoiesis in healthy adults, adults with induced hemolytic anemia and in early postnatal *Bothrops jararaca* (Bj) snakes. **Methods:** Snakes recently captured from nature during spring/summer were sent to Instituto Butantan and separated into groups: healthy adults (HA, n=7) and adults with induced hemolytic anemia (IHA, n=4) by saponin *s.c.* injection (7.5 mg/ml/kg). Animals were anesthetized with thiopental (30 mg/kg), and the spleen was removed, fixed in Bouin solution and embedded in paraffin or historesin. Blood was drawn to determine pro-erythrocyte counts and some hematological parameters in IHA. **Results and Discussion:** Adult Bj spleen is closely associated with the pancreas. However, it is enclosed by a fibrous capsule of connective tissue, which is rich in collagen and blood vessels, clearly separating it from pancreas. This capsule penetrates the splenic pulp, forming septa, called capsule/septal tissue. The white (WP) and red (RP) pulp are distinct and evident, but there is not a clear demarcation between them. RP is highly vascularizated, with vessels and sinusoids, and with many collagen fibers. Differently, WP is basically composed by lymphoid tissue, mostly lymphocytes, with a few vessels and fibers. These characteristics are also present in other species of snakes. WP and RP ratio is variable, and seasonality seems to be an important factor for it. Spleen can store thrombocytes and granulocytes, and considerable amount of heterophils were seen in snakes' RP spleen, as previously reported in the alligator *Alligator mississippiensis*. About erythropoiesis, there was no activity in HA. However, an increase in immature cells was found, which were similar to erythroblasts in IHA, whose pro-erythrocytes levels reached 7 to 17%. These cells were found in WP, and additional staining and electron microscope analysis should be undertaken to identify them. Spleen from the early postnatal period will also be studied.

Supported by: FAPESP, CNPq

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01 a 03 de Dezembro de 2010, São Paulo, SP

7.11 Genotoxic and cytotoxic effects of 7,12-dimethylbenz(a)anthracene (DMBA) on bone marrow cells from mice genetically selected for inflammatory response

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Introduction: DMBA is a genotoxic agent that reacts directly with DNA, inducing cytotoxicity. Previous studies have reported that DMBA-induced bone marrow (BM) toxicity is p53-dependent in vivo. Two lines of mice genetically selected for maximal (AIRmax) or minimal (AIRmin) local acute inflammatory response (AIR) to a non-immunogenic substance (Biogel) have shown different sensitivity to DMBA toxic effects. Our laboratory reported significantly decreased BM cellularity and decrease in proliferation capacity in AIRmin mice, following DMBA administration. **Objectives:** We investigated the genotoxic and cytotoxic effects of DMBA treatment on the BM of AIR mice. **Methods:** AIRmax and AIRmin mice were treated *in vivo* with a single i.p. dose of 50 mg/kg DMBA in olive oil. BM cells were used in these studies. Flow cytometric analysis was used to determine apoptosis/necrosis levels in cells stained with propidium iodide and annexin V. DNA damage was assessed by the alkaline single-cell gel electrophoresis (comet) assay and expressed as tail moment. Total RNA was extracted to quantify the expression levels of the poly (ADP-ribose) polymerase family member 1 (*parp-1)* involved in DNA repair, by real-time PCR. The p53 protein levels were determined by Western blot analysis. **Results and Discussion:** The kinetics of cell repair was measured at various times after DMBA treatment over a 24-h period. DMBA treatment induced an increase in the tail moment at 2 h in AIRmax (8.0±0.14 versus 0.17±0.09 control mice) and AIRmin (16.2±0.4 versus 0.11±0.05 control mice). The removal of DNA lesion was observed at 4h in AIRmax and at 8h in AIRmin BM cells. Additionally, 24 h after DMBA treatment, the percentage of necrotic cells increased significantly ($p < 0.01$) in AIRmin mice only (25.1±9.2 versus 8.4±4.7 control mice). No significant effects were observed in the percentage of apoptotic cells. The *parp-1* gene showed a 3-fold increase in mRNA expression in AIRmax cells after 12 h of DMBA treatment. Futhermore, p53 protein level was 7.7-fold lower in cells of AIRmax and 6-fold higher in AIRmin mice after 24 h of DMBA treatment, when compared to their controls. Our results demonstrated that DMBA produces long-lasting genotoxicity and cytotoxicity in BM cells in AIRmin mice only. p53 protein is increased in response to the DNA damage induced by DMBA followed by increased necrotic cell numbers in AIRmin mice. On the other hand, AIRmax mice showed a decreased p53 protein level, increased PARP-1 expression and larger capacity for removal of DNA lesions, which suggests that it has an efficient DNA repair mechanism.

