Meeting Abstracts

VIII São Paulo Research Conferences - Cancer 2007: From Molecular Biology to Treatment Meeting Abstracts

PT.001

Causal Relation Between TIMP1 Gene Demethylation and *Anoikis* Resistance Along Melanocyte Malignant Transformation

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Background: Although *anoikis* resistance has been considered a hallmark of the malignant phenotype, the causal relation between neoplastic transformation and anchorage-independent growth remains undefined. To study this correlation, we developed an experimental model of murine melanocyte malignant transformation, where melanoma cell lines (as Tm1 and Tm5) were established after submitting a non-tumorigenic melanocyte lineage (melan-a) to sequential anchorage blockade cycles. Melan-a sublines, submitted to 2, 3 and 4 deadhesion cycles, were also established, showing progressive *anoikis*-resistance, and representing distinct phases of tumor progression. Gene expression analysis showed an up-regulation of Timp1 in melanoma compared to melanocyte lineages. Although described as a MMP inhibitor, this protein has been recently associated with *anoikis* resistance in human breast epithelial cells. These data prompted us to investigate whether Timp1 expression is regulated by DNA methylation and is related to the acquisition of the *anoikis*-resistant phenotype in our model. **Methods and Results:** While melan-a cells express low levels of Timp1, all cell lines derived from melan-a express high levels of this protein. After 5AZAdC treatment, increased levels of Timp1 mRNA were observed in melan-a cells, suggesting that in these cells Timp1 expression is suppressed by promoter hypermethylation. Ms-SNuPE analysis has shown an increased demethylation in Timp1 gene along melan-a carcinogenesis. Overexpression of Timp1 in melan-a cells resulted in increased survival in anchorage-independent conditions, but was unable to transform these cells. In addition, latency time for tumor appearance was reduced in Timp1-transfected melanoma cells, indicating that this molecule may be associated with tumor aggressiveness. **Conclusions:** Our results show an increment in Timp1 expression along the carcinogenesis, resulting from progressive gene demethylation, which is causally associated with an increase in *anoikis*-resistance but not with the acquisition, by itself, of a fully transformed malignant phenotype. FAPESP and CAPES

PT.002

New and Recurrent Chromosomal Abnormalities Identified by Spectral Karyotyping (SKY) Analysis in Leukemias and Lymphomas

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Background: Cytogenetic investigation of hematological neoplasias has significantly increased our knowledge of the basic genetic mechanisms involved in leukemogenesis. However, G-banding analysis of subtle chromosomal rearrangements or complex karyotypes with multiple markers can be inadequate due to poor chromosome morphology and/or an insufficient yield of analyzable metaphases. Spectral karyotyping (SKY) has been developed to unambiguously display and identify all chromosomes at one time using a spectrum of 24 different colors. **Objectives:** We applied SKY to a panel of 11 cases of leukemia [T-prolymphocytic leukemia, T-PLL (1); Acute lymphoblastic leukemia, ALL (4), Acute myeloid leukemia, AML (4), Chronic myeloid leukemia, CML (2)], two cases of mantle lymphoma (MCL) and one case of MDS, associated to Behçet disease. All samples presented abnormal G banded karyotypes. SKY analysis was performed to confirm all abnormalities, previously seen by G-banding, identify cryptic translocations and better

define complex chromosomal. All karyoptypes were described according to ISCN (2005). **Results:** Of the 94 chromosomal abnormalities described, 42 were only detected by SKY, one abnormality was redefined and 51 were confirmed. Among the samples studied, the T-PLL showed a complex karyotype with two translocations involving both sex chromosomes, a marker and a ring chromosome. SKY confirmed the t(X;14)(q28;q11) and t(Y;14)(q12;q11) and identified the ring and marker chromosomes. In AML M5, SKY reveled a complex translocation involving chromosomes 3, 10 and 11, t(3;11;10)(q27;q23;p12) and confirmed the extra copy of chromosomes 4, 5, 11 and 16. In two cases of CML in blast crisis, SKY identified the t(2;14)(p12;q32), among 78 chromosomes and two Ph chromosomes in one case, with lymphoid morphology. The other case exhibited a t(5;17)(q13;q21), previously described as del(5)(q31), by G-banding analysis. A rare case of acute basophilic leukemia, Ph+, SKY identified a complex rearrangement among chromosomes 1, 5 and 22, t(1;6), t(1;17), t(5;6), and t(8;13) and confirmed del(4)(q21). **Conclusion:** We conclude that SKY considerably enhances the accuracy of karyotype interpretation. The prognostic value associated to the new abnormalities observed is not well established yet. However, the breakpoints described may identify genes associated to the leukemogenesis, or in contribution for the maintenance of aggressive phenotype in these patients.
Financial support: FAPESP (Proc. 07/52462-7)

PT.003

Evaluation of Clastogenic and Genotoxic Effects by *Ilex Paraguaiensis* (Mate Tea) Infusions in Male Wistar Rats

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The habitual use of mate, a hot infusion of *Ilex paraguariensis* dried and minced leaves, has been related to the increase in incidence of oral, laryngeal and esophageal cancers in southern part of Brazil, particularly in the state of Rio Grande do Sul. Some studies point to a mutagenic and genotoxic effects of mate in *Salmonela typhimurium* and *Escherichia coli* assays. Thus, the present study evaluated the potential mutagenic and/or genotoxic effects of mate *in vivo* bone marrow micronucleus test and comet test. Male Wistar rats were allocated into four groups: G1- treated with i.p. doses of 80 mg/kg diethylnitrosamine (DEN, positive control), G2 and G3- treated with mate infusion (20g leaves/L) and with *Camellia sinensis* (green tea) infusion (20g leaves/L) during eight weeks, respectively, and G4- no treatment (negative control). After four twenty four hours after the last dose of DEN, samples from peripherial blood and bone marrow were collected to comet and micronucleus tests. Differently from G1 group, mate and green tea treatments did not induced DNA damage when compared to the negative control (G4 group). Therefore, the results suggest that mate tea did not present mutagenic or genotoxic effects. Probably, thermal injury due to the consumption of very hot mate tea is the major risk factor to the development of esophageal cancer in southern region of Brazil. CAPES

PT.004

HPV Oncoproteins Expression, Iron Endocytic Pathway and Mitochondria in Mammalian Cells

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Background: Infection for certain Human Papillomaviruses (HPVs) represents the major risk factor for the anogenital cancer. The high-risk E6 and E7 oncoproteins drive cell proliferation through the association with PDZ domain proteins and Rb, and contribute to neoplastic progression, whereas E6-mediated p53 degradation prevents the normal repair of mutations in the cellular genome. Clathrin-mediated endocytic pathway seems to be a probable alternative entrance for HPV into the cells. **Objectives:** To evaluate the distribution of E6 and E7 oncoproteins, mitochondria, transferrin receptors (TfR), transferrin (Tf) and ferritin (Fe) for the iron endocytosis mediated by clathrin in human and animal cells, as alternative pathway for HPV infection. **Methods:** Cells transformed by HPV (HeLa, SiHa) were used as positive controls, while HPV-negative cell lines (CHO, 293T) were transfected with the pLXSN vector (complete sequence of E7 gene) using GeneJuice® Transfection Reagent (Merck). Primary antibodies against TfR, Tf and Fe (Dako), E6 and E7 (Oncogene) and secondary antibodies conjugated with AlexaFluor® Dyes (Molecular Probes) were applied in immunofluorescence assays. Western blotting method was used as control of proteins expression. **Results:** The

antibodies recognize the E6 and E7 proteins in HeLa and SiHa, in the cytoplasm and nucleus, and in pLXSN vector transfected cells CHO and 293T, only in the cytoplasm. TfR was detected at the plasma membrane of cells, as well as the Fe was intensely labeled in the cytoplasm, nucleus and mitochondria. Co-localizations of E6 with mitochondria were detected in HeLa and SiHa HPV transformed cells. **Conclusion:** The great amount of iron suggests a participation of this element in the HPV cells transformation. The co-localization of E6 with mitochondria constitute a new data, which also suggests be related to the intracellular iron pathway and in the process of cellular transformation.
*CAPES, §CNPq, ¨FAPESP Fellowships.
Supported by: FAPESP, Butantan Institute, Butantan Foundation and Ludwig Institute.

PT.005

Contribution of Epigenetic Mechanisms in Melanocytes Transformation Associated with Anchorage Blockade

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Background: A model of melanoma genesis was developed in our laboratory, where different melanoma cell lines were obtained as result of sequential anchorage blockade cycles of a non-tumorigenic melanocyte lineage, melan-a. Melan-a cells sublines submitted to 2, 3 and 4 deadhesion cycles were also established, representing distinct phases of tumor progression. **Objectives:** We have been interested in determining the contribution of epigenetic mechanisms in melanocytes malignant transformation induced by sequential anchorage blockade. **Methods and Results:** Results of our laboratory showed significant differences in global DNA methylation, and in DNA methyltransferases and histone methyltransferase EZH2 expression during melan-a transformation. There is a progressive increase in 5-methylcytosine content in melan-a cells maintained in suspension and a gradual genomic hypomethylation in 2C, 4C and melanoma cell lines 4C3, 4C11, Tm1, Tm5. Decrease both in dnmt3b and EZH2 expression was also observed in melanoma cells. In addition, we observed differences in methylation level of repetitive DNA sequences, like IAP and A-repeats. Expression of specific genes was also altered by DNA methylation in this model, like $p19^{ARF}$ and its upstream regulator dapk1. The expression of these genes were lost in melanoma cells, and reversed by *in vitro* cells treatment with aza-deoxycytidine associated with trichostatin A. The tumor suppressor gene $p19^{ARF}$ antagonizes the E3 ubiquitin protein ligase activity of mdm2 to activate p53 that, in turn, induces p21 expression and cell cycle arrest. We showed an increase of p53 expression in 2C and 4C and its lost in melanoma cell lines. On the other hand, mdm2 expression is increased only in melanoma cells. Interestingly, p21 expression is also increased in melanoma cell lines, suggesting a p53-independent function for this protein. These results show a perturbation of methylation profile along the malignant transformation process, which is already detectable few hours after melan-a deadhesion. This alteration may be sufficient to explain the acquisition of a malignant phenotype, since key regulatory genes have their expression levels modified.
Supported by FAPESP and CNPq

PT.006

Oxidative Stress Modulates DNA Methylation Along Melanocyte Malignant Transformation

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Background: Different melanoma cell lines (4C3, 4C11, Tm1, Tm5) were established after submitting a non-tumorigenic murine melanocyte lineage, melan-a, to sequential anchorage blockade cycles. Melan-a cell sublines submitted to deadhesion cycles (2C, 3C and 4C) were also established, representing distinct phases of tumor progression. **Objetives:** We have been interested in elucidating the participation of DNA methylation, an important epigenetic mechanism, in melan-a malignant transformation and its relation to oxidative and/or nitrosative stress resulting from adhesion blockade. **Methods and Results:** Increased intracellular oxidative (H_2O_2, O_2^- and MDA) and nitrosative stress (NO) were observed in melan-a cells submitted to adhesion blockade. In addition, anchorage impediment leads to a decrease in homocysteine intracellular concentrations, which could lead to alterations in DNA methylation reactions. In fact, significant alterations in global DNA methylation levels were observed along melan-a transformation: progressive increase in 5-methylcytosine content in melan-a cells maintained in suspension and gradual genomic hypomethylation in 2C, 3C, 4C and melanoma cell lines. In parallel, increased Dnmt1 and 3b expression was observed

in melan-a cells submitted to deadhesion, whereas 2C, 4C and melanoma cell lines lost the expression of *de novo* methyltransferase Dnmt3b. Searching for a relationship between oxidative stress and DNA methylation alterations, we found that the antioxidant N-acetylcysteine and NO synthase inhibitor, L-NAME, were capable of preventing both the increase in global DNA methylation and in Dnmt1 and 3b expression induced by melan-a adhesion blockade, suggesting that stress resulting from adhesion impediment can modulate DNA methylation. Interestingly, NO levels were not inhibited by L-NAME in non-adherent melan-a cells, but it totally impairs O_2^- production. **Conclusions:** Oxidative stress observed during melanocyte anchorage blockade seems to modulate global DNA methylation levels and may directly contribute to the acquisition of an anoikis resistant phenotype.
Supported by FAPESP and CNPq

PT.007

Characterization of Novel Genes Differentially Expressed in Breast Cell Lines Expressing Different Levels of c-erbB2

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Overexpression of the oncogene c-erbB2 is observed in 30% of breast tumors and is considered an adverse prognostic factor for breast cancer. However, the molecular mechanisms associated with c-erbB2 overexpression are not yet understood. In this work, transcriptional changes associated with c-erbB2 overexpression were investigated using Massively Parallel Signature Sequencing (MPSS). MPSS is a powerful technique for genome-wide gene expression analysis, which relies on the production and quantification of short tags proximal to the 3′ end of transcripts. Over 24 million MPSS tags, representing aproximately 24,000 different genes, were generated for a immortalized mammary luminal epithelial cell line (HB4a) and for a variant cell line (C5.2) transfected with c-erbB2. After establishing reliable correlations between tags and known human transcripts, 9% of the tags could not be associated with a known transcripts and were designated orphan tags. Among the orphan tags there were many that showed differential expression between HB4a and C5.2 cell lines and probably represent novel genes regulated by c-erbB2. In order to further characterize these novel genes, we have extended 83 orphan tags towards the 3′ end of the corresponding mRNA molecule using a methodology named GLGI (Generation of Longer cDNA fragments for Gene Identification). Forty one 3′ cDNA fragments were obtained and similarity analysis using BLAST allowed the identification of 13 transcripts putativelly regulated by c-erbB2. Differential expression of 5 out of these 13 transcripts was confirmed by Real-Time PCR. Among these 5 valitaded transcripts there are 4 that are overexpressed in the C5.2 cell line. The expression pattern of these 4 transcripts was analyzed by Real Time PCR in 11 c-erbB2 positive and in 12 c-erbB2 negative breast tumor samples. Besides, the impact of Herceptin treatment on the differential expression of 2 out of the 4 genes was also investigated. Now the new transcripts putatively regulated by the oncogene c-erbB2 will be investigated functionally.
Ludwig Institute for Cancer Research and FAPESP

PT.008

Functional Characterization of the Tumor Antigen CTSP-1

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Background: Cancer-testis (CT) antigens are immunogenic proteins expressed in normal gametogenic tissues and in different types of tumors. So far, more than 80 CT antigens, organized in 44 distinct gene families, have been identified; however, the biological functions of these proteins are still largely unknown. Recently, we identified a novel CT antigen, named CTSP-1, which is frequently expressed in a wide variety of tumors. Antibodies against CTSP-1 were detected in 10% (14/141) of plasma samples from patients with a wide spectrum of tumors. **Objective:** To characterize the biological function of CTSP-1 in tumor cells. **Methods:** [1] The full-length CTSP-1 was used as bait to screen a prostate tumor cDNA library in a yeast two-hybrid (Y2H) assay. [2] The mRNA and the protein expressions of 8 putative CTSP-1 binding partners were analyzed by RT-PCR and immunoblotting, respectively, in 4 different tumor cell lines (A2058, H1155, A172 and DU145) that express the CTSP-1 transcript. Immunoprecipitation and pull-down assays are currently being used to validate the

protein interaction information obtained from the Y2H screening. **Results:** [1] using the CTSP-1 as a bait, we identified 156 clones positive for both the reporters genes (lacZ and LEU2) analyzed in the Y2H assay. DNA sequencing of the clones resulted in the identification of 51 potential CTSP-1 binding proteins. Eight out of eleven selected candidates (BACH-1, CTCF, MDFI, PSMC-3, RSF-1, RYBP, YWHAB and YWHAZ) had their interaction with CTSP-1 confirmed in yeast. These candidates play important roles in tumorigenesis and a significant fraction of them is capable of regulating gene expression. [2] The mRNA and the protein expression analyses revealed that the 8 candidates are expressed in all four CTSP-1 positive tumor cell lines. **Conclusions:** The preliminary results indicate that CTSP-1 may play an important role in tumorigenesis by regulating gene expression as has been decribed for other CT antigens.
LICR and FAPESP

PT.009

Genomic Instability on the Progression of Human Actinic Keratosis to Squamous Cell Carcinoma

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Background: Genomic instability has been widely found in cancer cells. These alterations often affect microsatellites that may show variation on the number of internal repeats (*Micro Satellites Instability, MSI*), or total loss of repeats (*Loss Of Heterozygosity, LOH*). Anonymous repeats, revealed by RAPD technique (*Random Amplified Polymorphic DNA*) are also involved in DNA instability. Due to variations among individuals and abnormal tissues, these parameters serve as polymorphic markers. Squamous cell carcinoma (SCC), originated from keratinocytes in the squamous cell layer, can derive from benign actinic keratosis (AK) lesion. Since most AKs and SCCs occur on chronically sun-exposed sites, UV damage is considered the major cause of such genetic injuries. Previous work showed alterations in both microsatellites and RAPD patterns of skin cancers. **Objectives:** To investigate the genomic instability in AKs and SCCs, on the attempt to find molecular genetic markers. **Methods:** Genetic alterations in AKs and SCCs were investigated by RAPD and microsatellites analyses. DNA was obtained from Brazilian patients diagnosed and treated in the School of Medicine of University of Sao Paulo Out Clinics Hospital. Eight AKs, 24 SCCs, and 4 BCCs, matched to normal skin tissues and/or leukocytes were studied. Microsatellite patterns were obtained with *primers* specific to amplify D6S251, D6S252, D9S15, D9S50, D9S52, D9S180, D9S196, D9S280, and D9S287, in search of LOH and MSI. The RAPD primers used were: OPA 2, 7, 13 and 17, OPB 8, 13, 17 and 19. **Results**: D6S251 microsatellite was altered (LOH) in one AK and 5 SCC samples, a statistically significant difference (*p=0.0398*). DNA *fingerprints* obtained with RAPD *primers* maintained similarity between control and tumor patterns in decreasing number of samples, according to their histological degree of differentiation (27 % AK, 24 % SCC I, 9 % SCC II, and zero SCC III). **Conclusion**: These findings support the assumption of AK progression to SSC, and suggest D6S251 microsatellite and RAPD patterns to be potential tools in for diagnosis and prognosis.
Financial support: FAPESP

PT.010

A Polymorphism in the Matrix Metalloproteinase-9 Gene is Associated With Cell Differentiation in Squamous Cell Carcinoma in Mice