Supported by: CAPES, CNPq

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01 a 03 de Dezembro de 2010, São Paulo, SP

7.12 Lessons from nature: biological versatility of crotamine – a cationic peptide from the venom of the South American rattlesnake

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Introduction: Natural biodiversity of substances isolated from microorganisms, plants, animals or insects can serve as a prototype for designing biopharmaceutical products with a variety of specific properties. Animal venoms were used by humankind to cure diseases since ancient times. Several toxins and their derivates isolated from the venom are currently used in the clinic and diagnosis, while others appear as a basis for the development of new drugs with potential application for incurable human diseases, such as cancer and others. **Objectives:** This work focused on crotamine, a small basic toxic polypeptide found in the venom of the South American rattlesnake *Crotalus durissus terrificus*, which was discovered more than 50 years ago. Despite the time, the exceptional biological versatility of crotamine was demonstrated only in the past six years. **Methods:** The novel and exquisite biological activities of crotamine were accomplished using very low, micromolar, concentration of crotamine peptide and refined instrumention, such as confocal microscopy analysis in combination with culture of selected lines of actively proliferative (replicating) cells. Moreover, a multidisciplinary study based on structural reduction of crotamine proved to be successful in maintaining the natural cell penetrating and nuclear homing abilities. **Results and Discussion:** Here, we have shown (*i*) crotamine's cell penetrating ability, which allows it to pass through cell membranes and to accumulate preferentially in the nucleus; (*ii*) its property of intracellular vesicle tracking and ability to serve as a cell cycle marker, including interaction with chromosomes, nucleoli and centrioles, and (*iii*) its capability of delivering DNA into different mammalian cells *in vitro*. More recently, the antimicrobial action and possible selective anti-tumor activity of crotamine have been also suggested. Recent knowledge about crotamine may lead readers to examine the paradigmatic way of discovering novel and unpredictable properties of "old" toxins. Multidisciplinary studies used for crotamine investigation placed it in a rare category of versatile biomolecules, in which concentration, molecular target preference, structural ancestrality and specificity toward biological membranes play an integral role. The data demonstrated that crotamine is a druggable peptide ready to be employed as imaging agent for detecting dividing cells, intracellular delivery system for hydrophilic biomolecules, and alternative chemotherapeutic compound for aggressive types of cancer. The strategy of discovering crotamine properties can be stepwise dissected and followed for the investigation of undisclosed and unpredictable biological activities of other potentially useful natural toxins and drugs.

Supported by: FAPESP

7.13 Long-term culture of adherent mouse embryonic stem cells

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Introduction: Innumerous protocols, using the mouse embryonic stem (ES) cells as a model for in vitro study of neuron functional properties and features, have been developed. Most of these protocols are short-lasting, which therefore does not allow a careful analysis of neuron maturation, aging, and death processes. **Objectives:** We describe here a novel and efficient long-lasting protocol for in vitro ES cell differentiation into neuronal cells. **Methods:** It consists of obtaining embryoid bodies (EBs), followed by induction of neuronal differentiation with retinoic acid of non-adherent EBs (three-dimensional model), which further allows their adherence and formation of adherent neurospheres (AN, bidimensional model). **Results and Discussion:** AN can be maintained for at least twelve weeks in culture under repetitive mechanical splitting providing a constant microenvironment (in vitro niche) for the neuronal progenitor cells avoiding mechanical dissociation of AN. The expression of neuron-specific proteins, such as nestin, sox1, beta III-tubulin, MAP2, NF-M, Tau, NeuN, GABA and 5-HT, were confirmed in these cells maintained for three months with several splittings. Additionally, expression pattern of microtubule-associated proteins, such as Lis1 (lissencephaly) and Ndel1 (nuclear distribution element-like), which were shown to be essential for differentiation and migration of neurons during embryogenesis, was also studied. As expected, both proteins were expressed in undifferentiated ES cells, AN, and non-rosette neurons, although showing different spatial distribution in AN. In contrast to previous studies, using cultured neuronal cells derived from embryonic and adult tissues, only Ndel1 expression was observed in centrosome region of early neuroblasts from AN. Mature neurons, obtained from ES cells in this work, display ion channels and oscillations of membrane electrical potential, typical of electrically excitable cells, which is a characteristic feature of functional CNS neurons. Taken together, our study demonstrated that AN represent a long-term culture of neuronal cells, which can be used to analyze the process of neuronal differentiation dynamics. Thus the protocol described here provides a new experimental model for studying neurological diseases associated with neuronal differentiation during early development; it also represents a novel source of functional cells that can be used as tools for testing the effects of toxins and/or drugs on neuronal cells.

Supported by: FAPESP

XII Reunião Científica Anual do Instituto Butantan
01 a 03 de Dezembro de 2010, São Paulo, SP

7.14 Intense acute inflammation affects the number of bone marrow progenitor cells in emergency granulopoiesis in mice

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Introduction: The production of leukocytes in bone marrow (BM) is crucial for innate and adaptative immunity. Developmental commitment to a particular blood-cell lineage occurs by the sequential differentiation of hematopoietic stem cells (HSCs) into multipotential progenitors and terminally differentiated cells under the action of hematopoietic cytokines and transcription factors. Most knowledge comes from the steady-state process of leukocyte production, although recent studies have demonstrated that acute inflammation alters this process in the bone marrow (emergency granulopoiesis), accelerating myelopoiesis over lymphopoiesis. Mice genetically selected for high (AIRmax) or low (AIRmin) acute inflammatory response to Biogel P100, represented by the number of infiltrated granulocytes in inflammatory exudates, constitute a suitable model to study normal and emergency granulopoiesis. **Objectives:** The aim of this study was to comparatively evaluate in AIRmax and AIRmin, the effects of acute inflammation on three populations of progenitor cells in BM: hematopoietic stem cell (HSC), common myeloid progenitors (CMP) and granulocyte-macrophage progenitors (GMP), and the differentiation capacity of BM cells into neutrophils. **Methods:** Progenitor BM cell populations were characterized by flow cytometry in normal mice and 24 h after sc injection of Biogel using a combination of antibodies directed to specific surface molecules. Proliferation and neutrophil differentiation were evaluated in 5-day liquid cultures of BM cells stimulated with G-CSF plus IL-3 combined with all-trans retinoic acid (ATRA). **Results and Discussion:** FACS analysis revealed 4% HSCs ($lin^-sca\text{-}1^+ckit^+$ cells) and 11% GMPs ($ckit^{high}Fc\gamma RIII/II^{high}CD34^{high}$ cells) in BM cells from normal mice of both lines. CMPs ($ckit^{high}Fc\gamma RIII/II^{low}CD34^{high}$) were higher in AIRmin (5%) than in AIRmax (2.5%) BM cells. At 24 h after Biogel treatment, the percentage of HSCs, CMPs and GMPs increased 2-fold in AIRmax, reaching 8, 5 and 20%, respectively. In contrast, the percentages were maintained in AIRmin mice. On day 5 of culture, BM cells from AIRmax mice showed higher proliferation in normal ($6.06\pm1.29 \times 10^5$ cells/ml) as well as in Biogel-treated mice ($8.83\pm0.02 \times 10^5$ cells/ml) compared to AIRmin (0.90 ± 0.20 and $2.38\pm0.19 \times 10^5$ cells/ml, respectively). AIRmax BM cultures had 60% mature neutrophils ($CD38^-GR1^+$ cells) and AIRmin, 35%. Inflammation-induced extrinsic factors, such as cytokines, probably modulate cell-intrinsic factors, which can trigger distinct mechanisms toward self-renewal of HSCs and consequent differentiation into granulocytes in these lines.

Supported by: CNPq

XII Reunião Científica Anual do Instituto Butantan
01 a 03 de Dezembro de 2010, São Paulo, SP

7.15 Testing the potential of adipose tissue-derived stem cells in the treatment of equine endometriosis

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Introduction: Endometriosis is a progressive and irreversible disease which is defined as active or inactive periglandular and stromal endometrial fibrosis, including glandular alterations within fibrotic foci. Modifications induced by the disease alter the surface of the endometrium which, as a consequence, lead to infertility (impaired embryo implantation). Conventional treatments do not reduce the fibrotic process or even help to restore fertility. Stem cell (SC) therapy in horses is a promising tool for tissue reconstruction, which also provides additional benefits, such as anti-inflammatory and immunosuppressive activity. To the best of our knowledge, there is no information about the use of stem cells derived from equine adipose tissue, for the treatment of endometrosis. **Objectives:** The aim of the present work was to test the use of equine heterologous adipose tissue-derived stem cells to reduce the inflammatory process and to remodel periglandular fibrotic tissues affected by endometrosis. **Methods:** Equine heterologous adipose tissue-derived stem cells, previously obtained and characterized by our group, were used. Six estrus synchronized mares suspected of endometrosis (four for treatment and two for control) and three normal mares, also syncronized, were used. The diagnosis had been established by histomorphological and immunohistochemical analyses, performed using formalin-fixed, paraffin-embedded uterine biopsies. The sections were stained with hematoxylin/eosin and alcian blue, as well as anti-CD 10, anti-cytokeratin (CK) 22, estrogen receptor, progesterone receptor and anti-α-actinin antibodies. Equine undifferentiated stem cells were stained using a vital dye (Vybrant CFDA-SE Cell Tracer Kit®). These cells were infused into the uterus of the four treatment mares. Each one of these animals received $2.5x10^7$ stem cells. The two control mares were infused with a placebo. After 7, 15, 25 and 35 days, new uterine biopsies were obtained and remodeling of uterine tissues by stem cells was analyzed following protocol above. **Results and Discussion:** Histological changes were observed in the uterine biopsies of the 4 treatment mares before treatment, which confirmed the presence of fibrotic periglandular tissue in affected animals. Analysis performed 7 days after treatment confirmed the presence of injected cells in the uterine tissues, by red fluorescent signal provided by vital dye, while control animals did not show fluorescence. No signs of immunologic reaction in response to application of cells were recorded, since the presence of T-cells infiltrating at the site of cell graft was not observed. The presence of equine stem cells within uterine tissues was confirmed. Further investigations are needed in order to prove the remodeling of uterine tissue by stem cell differentiation.