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Inflammation and Repair.the metalloproteinase-9 (MMP-9) is an important enzyme during the basement membrane degradation, an essential step for cancer invasion. Variations in the MMP-9 gene promoter can modify its expression, and consequently interfere on the carcinogenesis process. The aim of this study was to test the hypothesis that the CA repeat polymorphism in the MMP-9 gene promoter interferes on the squamous cell carcinoma development. Ninety-four hairless mice were submitted to chemically induced skin carcinogenesis (DMBA 0,5%), once a week, during 16 weeks. At 17th week, the animals were sacrificed and the lesions biopsed. After the routine laboratorial processing, the histopathologic evaluation (stage of invasion, pattern of invasion and differentiation grade) was performed. At the biopsy, 0.5cm of each animal tail was collected for DNA extraction and evaluation of the CA repeat polymorphism in the MMP-9 gene promoter. The data was analyzed by chi-square test to evaluate whether the MMP-9 alleles or genotypes were associated with histopathological features. There was an association of the allele with 25 CA repeats with moderately differentiated lesions. The data suggest

that the MMP-9 gene polymorphism is related to the reduction on histological differentiation in squamous cell carcinoma induced by DMBA in hairless mice skin.
CAPES (2730/05-7)

PT.011

Acute Oxidative Stress Decreases Telomerase Activity in Walker Tumor Cells And Increases TERT Expression in SCC-25 Tumor Cells

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Background: Telomerase activation and telomere stabilization are important steps in tumorigenesis. Inhibition of telomerase and telomere shortening below a critical length triggers apoptosis in various cell types. In this regard, we have recently shown that oxidative stress induces apoptosis in Walker and SCC-25 tumor cells, through calcineurin activation and mitochondrial permeability transition, respectively. **Objectives:** In the present study we analyzed telomerase expression and activity in both Walker and SCC-25 cells submitted to oxidative stress. In addition, we verified the possible involvement of p53 in the process of cell death. **Methods:** Walker and SCC-25 tumor cells were exposed to 4 mM H_2O_2 for different periods of time and apoptosis was analyzed by annexin V staining. The levels of the catalytic component of telomerase (TERT) were determined by Western blotting. Telomerase activity was quantified using TRAP (Telomere Repeat Amplification Protocol). p53 and TERT expression levels were measured by quantitative RT-PCR. **Results:** Treatment with H_2O_2 for 3 h induced ~40 and 25% of apoptosis in Walker and SCC-25 cells, respectively. The p53 inhibitor pifithrin-alpha (PFT) caused a 2.0-fold decrease in the number of apoptotic Walker cells, but did not affect SCC-25 apoptosis, since these cells are $p53^{null}$. Apoptosis was accompanied by an 8.8-fold decrease of telomerase activity in Walker cells, which was partially prevented by PFT and by the Ca^{2+} chelator, BAPTA. In H_2O_2-treated SCC-25 cells there were no alterations in telomerase activity but they showed a 2.0-fold increase of TERT expression. **Conclusions:** (1) These results suggest that the concerted action of p53 and $[Ca^{2+}]_{cyt}$ decreases telomerase activity of Walker cells submitted to oxidative stress. (2) Increased TERT expression could be an attempt to extend proliferate lifespan of SCC-25 cells after oxidative stress.
Supported by CNPq, FAPESP, and FAEP/UNICAMP.

PT.012

Study of Tp53 Germline Mutation R337h in Adrenocortical Tumor Patients Treated at Centro Infantil Boldrini

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Background: In the southern and southeastern regions of Brazil, the incidence of childhood adrenocortical tumors (ACT) is 10 to 15 times higher than that in other parts of world. ACTs are very aggressive neoplasms that do not respond to standard chemotherapeutic agents. Several studies have shown an association between the development of these tumors and R337H, a germline mutation in the tumor suppressor gene *TP53*. This mutation at codon 337 within exon 10 results in an arginine to a histidine substitution. Previous studies in southern Brazil have revealed a strong association between the presence of germline TP53 R337H and ACT. **Objective:** To determine whether this *TP53* mutation is also associated with pediatric ACT in another geographic region of Brazil, children with ACT referred to the Centro Infantil Boldrini (Campinas/ Brazil) for treatment between 1982 and 2007 were studied. **Methods:** Peripheral blood of ACT patients was collected in EDTA and mononucleated cells were separated by Ficoll gradient. The DNA was extracted with Wizard kit (Promega), TP53 exon 10 was amplified by PCR (polymerase chain reaction) and analyzed by RFLP (restriction fragment length polymorphism). The mutation abolishes a *Hha I* restriction site within the amplicon. The products of *Hha I* endonuclease activity were resolved on 2% agarose gel stained with ethidium bromide. **Results:** Eighty-three ACT patients from São Paulo state and south region of Minas Gerais were treated at Boldrini Hospital. Fifty-one of those cases had blood samples available. We found forty-six R337H heterozygous mutants, corresponding to a frequency of 90%. **Conclusions:** We found a high frequency of the *TP53* R337H mutation among children with ACT. Currently we are screening for the mutation in patient's relatives. Close monitoring of infant carriers may enhance their chances of early tumor detection and better overall survival.

PT.013

ANKHD1 Gene Expression is Modulated by Chromatin Remodeling Agents in Patients Stromal Cell

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Background: The Ankyrin repeat and KH domain containing 1 protein (*ANKHD1*) is a novel human gene, homologue of Drosophila MASK (Multiple ankyrin repeats KH domain), which is known for its crucial role in photoreceptor differentiation, cell survival, and proliferation. It was demonstrated an upregulation of ANKHD1 mRNAs expression during myeloblastic and erythroblastic differentiation. ANKHD1 Upregulation was also observed in several leukemias cell lines and in bone marrow samples of leukemia patients when compared with normal bone marrow, suggesting that ANKHD1 could be involved in progression of malignant process in hematopoietic cells. Hypermethylation and acethylation contribute to the malignant process and chromatin remodeling agents can be used as therapeutic strategy in cancer. **Objectives**: In order to assay the role of ANKHD1 in MDS (myelodysplastic syndromes), a drug of known therapeutic and demethylator properties, Decitabine (DAC), was utilized to check ANKHD1 expression alterations in MDS samples. **Methods:** Mononuclear cells from bone marrow samples of 4 MDS patients were treated with DAC (1μM e 5μM) during 72h. Real Time PCR assays were used to analyze the differential expression of *ANKHD1*. **Results**: We have shown that *ANKHD1* transcript levels are upregulated in MDS samples after treatment with DAC in a dose-dependent way (2 to 3 fold increase). **Conclusion**: These data indicate that DAC permits the activation of ANKHD1 directly or indirectly for demethylation of inactive promoters in MDS and suggest that this protein could be involved in the regulation of different cellular process important for the progression of myelodysplastic syndromes.

Supported by: FAPESP

PT.014

Class 1 Histone Deacetylases Gene Expression in Childhood Acute Lymphoblastic Leukemia

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Background: Acute Lymphoblastic Leukemia (ALL) is a heterogeneous disease characterized by the accumulation of immature lymphocytes in the bone marrow with distinct biologic and prognostic groups. In addition to genetic alterations, epigenetic processes play an important role in carcinogenesis. Among the epigenetic mechanisms, the acetylation is crucial for chromatin regulation structure and transcriptional activity. In general, the histones acetylation is stimulated by the enzyme histone acetyl transferase (HAT), which is associated with nucleosome remodeling and transcriptional activation. In another hand, the deacetylation process is drived by the enzyme histone deacetylase (HDAC), which is associated with the chromatin condensation and transcriptional repression. Abnormal expression of HDACs is associated with carcinogenesis and has revealed a promising field to stratify the risk characterization and treatment of cancer. The investigation of these expression profiles may represent an important clinical factor in diagnosis and management of hematological malignances. **Objectives:** Correlate the expression profile of the class 1 HDACs (1, 2, 3 and 8) genes in ALL of childhood with other prognostic and clinical features of the patients. **Methods:** Fourty seven samples obtained from bone marrow patients and more ten samples of healthy donors were analyzed for HDACs (1, 2, 3 and 8) gene expression by TaqMan real-time polymerase chain reaction. The results of the relative quantification were correlated with age, risk group, initial blast cell count and immunophenotype using the Fisher and Mann Withney tests. **Results:** It was observed no relationship between HDACs 1, 2, 3 and 8 expressions with the followed features: age, initial blast cell count and risk group. Only HDAC2 showed a higher expression in patients CALLA- immunophenotype (p=0,04). However, there was a higher expression of HDAC2 (p=0,007), HDAC3 (p=0,014) and HDAC8 (p=0,002) in ALL samples when compared with health donors. **Conclusion:** The ALL group showed a higher expression of HDACs 2, 3 and 8 than healthy donors, suggesting that these genes may be involved in leukemogenesis. However, the overexpression of Class 1 HDAC genes did not correlate to the prognostic and clinical characteristics of patients with ALL. It is necessary to perform functional investigation to confirm these results.

CNPq

PT.015

Methylation Pattern Characterization of *MDR1* Gene Promoter Region at Acute Myeloid Leukemia Patients

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Background: *MDR1* gene encodes the protein Pgp-1 (P-glycoprotein 1) associated with the development of resistance against chemoterapic drugs in different tumors. Pgp 1 expression is detected in 40% of patients with Acute Myeloid Leukemia (AML), and in 70% of the relapsed patients. Different studies showed an association between an increased *MDR1* transcription and the presence of 5′ - CpG – 3′ hypomethylated sites at the proximal promoter region in AML patients. **Objectives:** To examine the association between the methylation pattern at the *MDR1* proximal promoter region in AML cells from patients and the Pgp 1 expression (amount of protein) and activity. **Methods:** DNA was isolated from peripheral blood of 13 AML patients. Pgp 1 activity and expression was analyzed by flow cytometry. To analyze the methylation pattern DNA was treated with Sodium Bisulfite using the "CpGenome™ DNA Modification Kit" (Chemicon). Promoter region was amplified by PCR using specific primers and then directly sequenced. All procedures followed ethical guidelines approved by the local Ethics Committee (INCa). **Results:** We examined 11 CpG sites in proximal promoter region and all patients showed most sites unmethylated. The CpG sites located at –135, -111 and –106, from the transcription start site, were not methylated in all patients (100%), and sites located at –57, -30 and +4 showed a minor methylation status (~80%); additionally in five patients all CpG sites were not methylated. We did not found any association between Pgp 1 expression and activity.

Swiss Bridge Foundation; FAF, Brazil; CNPq, Brazil; Ministry of Healthy, Brazil

PT.016

Different Evolutionary Strategies for the Origin of caspase-1 Inhibitors

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Caspase-1 (CASP1, IL-1B converting enzyme) is involved in both cytokine maturation and apoptosis. In addition to its pro-inflammatory properties, IL-1B contributes to tumorigenesis by influencing angiogenesis, proliferation and metastatic capacity of many tumors. The active CASP1 is produced by proteolytic cleavage of its pro-domain, which contains the CAspase Recruitment Domain (CARD). COP1, INCA and ICEBERG are all CARD-only proteins that are mapped at chr11q22.3 and use their CARD domain to inhibit CASP1 activation. Here, we show that CASP1 inhibitors are products of a series of gene duplications that occurred in the human lineage after the divergence between human and mouse. We have maped all duplications at this locus and proposed a chronological order for them. At protein level, COP1, INCA and ICEBERG share 91%, 83%, and 53% identity to CASP1, respectively. Based on these identities and the alignment information, the first duplication event involving CASP-1 gave rise to ICEBERG and the second originated INCA. The third duplication involved INCA and gave rise to a new gene (or pseudo-gene) represented by GenBank entry CB985891 since there are regions specific to both CB985891 and INCA. A more recent duplication which involved a shorter fragment of CASP1 originated COP1. The exon-intron structure of these inhibitors indicated that although INCA, CB985891 and ICEBERG duplicated regions contain the entire CASP1 sequence, all cDNAs sequences from these genes encode shorter ORFs corresponding to the CARD domain due to the presence of stop codons at different positions within each gene and that were probably created after gene duplication events. A cytosine (C) to adenosine (A) substitution at exon 2 of ICEBERG created an TAA; a C to timine (T) substitution at exon 3 of INCA created TAG and a mismatch mutation at C to guanine (G) created an TAG for COP1 at exon 3. We compared the conservation of these inhibitors in primates, since mouse genome contains none of the CASP1 inhibitors. INCA, CB985891 and COP1 are present at both Chimpanzee and Rhesus, however ICEBERG is absent in the Chimpanzee genome. Finally, we found that the majority of the inhibitors stop codons are conserved among these primates.

Supported by FAPESP/LICR

PT.017

Subpopulation Analyzes of Melanoma with Characteristics of the Stem Cells and its Correlation with Malignant Tumors

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Introduction: The process of tumorigenesis in melanoma elapses of imperfections in the mechanisms that control the growth and the cellular proliferation. During the tumoral growth some cells of cancer adopt different characteristic of the original cells and these cells determine the evolution of the cancers inducing more invasive tumors and more malignant. **Objective:** To compare phenotypic and malignity of the murine melanoma cells with a subpopulation originated of them after long periods of culture. **Methods**: We analyze the expression of c-kit and Sca-1 by flow cytometry, (Facs Calibur with Cell Quest Software, BD, Bioscience, San Jose, USA). The cellular proliferation was evaluated using neubauer chamber. The malignity of these cells, *in vivo*, was verified following the tumoral progression. Phenotype differentiated was performed by optical microscopy, (Light inverted microscope Nikon T300 Diaphot, Japan) and photographed with video camera (Sony ExwareHad DSP Color Video Camera SSC-DC54A), connected to the microscopy. **Results**: Melanoma cells maintained for long periods in culture presented a subpopulation with different phenotype and are more malignity of the original population. These cells express stem cell markers such as c-Kit (65%) and Sca-1 (10%), present more pigmentation, proliferation and malignity than original population. **Conclusion**: Our results indicate that long periods of melanoma culture generate cells with different properties from the originals cells. We believe that the best characterization of these cells can assist in the better understanding of the tumoral progression and it will help the development of future therapies.

CAPES

PT.018

Dysregulated Expression of Hox Genes in Oral Squamous Cell Carcinoma Compared to Normal Oral Mucosa

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Background: It is becoming widely accepted that normal development and tumorigenesis are influenced by the same morphogenic processes. Transcription factors that orchestrate developmental organogenesis, such as homeoproteins, can often contribute to tumorigenesis. The HOX family of homeoproteins plays important roles in development, in part by controlling proliferation, differentiation, and apoptosis. Expression of HOX genes is associated with many cancers including those of lungs, kidneys, and ovaries. This suggests that misexpression of HOX genes, after development is complete, may promote tumorigenesis by aberrantly activating transcriptional programs that normally contribute to organogenesis. **Methods:** We analyzed the expression levels of the 39 members of the HOX gene family in 10 pairs of normal oral mucosa and oral squamous cell carcinoma (OSCC) obtained from the same patient. Total RNA were isolated and subjected to the semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) "duplex" method with specific primers for each of the HOX family members and for the control gene GAPDH. The samples were electrophoresed in 8% polyacrylamide gel and resultant bands were quantified by densitometry. **Results:** None of the samples (either normal oral mucosa or OSCC) expressed the members A6, A9, B6, B8, B9, C4, C5, C11, and D12. Most of the members were silenced in the normal oral mucosa, with exception of A1, D8, D9 and D10. The members A1, A4, A5, A7, A10, B7, C4, C5, C6, C8, C9, C10, C11, C13, D1, D4, D9, D10, D11 e D13 were significantly overexpressed in OSCC when compared with normal oral mucosa. **Conclusion:** These results suggest that a dysregulated expression of specific members of HOX genes may be related to the tumorigenesis and/or tumor progression of the OSCC.

Financial support: FAPESP and CAPES.

PT.019

Identification of Germline Mutations in the VHJ Gene of Families with the Von Hippel-Lindau Disease

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Tumor development results from a series of genetic changes, which may involve the activation of proto-oncogenes, as well as the inactivation of tumor suppressor and DNA repair genes. The loss of function by germline mutation in these genes leads to hereditary cancer syndromes, such as the syndrome of von Hippel-Lindau. This syndrome of autosomal dominant

pattern is caused by germline mutations in the VHL gene, and predisposes the patient to the development of a variety of malignant and benign tumors in various organs. Symptoms may appear since the first years of life and include: retinal and central nervous system haemangioblastomas, phaeochromocytoma, renal cell carcinoma, as well as multiple cysts of kidneys, pancreas, liver and epididymis. The molecular analysis of germline mutations is a tool that enables the early diagnosis of affected relatives without clinical symptoms of the disease (clinically asymptomatic). The disease clinical and molecular diagnoses are based on criteria that consider the family history and the clinical presentation of the lesions. In this work, mutation analysis were performed using peripheral blood genomic DNA and the exons of the VHL gene were submitted to PCR amplification using specific primers. The PCR products were then sequencing. Among 19 families so far studied, 12 different mutations were identified in probands (small deletions, frame shift, nonsense, missense and splice site). The predictive test has been applied to asymptomatic relatives under risk and among 14 relatives studied, four were mutation carriers and were included in lesions screening program. The mutation was not detected in three families by sequencing, and the MLPA methodology will be used to allow the detection of large deletions and insertions. Besides the contribution to a deeper knowledge of the disease in the Brazilian population, the present study also offers the possibility of an early diagnosis which is important in the assessment of risks and in the guidance of preventive measures; all of these factors resulting in the improvement of the attention to VHL patient's families.

Ministério da Saúde e Instituto Nacional de Câncer

PT.020

Presence of HPV DNA in Pelvic Lymph Nodes of Invasive Cervical Carcinoma with and Without Metastasis – an Evidence of HPV Propagation?

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Background: The progress of cervical tumor involves a complex multistep that can result in invasion and metastasis. The Human Papillomavirus (HPV) have been considered the primary etiologic agent of cervical tumorigenesis, however the evidence of HPV in lymph nodes is still unclear and could represent a mechanism to infected distant anatomic sites. **Objetive:** Verify the frequency of HPV DNA in pelvic lymph nodes with and without metastasis of cervical cancer. **Methods:** Forty patients with invasive cervical carcinoma were stratified into two subgroups according histological diagnosis: 18(45%) and 22(55%) out of 40 patients were diagnosed, respectively, as invasive cervical cancer with and without metastasis to pelvic lymph nodes. In addition, pelvic lymph nodes of both cervical cancers with and without metastasis were also evaluated. Paraffin-embedded invasive cervical lesions and pelvic lymph nodes were submitted nested PCR method in order to detect and type HPV DNA. **Results:** 12 (54.5%) out of 22 invasive cervical cancer without metastasis and 3(13.6%) of their pelvic lymph nodes presented HPV DNA. Out of the 3 lymph node HPV-positive, 1(33.3%) presented HPV DNA only in lymph node and 2(66.7%) in both invasive cervical cancer and lymph node. Otherwise, 14(77.8%) out of 18 invasive cervical cancer with metastasis and 7(38.9%) of their pelvic lymph node were positive to HPV. Out of the 7 lymph node HPV-positive, 1(14.3%) presented HPV DNA only in lymph node and 6(85.7%) in both invasive cervical cancer and lymph node. **Conclusions**: These results showed the high frequency of the HPV infection in pelvic lymph nodes. Surprisingly, we found HPV DNA in pelvic lymph nodes without metastasis, might indicating that HPV infection could be propagate throughout of pelvic lymph nodes.

CAPES, CNPq, FAEPA, FAPESP

PT.021

Cytogenetic Instability Revealed by Micronuclei Formation in Liver Benign and Pre-Malign Lesions Compared with Hepatocellular Carcinoma from Virus C Infected Patients

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Background: There is lack of substantial cytogenetic data in early events of hepatocarcinogenesis. **Objectives:** This fact led us to evaluate chromatinic losses by micronuclei (MN) formation in chronic hepatitis parenchyma (CHP), cirrhotic nodules: regenerative – NRs, macroregenerative - MRNs, low grade dysplastic – LGDNs, HGDNs - high grade dysplastic - HGDNs and tumoral nodules (TNs) of hepatocellular carcinoma (HCC). **Methods:** The analysis of mononucleated

hepatocytes from liver samples of patients with Hepatitis C virus were performed using Feulgen-Fast-green dying techniques as follows: CHP (8 cases); RNs, MRNs, LGDNs, HGDNs and TNs (18 cases). Twelve control liver samples of healthy organ donors were included. Thus, 2000 hepatocytes, per area, in formalin-fixed and paraffin-embedded specimens were analyzed. The frequencies (‰) of micronucleated hepatocytes (MN-Heps) were established by the Students test, taking in consideration a significance level of P≤0.05. **Results:** The mean rates of MN-Heps from RNs, MRNs, LGDNs, HGDNs and TNs were higher and statistically significant than the normal parenchyma, P<0.05. The same results were found in relation to the mean rate of MN-Heps from LGDNs and HGDNs of all cases of hepatic cirrhosis when compared to CHP. Conversely, the comparison among HGDNs, LGDNs and RNs and MRNs did not present significant differences (P>0.05). Although we have detected chromatinic losses by MN in chronic hepatitis parenchyma, it is considered similar to normal parenchyma. By the other side, our data showed that HGDNs and LGDNs as well as, RNs and MRNs from HC or HC - HCC related cases are cytogenetically abnormal. **Conclusion:** chromatinic instability already present in regenerative cirrhotic nodules (Cancer Genet. Cytogenet., 2004) and also found in low and high grade dysplastic nodules might contribute to the development of HCC. This work was supported by grants for: FAPESP, Alves de Queiróz Family Fund for Research, Sense Ind. Com. Ltda.

PT.022

Germline Mutation Characterization in Brazilian Hereditary Non-Polyposis Colorectal Cancer and Breast Cancer Families

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Background: Hereditary non-polyposis colorectal cancer (HNPCC) represents about 3-8% of all cases of colorectal cancer (CRC) and it is a dominantly inherited cancer predisposition syndrome with 80% of penetrance. According to the InSIGHT´s Database approximately 50% and 40% of mutations responsible for Lynch Syndrome have been found in *MLH1* and *MSH2* genes, respectively. Hereditary breast cancer (BC) is thought to account for 5-10% of all BC cases and is associated with *BRCA1* and *BRCA2* genes mutations. **Objective:** To identify germline mutation in *MLH1, MSH2, BRCA1* and *BRCA2* genes in patients whose criteria for inherited cancer predisposition were fulfilled. **Method**: The population involved in this study had been treated in the Hospital A.C. Camargo which represents 100 patients with CRC (clinical suspect of Lynch Syndrome-Amsterdam I/II or Bethesda) and BC (breast-colon syndrome). By now 60 patients were convoked to peripheral blood collection, being 32 with CRC and 28 with BC. DNA extraction, generation of amplicons (20 amplicons to each *MLH1* and *MSH2* genes and 10 to *BRCA1*), screening by DHPLC (Denaturing High Performance Liquid Chromatography*)* experiment followed by automatic sequencing for such suspected mutation cases were performed. **Results**: One normal DNA sample was used as reference. The fragments were amplified and the absence of mutation was confirmed by automatic sequencing. From the 43 genomic DNA already extracted, 10 patients with CRC and 5 with BC were screened by DHPLC. By the time, 135 amplicons were analyzed by DHPLC, such as 8 exons from *MLH1* (exon 2, 4, 6, 8, 10, 12-I, 14 and 18), 5 exons from *MSH2* (exon 3-II, 11, 12-I, 13 and 14-II) and 1 exon from *BRCA1* (exon 3). From those, 26 amplicons suspect of mutation profile was detected, such as alteration in gene *MSH2* for all 10 patients in exons 12-I and 13, 2 patients in exon 3-II and only one in the exon 11. For the gene *MLH1*, 2 patients showed suspect mutation profile in the exon 8 and 1 patient in the exon 2. The suspected mutation will be verified by automatic sequencing.
FAPESP and CAPES

PT.023

The Clinical Genetics and RB1 Mutation Spectrum od Brazilian Retinoblastoma Families

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Background: Retinoblastoma is a malignant tumor of the developing retina occurring in early childhood, resulting from dysfunctional mutations affecting both alleles of the tumor suppressor, *RB1* gene. Constitutive mutations confer a poor prognostic and increased risk of secondary tumors. **Objectives:** The aim of the present study was to identify genetic abnormalities in the *RB1* gene in retinoblastoma Brazilian patients and to a possible genotype/phenotype correlation. **Methods:**We analyzed 90 patients and their families

with different clinical presentations of retinoblastoma who were referred to the Instituto Nacional de Câncer (Rio de Janeiro, Brazil). DNA from leukocytes of all patients was isolated and tumor tissue was analyzed in one patient. The 27 exons and flanking intron regions and promoter region of *RB1* were amplified, purified, labeled and sequenced. We also analyzed three microsatellite markers located in intron 2 of *RB1* or tightly linked to this gene. **Results:** Germline mutations resulted in: premature termination codons, missense and splice site mutations and polymorphisms. Conventional cytogenetic analysis of lymphocytes showed rearrangements in two bilaterally affected patients. In one of them, a 13q14 deletion coexisted with both alleles shown by DNA sequencing and microsatellite analysis of blood and tumor DNA. In the other, a ring chromosome 13 was present. In a non-affected brother of a bilaterally affected patient we found three intragenic microsatellite alleles, two corresponding to maternal alleles, and a third of a paternal allele. **Conclusion:** The majority of mutations here identified was *de novo*, mainly C to T transitions, and are associated with an early age at diagnosis. Chromosome alterations here detected probably involve variable expressivity and penetrance in retinoblastoma patients by chromosome mosaicism. The presence of three microsatellites alleles in a non-affected individual might have resulted from a recombination event between the upstream and downstream regions of this microsatellites of *RB1* intron 2. The mutation analysis presented here may provide a basis of the germline mutation spectrum of *RB1* in Brazilian retinoblastoma patients.

CAPES, CNPq, FAF

PT.024

The Impact of the Clinical and Genetic Screenings on the Management of the Multiple Endocrine Neoplasias Type 1 and Type 2: Ten Year Experience of the Endocrine Genetics Unit, Hospital das Clínicas, FMUSP

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Multiple endocrine neoplasia type 1 (MEN1) and type 2 (MEN2) are autosomal dominant diseases characterized by the presence of concomitant tumors in: parathyroid, pituitary and endocrine pancreas (MEN1); medulla of the thyroid, parathyroid and medulla of the adrenal (MEN2). The MEN1 syndrome is caused by inactivating mutations in the *MEN1* tumor suppressor gene, while the MCT/MEN2 syndrome is caused by activating mutations in the *RET* proto-oncogene. Multiple endocrine neoplasias are still underdiagnosed or late-diagnosed condition in many areas of the world, including Brazil. A clinical screening program focusing both MEN1 and MEN2 started 10 years ago at Hospital das Clínicas of the School of Medicine of the University of São Paulo. Few years later a genetic screening of *RET* and *MEN1* genes was implemented. The present study reports a retrospective analysis focusing the impact of this screening program on the management of patients with MEN and their at-risk relatives. Over the past ten years, fifteen kindreds with MEN1 including 161 at-risk family members and twenty-two kindreds with MCT/MEN2 including 178 at-risk family members were diagnosed and treated in our Institution, many of this patients were from other regions of Brazil and came to SP because of the screenings. Six different mutations in the codons 611, 620, 634, 791, 918 were identified in the mutational hot-spots of the *RET* gene. Twelve different mutations were identified scattered through the *MEN1* gene. In accordance to the international Consensus for treatment of MENs, *RET* mutation carriers were indicated to preventive thyroidectomy and *MEN1* mutation carriers were indicated to periodic clinical surveillance. Our data supports the benefits of the genetic screening in the management of patients with MEN1 and MEN2 syndromes.

FAPESP, FFM

PT.025

Ret Genetic Screening of Patients with Familial Medullary Thyroid Carcinoma/MEN2 Syndrome: Expanded Analysis in a Family with no Mutation in the *RET* Hot-Spot Exons

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Context: Multiple Endocrine Neoplasia Type 2 (MEN2) is an autosomal dominantly inherited neoplasic disorder that involves medullary thyroid carcinoma (MTC), pheochromocytoma (PHEO) and parathyroid glands (HPT). Importantly, it was reported a high frequency (95%) of activating mutations in the proto-oncogene *RET* of index patients with a familial history of CMT. After the identification of a *RET* germline mutation in an MEN2 index case, the genetic screening permits the identification of mutation carriers within the at-

risk family members. According to the international Consensus for treatment of MENs, RET mutation carriers should be indicated to precocious and curative total thyroidectomy. Twenty-three families with familial MTC/MEN2 were referred to the laboratory of the Endocrine Genetic Unit at FMUSP to *RET* genetic analysis. Germline mutations were identified in twenty-two of these families (95%), while one family presenting with FMTC had no mutation in the 6 hot-spot exons (10,11,13,14,15,16) of the *RET* gene. **Objective:** We sought to expand our genetic investigation in this rare and specific case. **Methods:** DNA extraction, PCR and automated sequencing. **Results:** We identified no germline pathological variants in the entire coding regions (21 exons) and the intron/exon boundaries of the *RET* gene of this index case. Further, we found no somatic mutations in the mutational hot-spots of the tumoural tissue. **Discussion:** Our data confirmed the presence of a pathological germline mutation in the great majority of the familial cases with CMT/MEN2A. Also, we described a family presenting with FMTC apparently caused by genetic events not able to be identified by DNA sequencing, as large insertion/deletion, or caused by genetic events other than RET proto-oncogene. We are investigating by real time-PCR a possible insertion/deletion in this case.
FAPESP, FFM

PT.026

Search for *MEN1* Germline Mutations in Patients with Usual and Unusual Clinical Features of Multiple Endocrine Neoplasia type 1

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Introduction: Multiple endocrine neoplasia type 1 (MEN1; OMIM 131100) is an autosomal dominant inherited tumor syndrome caused by inactivating mutation in the *MEN1* tumor suppressor gene. MEN1 is clinically characterized by concomitant occurrence of parathyroid, endocrine pancreas, and pituitary tumors. Patients from Hospital das Clínicas (HC-FMUSP, SP) with a) tumors in at least two main MEN1-related glands, b) HPT at young age (< 30y), c) multiglandular HPT or d) parathyroid hyperplasia are routinely referred to molecular diagnosis at our Institution. Recently, we have reported the identification of 12 novel germline *MEN1* mutations identified during a genetic screening program that involved 14 families with MEN1 syndrome and 141 at-risk individuals (Toledo RA, 2007). **Objective:** To continuing and expand the *MEN1* screening project in our Institution. **Patients:** Five new cases fulfilling the criteria to *MEN1* genetic screening were referred to our laboratory. **Methods:** DNA extraction, PCR amplication of the entire coding region (2-10 exons) and intron/exon boundaries and automated sequencing. **Results:** We identified 2 different *MEN1* disease-causing germline mutations, L413R and R527X, in the two patients with the most common history of MEN1: early development of HPT followed by later development of pituitary and/or endocrine pancreas tumor. We found no mutation in a case presenting an unusual clinical feature: development of Cushing due to an ACTH-secreting pituitary adenoma followed by a late hyperparathyroidism. Further, we did not find pathogenetic alterations in two cases with parathyroid hyperplasia. **Conclusions:** High frequencies of *MEN1* gene mutations were detected in Brazilian families with classical diagnosis of MEN1 syndrome referred to genetic testing (> 90%). Seven novel germline mutations predicted to cause inactivation of the MEN1 tumour suppressor gene were identified during our genetic screening program. Patients with an unusual MEN1 clinical feature had no *MEN1* gene mutations. Our data underscore the importance of a systematic *MEN1* screening programme in Brazil.
FAPESP, FFM

PT.027

Loss of Heterozygosity Analysis in Tumors from Patients with MEN1 Syndrome

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Introduction : The *MEN1* gene, associated with the Multiple Endocrine Neoplasia type 1 syndrome (MEN1), codifies for a 610-amino acid protein named MENIN. Several tumor suppressor roles of MENIN have been disclosed so far, such as, a) cell cycle and cell growth control, b) transcription regulation, c) DNA repair, d) genome stability, e) apoptosis regulation, and f) endocrine cell proliferation. A *MEN1* germline mutation predisposes the genome to a second mutational event concerning MEN1-associated glands (parathyroid, pituitary and endocrine pancreas), causing loss of heterozygosity (LOH) of the 11q13 locus. The inactivation of MENIN is predicted to disrupt its tumor suppressor molecular pathways, thus leading to MEN1

tumorigenesis. These findings are consistent with the Knudson's 2-hit hypothesis for tumor suppressor genes. **Objective:** To investigate the loss of the wild type allele (LOH analysis) in DNA samples of tumors from patients with MEN1 syndrome.**Patients:** Twenty-one patients clinically and genetically diagnosed with MEN1. **Tumors:** Tumors occurring in: parathyroid, pituitary, pancreas and skin were studied. **Methods:** LOH analysis was carried out using a MEN1-flanking microsatellite marker (PYGM) and by as intragenetic single nucleotide polymorphism (SNP D418D). **Results:** We identify frequent 11q13 LOH and loss of the wild type allele in the most common MEN1-related tumors, occurring in parathyroid, pituitary and pancreas. Less frequent LOH was identified in the skin tumors.
FAPESP, FFM

PT.028

Glutathione S-Transferase Polymorphisms In Osteosarcoma Patients

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Background: The Glutathione S-transferase (*GST*) supergene family includes several genes that code enzymes involved in the detoxification of many xenobiotics agents, including carcinogens and anticancer drugs. The polymorphisms in these genes have been associated both with cancer susceptibility and anticancer drugs resistance. Osteosarcoma (OS) is the most common malignant bone tumor in children and adolescents. Moreover, multidrug resistance and poor clinical outcome are problems that reach almost 50% of osteosarcoma patients. **Objectives:** We investigated genotypic frequencies of *GSTM1*, *GSTM3*, *GSTP1* and *GSTT1* genes in 80 osteosarcoma patients and 160 normal control subjects. We also evaluated the relationship between genetic polymorphisms and clinical features of patients. **Methods:** *GSTM1* and *GSTT1* deletion polymorphisms were examined with polymerase chain reaction (PCR), while the polymorphism of 3 bp deletion in intron 6 of *GSTM3* and the +313A>G polymorphism of *GSTP1* were investigated using PCR-RFLP methods. **Results:** The *GSTP1* polimorphic allele was more frequent in patients (P=0.020). There was significant association between *GSTM1 null* genotype and relapse occurrence (P=0.034) and the presence of at least one *GSTM1* allele was correlated with high tumor necrosis grade in surgical specimens (P=0.055). Futhermore, the overall survival curve of *GSTM1* genotype groups showed a low survival rate of patients with *null* genotype (P=0.026). **Conclusion:** The homozygous polymorphic genotype of *GSTP1* probably plays an important role in the osteosarcoma tumorigenesis mechanisms. The *GSTM1 null* genotype is related to poor clinical outcome characterized by increased relapse ocurrence, while the presence of at least one *GSTM1* allele guarantees a good response to treatment and better survival. The findings of this study showed how *GSTs* polymorphisms can influence treatment response and osteosarcoma progression, providing more sources for searching new therapeutic targets and molecular markers to improve patients response and survival.
Supported by: GRAACC, FAPESP 04/12150-8 and 04/12146-0

PT.029

Functional Polymorphism of Matrix Metalloproteinase-9 Gene and Diffusely Infiltrating Astrocytomas

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Background: Matrix Metalloproteinase 9 (MMP-9) plays an important role in the growth, invasion and metastasis of tumors. This enzyme has a role in the degradation of extracellular matrix and the basal membrane, contributing to the infiltrative characteristic of malignant astrocytomas. The expression of MMP-9 in astrocytomas could be detected by different methods, and zymography analysis revealed that about 90% of the most malignant astrocytoma, glioblastoma (GBM), express MMP-9. The nonsynonymous single-nucleotide polymorphism (SNP) in a functional domain of MMP-9 gene may exert an impact in the expression level of the gene, as well as in the enzymatic activity of MMP-9, and thus contribute to the predisposition and aggressiveness of cancer. The genotypes with the 279R allele are significantly associated with the risk to develop lung cancer metastasis. **Objectives:** To analyze the genotype frequency of MMP-9 R279Q SNP in patients with diffusely infiltrating astrocytoma; to correlate the genotypes with the patients' following data: sex, age, histological grade and overall survival. **Patients and Methods**: 146 cases (24 low grade astrocytomas, 18 anaplastic astrocytomas and 104 GBMs) were matched by sex and age in a case control study. The peripheral DNA of blood samples were

extracted from all cases and controls and submitted to polymerase chain reaction (PCR). The genotype of R279Q SNP was determined by digestion with MspI. **Result and Conclusion**: The sex-age-adjusted OR was 1.13 for 279RQ genotype relative to the 279QQ genotype (95%CI, 0.52-2.43) and 1.25 for 279 RQ genotype relative to the 279QQ genotype (95%CI, 0.76-2.03). The different genotypes were not correlated to the malignant grade of astrocytomas or to overall survival in GBM patients when Kaplan-Meier analysis was carried out (p= 0.648). The frequencies of the genotypes in our population, RQ, 51.4%; QQ, 37%, and RR, 11.6%, were different from those found in the Japanese and Chinese population, which may explain the different results of correlation observed in other neoplasms. The R279 SNP of MMP-9 gene is not associated with the risk to develop diffuse astrocytomas.
FAPESP