Supported by: CAPES, FAPESP

XII Reunião Científica Anual do Instituto Butantan
01 a 03 de Dezembro de 2010, São Paulo, SP

7.16 Differential protein expression in snake venom gland in quiescent and activated stages

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Introduction: The venom gland of viperid snakes has a central lumen where the venom produced by secretory cells is stored. When the venom is lost from the lumen, the secretory cells are activated and new venom is produced. The production of new venom is triggered by the action of noradrenaline on both a- and b-adrenoceptors in the venom gland. We have shown that the protein composition of the venom gland changes during the venom production cycle. Noradrenaline released just after venom extraction regulates the activation of transcription factors and consequently regulates the synthesis of proteins in the venom gland, which is important to activate the venom gland for venom production. **Objectives:** The aim of this study was to further analyze the proteins of venom gland that are involved in venom gland activation by noradrenaline released just after losing venom from the lumen. **Methods:** Venom gland were obtained from female *Bothrops jararaca* snakes in the quiescent stage (N=3) and in activated stage (4 days after venom extraction, N=3). Extracts of these glands were prepared and the proteins were analyzed by two-dimensional gel electrophoresis (2-DE). For the first dimension, isoelectric focusing was done in precast IPG strips (18 cm, pH 3-10) at 20ºC. For the second dimension, the IPG strips were applied to 12.5% SDS-polyacrylamide gels. Gels were run in triplicate, stained with Coomassie Blue G for 4 days and the density of the spots was quantified using ImageMaster 2D Platinum 7. **Results and Discussion:** The 2-DE images of venom gland extracts showed stained spots with PI ranging from 4 to 10 and molecular mass ranging from 200 to 7 kDa. Comparison between venom gland in quiescent stage and in activated stage pointed out that different proteins are expressed. The analysis of the gel showed that 15 spots of protein were expressed in both stages, but only the density of two of them was significantly higher in the extract of venom gland in quiescent stage ($p < 0.05$). Specific spots were found in each stage. The presence of 260 spots was observed only in the extract of venom gland in quiescent stage, whereas 216 spots were observed only in the extract of venom gland in activated stage. The data obtained from 2-DE analysis demonstrates that a great variation in protein expression occurs between venom glands in quiescent and activated stage. These results are in accordance with our previous results using one-dimensional electrophoresis. The identification of these proteins allows us to understand the mechanism of venom gland activation with consequently new insights into the regulation of exocrine glands of mammals.

Supported by: FAPESP, CNPq

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01 a 03 de Dezembro de 2010, São Paulo, SP

7.17 Production of recombinant FGF2 18 kDa and 22.5 kDa in *Escherichia coli* to probe FGF intracellular signaling in mammalian cell lines

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Introduction: FGF2 (fibroblast growth factor 2), the prototype member of the FGF family, has multiple molecular species, sharing a C-teminal sequence of 155 amino acids, which are translated from different initiation sites of the same mRNA. The smaller of the family, FGF2 (18 kDa), is released to the extracellular milieu and binds to specific receptors (FGFR), initiating a complex array of signals with paracrine and autocrine functions. On the other hand, larger isoforms of FGF2 (21, 22, 22.5 and 34 kDa) are intracellular species, which are translated from alternative codons (CUGs) and remain inside the cell interacting with unknown partners to play still undefined intracrine functions. Recently, our group demonstrated that FGF2 (18 kDa) blocked the cell cycle and induced senescence in ras-dependent malignant cells, in spite of triggering mitogenic pathways ERK-MAPK and PI3K. However, FGF2 (22.5 kDa) triggers only the mitogenic pathway in the same cell lines. **Objectives:** Our objective was to produce the recombinant proteins: FGF2 (18 kDa) and FGF2 (22.5 kDa) with and without His or protein A tags. These recombinant proteins are important for later search of intracellular partners of both FGF2 18 or 22.5 kDa, aiming to elucidate FGF2 signaling in mammalian cells. **Methods:** Different *E.coli* strains were utilized (DH-10β, DH-5a and ArticExpress). These strains were transformed with cDNAs of FGF2 (18 and 22.5 kDa), which was subcloned in pET vector system. Selected colonies were grown in LB medium at 37°C and induced by different IPTG concentration (0.1-1.0 mM). Bacterial lysates were submitted to SDS-PAGE and Coomassie staining to visualize the best concentration of IPTG, and purification was done in the FPLC system. **Results and Discussion:** The expression of FGF2 (18 and 22.5 kDa) with protein A tag in *E. coli* DH-10β was successful on a small scale (10 mL) and, presently, biologic assays and larger scale production are underway. FGF2 (18 kDa), without tags, was cloned in pET-3d plasmid and expressed in *E.coli* (BL21) with 0.5 mM IPTG. Cell lysates were fractionated in an Akta FPLC system using a NaCl gradient. The fraction eluted at 1.7 M NaCl is under analysis using an MS/MS system.