PT.030

Analysis of Polymorphism of EGF and EGFR Gene in Diffuse Astrocytomas

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Background: Diffuse astrocytomas are the most frequent CNS tumors and are frequently associated to EGFR alterations, including over-expression, responsible for an increased mitogenic signaling cascade. Activation of autocrine EGF/EGFR loop creates a link between proliferation and the cell cycle machinery irrespective of the genetic abnormalities identified. Single nucleotide polymorphisms (SNPs) in EGFR and EGF may influence the control of gene expression and thus, be associated to an increased risk to develop astrocytomas. **Objective:** To analyze SNPs located at the promoter region (C-191A, G-216T), exon 21 (A2073T) in EGFR gene and polymorphism in 5' untranslated region of EGF (A61G), and to associate these SNPs with EGFR expression and overall survival. **Methods**: We conducted a case-control study to analyze DNA from peripheral blood from 193 cases and 200 controls. Polymorphisms were genotyped by PCR reactions followed by restriction fragment length polymorphism analysis. Evaluation of genotype frequency in cases and controls, and Hardy-Weinberg for genotype distribution were tested by c^2 test. Odds ratio (OR) and 95% confidence interval (95%CI) were calculated by logistic regression. EGFR gene expression analyses was carried out by Real Time PCR and correlation to overall survival was analyzed by Kaplan-Meier. **Results:** The analysis of genotype frequency showed no significant differences between cases and controls for EGFR SNPs [A2073T (p=0.13), C-191A (p=0.73), G-216T (p=0.38)] and EGF SNP [A61G (p=0.95)]. No differences in the OR were observed for the four SNPs analyzed separatedly or in combination. No significant differences of EGFR expression and the overall survival time were observed for the different genotypes of EGFR and EGF genes. **Conclusion**: Our study showed that EGFR and EGF SNPs are not associated with an increase in the relative risk of astrocytoma compared with the normal population. The SNPs do not influence EGFR gene expression, and additionaly there is no correlation.
FAPESP, CAPES

PT.031

Polymorphisms of Kiss-1, Ninjurin and Tax1bp1 in Head and Neck Cancer Patients

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Background: Head and neck cancer is a major global threat to public health with 200,000 new cases diagnosed worldwide on an annual basis. It is the sixth most common type of cancer among developing countries. Tobacco chewing, alcohol consumption, and smoking seem to be the most important risk factors. **Objectives:** to assess the sociodemographic profile, tobacco and alcohol use, to evaluate single nucleotide polymorphisms (SNPs) from KISS-1 (G→A), NINJURIN (A →C) and TAX1BP1 (T→ A) genes in patients with head and neck cancers, and to compare their frequencies within a control population. **Methods:** We analyzed 191 cases comparing to 175 controls. DNA was extracted from lymphocytes of peripheral blood for the investigation of NINJURIN and TAX1BP1polymorphisms using Polymerase Chain Reaction (PCR) and enzyme digestion and KISS-1 polymorphism using SSCP-PCR. Statistical analysis was achieved by the Fisher Exact and Chi Squared tests, with P-values <0.05 being considered statistically significant. **Results:** The majority of patients were constituted by men (84.82%), caucasoids (87.77%), mean age was 68 years, 91.10 % smoked, 77.49 % used alcohol. The

most frequent primary site was oral cavity (38.36 %). We did not find statistical differences in genotyping frequencies for the KISS-1 gene (P=0.09) and TAX1BP1 gene (P=0.1748), among patients and the control group, but statistical differences were found in NINJURIN gene (P=0.05). **Conclusion:** The polymorphism of NINJURIN gene seem to be associated with head and neck cancer. The understanding of the mechanisms involved in the tumorigenic process can contribute for the development of individualized treatments and disease prevention.
CNPq, FAMERP - FUNFARME and FAPESP.

PT.032

Influence of Genetic polymorphism in Glutathione –S- Transferase P1 on Survival and Treatement in Patients with HNSCC

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Background: Glutathione-S-transferase P1 (GSTP1) detoxifies a wide range of endogenous and exogenous carcinogens and anticancer agents such as cisplatin. GSTP1 has a polymorphic site at codon 105 (exon 5), where an adenosine-to-guanine (A-G) transition causes an isoleucine-to-valine substitution (I105V). This polymorphism is associated with alterations in enzyme activity and may be a determinant of cisplatin response and survival of treated patients **Objective:** Our intention was to evaluate the association of GSTP1 polymorphisms and treatment outcome in head and neck squamous cells carcinoma (HNSCC) patients. **Patients and Methods:** GSTP1 was genotyped by a PCR-RFLP method, in a prospective cohort of 49 patients diagnosed with inoperable HNSCC treated with exclusive platin based concomitant chemoradiotherapy. **Results:** The 49 patients were genotyped and categorized as a wild or polymorphic. No significant association was noted between GSTP1 polymorphism and treatment response (p=0.27, Fisher's Exact Test). The patients with a polymorphic genotype showed better overall survival than wild type patients, however without statistical significance difference (OS: 14 months, 95% CI: 13 – 16; p=0.074, Log Rank). Similar results were found in survival free progression (SFP: 14 months, 95% CI: 13 – 16; p=0.085, Log Rank). **Conclusions:** GSTP1 polymorphism may be related with better SFP and OS in patients with HNSCC treated with cisplatin based chemoradiotherapy.
Supported by Capes

PT.033

Human Papilomavirus (HPV) and Codon 72 TP53 Gene Polymorphism in Oral Squamous Carcinoma

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Background: The oral squamous cells carcinoma represents 90% of all the malignant tumors that affect the oral cavity. The infection for HPV (Human Papiloma virus) demonstrated a relevant factor in the development of the oral carcinoma, as well as the polymorphism of the codon 72 of the gene *TP53*, whose the transcription of the tumoral suppressor protein p53 is modified. The degradation of the protein occurs in function of the viral oncoprotein E6 interation with the ubiquitin-proteassome proteolytic system. **Objectives**: The objective of this work was to identify the presence of the HPV in the oral carcinoma and to verify the association with *TP53* (codon 72) polymorphism. **Methods**: The experimental group was composed of 24 patients with oral squamous carcinoma who were attended in the Cancer's Hospital of Pernambuco. It had been studied 21 controls, which had been grouped in accordance with the familiar and ambient proximity of the patients. The biological material was gotten by the exfoliation of the oral mucosa and the DNA extracted through the method of Salting Out. The detection of the HPV was carried out by PCR having used itself primers GP5+ GP6+. The identification of the polymorphism G-allele was made by PCR-RFLP. **Results**: The results had been negative for the HPV presence. It was not observed association between the presence of the polymorphism and the oral squamous carcinoma in patients (X^2=4.048; p=0.132). The results had also confirmed that men (X^2=5.88; p=0.01) and

smokers (X^2=8.84; P=0.002) presented a larger frequency of positive diagnosis for the oral carcinoma, as well as for the age of the studied individuals (X=5.88; p=0.01). **Conclusion:** The results not suggest an association with the *TP53* (codon 72) polymorphism and HPV infection with the oral squamous carcinoma. The results for the sex and smoking are in according with literature.

PT.034

Folate Gene Polymorphism and Risk of Squamous Cell Carcinoma of Head and Neck

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Background: Squamous cell carcinoma of head and neck (SCCHN) occurs due to an accumulation of aberrations in the DNA, which affects gene function and expression. Alterations in the folate metabolism interrupt the DNA synthesis, leading to the hypomethylation and subsequent activation of proto-oncogenes and/or hypermethylation of the DNA that inactivates tumor suppressor genes. Mutations in the *methionine synthase* gene *(MTR)*, which takes part in the folate pathway, contribute to the increase of plasma homocysteine levels, and defects in MTR activity may be important in tumorigenesis. A common polymorphism of the *MTR* gene, an A-to-G transition in nucleotide 2756, has been associated with SCCHN. The allele frequencies for this variant in healthy Brazilian individuals were about 0.7-0.8 and 0.2-0.3 for A and G, respectively. **Objective:** To assess the frequency of the *MTR* A2756G polymorphism in patients with oral squamous cell carcinoma and compare it with those found in healthy controls. **Methods:** DNA was extracted from peripheral blood leukocytes of 115 patients with SCCHN and 125 healthy controls. The *MTR* A2756G polymorphism was investigated by Polymerase Chain Reaction (PCR) and enzyme digestion with Hae III. Statistical analysis was performed using Fisher's Exact Test, with P-values <0.05 considered as statistically significant. **Results:** There was no statistical difference in genotype frequencies for the *MTR* polymorphism (P = 0.8378) between patients and controls. In patients, the frequency of allele A was 0.79 and of allele G 0.21; in the control group, the frequency of allele A was 0.80 and of allele G 0.20. There was no difference in allele frequency between the groups (P = 0.8610). **Conclusion:** No statistical evidence was found indicating that this polymorphism has an influence on SCCHN. However, the potential effect of the *MTR* A2756G polymorphism deserves further investigation in similarly designed studies.

CNPq, FAMERP/FUNFARME

PT.036

Thymidylate Synthase Polymorphisms and the Risk for Development of Acute Leukemia in Childhood

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Background: Leukemia is the most common malignancy diagnosed in children, corresponding 30% of pediatric cancer. The acute leukemia (AL) subtypes are often characterized by genetic alterations such as scattered mutations and chromosomal alterations, including translocations, inversions and deletions. Folic acid is an important vitamin for cell division and maintaining homeostasis. A deficiency of folic acid results in a number of cellular disorders. Bioproducts of this metabolism take part in processes of synthesis and repair of DNA, a vital mechanism in the prevention of chromosomal abnormalities. Thymidylate synthase (TS), present in the folate metabolism, is a key enzyme in the DNA synthesis and cellular proliferation. Common 28-bp tandem repeat polymorphisms (3R or 2R) in the *TS* promoter enhancer region have been reported and are supposed to contribute with the normal DNA synthesis protecting against the leukemogenesis. The frequency of the 2R/2R polymorphism in Southeast Brazilian population is 17.7% and 3R/3R is 26% and there are not studies in Northeast Brazilian population. **Objectives:** The aim of this study was to investigate the association between *TS* tandem repeat polymorphisms with the risk of children acute leukemia. **Methods:** Samples from a series of 116 children with AL were subjected to genetic polymorphism analysis in a case-control study. The control group consisted of 59 subjects with no previous history of cancer. The *TS* tandem repeat

polymorphisms were determined by polymerase chain reaction assay. **Results:** The genotyping distribution was in accord Hardy-Weinberg equilibrium in both groups. No associations were observed in Odds Ratio analysis ($OR_{3R3R/2R2R}$ = 0.66; p = 0.52; CI = 0.26-1.67). **Conclusion:** The results not indicate association between TS tandem repeat polymorphisms with the risk of the acute leukemia.
CAPES

PT.037

Polymorphisms in Prostate-Specific Antigen Gene (PSA) Cyitocrome P4501A1 Gene and the Susceptibility To Prostate Cancer

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The prostate cancer is the second principal cause of death by cancer in men. Gene-environment integration have been the focus of several recent studies about human cancers occurrence, and an association between the risk and the polymorphisms of the *CYP1A1(A^{462}-G)* gene has been reported in several types of tumors, among them prostate cancer. Recent studies have indicated that the prostate-specific antigen (PSA) gene polymorphisms may be associated with the risk of prostate cancer. *PSA* gene promoter has a polymorphic ARE sequence, and one polymorphism commonly described is the transition G→A in the positions -158. This polymorphism may be associated with higher PSA levels and increase of prostate cancer risk. We have investigated the potential functional significance of the polymorphisms *CYP1A1(A^{462}-G)* and *PSA(G^{-158}A)* and their associations with prostate cancer in an Caucasian population. In this study were analyzed 55 DNA samples from patients that presented high levels of PSA (≥4ng/ml) and histological confirmation of prostate cancer and 55 controls cancer-free with normal level PSA (<2ng/ml). The patients were matched to health controls, by age, ethnic group, smoking and drinking status. The genotypes of *CYP1A1(A^{462}G)* and *PSA(G^{-158}A)* where determined by PCR-restriction fragment length polymorphism analysis (RFLP) with the enzymes *MspI* and *NheI*, respectively. The *CYP1A1 G/G* genotype (OR, 0.36; IC 95% = 0.12-1.10) was not statistically significant for the risk of prostate cancer. The association study of the *PSA G^{-158}A* polymorphism also did not demonstrate differences between patients and controls (OR, 0.92; IC 95% = 0.42-2.02). In this study, it was not found differences statistically significant between the polymorphisms *CYP1A1(A^{462}G)* and *PSA(G^{-158}A)* and the increased risk for developing prostate cancer. These results are partials and we believe that after the raise of the sample number it will be possible establish a clearer relationship between these polymorphisms and cancer susceptibility to the prostate cancer.
Capes/CNPq

PT.038

Cross-Talk Between Insulin and estrogen Signaling Systems in the MCF-7 Breast Cancer Cell Line

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Background: Obesity has been consistently shown to increase rates of breast cancer in postmenopausal women. The hormonal changes associated with obesity are considered to be responsible for these adverse effects, with particular emphasis being placed on the increased production of peptide and steroid hormones, such as oestrogens and insulin. **Objectives:** In this study, we investigated the possibility of direct interactions between insulin and 17β-estradiol (E2) action in the breast cancer MCF-7 cell line, focusing on some key intermediate steps in the PI3K/Akt/mTOR signaling pathway. **Methods:** Western blotting and MTT cell proliferation assays were conducted on MCF-7 cells to evaluate the cross-talk between insulin and estrogen signaling pathways. **Results:** Our data show that insulin and E2 alone were able to increase proliferation of MCF-7 cells and to produce molecular activation of the Akt/mTOR signaling pathway. However, combined administration of insulin and E2 not only led to a significant increase in MCF-7 cell proliferation, which was abrogated by rapamycin administration, but also provoked a quantitative potentialization of molecular signaling through the Akt/mTOR pathway. **Conclusion**: We provide evidence for a direct and positive cross-talk between insulin and estrogen signaling at the level of Akt/mTOR pathway the MCF-7 breast cancer cells. This mechanism may serve to potentiate the activity of both the insulin and estrogen pathways and to increase stimulation in physiological processes, such as cell growth and proliferation.

FAPESP

PT.039

Time-Dependent Effects of a Novel Ruthenium Complex Containing the Non-Steroidal Anti-Inflammatory Drug (NSAID) Ibuprofen on the MRNA Expression of Proteins Involved in Cell Cycle Control, Angiogenesis and Invasion in C6 Rat Glioma Cells in Vitro

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Introduction: Malignant gliomas have poor patient prognosis due to their highly invasive nature and their resistance to conventional radiotherapy and chemotherapy. The NSAIDs are known cyclooxygenase-2 inhibitors and have anti-tumour effects. Ruthenium containing compounds have been shown to exhibit anti-tumour activity, which involves binding to both DNA and proteins. The novel ruthenium-ibuprofen complex (RuIb) significantly inhibits proliferation of C6 rat glioma cells *in vitro* at a concentration of 100µM after 72hrs of treatment. **Objectives:** The aim of the present study was to identify specific targets of RuIb action and the time-dependence of these changes by analyzing the mRNA expression profiles of proteins of importance for glioma proliferation and invasion. **Methodology:** RT-PCR was performed using specific primers in order to study the mRNA expression pattern of proteins after 100mM RuIb exposure *in vitro* at 3, 6, 12, 24, 48 and 72hrs of treatment. **Results:** RuIb decreased the expression of cyclin D1, EP1, EP4 (48hrs), PPARg, pRb and increased the expression of Bax, Bcl-2, p21, p27, p53, p65, c-myc, E2F1, ERK2, nm23a and b, VEGF-A, Flt-1 and EP4 (6hrs). C6 cells did not express p16 and COX2, under control conditions or after RuIb exposure. **Conclusions:** The alterations in the expression of cell cycle and apoptosis-related proteins indicate that RuIb has multiple targets, which translate into the inhibition of proliferation and induction of cell death.

FAPESP; CNPq

PT.040

Obtainment Of Human Adrenal Tumor Cells Culture

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Objectives: Adrenocortical carcinoma is a rare malignant neoplasm with extremely poor prognosis. The diagnosis of malignancy is mainly based on histopathology and immunohistochemistry. To date, the molecular pathogenesis of adrenocortical tumors is still poorly understood. In this context, the objectives of this study were to isolate and maintain cell cultures of human adrenocortical hyperplasia, adenomas and carcinomas, as well as to produce experimental systems models for comparative analysis of gene expression profiling, intracellular signaling, proliferation and cell death of these cell cultures. **Material and Methods:** The adrenal tumors fragments were obtained from patients of the Hospital das Clínicas and transported in DMEM with antibiotics and then dissected from fat and necrotic tissues. Fragments digestion was performed using different protocols relating to fragments aspect: 1- Collagenase digestion (2-4mg/ml) and DNaseI (1µg/ml); 2- Cold 2% Trypsin, mechanical digestion and DNaseI (2,5µg/ml); 3- Collagenase digestion, DNaseI and Percoll gradient; 4- Explants technique. The cultured cells were characterized by H&E staining, cytoskeleton proteins imunocytochemistry and steroidogenic enzymes expression by RT-PCR. Steroid hormone levels were analyzed by RIA in the cell culture medium and IGF2, IGF1R and FGF2 gene expression were assessed by real-time PCR. **Results:** Twelve adrenal tumors fragments were received up till now and eight cell cultures were obtained from: 1 leiomiosarcoma, 1 mielolipoma, 1 adrenocortical adenoma, 3 adrenocortical hyperplasias and 2 adrenocortical carcinomas. The HSD3B2, CYP11B1 and CYP21B1 gene expression were detected in T7 cell culture adenoma. In addition, after 5 days in culture, the medium of T7 cells presented elevated steroid production: cortisol (238 µg/dL), testosterone (1098 ng/dL), 17OHP (> 20 ng/mL), DHEA (8.9 ng/mL) as well as in T12 cell culture medium (carcinoma): 17OHP (0,5 ng/mL), DHEA (8.8 ng/mL). **Conclusions:** The collagenase plus DNase I protocol was the most effective added with Percoll gradient. In addition, our experience shows that the proceeding decisions must be taken accordingly to aspect, consistency, coloration, presence of blood / necrotic tissue of tumor fragments.