Supported by: FAPESP, CNPq

7.18 Effect of protease-activated receptor-2 activating peptide on B1 cell spreading and its modulation by the C-terminus of the calcium-binding protein S100A9

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Introduction: B1 cells, found in the peritoneal cavity of mice, are able to differentiate into phagocytes. They show similar functions compared to macrophages, suggesting their importance as a new mononuclear phagocyte in the inflammatory process. A number of serine proteases display diverse extracellular and intracellular functions during inflammation via protease-activated receptors (PARs). We have shown that PAR-1 and -2 influence the spreading and phagocytosis by adherent peritoneal cells in mice, and that the C-terminal peptide from murine S100A9 (mS100A9p) inhibits the increment induced by PAR-1 activating peptide in these events. To date, however, the participation of PARs and mS100A9p has not been studied in B1 cell function. **Objectives:** In this work, we evaluated the ability of synthetic PAR-1 (PAR_1-AP) or PAR-2 (PAR_2AP) activating peptides to interfere with B1 spreading, and the putative modulatory effect of mS100A9p on this phenomenon. **Methods:** B1 cells obtained from stationary cultures, originated from the peritoneal cavity of Swiss mice, were cultivated and plated on glass coverslips ($2x10^5$ cells/coverslip) in 0.5 mL of R10 medium/well (control) in order to carry out spreading assays. In addition, B1 cells were incubated in R10 medium containing PAR_1AP (20 or 40 mM/well) or PAR_2AP (5, 10, 20 or 40 mM/well). The effect of the reverse PAR_2AP peptide (5 or 10 mM/well) was also evaluated in the presence or not of PAR_2AP. In addition, B1 cells were treated with mS100A9p (1.17 or 2.35 mM/well) during the spreading period (24 h) in the presence or not of PAR_2AP (5 mM/well). Data were generated by evaluating cells by phase contrast microscopy. **Results and Discussion:** The results demonstrated that only PAR_2AP, at all concentrations tested, increased the spreading ability of B1 cells, and that this effect was blocked by its reverse peptide, suggesting the specificity of this receptor on B1 cell spreading. The reverse peptide of PAR_2AP *per se* did not interfere with B1 spreading. However, mS100A9p inhibited not only cell spreading in the control group, but also the increase in spreading induced by PAR_2AP. These findings demonstrate for the first time that B1 cells respond to the PAR-2 agonist, and that this effect is modulated by mS100A9p. The model used here, in conjunction with the data shown, may be used as a tool for providing a better understanding of the involvement of PARs and B1 cells in the pathophysiology of the inflammatory process, as well as for using mS100A9p as a modulatory molecule to control the function of inflammatory cells.

Supported by: FAPESP, Fundação Butantan

XII Reunião Científica Anual do Instituto Butantan
01 a 03 de Dezembro de 2010, São Paulo, SP

7.19 Brazilian advances in stem cell technologies: first report about successful and efficient production of induced pluripotent stem cells

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Introduction: Induced pluripotent stem (iPS) cells have emerged as an alternative to human ES cells, which allows us to overcome ethical problems of embryo destruction and to obtain patient matched pluripotent stem cells for future therapies. The marathon of iPS cells started when Yamanaka in 2006 derived these cells by forcing expression of certain genes in fibroblasts, reversing them into the pluripotent state similar to that of embryonic stem (ES) cells. iPS cells can be generated by reprogramming human terminally differentiated cells and precursors by the overexpression of specific genes, such as Oct3/4, Nanog, Sox-2, c-Myc, Klf-4, which normally express in early development. However, fibroblasts show low efficiency and slow reprogramming compared to other somatic cell types. Indeed, more immature somatic cells and cells isolated from younger organisms have shown higher efficiency of reprogramming. Previously, our group isolated a unique population of immature dental pulp stem cells (IDPSC) from deciduous teeth, which we used as a source for iPS cells generation. IDPSC is a very attractive cell type that can be easily isolated from an assessable tissue of young patients. IDPSC show fibroblast-like morphology, retain characteristics of adult multipotent stem cells and express at least one transcription factor, Oct4. **Objectives:** The aim of the present work was to derive induced pluripotent stem cells from human immature dental pulp stem cells. **Methods:** Using four of Yamanaka's factors, we easily derived iPS from IDPSC, which were named IDPS-iPS. Reprogramming of IDPSC occurred under feeder-free conditions, allowing simple pluripotent colony harvest and avoiding future problem of zoonoses. In order to evaluate teratoma formation, one million iPS cells were injected into nude mice. These reprograming cells were characterized by RT-PCR and immunofluorescence using some specific markers. Karyotype analyses were also performed. **Results and Discussion:** Such reprogrammed cells display all characteristics of human ES cells especially with respect to differentiating capacity. Multiple small colonies were already observed five days after injection. IDPS-iPS cells generated teratomas after intramuscular injection into nude mice demonstrating a wide range of differentiated tissues. It was reported that the suppression of p53 gene increased efficiency of reprogramming, suggesting its use as an improvement tool. Although IDPSC strongly express p53, they show high reprogramming efficiency (2.8%). The suppression of p53 is dangerous because iPS cells carrying genetic aberrations, although showing normal iPS cell morphology, can be obtained. Thus, IDPSC-derived iPS cells show an advantageous cell type for future therapies, since they do maintain expression of p53, while control human ES cells do not, suggesting that IDPSC could be safer for application in stem cell therapies.

Supported by: FAPESP

7.20 Immature dental pulp stem cells: biotechnological product ready to go through stem cell-based therapy market

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Introduction: Human immature stem cells from dental pulp (IDPSC) are originated from the neural crest and express embryonic, mesenchymal and epithelial stem cell markers in vitro. They appear to be less problematic, as they are not ethically controversial and painful source. In addition, IDPSC are widely multipotent, not tumorigenic, and maintain their ''stemness'' through several serial passages. Because of short population doubling time, these cells can be scaled up to large numbers. Due to these characteristics, they are considered the most promising tool for regenerative medicine and tissue engineering. **Objectives:** Aiming at establishing a cell bank of IDPSC, we compared IDPSC isolated at the very beginning (early) of dental pulp tissue culture and after three months (late). **Methods:** The phenotype and proliferative potential and also differentiation capacity were evaluated, when IDPSC were cultured in different media. Immunofluorescence and RT-PCR analyses were performed using mesenchymal/embryonic and epithelial stem cell markers. IDPSC were submitted to neural/chondrogenic and myogenic differentiation and were confirmed by morphological, immunofluorescence and specific staining. **Results and Discussion:** Our data demonstrate that IDPSC can be easily isolated and successfully expanded *in vitro*, while maintaining their undifferentiated status, preserving their properties even after cryopreservation. Furthermore, dental pulp tissue can be maintained for a long time in culture providing an unlimited source of IDPSC for future therapeutic applications. We also demonstrated that no significant changes in proliferative potential, stem cell marker expression profile and differentiation capacities in both, early and late isolated IDPSC populations, were observed. These data can contribute significantly to the practical application of IDPSC in regenerative medicine. Therefore, this report describes that the hurdles of adult stem cell technologies have been overcome in the case of IDPSC. These stem cells represent a biotechnological product ready to go through the stem cell-based therapy market.

Supported by: FAPESP

7.21 HPV genotype distribution in women seen for routine examination in health department, Ouro Preto, MG, Brazil

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Introduction: 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 clinical progression of cervical cancer include the involvement of high-risk viral types, viral load, integration of viral DNA in the host chromosome and interaction with different co-factors. **Objectives:** We investigated the types of HPV in women of the city of Ouro Preto, MG, relating to HPV cytological alterations and cervical cancer development. **Methods:** Patients received at the City Health Department for routine gynecological were submitted to anamnesis for assessing socio-demographic characteristics, including family, sexual and reproductive history. Cervical samples were collected for cytological examination and molecular analysis (HPV). Detection and typing were performed by polymerase chain reaction with primers MY09/11, RFLP and sequencing. **Results and Discussion:** We evaluated 569 patients, the majority being from the urban area (63.6%), married (63.4%), with only 1 sexual partner (49.6%). The overall HPV prevalence was 23.4% and 56 women (10%) showed cellular changes in the Papanicolaou test: 33 women had cellular atypia of undetermined significance possibly not neoplastic (ASCUS), 8 showed no cellular atypia discarding injury of high degree(ASCH), 1 had non-neoplastic glandular cells (AGC-SOE), 13 had squamous intra-epithelial lesion of low-grade(LSIL), and 1 had squamous intra-epithelial lesion of high-grade(HSIL). A total of 97 women had viral type identified by PCR-RFLP, and 15 had viral type identified by sequencing. Among the patients, 60% had infections with oncogenic HPV high risk, 21% with low risk HPV, and 3% of indeterminate oncogenic risk HPV, while 16% had multiple viral infections. The most common HPV types were HPV 16 (34.6%), HPV 6 (9.8%), HPV 18 (6%), HPV 53 and HPV 61 (5.3%).The data show the presence of different viral types in the city of Ouro Preto in women with or without cytological alteration, and cytological studies indicate that prevalence studies should be conducted prior to vaccination approaches, for the survey and understanding of the determinants of the development and progression of cervical cancer.