FAPESP, PRP-USP, CNPq

PT.041

Effects of Ciliary Neurotropic Factor on Cellular Proliferation and Gap Junction Communication of Human Glioma Cell line

NG97 In Vitro

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Background: Ciliary Neurotrophic Factor (CNTF) is involved in astrocyte differentiation. In some glioma cell lines CNTF is able to reduce the proliferation rate and enhance the connexin43 (Cx43) expression. **Objectives:** The aim of this study was to investigate the effects of CNTF on NG97 cells, a human glioma cell line established in our laboratory from a tumor diagnosed as a grade III astrocytoma. **Methods:** NG97 were cultivated in supplemented RPMI 1640 media, with or without CNTF. The growth curve was obtained by plating treated and control NG97 cells in 24 well plates (10^4cells/mL/well) and counted daily for 11 days, using the trypan blue dye staining to identify viable cells. To investigate the cell cycle, NG97 cells were fixed in ethanol 70% and then by propidium iodide staining protocol. Functional gap junction was analyzed by the fluorescent dye transfer assay (Calcein-AM and DilC18) and evaluated with a flow cell cytometer FACscalibur®. **Results:** The culture of NG97 cells treated with CNTF exhibit a growth curve lower than the control ones. The differential cell counting performed by trypan blue dye does not reveal difference between the culture conditions, suggesting an arrest of the cell cycle. Moreover, an increased number of NG97 cells in G1-S cycle at CNTF condition were observed by propidium iodide analysis. Functional gap junction investigation evidenced that the cells treated with CNTF showed an increased dye transfer when compared with non-treated ones. **Conclusion:** Our data suggest that CNTF was able to modulate the growth of human glioma cell line NG97 in culture and this effect could be linked to the control of junctional communication. Further studies are on going to investigate the underlying molecular mechanisms involved in these effects of CNTF.

Supported by FAPESP 05/55995-0

PT.042

Response to EGFR Inhibitors in Fibroblast Cell Lines and its Association with Germline Polymorphism

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Background: Only a small number of cancer patients benefit from therapy with EGFR inhibitors. It is therefore important to understand the mechanism of action of these drugs and to find predictive markers for drug response. Although somatic mutations and gene amplification have been correlated with the efficacy of EGFR-targeting therapy, cancer cells and/or patients with normal EGFR expression are also sensitive to these drugs. We then aim to further understand the mechanism underlying the predisposition to sensitivity to EGFR inhibitors in germline cells. **Methods:** We chose 70 human normal fibroblasts cell lines as an *in vitro* model. Cytotoxicity studies were performed on these cells using two EGFR inhibitors, gefitinib (G) and AG1478 (AG). Cells were incubated with serial concentrations of the drugs (10, 20, 40 and 60 mM for G and 5, 10, 15 and 20 mM for AG) dissolved in DMSO. Growth inhibition was measured by Alamar Blue. Three polymorphisms, -216G/T, R497K and intron 1 $(CA)_n$, were genotyped in these cells. EGFR expression was measured with real-time PCR. **Results:** There was considerable variability in drug response in a dose-dependent manner among these cells. The two drugs behaved quite differently. The fibroblasts had a much more variable response to G (mean of coefficient of variance of survival rates under all 4 concentrations, 61%) when compared to the response to AG (26%). Drug response was not correlated with EGFR expression. A significant correlation was observed between the R497K polymorphism and the survival rate of cells treated with AG at 5 μM ($p<0.01$) and 10 mM ($p<0.05$) with higher growth inhibition in K allele-carriers. No correlation was observed between any of the three EGFR polymorphisms and G cytotoxicity. **Conclusions:** These observations suggest that the cytotoxic effects of G and AG are due to different mechanisms, and that other genes such as transporters may also contribute to drug response. The R497K polymorphism may be useful as a predictive marker for response to AG and/or similar agents.

Financial support: Capes

PT.043

Expression of Genes Belonging to Glycerolipid Metabolism and Cytokine-Cytokine Receptor Interaction Processes in Samples of Normal, Intestinal Metaplasias and Adenocarcinomas of the Stomach and Esophagus

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We demonstrated that, in intestinal metaplasia of gastric or esophageal mucosa (Barrett's disease), a set of genes functionally related to glycerolipid metabolism and cytokine signaling have altered expression. Whereas signaling favoring inflammation is augmented, expression of genes involved in peroxilipid and aldehyde metabolism is diminished. To validate these observations, we used Q-PCR and measurer expression of the 9 genes responsible for alterations in these two pathways: IL1R2, CCL20, CCL18, INHBA, IL4R, IFNAR2 (citokyne), AKR1B10, ALDH3A2, ADH1B, DGKQ and CDS1 (glycerolipid). mRNA levels were determined in 24 samples used in the array experiments and in 20 new, independent samples. There were 3 samples representing normal mucosa, 5 for inflammatory stage, 15 for intestinal metaplasia, and 21 for adenocarcinomas. For each gene, we determined the expression level based on ΔC_T as compared to the control and determined arbitrary units to determine expression in relation to normal mucosa. The differential expression of all 9 genes was validated, with statistical significance for 7 genes. Our data confirm that inflammation in patients with chronic intestinal metaplasia might leads to higher levels of aldheydes. Considering the potential of aldehydes for DNA damaging, this pathway could favor oncogenic transformation and hence, these enzymes might be targets for preventing adenocarcinomas of the gastroesophageal mucosa.
CNPq

PT.044

Role of the Kinin B1 Receptor in Murine Melanoma Cells Proliferation

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Background: Melanomas are aggressive, recurrent and frequently metastatic tumors, therefore conferring a poor prognosis. Neoplasic tissues show high proliferation rate mainly due to up-regulation of survival and mitogenic pathways. It is also well established that an inflammatory microenvironment is crucial for tumor progression for secreting factors which stimulate malignancy growth. The Kallikrein-kinin-system (KKS) is classically involved in regulating vascular and inflammatory events. Such effects are mediated by Bradykinin (BK) and Kallidin (KD), peptides that act on the G-protein coupled receptor B2. Moreover, BK and KD are also cleaved by carboxypeptidases, generating desArg^{9}-BK (DABK) and desArg^{10}KD (DAKD), respectively. DABK and DAKD bear high affinity to the B1 receptor. **Objectives**: Investigate the role of the B1 receptor in murine melanoma cells proliferation, by analyzing the KKS components expression, cell viability rate after B1 agonist stimulation and the activation of a kinase involved in proliferation (ERK1/2) after agonistic stimulation. **Methods**: The murine melanoma cell line Tm5 was cultured in RPMI medium supplemented with 5% FCS. Expression analyses of KKS components were performed by RT-PCR. The colorimetric MTT assay was used to measure cell viability in groups treated with vehicle or DABK (1μM) for 24, 48 and 72 hours. ERK1/2 phosphorylation was accessed by western blotting using specific antibodys anti-ERK and anti-phospho-ERK after stimulation with vehicle or DABK (1 μM) for 10, 30, 60 or 180 minutes. **Results**: Tm5 cells constitutively express B1 receptor, angiotensin-converting enzyme (ACE), which cleaves BK and KD into inactive metabolites, and do not express B2 receptor mRNAs. DABK-stimulated cells showed an increase in proliferation rate as a higher number of viable cells was measured after 72h. ERK1/2 phosphorylation was increased from 10 to 60 minutes after DABK stimulus returning to basal levels after 180 minutes. **Conclusion**: Besides the classical involvement in vasodilatation and inflammation, our results point to a direct participation of the kinin B1 receptor in melanoma cells proliferation ruled by ERK1/2 phosphorylation.
FAPESP, CNPq, CAPES, FAEPA

PT.045

Modulation of the IRS/PI3K/AKT/MTOR Pathway in Pre-Clinical Experimental Model of Prostatic Tumor Under a Hyperlipidic Diet

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Background: Diseases such as Obesity, Type II Diabetes Mellitus and Metabolic Syndrome are correlated to an elevated predisposition in some types of cancer, to aggressiveness increase in others and share a common state of hyperinsulinemia. One of the signaling pathways associated with carcinogenesis, the IRS/PI3-kinase/Akt/mTOR pathway is also the main insulin signaling pathway. The molecular mechanisms involved in the increased aggressiveness of hyperinsulinemic prostate cancer patients are still unknown. **Objectives:** Therefore, the objective of the present work is to investigate the effects of hyperinsulinemia on the tumor growth and to characterize the IRS/PI3-kinase/Akt/mTOR pathway in PC-3 xenografts on SCID mice. **Methods:** SCID mice were fed with a high-fat diet for eight weeks and the insulin resistant animals received 10^6 PC-3 cells subcutaneously. The tumour growth was measured daily for one month. **Results:** Our results show that

high-fat diet-induced insulin resistant mice had a larger tumor growth compared to the control group. We also noticed an increase in IRS-1 phosphorylation, IRS-1/PI3K association, Akt phosphorylation and mTOR activity in the PC-3 xenografts after acute insulin treatment on the high-fat diet animals, however in these animals the activation of this signaling pathway was reduced in classic peripheric tissues involved in insulin sensitivity (liver and muscle). **Conclusion:** Our data also show that the IRS/PI3-kinase/Akt/mTOR pathway directs the metabolic signals to tumor growth and suggests that the increase in the activation of this signaling pathway is involved in the aggressiveness increase of the prostatic tumors in hyperinsulinemic situation.
FAPESP

PT.046

ARHGAP21 Inhibits Glioblastoma Cell Migration by Inactivating Fak and PKCz

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Glioblastoma multiforme is a highly aggressive and the most common glial tumor type and, although there have been advances in treatment, the average survival expectancy is of only 12-15 months. Several genes have been shown to influence glioblastoma progression. In the present work we demonstrate that the expression of the RhoGTPase Activating Protein 21 (ARHGAP21) in glioblastoma cell lines correlates with a lack of invasiveness. The opposite was found for the expression of Focal Adhesion Kinase (FAK), a key component in the control of actin dynamics. On T98G, a glioblastoma derived cell line, ARHGAP21, FAK and the protein kinase C isotype zeta (PKCz) were found to interact. ARHGAP21 depletion by shRNAi on those cells increased (1) the FAK and PKCz phosphorylation states, (2) Cdc42 activity, (3) the production of metalloproteinases 2 and 9 (MMP-2 and MMP-9) and (4) cell migration rates. Those modifications were shown to be mainly due to the loss of ARHGAP21 action over FAK and PKCz and, consequently, the activation of downstream effectors. These results are extremely important, not only because they suggest that ARHGAP21 might act controling glioblastoma aggressiveness, but also because they indicate that ARHGAP21 might be a master regulator of migration in different tissues, and as such have a crucial role in controlling the progression of different tumor types.
FAPESP and CNPq

PT.047

ARHGAP21 is Upregulated by Decitabine Treatment in Bone Marrow Mononuclear Cells From Patients with Myelodysplastic Syndromes: Positive Correlation with β-*Catenin Expression*

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Background: ARHGAP21, a negative regulator of RhoGTPase signaling pathways, is a component of cell-cell junctions that controls β-catenin recruitment. The Myelodysplastic Syndromes (MDS) encloses a clonal hematopoietic stem cell disorders characterized by ineffective hematopoiesis and by a high incidence of progression to acute myeloid leukemia (AML). Decitabine (DAC) has significant therapeutic value for the treatment of patients with MDS and AML, being able to induce differentiation and inhibition of growth of leukemic myeloid cells. Recently, β-catenin has been described as a target for DAC treatment in AML and MDS with del(5q). **Objective:** to evaluate the expression of *ARHGAP21* during MDS progression, analyzing *ARHGAP21* as a target for DAC treatment. **Methods:** Myeloid cell lines, 65 bone marrow samples of patients with MDS and acute leukemias, including 13 MDS, 5 AML derived from MDS (AML/MDS), 37 *de novo* AML and 10 acute lymphoblastic leukemia (ALL) , and 5 normal hematopoietic tissues were analyzed in this study. Real-time PCR assays were used to determine the differential expression of *ARHGAP21* and β-*catenin*. Localization of ARHGAP21 and β-catenin were obtained using confocal microscopic analysis. **Results**: *ARHGAP21* levels were upregulated in myeloid cell lines and in samples of patients with AML/MDS, *de novo* AML and ALL when compared with normal bone marrow samples, showing positively correlation with blast cell counts in bone marrow cells of patients. DAC treatment promotes upregulation of *ARHGAP21* and β-*catenin* in bone marrow mononuclear cells (MNCs) derived from MDS patients. In addition, we showed that ARHGAP21 is preferentially localized in the cytoplasm of the MNCs, but after DAC treatment this protein translocates into the nucleus. **Conclusion**: *ARHGAP21* is overexpressed in AML and ALL cells. The upregulation of *ARHGAP21* in MNCs by DAC treatment, in parallel with β-*catenin* expression, and its translocation cytoplasm-nucleus, suggest that ARHGAP21 may be involved in the abnormal phenotype of MDS cells, being a candidate for anti-tumor therapy.
FAPESP, CNPq.

PT.048

ROS Mediate FGF-2 Senescence-Triggering in Ras-Transformed Mouse and Human Cells

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Background: FGF2, the founder of the large FGF family, plays key roles in a number of tissues as paracrine, autocrine and intracrine regulators of mitogenesis, cellular differentiation, organ morphogenesis and tissue repair. But, FGF2 roles in oncogenesis remain obscure or, even, controversial. **Objective:** Our goal is to analyze the death and/or senescence induced by recombinant FGF-2 (18 kDa) in Ras-transformed mouse and human cells, aiming to investigate whether ROS signaling is relevant in this phenomenon. **Methods:** Cell cycle and death were analyzed by FACS. Extracellular superoxide release assayed by lucigenin-enhanced chemioluminescence. Intracellular ROS measured by flow cytometry using hydrowthidine. Cell death induced by FGF-2 estimated by colony growth in clonogenic assays. Ras expression monitored by Real-time PCR and Western-blot. **Results:** In the mouse Y1 adrenal line of Ki-Ras-driven malignant cells, FGF2 promotes G_1 phase progression and S phase entry, but cell cycle is trapped at S phase and proliferation is irreversibly blocked, characterizing a form of senescence. Coherently, FACS analyses confirm that FGF2 does not cause apoptosis or necrosis in Y1 cells. Likewise, in the human HEK-ER:RasV12 line, activation of ER:RasV12 with OH-tamoxifen renders cells malignant and prone to senescence upon FGF-2 treatment. In addition, FGF-2 stimulates Y1 cells to produce intracellular ROS and to release superoxide anions into intracellular medium. Furthermore, the ROS scavenger NAC protects Y1 cells from senescence induced by FGF-2, implicating ROS in senescence triggering by FGF-2. **Conclusion:** ROS signaling likely mediates senescence-triggering by FGF2 in Ras-dependent malignant cells. Acknowledgements: We thank Dra. Elaine Hatanaka and Prof. Rui Curi (Instituto de Ciências Biomédicas, USP), for helping with FACS and ROS analyses, and Prof. Christopher M. Counter (Duke University, North Caroline, USA), for providing the HEK-ER:RasV12 cells.
Supported by: FAPESP and CNPq

PT.049

The NFAT1 Transcription Factor Inhibits Cyclin and Expression, Cell Cycle Progression and Tumor Growth in Vivo

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Background: The NFAT (Nuclear Factor of Activated T cells) family of transcription factors plays a central role in the regulation of several genes related to the immune response and has been recently implicated in the proliferation and differentiation of numerous cell types. However, the specific role of the major family member, NFAT1, in lymphocyte proliferation remains to be elucidated. Previous analyses indicated that NFAT1-deficient mice display lymphocyte hyperproliferation, shortened cell cycle duration, and cyclin overexpression. **Objectives:** Evaluate the putative role of the NFAT1 transcription factor in the regulation of lymphocyte proliferation. **Methods and Results**: Our results show that B-lymphocytes represent the major cell population responsible for the hyperproliferative phenotype observed in the lymph node of NFAT1-deficient mice. Upon BCR stimulation, NFAT1-deficient B cells proliferate more and present shortened cell cycle duration, however no differences were observed when these cells were stimulated through toll-like receptors. Given that the c-myc family of transcription factors is upregulated in some B cell lymphomas, we analyzed its expression in these cells. Interestingly, c-myc expression was not altered in NFAT1-deficient B cells. In contrast, cyclin E was significantly upregulated in NFAT1-deficient B cells stimulated with PMA plus ionomycin. In an attempt to validate the role of NFAT1 in B cell proliferation, we silenced NFAT1 expression in a B cell line. In fact, silencing of NFAT1 increased the proliferation rate of the B cell line. Futher analyses demonstrated that ectopic expression of NFAT1 in CHO cells inhibited cellular proliferation, cyclin E expression, colony formation *in vitro* and tumor growth *in vivo* when these cells were injected subcutaneously in Balb/c nude mice in a model of tumorigenesis. **Conclusion:** Together, these results indicate NFAT1 as a negative regulator of cyclin E expression in B cells. Also, inactivation of NFAT1 transcription factor could lead to B lymphocyte malignancies, since deregulated cyclin E expression has an established role in tumorigenesis.
Financial support: FIRCA/NIH, ICGEB, CNPq, CAPES

PT.050

Transcriptional Regulation of the *c-Myc* Proto-Oncogene by NFAT1

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Background: The Nuclear Factor of Activated T Cells (NFAT) family of transcription factors, encompasses four proteins (NFAT1-4) that are regulated by the

calcium/calcineurin signaling, and a more distant relative, NFAT5, regulated by tonicity. It has been demonstrated that the NFAT proteins are expressed in a diversity of tissue types and the members of this family have different regulatory functions, including cell cycle regulation, apoptosis, cell development and differentiation, and angiogenesis. Recent data demonstrated a role for NFAT proteins several human malignancies, such as lymphoma, breast, pancreatic and colon cancer. Moreover, the *c-Myc* proto-oncogene, a conserved nuclear protein that is fundamental to promote cell growth, proliferation and cell death, is also deregulated in these types of cancer. Previous data from our lab suggested that lymphocyte from NFAT1-deficient mice presented deregulated expression of *c-MYC* mRNA when compared with the wild type mice assessed by RNase protection assays (RPA), raising the possibility that NFAT1 play a role in control *c-Myc* expression. **Objectives:** Evaluate whether the NFAT1 transcription factor directly regulates the expression of *c-Myc* proto-oncogene. **Methods and Results:** Bioinformatic analysis demonstrated that the human *c-Myc* promoter presented seven putative NFAT element-binding sites. To further evaluated these elements we performed an eletrophoretic mobility shift assay (EMSA) for all these seven sites. In fact, NFAT1 protein was able to bind to three of these element-binding sites, including the most proximal site, which has already been demonstrated to be a binding site for NFAT2 protein. Additionally, a chromatin immunoprecipitation assay (ChIP) demonstrated that NFAT1 direct bind to *c-Myc* promoter *in vivo* in mouse lymphocytes. To characterize this regulation pathway, fragments of the human *c-Myc* promoter were inserted upstream in a luciferase reporter gene and are being assayed for NFAT1 responsiveness upon stimulation in Jurkat T cells. **Conclusion:** Our findings suggest that NFAT transcription factors play a role in regulation of *c-Myc* expression and might be related with cell malignancies.