Supported by: CAPES, CNPq, PRONEX, FAPESP

7.22 Analysis of codon 72 polymorphism of *p53* gene in women from Ouro Preto, Minas Gerais

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Introduction: The p53 gene (17p13.1) has 11 exons, where the first is not transcribed. The p53 protein has 393 amino acids, 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. The p53 protein 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. 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:** The aim of the study was to determine the frequency of polymorphism in codon 72 of the p53 gene in 348 randomly selected women undergoing routine gynecological examination for HPV detection. **Methods:** Analysis was performed in cervical samples obtained from 482 women of Ouro Preto, Minas Gerais. The polymorphism at codon 72 of exon 4 of gene p53 was determined, using the PCR technique, 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% (197), Arg/Pro 47% (228) and Pro/Pro 12% (57). HPV was found in 13.0% (65) of patients; 9.0% (42) showed relevant alterations in cytological examination. Correlating cytological alteration and the genotypic frequencies, it was possible to determine the following. ASC-US: Arg/Arg 14.0% (6), Arg/Pro 23.0% (10), Pro/Pro 17.0% (7). LSIL: Arg/Arg 12.0% (5), Arg/Pro 10.0% (4), Pro/Pro 7.0% (3). ASC-H: Arg/Arg 10.0% (4), Arg/Pro 5.0% (2) Pro/Pro 2.0%(1). The genotype Arg/Arg was not more frequent in samples verified as ASC-US and LSIL, but was more frequent than samples cytologically showing a more severe diagnosis.

Supported by: CNPq, FAPESP, PRONEX, FACEPE

XII Reunião Científica Anual do Instituto Butantan
01 a 03 de Dezembro de 2010, São Paulo, SP

7.23 Epithelial lineage fate of human immature dental pulp stem cells

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Introduction: Human immature dental pulp stem cells (IDPSC) are of neural crest origin, and they express *in vitro* a set of human embryonic stem (ES) cell, and mesenchymal and neuroepithelial stem cell (MSC and NESC) markers. We already showed that they were able to reconstruct corneal epithelium of the burned eye surface in an animal model, producing a complex tissue composed of five different cell types, suggesting their capacity to respond adequately to environmental signals and to differentiate into the tissue of interest. **Objectives:** In order to extend our knowledge about epithelial lineage fate of IDPSC, we tested the capacity of these cells to populate germinal epithelium. How can this fate of IDPSC be influenced after transplantation into the testis of fertile (normal environment) and infertile (deficient environment) mice? **Methods:** IDPSC from patients of both sexes were used. Different IDPSC transplantation experiments were performed. First, a cell suspension (10^5 cells) was stained with Vybrant CM-DiI. Additionally, LacZ or GFP gene reporters were introduced into IDPSC. In addition, cells were injected into the testis of fertile mice. The mice were killed after three, five and nine days. Second, to destroy endogenous spermatogenesis, the recipients were irradiated and cells were injected directly into seminiferous tubes. Mice were killed three months after injection. Control mice were injected with physiologic solution. Thin whole-testis sections were prepared. Presence of IDPSC in mouse testis was detected by Vybrant CM-DiI and by expression of the enzyme β-galactosidase or GFP by confocal microscopy. In addition, fluorescent *in situ* hybridization (FISH) analysis using a probe for human sex chromosome was carried out. **Results and Discussion:** Our data demonstrated that after IDPSC transplantation into testis of fertile mice, fluorescent signals were observed within cross-sections in several seminiferous tubules (ST). In fertile mice, the process of population and proliferation was quick. Three days after transplantation, hIDPSC were detected in Leyding and Sertoli cell compartments. On the 5^{th} and 9^{th} days, these cells formed fluorescently labelled clusters similar to those defined as germ cell associations within mouse ST. FISH analysis confirmed this finding and one X chromosome signal was detected in adjacent nuclei of cells with human sperm-like morphology. Three months after injection in ST of infertile mice, expression of GFP or β-galactosidase was demonstrated in supporting cells and in the lumen. Control mouse ST (without hIDPSC) did not show any fluorescence or staining. We determined if differentiation of IDPSC can be a result of the fusion between mouse and human cells. Fusion between cells was observed at a low frequency, and it was only between cytoplasm and not nuclei. We used four different approaches in order to provide clear evidence of hIDPSC survival, proliferation and differentiation in mouse testis. In contrast to previous studies, which used human spermatogonial stem cells able to survive only in mouse testis, we demonstrated that IDPSC showed colonization of different compartments of mouse testis. This work also confirms our previous observation that IDPSC show a strong epithelial lineage fate.