Financial support: FIRCA/NIH, ICGEB, CNPq, CAPES

PT.051

NFAT Family of Transcription Factors as Oncogene and Tumor Suppressor

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Background: Nuclear factor of activated T cells (NFAT) was first described as a major activation and differentiation factor in T cells. NFAT family comprises five different proteins, NFAT1-5. Several studies *in vitro* suggest NFAT family members as redundant transcription factors. However, NFAT1-/- mice present an opposite phenotype when compared to NFAT2-/- mice, suggesting opposite roles for different NFAT members. In fact, NFAT1-/- mice are viable and present lymphocyte hyperproliferation whereas NFAT2-/- mice are embryonic lethal and lymphocytes from RAG1-/- chimeric mice showed reduced proliferation. Also, it has been shown that NFAT downregulates expression of CDK4 and cyclin A2 genes, and induce expression of cyclooxygenase-2, c-myc and cyclin D1 allowing tumor growth and survival. These data suggests that NFAT might have an important role in cell cycle regulation and tumorigenesis. **Objectives:** Analyze the specific roles of NFAT1 and NFAT2 proteins in cell cycle control and cell transformation. **Methods and Results:** We constructed retroviral vectors containing constitutively active (CA) forms of NFAT1 or NFAT2 proteins. NIH3T3 fibroblasts expressing CA-NFAT1 showed a remarkable reduction in proliferation, increased apoptotic cell death and cell cycle arrest when compared to control. Furthermore, CA-NFAT1 showed a capacity to suppress proliferation, form colonies in soft agar and reduce focus forming in focus-forming assay in H-RasV12 transformed NIH3T3. Conversely, NIH3T3 expressing CA-NFAT2 showed increased proliferation capacity when comparing to control, and also lost of contact-mediated growth inhibition and anchorage-independent cell growth. CA-NFAT2 is also able to induce cell proliferation and inhibit cell death in privation of growth factors and induce tumor growth in nude mice. NFAT1 induced phenotype was mapped at the TAD c-terminal domain. **Conclusion:** This is the first time that a dichotomy role of NFAT family members in cell cycle and transformation is described in the same cell. Taken together, our results corroborate the oncogenic potential of NFAT2 already proposed and suggest a tumor suppressor role for NFAT1.

Financial support: FIRCA/NIH, ICGEB, CNPq.

PT.052

K-RAS Activation UP-Regulates Expression of FGF-Receptors in Mouse Y1 Adrenal Tumor Cell Lines

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Background: FGF2 (Fibroblast Growth Factor 2), a classical mitogen, displays anti-proliferative effects upon Ras-transformed cells, whose mechanisms and biological significance remain undefined. The FGF2 signaling system comprises 5 FGF-TyrKinReceptors (FGFRs), except for the FGFR-5 that lacks the TyrKin domain. FGFRs activate various mitogenic pathways

such as Ras/ERK, PI3K/Akt and PLC/PKC. Specificity differences among FGFRs are still under debate. The mouse Y1 adrenal malignant cell line carries amplified K-Ras oncogene and, consequently, presents high basal [K-Ras-GTP]. We have previously shown that FGF2 triggers senescence in Y1 cells. But, [K-Ras-GTP] reduction by way of a dexamethasone-inducible Ras^{N17} dominant negative mutant renders Y1 cells completely resistant to FGF2. It is still unknown whether the expression profile of FGFRs changes with reduction in [K-Ras-GTP]. **Objectives:** To investigate whether expression profile of FGFRs depends on [K-Ras-GTP]. **Methods:** Anti-proliferative effects of FGF2 in Y1 cells were estimated by clonogenic assays. Ras^{N17} protein expression was analyzed by Immunoblot. FGFRs 1-5 mRNA expression was analyzed by quantitative RT-PCR. **Results:** Y1 cells express the following FGFRs: 1IIIc, 2IIIc, 3IIIc and 5. In Y1 cells, the specific inhibitor of FGFRs-TyrKin PD173074 completely abolishes FGF2-induced senescence, showing that FGFR-5 is irrelevant for this cell response. Three clones (R1.2, R1.6, and R1.8) were analyzed. Dexamethasone induction of Ras^{N17} protein led to a relative reduction in all three FGFRs, namely, 1IIIc, 2IIIc and 3IIIc. However, the levels of FGFR1IIIc in all $Y1Ras^{N17}$ sublines were much higher than in parental Y1 cells. **Conclusion:** Y1 cells express FGFR-1IIIc, 2IIIc, 3IIIc and 5. In Y1 cells, FGF2-senescence triggering depends on activation of FGFRs-TyrKin. Reduction in [K-Ras-GTP] correlates with relatively lower levels of expression in all three FGFR-1IIIc, 2IIIc and 3IIIc.

Supported by CNPq and FAPESP.

PT.054

Mechanisms of Resistance to FGF2-Anti-Proliferative Effects in Murine Cell Lines Transfomed by Ras Oncogene

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Background: FGF2 (Fibroblast Growth Factor 2), a strong growth factor for normal cells in primary cultures and immortalized non-tumorigenic cell lines, has been shown to block proliferation and trigger senescence in Ras-transformed murine cell lines. It is largely known that amplification and gain of function mutations in ras proto-oncogenes are frequent genetic lesions in human cancers of bad prognostic, which make these previous observations of great interest. **Objectives:** This work aimed to investigate mechanisms of resistance to novel FGF2-anti-proliferative effects in murine cell lines transformed by ras and highly resistant to apoptosis. **Methods:** Two mouse malignant cell lines were used: Y1 adrenal cell line carrying amplified K-ras and over-expressed K-Ras protein and, 3T3-B61, obtained by transfection of immortalized Balb-3T3 fibroblasts with the H-rasV12 oncogene. To elucidate mechanisms of resistance to FGF2 action, we selected, isolated and characterized clonal sublines resistant to FGF2 from both Y1 and 3T3-B61 parental lines. **Results:** We show that FGF2-resistant clones are rare normal-like revertant sublines that have lost K-ras amplified sequences and H-rasV12 transgene and, consequently, no longer display Ras over expression. These sublines are dependent on FGF2 for growth, do not grow in suspension cultures and exhibit low or non tumorigenicity in Nude mice. These results show that FGF2 exerts a strong selective pressure against ras-transformed cells, inducing senescence and irreversibly blocking proliferation. **Conclusions:** In this work, we show that FGF2 selectively targets Ras pathologic expression, in such a way that only those cells that loose ras overexpression are capable of surviving FGF2 treatment. Elucidating the mechanism of action of FGF2 over these cells will bring important information for the comprehension of the biology of tumors dependent on ras oncogenes and, possibily, also for therapy of such tumors.

Supported by FAPESP and CNPq.

PT.055

Temperature Regulates Alternative Splicing in *RIPK2* Gene

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Receptor-interacting proteins (RIP) are a family of serine/threonine kinases, which integrate extra and intracellular stress signals caused by different factors, including infections, inflammation, cellular differentiation and DNA damage. Both pro-survival, inflammatory and immune responses as well as death-inducing signaling pathways can be initiated by the RIP kinases. Multiple isoforms of *RIPK1*, *2*, *3* and *5* transcripts are generated by alternative splicing. Their precise functions and significance remain to be determined. Since RIP kinases integrate extra and intracellular stress signals and initiate different signaling cascades, environmental changes may affect isoform

expression with potential biological implications. In the present study, homology modelling of two RIP2 isoforms was performed using the MODELLER software. Pairwise sequence alignment and assessment of statistical significance, multiple alignment and additional functions were also performed via the AMPS (Alignment of Multiple Protein Sequences) package. Structural analyses were performed using the INSIGHT II software. In addition, we investigated the expression of *RIPK2* transcripts in human normal cells from *post mortem* tissues of brain, heart, kidney, larynx, liver, lung, testis and tongue, in human squamous cell carcinoma of the oral cavity and in canine mammary tumors. In order to determine if both isoforms are regulated at the pre-translational level in response to stress conditions, we analyzed their expression upon heat/ cold exposure and acidic and osmotic shock in three human tumor cell lines (Fadu/HTB-43, Siha/HTB-35 and HeLa/CCL-2). We observed that the ratio isoform 1 / isoform 2 is regulated in response to temperature stress and demonstrated that both variants are present in all tissues analyzed. Such widespread expression in multiple organ systems supports a relevant functional role for RIP2 isoforms in normal tissue homeostasis. Despite overall low sequence identity among the complex structures of the homologue RIP2 proteins, the active sites of isoforms 1 and 2 are structurally similar and reasonably well conserved. The models obtained for the catalytic domains of isoform 1 and 2 showed good quality regarding to the stereochemistry and atomic contacts. Because 10 phosphorylation sites predicted for isoform 1 are probably absent in the isoform 2 sequence, phosphorylation of the latter may be less effective in inducing a response evoked by the same stimulus.

Financial Support: FAPESP, Ludwig Institute, CNPq, CAPES.

PT.056

Analisys of Gene Expression Pattern of TA*p73* and ΔN*p73* Isoforms in Samples from Patients with Acute myelogenous Genous Leukemia

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P73, a homologue to the tumor suppressor gene *p53*, is involved in tumorigenesis, though its specific role remains unclear. The gene has two distinct promoters which allow the formation of two protein isoforms with opposite effects: full-length transactivating TA p73 shows pro-apoptotic effects, while the shorter ΔNp73, which lacks the N-terminal transactivating domain, and exert dominant-negative function towards p53 and TAp73 activity. In the present study, we have investigated, by Real Time PCR, the gene expression pattern of TA*p73* and ΔN*p73* in 147 samples derived from patients with *de novo* AML and 22 samples of CD34+ hematopoietic progenitor cells purified from bone marrow of healthy donors. We detected a significantly higher expression of TA*p73* and ΔN*p73* genes in AML samples compared to hematopoietic progenitor samples from healthy. Chromosomal translocation carrier samples (*PML-RAR*α, *AML1-ETO* and *CBF*β*-MYH11*) presented significantly higher levels of the TA*p73* form. On the other hand, that negative samples for these rearrangements had expressed higher levels of the truncated form ΔN*p73*. After that, we performed an *in vitro* cytotoxicity assay to evaluate the ARA-C effects associated to the *p73* isoforms gene expression pattern. In the presence of the ARA-C drug, the primary leukemic cells that presented a higher proportion ΔN*p73* / TA*p73* were significantly more apoptosis-resistant. Besides, the protein expression assay corroborated the gene expression results, *i. e.*, samples with higher proportion ΔN*p73* / TA*p73* were more apoptosis-resistant in the presence of ARA-C. Our results suggest an association between the presence of *PML-RAR*α, *AML1-ETO* or *CBF*β*-MYH11* chromosomal translocation and a higher TA*p73* gene expression compared to ΔN*p73* isoforms expression in AML samples. Furthermore, the relative expression pattern of the ΔN*p73* isoforms may be associated to differential responses to pro-apoptotic stimuli and treatment.

Fundação de Amparo a Pesquisa do Estado de São Paulo - FAPESP

PT.057

A Lentiviral Vector with Expression Controlled by E2F-1: a Potential Tool for the Study and Treatment of Proliferative Diseases

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Objective: One of the fundamental characteristics of tumor cells is the deregulation of the Rb-E2F axis of cell cycle control. When free of Rb, E2F-1 acts as a transcriptional activator that promotes cell division. The promoter of the E2F-1 gene is controlled in a cell cycle-dependent manner due to the activity of E2F family members. We sought to take advantage of this in order to restrict the expression from viral vectors to proliferating cells, such as in tumors. **Methods**: The FE2FLW vector was constructed by inserting the promoter of the E2F-1 gene, previously isolated by PCR, as well as the cDNA for the luciferase reporter

gene into the FUW lentiviral backbone. Alterations in reporter activity were then assessed in primary cardiomyoblasts, NIH3T3 or HT1080 cells transduced with the FE2FLW vector and growing asynchronously or upon serum starvation, contact inhibition or drug treatment to induce G1 arrest. Forced expression of an E2F-estrogen receptor fusion proteins was used to evaluate the participation of different E2F family members in controlling E2F-1 promoter activity in the presence of the inducing agent, 4-hydroxytamoxifen (4OHT). Expression from the FE2FLW vector was also examined in response to forced expression of p53. **Results**: We observed sharply decreased reporter activity in cells transduced with the FE2FLW vector and halted in the G1 phase, yet luciferase activity was regained when the cells were released from the G1 arrest. Fusion proteins consisting of E2F-1, 2 or 3 with the estrogen receptor translocated to the nucleus upon treatment with the inducer, 4OHT, but only E2F-1 was shown to activate expression from the FE2FLW vector. Our lentiviral vector was also useful as a reporter of the response to p53 in PC3 cells, since expression of p53 lead to cell cycle arrest and a proportional reduction in reporter activity. **Conclusion**: The FE2FLW lentiviral vector offers transgene expression controlled in a cell cycle-dependent manner. This vector may prove useful for therapeutic approaches where tumor specific transgene expression is desired or as a reporter of cell proliferation.

FAPESP, CNPq

PT.058

Development of an Adenoviral-Vector with Robust Expression Driven by p53

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Background: Cancer Gene therapy is based on the utilization of vectors which offer efficient gene transfer and reliable expression of the therapeutic gene. Adenoviral vectors have been used in *in vivo* protocols due to their high titer and transduction efficiency. However, appropriate control of transgene expression remains a topic of great interest. Many different strategies have been described for modulating transcription, yet most have some drawback, such as low levels of transgene expression. **Objectives:** The aim of this study was to develop a new vector for gene therapy in which transgene expression is regulated by the tumor suppressor p53. Since p53 can be induced by radiotherapy and chemotherapy, this new vector could be used in combination with conventional therapies. **Methods and Results:** The PG element, which consists in 13 tandem copies of a p53 binding site, was isolated from PG13-CAT (Science,1991,252(5013):1708-11), and used in the construction of a variety of chimerical promoters in plasmid-based vectors encoding the lucifecase (luc) reporter gene. The most efficient chimerical promoter, called PGTx□, offered negligible expression in the absence of p53, but luc activity was induced 50-fold in the presence of p53, an peak expression level that was 5 fold higher than that mediated by the CMV promoter. The synthetic promoter was inserted in an adenoviral vector, originating the AdPG-luc. Viral stocks of AdPG-luc were used to transduce cell lines harboring temperature-sensitive alleles of p53, revealing an induction up to 700-fold in presence of transcriptionally active p53. In cells harboring wild type p53, expression from the viral vector was induced 8-fold upon treatment with sub-toxic doses of doxorubicin. **Conclusion:** We developed a new adenoviral vector with robust expression and tightly regulated transcription controlled by p53. Since sub-toxic doses of drug were able to induce substantial viral expression, this vector may be of great interest for combining genetic and pharmacologic treatment modalities. We envision that such synergism may permit the reduction of drug dose while enhancing its therapeutic effect.

Supported by FAPESP and CNPq

PT.059

Gene Therapy Combining Interferon-β and p53 in a Mouse Model of Malignant Melanoma

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Background: p53 is a tumor supressor gene that, once activated, acts as a trancriptional regulator by binding to the promoter sequences of various targets genes, such as those involved in apoptosis, growth arrest and senescence. The loss of p53 and evading recognition by the immune system are two common factors that contribute to tumors formation. IFN-beta has potent antiproliverative activities and has been recently shown to stimulate p53 at the transcriptional level. The influence of IFN-beta on p53 expression translates into significant enhancement of apoptosis and reduction of chemotherapeutic dosages needed to destroy tumor cells in vitro. Furthermore, IFN-beta is capable of stimulating both the innate and adaptive immune responses, making it a critical mediator of antitumoral and antiviral response in vivo. **Objective:** To determine whether adenovirus-mediated transfer of IFN-beta and p53 would be an effective treatment for malignant melanoma (B16F10) in vivo and to ascertain

if a protective antitumor response can be triggert by the immune system, serving to prevent secondary tumors and metastatic foci. **Methods:** Use a novel adenovirus gene transfer approach that combines the functions of the promoter and the transgene in order to achieve superior transgene expression. In this system, the cDNAs of IFN-beta and p53 are under transcriptional control of a p53-reponsive promoter. In this way, a positive-feedback regulatory mechanism is established where the activities of p53 are used to drive both viral expression and to bring about tumor inhibition. This vector will be used to evaluate tumor inhibition in vivo in an Ad-permissive model of malignant melanoma in C57BL/6 mice. **Results:** Melanoma cells are relatively resistant to adenovirus vector mediated gene transfer due to the low expression of Coxsackie-adenovirus receptor (CAR), which acts as the principal Ad-receptor. Therefore, extremely high doses of Ad are required for effective gene therapy against melanoma. In order to render or model system susceptible to Ad infection, B16 cells were transduced with CAR-expressing retrovirus. These cells were analyzed for lacZ expression after transduction with AdCMVLacZ. A high percentage of positive cells were obtained at a multiplicity of infection (MOI) of 100. Thus our model system is ready for testing with the bicistronic vector. **Conclusions:** Previous results from our laboratory indicate that the bicistronic approach may be an improvement over the introduction of a single therapeutic gene. We hope to confirm that the combined multi-gene treatment plus auto-regulatory expression mechanism of this vector provide an effective anti-tumor response both at primary and metastatic foci.

FAPESP, CNPq

PT.060

Exploring the Role of p53 in the Anti-Tumor Response to Interferon-Beta

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Background: As the literature and our results show, p53 is an important factor for the control of cell proliferation and induction of apoptosis. Interferon-beta (IFNβ) has an important role in modulation of the immune response, participates in the creation of anti-tumor immunologic memory and also has an impact in the proliferative and apoptotic potential of tumor cells. Recently, data in the literature has shown that IFNβ activates transcription of p53 and that some components of IFN system affect its function via the p53/p14arf pathway. **Objectives:** To evaluate the role of p53 in the response to IFNβ in a melanoma model. **Methods and Results:** The pCMVmIFNβ and pCMVp53 vectors are being used to express IFNβ and p53, individually or together, in B16 cells (mouse melanoma). The function of these cDNAs has been measured through luciferase assays where a reporter construct (IFNβ-Luc, IFNα-Luc, IRF7-Luc and PGTxβ-Luc) was included. We hoped to identify a cooperation between IFN and p53 in activation of IFN or p53 responsive promoters, but have instead observed antagonism. To better explore this surprising result, we have generated new tools for the study of p53's role in the response to IFNβ. We have knocked-down the endogenous p53 in B16 cells using the LMP-p53.1224 vector (kindly provided by Scott Lowe, CSHL, USA), which encodes an shRNAmir specific for destruction of the murine p53 transcript. Reduction in p53 protein level, as well as that of the p53 target gene p21Waf1, has been confirmed by Western blot. Functional loss of p53 activity in these cells has been demonstrated using a p53-responsive reporter construct, pPGTxβ-Luc. In addition, we are constructing adenoviral vectors for p53 or IFNβ gene transfer. Once they are ready, these vectors will be applied to the parental or modified B16 cells and the role of p53 in mediating the response to IFNβ will be evaluated. **Conclusion:** Using these tools, we will explore the role of p53 in the response of B16 cells to IFNβ. This project will serve as a foundation for the development of new gene therapy strategies for melanoma.

FAPESP, CNPq

PT.061

Restoration of Both p16 and p53 Using Only One Adenovirus Leads to Better Tumor Regression Than Either p16 or p53 Alone

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Background: To instigate tumor growth, mammalian cells tend to lose both p16 and p53 tumor suppressor genes, two important steps towards uncontrolled division, while escaping from death and senescence. Considering the key role of simultaneous inactivation of p16 and p53 in tumorigenesis, restoration of these two genes could lead to greater efficacy of cancer gene therapy. **Objectives**: Investigate the effect of the restoration of both p16 and p53 mediated by the bicistronic adenovirus Adp16IRESp53 on proliferation of H358 human lung carcinoma cells *in vitro* and using a subcutaneous xenograft tumor model in nude mice. **Methods**: *In vitro* assays include growth curves, cell

cycle analyses (FACS-PI), senescence staining, viability and apoptosis assays. For analysis of consequences of *in situ* administration of Adp16IRESp53 on tumors size, tissue sections were stained with H&E, probed for proliferation markers Ki67 and BrdU, or an indication of apoptosis by the TUNEL assay. **Results:** The Adp16IRESp53 promoted strong inhibition of proliferation and extensive cell death (90%) in H358 cells. In comparison, monocistronic vectors delivered individually or in combination were markedly less effective. For example, Adp16+Adp53 yielded only 39% cell death. These results confirm the powerful anti-proliferation effect of the bicistronic configuration. Data from H358 tumors treated in nude mice followed the same pattern and also indicated that Adp16IRESp53 was a better anticancer agent than either Adp16 or Adp53 alone. **Conclusion:** Our results support the concept that, since the rise of cancer is a multi-step process, the treatment of this disease with tumor suppressor gene therapy will depend on the restoration of multiple tumor suppressor genes. These results also indicate that the bicistronic delivery of p16 and p53 is a promising cancer gene therapy strategy with a broad therapeutic window.
Financial Support: FAPESP, CNPq.

PT.062

Strategic Promoter/Transgene Combination in Viral Vectors: Enhancing p53/p19 Expression and Anti-Growth Effect

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Objectives: We have developed a novel gene transfer approach that combines the promoter and transgene functions. For this, the LTR of a MoMLV-based vector was modified with the addition of a p53-responsive element, creating the pCLPG vector,which in the presence of p53, offers superior transegene expression. Inclusion of the p53 cDNA establishes a positive-feedback expression mechanism resulting in enhanced inhibition of tumor cell proliferation. A bicistronic vector that contains both the p19Arf and p53 cDNAs was build under transcriptional control of the p53-responsive promoter. In this way, the activities of two powerful tumor suppressor genes would be united in order to both drive viral expression and inhibit tumor growth. In this preliminary study, we assayed vector expression as well as the tumor inhibiting activity of p19Arf, p53 or the combination when expressed from the p53-responsive vector. **Methods and Results:** In a colony formation assay, we observed that the C6 rat glioma cell line (p53+, p19Arf-) was inhibited by 90% when treated with either the pCLPGp19Arf or pCLPGp19IRESp53 vectors and by 50% with the pCLPGp53 virus, suggesting that the presence of p19Arf was sufficient to inhibit proliferation. Reporter assays to measure p53 transcriptional activity revealed that, in C6, p53 activity was induced upon the introduction of either p19Arf or exogenous p53. In contrast, the B16 mouse melanoma cell line (p53+, p19Arf-) was quite resistant to the introduction of p19Arf alone, but the combination of p19Arf plus p53 in the bicistronic vector was able to block proliferation by 40%. Introduction of exogenous p53 in B16 cells did not induce reporter activity, yet introduction of p19Arf resulted in activation of the endogenous p53.At this time we are examining the cell cycle response upon introduction of these pCLPG viruses. Currently, we are seeking to confirm the expression profile of these vectors by Northern blot analysis. **Conclusion:** These results suggest that the bicistronic approach may be an improvement over the introduction of a single therapeutic gene. Introduction of p19Arf may enhance p53 activity and lessen the impact of mdm2.
FAPESP

PT.063

Resistance of Human Glioma Cells to Different Chemotherapeutical Agents is Determined by DNA Repair Efficiency and Apoptosis Induction Ability : Role of P53

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Astrocytic tumors are the most common primary brain tumors and among these, glioblastoma multiformae is the most malignant form. Therapy consists of surgical resection to a safely possible extent followed by radiotherapy with adjuvant chemotherapy. Current protocols of chemotherapy are made on the basis of chloroethylating nitrosoureas such as ACNU and BCNU, and methylating agents such as temozolomide (TMZ). To this moment there is no predictive marker for deciding which chemotherapeutical regimen will be administered to a determined patient, and acquired resistance to treatment is a major cause of failure

in glioma therapy of human populations. To shed a light on this subject, we treated wild-type and p53 mutant human glioma cells with ACNU, that induce DNA interstrand crosslinks (ICLs) and with TMZ, that induce methylating lesions in DNA. The data showed that while p53 mutant cells are resistant to TMZ treatment, they are much more sensitive than wild type cells to ACNU treatment. Interestingly, while in p53 wild-type cells ACNU induced exclusively apoptosis, in p53 mutant cells there was a large induction of necrotic cells. We propose that the sensitivity on p53 mutant cells to ACNU is due to a deficient repair of ICLs, induced by ACNU. On the other hand, the data also indicated that the resistance that these mutant cells present to TMZ treatment is due to a lack of FAS expression after treatment, which was dependent on functional p53. Interestingly, for both agents (ACNU and TMZ) p53 wild-type cells preferentially underwent apoptosis trough the extrinsic pathway, while p53 mutant cells utilized exclusively the intrinsic pathway. Moreover, the primary DNA lesion after both agents is O^6-alkylguanine, since expression of the specific DNA repair enzyme MGMT totally inhibited cell death after ACNU or TMZ. The results indicate that p53 is a predictive marker of therapy and, therefore, the p53 status of the tumor tissue upon resection should be assessed. It also indicates that p53 mutated gliomas should be treated with CNUs instead of TMZ or other methylating drugs, provided MGMT is not expressed or inactivated.

DFG, FAPESP and CAPES

PT.064

Concomitant Downregulation of Survivin and Xiap by Imatinib is Capable to Decrease Apoptosis Resistance in CML Cell Line

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Background: Chronic myelogenous leukemia (CML) is a myeloproliferative disorder caused by excessive granulopoiesis due to the formation of the constitutively active tyrosine kinase BCR-ABL. The BCR-ABL induces hematopoietic cell transformation and protects cells from apoptosis. However, the mechanisms whereby BCR-ABL blocks apoptosis are poorly defined. Imatinib, an inhibitor of BCR-ABL, is able to overcome resistance and induces apoptosis in CML cells. **Objective:** To verify whether the inhibitor of apoptosis protein (IAP) family, in particular Survivin and XIAP, are regulated by BCR-ABL. **Methods:** For this purpose, K562 cell line (CML blast crisis, BCR-ABL positive) was treated with Imatinib and with arsenic trioxide (As_2O_3) which has a mechanism of apoptosis induction independent of BCR-ABL inhibition. Cell viability was evaluated by MTT, while the induction of apoptosis was verified by annexin V staining and further flow cytometer analysis. To evaluate the effect of Imatinib and As_2O_3 in the modulation of Survivin and XIAP expression, Western blot was performed. **Results:** Both drugs were capable of decreasing cell viability and inducing apoptosis in a concentration- and time-dependent way. Interestingly, the treatment of K562 cell line with Imatinib at IC_{50} (concentration capable of inhibiting 50% of cell viability) decreased the expression of Survivin and XIAP, significantly, when compared with control (80% and 60%, respectively). On the other hand, the treatment with As_2O_3 at IC_{50} did not change the expression of these proteins. **Conclusion:** Our results indicate that the expressions of the antiapoptotic proteins Survivin and XIAP are regulated by the activity of BCR-ABL, since its inhibition by Imatinib is able to decrease the levels of these proteins and it was correlated with the induction of apoptosis. Moreover, As_2O_3, instead of inducing apoptosis in K562 cell line, was not able to modulate the expression of IAPs, reinforcing the idea that the expression of these proteins is dependent of BCR-ABL activity.

FAPERJ, CAPES, FINEP, SwissBridge Foundation

PT.065

Resveratrol and Quercetin Decrease the Cell Number and Viability in Several Glioma Lineages

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Background: Glioma is the most common brain tumor and has a dismal prognosis despite the use of surgery, chemotherapy, and radiation therapy. There is an obvious need for the development of novel therapeutic agents. Recent studies have suggested that diet-derived phenolics, such as resveratrol and quercetin, may play a beneficial role in inhibiting, reversing or retarding tumorigenesis. **Objectives**: The goal of this work is analyze the capacity of resveratrol (R) and quercetin (Q) to induce cell death in 4 gliomas cell lines – rat C6, human U87 and U138 and mouse GL261. **Methods**: The gliomas were treated for 24, 48 and 72h with the drugs in the following doses (uM): R -10, 25 and 50; Q – 25, 50, 100; and the combination of R10uM and

Q25uM. The cell viability was analyzed by MTT assay and the number of cells was determined by counting in a hemocytometer. To elucidate a molecular mechanism of action we analyzed the effect of R10uM and Q25uM in the expression survivin, an inhibitor of apoptosis, and Bax, a protein involved in the liberation of citochrome C by Real-Time RT-PCR in C6 after 4h of treatment. **Results**: In all lineages tested, resveratrol decreases the number of cells and its viability. These results were more expressive in C6 and GL261 than U138 and U87. When cells were incubated with quercetin, there was a decrease in cell number and viability, which was the same in all lineages, but the effect of quercetin was smaller when compared to resveratrol. These drugs had no additive effect in C6 and GL261 but its effect were additive in the human gliomas. Both drugs increased Bax expression but only resveratrol increased survivin expression. **Conclusion**: These results indicate that resveratrol as well as quercetin were effective in decreasing tumor cell number and viability with resveratrol being more effective. These drugs also increased the expression of Bax that might induce apoptosis and resveratrol increased survivin that inhibits the apoptotic process. This effect could be an early response to an insult stimulus. More studies are under way in order to better identify the molecular differences between the lineages and discover a possible mechanism of resveratrol and quercetin resistence and also its mechanisms of interaction.
Financial Support: CNPq, FAPERGS.

PT.066

Apoptosis Modulation in Gliomas by RNA Interference and Transgene Expression Using Lentiviral Vector

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Background: Gliomas are the most common tumors on CNS and present a poor prognostic due to invasion and resistance to radio and chemotherapies. Several genes may be responsible for this resistance. We chose to study three tumor suppressor genes, p53, PTEN and Bax, that are commonly supressed in gliomas, and three genes that are commonly over-expressed in gliomas, Bcl2, survivin and XIAP. These proteins are inhibitors of apoptosis, therefore increasing resistance to cytotoxic treatments when over-expressed. **Objectives**: The aim of this work is to evaluate the expression of genes involved in apoptosis regulation that are commonly altered in several glioma cell lines and to analyze the effect of the silencing of survivin and XIAP, two inhibitors of caspases, on cell death. **Methods:** shRNA-containing lentiviral plasmids were acquired from a commercial library (MISSION™ shRNA Library, Sigma – Aldrich). Gliomas were transduced with lentivirus containing the shRNAs and selected with puromycin. The expression of the silenced genes was analyzed by RT-PCR. The transduced cells were treated with doxorubicin, a DNA alkylating agent. Cell viability was measured by MTT assay. **Results:** shRNAs against survivin induced a reduction of 30% in the expression of this gene in U87 glioma cell line. Preliminary results indicate that cells with reduced survivin expression are more sensitive to doxorubicin, indicating that survivin is a good target for glioma sensitization to chemotherapic agents. Silencing of XIAP produced significant basal cell death 48h after viral transduction and puromycin selection indicated that the transduced cells were not viable. **Conclusion:** Silencing of inhibitors of apoptosis, such as XIAP and survivin, are good targets for the sensitization of gliomas to cell death, and a potential strategy for glioma therapy.
CNPq, FAPERGS

PT.067

Studies on the Mechanisms of PC-3 Prostate Cancer Cell Death by Simvastatin

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Background: The hydrophobic statin simvastatin is a 3-hydroxy-3-methylglutaryl CoA reductase inhibitor widely used in the treatment of hypercholesterolemia and seems to have anticancer effect. Androgen-independent PC-3 prostate cancer cell line is commonly used as cell culture model of advanced prostate cancer. **Objectives:** In the present study, we analyzed the mechanisms of simvastatin toxicity to PC-3 cells. **Methods:** The sensitivity of PC-3 cells to simvastatin was investigated using trypan blue assay. Characterization of apoptosis and necrosis was proceeded by annexin V and propidium iodide staining respectively, and cytosolic free Ca^{2+} concentrations ($[Ca^{2+}]$ cyt) were measured with Fluo-3AM fluorescent probe in a FACSCalibur flow cytometer. Mitochondrial membrane potential was estimated by fluorescence changes of safranine O using a spectrofluorometer. Oxygen consumption was measured using a Clark-type oxygen electrode. **Results:** Simvastatin (0.1-100 μM) inhibited PC-3 cell proliferation. At 10 and 60 μM simvastatin induced apoptosis and necrosis respectively. A 3-fold increase

in PC-3 [Ca^{2+}] cyt was observed during either apoptosis or necrosis. Both apoptosis and [Ca^{2+}]cyt increase were totally prevented by the addition of 100 μM mevalonate. This suggests that the inhibition of cholesterol synthesis is followed by Ca^{2+} mediated apoptosis. Necrosis was associated with decrease in respiration and mitochondrial membrane potential. Both necrosis and mitochondrial dysfunction were partially prevented by 0.5 μM cyclosporine A (permeability transition pore [PTP] and calcineurin inhibitor), 0.5 μM FK506 (calcineurin inhibitor), and 0.5 μM bongkrekic acid (PTP inhibitor). None of these treatments prevented the increase in [Ca^{2+}]cyt, but chelation of the [Ca^{2+}]cyt with 2.5 μM BAPTA-AM protected PC-3 cells from necrosis. It is proposed that rise in [Ca^{2+}]cyt is one of the first steps in the process of cell necrosis and is followed by activation of the calcineurin pathway. **Conclusion:** We may conclude that simvastatin-induced apoptosis in PC-3 cells is dependent on the mevalonate pathway whereas necrosis is dependent on calcineurin pathway with mitochondrial dysfunction.
Supported by FAPESP and CNPq.