Supported by: FAPESP

XII Reunião Científica Anual do Instituto Butantan
01 a 03 de Dezembro de 2010, São Paulo, SP

7.24 Immunochemistry analysis of primary cell lines obtained from BPV-related lesions

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Introduction: Bovine papillomavirus (BPV) is a group of DNA oncoviruses from the family *Papillomaviridae*, which affect cattle, causing warts (papillomas and fibropapillomas), and cancer in the alimentary tract and urinary bladder associated with co-factors. BPV is the first of this group reported to infect species other than cattle (horses and donkeys), and it is also considered an excellent model for comparative studies (as comparing to HPV). **Objectives:** We present here a study of morphological characterization of primary cell lines from skin papilloma, esophagus papilloma and bladder mucosa of animals affected by BPV, which evaluated the expression of cell type-specific intermediate filaments and the presence of BPV-1 proteins. **Methods:** To establish cultures, 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% CO_2. All the 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. The cultured cells were also labelled with the cell type-specific antibodies anti-vimentin and anti-pan-cytokeratin (Sigma) for cytological characterization and with anti-BPV-1 antibody (BPV-1 H8 Abcam plc, Cambridge). **Results and Discussion:** The animals showing lesions were confirmed as BP-positive for BPV-1, BPV-2, and some for BPV-4. All cell lines were stained 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. The cell lines were also stained by anti-BPV-1, mainly in the perinuclear region. This double labelling feature demonstrated in the cell lines has already been 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 altered expression, which could indicate the expression of this filament as a possible element for diagnosis in cancer. The positive staining for BPV-1 agrees with the PCR results, indicating viral presence in the cell lines, at least the major segment capsid protein is stained by this antibody.

Supported by: CNPq, FAPESP

XII Reunião Científica Anual do Instituto Butantan
01 a 03 de Dezembro de 2010, São Paulo, SP

7.25 Discussing chromosome aberrations related to BPV in peripheral lymphocytes of cattle (*Bos taurus*)

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Introduction: There are 10 bovine papillomavírus (BPV) types that in synergy with carcinogens such as quercetin can trigger cellular changes. Host chromatin alterations induced by viruses, mainly due to the expression of viral oncogenes, per se significantly increase the number of cells with abnormal centrosomes leading to mitotic aneuploidy and other structural and numerical aberrations. **Objectives:** This study aimed to evaluate chromosomal aberrations resulting from this instability in host chromatin of BPV-infected cells. **Methods:** Peripheral blood samples were collected from *Bos taurus* females. Viral detection was performed using PCR, and lymphocyte cultures were established for cytogenetic analysis. A total of 61 animals were analyzed in a "blind-test" in which slides were submitted to conventional staining, C-banding and silver nitrate staining to observe centromere structure and the nucleolar organizer regions (NORs). Twenty-eight (28) animals were verified as non-infected by BPV. Thirty-three (33) animals showed the presence of BPV. The BPV-infected group was divided into two groups: symptomatic (severe cutaneous papillomatosis) and asymptomatic. **Results and Discussion:** The symptomatic group (17 females) exhibited an average of 42.71% aberrant cells/ individual. The asymptomatic group (16 animals) exhibited an average of 40.19%. The analysis included 50 to 100 cells/sample, total of 2203 cells: 918 with one or more chromosomal aberrations. The chromosomal aberration rate of the control group was 4±2. The chromosomal aberrations identified in this study were: centric association (CA), acentric fragment (AF), telomeric association (TA), telomeric association with a single chromatid (TAcr), chromatid breaks (CtB), chromosomal breaks (CmB), gaps; aneuploidy, polyploidy, addition or loss of chromosomal segment (add or del) and early chromatid separation (EcrS). The AT, ATcr, add or del SPcr are not frequently observed in bovine cells infected with BPV. However, "in vitro" HPV-infected human cells have been described showing similar alterations. Gaps were very frequent in this study; however, we did not consider the gaps as an important chromatin lesion, due to eventual subjective analysis. The differences observed compared to the control support the inclusion of these data. The analysis of NOR localization in autosomal distal telomeres, emphasizes that the centric association described here is related to BPV actions on proximal telomeres. Associations of distal telomeres are not stable and thus difficult to detect.

Supported by: CNPq, FAPESP