PT.068

Effect on the Snake Venom Toxin Jararhagin on the Expression of Adhesion, Tumor Suppressors and Apoptotic Genes in Human Malignant Melanoma Cell

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Background: Several toxins have been tested on cancer cells as potential therapeutical tools. The toxin jararhagin, present in the *Bothrops jararaca* venom, reduces melanoma cells growth, migration and invasion *in vitro*, and reduces the number of metastasis *in vivo* in mice models. However, the cellular pathways affected by this toxin are still unknown. **Objectives**: To evaluate the effect of the jararhagin on the expression of genes related to adhesion, tumor suppression and apoptosis in human SKmel-28 melanoma cells. **Methodology**: The SKmel-28 cells were treated with 30, 60 and 90 ng/uL of jararhagin for 24 h. Total RNA was reverse transcribed to obtain pools of cDNAs used for PCR amplification. The expression of integrin subunits α_1, α_4, α_5, α_v, β_1 and β_3; tumor suppressors CDKN1A and TP53, and apoptosis-related CASP1 and CASP3, was evaluated. In order to confirm the effect of jararhagin on the cell cycle, treated Skmel-28 cells were analyzed by flow cytometry in the same concentrations and exposition time. **Results and Discussion**: Jararhagin provoked a decrease on the expression of integrin subunits α_1, α_4, β_1 and β_3 in treated as compared to untreated tumor cells. However, the highly expressed integrins α_5, α_v were not affected by the concentrations tested. As to genes TP53, CDKN1A and CASP1, increased expression was found with 30 ng/uL jarharagin, whereas in case of CASP3 gene this increase was achieved with 60 ng/uL. Flow cytometry showed reduction on the number of tumor cells treated with jararhagin in G1, S and G2/M phases. These results agree with the increased expression of CASP genes, related to apoptosis, and tumor suppressors. **Conclusion**: The reduced expression of some integrins is in accordance with the morphological alterations and adhesion loss induced by jararhagin in Skmel-28 cells. Furthermore, the reduction on the number of cells in the different cell cycle phases is also in agreement with the increased expression of suppressor and apoptotic genes. Other gene expression studies will be performed to confirm the anti tumor effect of jararhagin.
FAPESP

PT.069

The Novel Cytokine PANDER/FAM3B Confers a Survival Advantage in Prostate Tumor Cells

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PANDER (Pancreatic-derived factor) is a novel cytokine that induces apoptosis in insulin-secreting b-cells. As gene expression analysis performed in paired clinical tissue samples revealed that PANDER expression is increased in late stage prostate cancer, we evaluated the role of this cytokine in tumor progression. As shown by cell growth analysis, PANDER over expressing DU145 prostate tumor cells proliferate 56±8 % more than vector alone-transfected cells. Comparison of cell viability (MTT assay) and DNA fragmentation analysis revealed that PANDER also provides a relative 52±13% survival advantage to the DU145 transfected cells, protecting them from apoptosis triggered by different stimuli such as staurosporine, TNF-a + cicloheximide and serum deprivation. These proliferative and survival advantages provided by PANDER are accompanied by an increased expression of Bcl-2 and Bcl-X_L anti-apoptotic genes. Additionally, an increased colony formation in soft-agarose was observed, suggesting the transfected cells acquired an enhanced tumorigenic capability. Thus, in contrast to its role in pancreatic b-cells, PANDER was capable to activate pro-survival mechanisms in DU145 prostate tumor cells. This novel function could be operating by Bcl-2-mediated inhibition of programmed cell death pathways and highlights a putative role for this cytokine in tumor progression.
Support: FAPESP, CNPq, FINEP

PT.070

Genes Downstream the Hyperactivated PI3Kγ Pathway in Acute Lymphoblastic Leukemia

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Background: Previous reports showed that components of PI3K/Akt/mTOR signaling pathway are frequently hyperactivated in acute lymphoblastic leukemia (ALL), contributing to chemotherapy resistant phenotype. To identify putative downstream effectors genes of the PI3K pathway, we synthesized a new specific PI3Kg inhibitor, the AS605240. PI3Kg is preferentially expressed on hematopoietic tissue. **Objectives:** The goal of this study is to identify genes affected by AS605240 treatment in different ALL cell lines. **Methods:** Nalm6, REH (B-ALL), CEM and Jurkat (T-ALL) cell lines were incubated for 48h with different concentrations of AS605240 (0,001 to 200 uM). Cell proliferation was measured by MTT assay and the IC50 dose for each cell line was calculated. Cells were then treated with the IC50 dose of AS605240 or control, during 6h, and total RNA was used for microarray analysis. In addition, primary ALL cells of B lineage were treated with AS605240 and used in microarray analysis for comparisons. Expression profiles were analyzed using the GSEA and CMap plataforms. **Results:** All cell lines investigated showed similar AS605240 sensitivity (IC50: 20uM). Presently, we have microarray data for Nalm6 and one primary ALL only. The AS605240 effect on primary ALL cells is not readily comparable to that of the ALL cell line (Nalm6). This may me due to the fact that primary ALL cells dye when in culture. Several genes were found to be upregulated/downregulated in Nalm6 after treatment, but fold activation values were relatively low. CMap analysis allowed us to confirm that AS605240 treatment is compatible to that reported for other PI3K inhibitors. Interestingly, AS605240 caused an effect similar to Geldanamycin. Gene set linked to miRNA and NUP8_HOXA9 pathways were the most highly enriched by AS605240, as deduced from GSEA analysis. **Conclusion:** Blocking of the PI3K pathway by AS605240 decrease ALL cell survival. Genes up- and down-regulated by AS605240 seem to be linked to regulation of transcription, cell growth, ubiquitin cycle and others. Interestingly, the effect of AS605240 was similar to the effect of Geldanamycin, an antibiotic shown to have antitumoral effects. It would be interesting to test the effect of AS605240 in conjunction to Geldanamycin. Capes, Fapesp

PT.071

Evaluation on the Cytotoxic Effect of Inkki and Ypvepfte Peptides in Human Cultures of Fibroblasts and Melanoma Cells

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Objective: Evaluate the cytotoxic effect of synthetic peptides INKKI and YPVEPFTE obtained from hydrolysis of casein cultures in human fibroblastic and melanoma B16F10 cells. These peptides stimulate activity phagocyte of resident macrophages, potentiate bradykinin action and stimulate proliferation of lymphocytes T and induce the cellular migration in the inflammatory exudates. **Methods and Results:** Primary cultures obtained from human fibroblasts of the normal skin and from murine melanoma B16F10 were kept in culture and after its confluence; the cellular suspensions had been platted in plate of culture of 96 wells, in the concentration of $5x10^5$cel/ml. The initial concentrations of treatment with 6,6 µg/µl peptides had been 0,0128 µg/µl, maintained for 24 hours in a humidified atmosphere of 5% CO_2 in air at 37ºC. The evaluation of the cytotoxic and proliferation effects was carried through by colorimetric method MTT ("3-(4,5-dimethylthiazol-2-y1)2,5-diphenyl tetrazolium bromide"), with 540 nm of absorbance. The analysis statistical was carried through by test Student and analysis of variance ANOVA for the program (Instat version 2.0), applying the level of significance $p< 0.05$. Our results had that peptides INKKI in the concentration of 6,6 µg/µ presented a significant cytotoxic activity with IC50% of 4.9ug/uL in melanoma cells B16F10. On the other hand, peptides YPVEPFTE in the same concentrations did not present cytotoxic activity. Both the peptides had not presented significant cytotoxic or proliferate activity in primary fibroblasts cultures. **Conclusions:** The results had shown that peptide INKKI presented selective cytotoxic activity in culture of melanoma cells B16F10, these results suggest that small peptides can permeable cellular membranes and specifically to present a cytotoxic effect in cultures of cells of melanoma B16F10.
FAPESP

PT.072

T-Cell Acute Lymphoblastic Leukemia (T-ALL) Cell Lines, But Not Primary T-ALL, are Sensitive to the CXCR2 Antagonist SB225002

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Background: Host factors play an important role in the development of Acute Lymphoblastic Leukemia (ALL). Previously, we found that ALL-stimulated bone marrow (BM) stromal cells present up-regulation of the interleukin-8 (IL8) gene. To explore the effect of IL8 in the ALL BM microenvironment, we synthesized a CXCR2 (IL8 receptor) antagonist called SB225002 (N-(2-hydroxy-4-nitrophenyl)-N´-(2-bromophenyl) urea) and two analogues of it: 4-Bromine (Br) and 3.4-Chlorine (Cl). SB225002 is also known to bind histamine receptors. **Objectives:** The aim of this study was to analyze the effects of SB225002 and two analogues in the viability of ALL cell lines and primary ALL cells in culture. **Methods:** Nalm6, REH, Jurkat and CEM cell lines were incubated for 48h with SB225002 (10-100mM) or its analogues (10 and 100mM). T-cells were also treated with IL8 (10 and 100ng/ml) or histamine (10 and 100mM). Cell proliferation was measured by the MTT assay. Primary ALL cells were co-cultured with human mesenchymal stem cell and treated with SB225002 (10 and 100mM) or IL8 (10 and 100ng/ml). Co-cultures were incubated for 48h and cell proliferation was measured by FACS analysis. **Results:** Jurkat and CEM (T-ALL) were affected by SB225002 at concentrations above 20mM (IC_{50} dose), while Nalm6 and REH (B-ALL) were resistant. Primary B- or T-cells were resistant to the action of SB225002. None of these cells were affected by the analogues Br and Cl. IL8 in conjunction or not with SB225002 had no effect on ALL proliferation/survival. Histamine at 100mM had a very little effect on T-cell line proliferation/survival. Interestingly, 100mM histamine was able to cause a 4- to 5-fold enhancement on the deleterious effect of a sub-dose of SB225002 (10mM). **Conclusion:** SB225002 has a deleterious effect exclusively against T-cell lines. Histamine synergistically interacts with SB225002, thus suggesting that its effect may occur via the histamine receptor. Lack of any effect of these molecules against primary ALL cells or B-cell lines is quite intriguing and illustrates the heterogeneous nature of all.

CNPq and FAPESP

PT.073

Subcellular Localization of Prohibitin and Nucleophosmin in Cisplatin-Treated Human Melanoma Cells

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Background: Melanoma incidence is increasing worldwide and represents a clinical challenge, as its treatment outcome is still poor (less than 30% of objective responses in advanced cases). The molecular bases for chemoresistance are poorly understood. A proteomic approach was conducted in our laboratory to identify differences in protein expression. Prohibitin, a mitochondrial chaperone and an E2F inhibitor, and nucleophosmin, a p53 regulator, were overexpressed after treatment with cisplatin. **Objectives:** Here, we have investigated prohibitin accumulation and subcellular compartmentalization in cisplatin-induced cell death in human metastatic melanoma cell lines. **Methods:** Different melanoma cell lines were treated or not with 25mM cisplatin for 24 hours. Western Blot and flow cytometry was conducted to show its overexpression and cell cycle phases. Immunofluorescence followed by confocal microscopy analysis was conducted to see its localization and accumulation using polyclonal and monoclonal antibodies to prohibitin and monoclonal antibody to nucleophosmin. **Results:** After treatment with cisplatin, prohibitin levels increased in the nucleus and cytoplasm of both sensitive and cisplatin-resistant cells. Immunofluorescence assays and confocal microscopy have shown distinct patterns of expression of prohibitin. Prohibitin was found in the cytoplasm associated to mitochondria and within nuclear compartments. In cisplatin-resistant cells, prohibitin was found around the nucleolus and spread over the nucleus in cisplatin-sensible cells. Nucleophosmin levels increased after treatment with cisplatin. While nucleophosmin was found within the nucleolus in cisplatin-resistant cells, in cisplatin-sensitive cells it was found dispersed in the nuclear space. Conclusions: Prohibitin may be involved in melanoma resistance, not only for its overexpression, but also due to its nuclear compartmentalization. Nuclear reorganization, as evidenced by both prohibitin and nucleophosmin compartimentalization, accompanies the cellular response to cisplatin towards survival.

Supported by FAPESP and CNPq.

PT.074

Antiproliferative Effect of Sodium Butyrate in Cell Lines of Non-Small Cell Lung Cancer

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Background: Lung cancer is the most common kind of malignancy. The Brazilian Cancer Institute (INCA) estimated 27.170 cases of lung cancer in 2006 for the Brazilian population. In the present work, we have compared the effects of two histone deacetilase inhibitors (HDACi), sodium butyrate and Trichostatin A, in two cell lines of non-small cell lung cancer, A549 and H460. Sodium butyrate acts on the regulation of cell survival causing arrest on cell proliferation and inducing differentiation and apoptosis *in vivo* and *in vitro*. **Methods**: The non-small cell lung cancer lines A549 and H460 of were treated with sodium butyrate (1mM, 3mM e 10mM) and Trichostatin A (0,02 µM; 0,2 µM e 1,0µM) for 24hours, 48hours and 72hours. At the end of each time point, cell proliferation was measured by counting the number cells in each condition. We also measured cell viability by MTT assay and apoptosis by Anexin V staining. To try to elucidate the mechanisms of sodium butyrate regulation, we have performed immunoblotting to access the levels and activation state of MAP Kinase family members and Epidermal Growth Factor Receptor (EGFR). In addition, EGFR gene expression was monitored by real time PCR. **Results:** Our experiments show that both sodium butyrate and Trichostatin A, decrease cell proliferation and viability in A549 and H460 cells. The cell line H460 was more resistant to the sodium butyrate treatment and did not present modifications in EGFR protein expression levels, but showed an increase in both ERK protein expression and EGFR mRNA levels. Trichostatin A caused a reduction in EGFR protein expression, but an increase in the mRNA levels in both cell lines. **Conclusion:** The A549 and H460 cell lines showed a time and dose dependent response to sodium butyrate, but H460 cells were more resistant to the treatment than A549 cells. These results, together with the up-regulation of the p-ERK levels, suggest that components of the JNK/MAPKs stress pathway are affected by sodium butyrate. The HDACis, sodium butyrate and Trichostatin A, cause an up-regulation of EGFR at the transcriptional level.

CNPq

PT.075

Sea Anemone Cytolysins Induce Cytotoxicity in Human Glioblastoma Cells

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Background: the search for new drugs and treatment approaches is of particular importance for malignant gliomas, which are lethal with the available medical care. In many organisms, cell death can be induced by cytolysins, which are proteins that can form pores in biological membranes. Perhaps by facilitating drugs to enter into the cytosol or by disturbing ionic balance, cytolysins might be used to kill glioblastoma cells. **Objective**: We investigate the cytotoxicity of toxin Bc2 and equinatoxin (EqTx-II), two sea anemone cytolysins, against U87 and A172 human malignant glioma cells. **Methods**: the cellular viability was determined by the MTT and LDH methods. We analyzed the cell morphology by optic microscopy. **Results**: toxin Bc2 (0.3-1 µg/ml) and EqTx-II (3-10 µg/ml) significantly reduced the viability of U87 and A172 glioblastoma cell lines in a dose dependent manner. In rat astrocytes, the decrease in cell viability induced by Bc2 (1 µg/ml) and EqTx-II (10 µg/ml) was about 3.5 and 2 fold lesser than in glioma cells, respectively. U87 cells treated with Bc2 (1 µg/ml) or EqTx-II (1 µg/ml) released 4 times more LDH than untreated cells. PD98059 (a MEK inhibitor), staurosporine (a broad spectrum PKC inhibitor) and KN-62 (a CaMKII inhibitor) decreased the cytotoxic effects of Bc2 and EqTx-II on U87 cells. We decide to test whether cytolysins could be used to increase the efficacy of conventional anti-cancer agents. Non cytotoxic concentrations of Bc2 (0.1 µg/ml) and EqTx-II (0.3 µg/ml) potentiated 10 to 300 fold the cytotoxicity induced by low dose concentrations of all classical chemotherapeutics agents tested: ara-C, doxorubicin and vincristine. **Conclusions**: the sea anemone cytolysins Bc2 and EqTx-II induced cytotoxicity in human malignant glioma cells, possibly by involving calcium, and the activations of MAPK/ERK and PKC pathways. Moreover, non-cytotoxic concentrations of these cytolysins potentiated the cell death induced by chemotherapeutic agents. These results are promising, since lower concentrations of chemotherapeutic drugs could reduce the adverse effects of chemotherapy.

Financial support: CAPES, CNPq and Faperj.

PT.076

ADAM23 Silencing in Breast Cancer is Associated with Capase-1 and DAPK-1 Downregulation and Resistence to Anoikis

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Background: In the absence of extracellular matrix ligands, signals from integrins and other adhesion molecules trigger apoptosis in normal epithelial cells (a process termed "anoikis"), regulating their fate in a ligand dependent manner. The achievement of anchorage independence is a decisive step for apoptosis resistance and for a successfully tumor cell spreading. These abilities confer an effort in the metastatic process. The ADAM23 protein - a member of the ADAM family (A Desintegrin And Metalloprotease) – lacks the common functional metalloprotease domain and has been shown to support integrin–mediated cell adhesion by binding to $\alpha_V\beta_3$-integrin. Clinically, we have recently demonstrated that the ADAM23 gene is frequently silenced in primary breast tumors, being strongly associated with the development of distant metastasis; functionally, we have been shown that ADAM23 knock-down cells display higher levels of active $\alpha_V\beta_3$ integrin and an increase of two fold in FAK phosphorylation levels, correlating with higher migratory and adhesive capacities when compared with ADAM23$^+$ cells. **Objectives:** Previous results motivated us to examine if ADAM23 silencing can render breast cancer cells to an apoptosis-resistant phenotype and to study the signaling pathways related with this resistance. **Results:** Eighty four apoptosis-relevant genes were screened by quantitative PCR gene microarray. The different mRNA expression pattern for two groups of cells - ADAM23 knock-down and ADAM23$^+$ cells (wild-type) - in adherent surfaces, were obtained. After filtering out genes which showed less than 4-fold change in expression level (81/84; 96%) and by eliminating genes that showed pvalue>0,05 (1/3), only 2 differentially expressed genes remained: Caspase-1, with a 18-fold decrease in ADAM23 knock-down cells (p=0,006), and DAPK-1 (Death-Associated-Protein-Kinase-1) with a 9-fold decrease in ADAM23 knock-down cells (p=0,03). Complementary, quantitative PCR showed that overexpression of ADAM23 in wild-type cells did not affect Caspase-1 levels, but induce a 4-fold increase in DAPK-1 levels, suggesting that increasing ADAM23 protein levels could facilitate death-related pathways. After that, cells suspended in medium with/without serum were assayed for anoikis. Both groups, ADAM23 knock-down and ADAM23$^+$ cells, displayed a similar death kinetics when were growing in medium with serum (11-14% apoptotic cells, after 48-96h), but ADAM23 knock-down cells were up to 30% more resistance to anoikis in SFM, after these same period. **Conclusions:** These results indicate that loss of ADAM23 can induces a rearrangement between survive and death signaling pathways, controlling the expression of specific pro-apoptotic genes. They suggest that the ADAM23 silencing during the progression of breast cancer can be associated with death resistance during tumor cells spreading.

Ludwig Institute for Cancer Research - Laboratory of Molecular Biology and Genomics

Support by FAPESP

PT.077

Biphosphinic Palladacycle Complex Induces Early Cell Death in Human Leukemia Cells Trigged by Ros Generation and Lysosomal Membrane Permeabilization

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Previous study reported by our group introduced a new organometallic class of antitumoral drugs called Biphosphinic Palladacycle Complexes (BPC). Here, we show that BPC causes cell death in K562, HL60 and Jurkat leukaemia cells but is ineffective in this respect in normal human lymphocytes. The IC_{50} values obtained for leukaemic elicited lysosome rupture of leukaemic cells in 5 h post exposure and triggers apoptosis cells post 5 h of BPC were 6 μM. BPC compounds show lysosomotropic properties, this drug in both cell lines, inducing chromatin condensation, mitochondrial potential dissipation, apoptotic bodies and DNA fragmentation. Interestingly, the lysosomal cathepsin B inhibitor, CA074, decreased markedly BPC-induced caspase 3 and 6 activation and protected the dissipation of mitochondrial potential induced by BPC also. Lysosomal membrane permeabilization induced by BPC in leukaemia cells correlated with the generation of reactive oxygen specie (ROS). The presence of ROS generation in BPC treated cells was consistent with the results demonstrating presence of membrane lipid peroxidation. Thus, following BPC treatment, lysosomal membrane rupture precedes cell

death and the apoptotic signalling pathway is initiated by release of cathepsin B in cytoplasm of leukaemia cells.

FAPESP, CNPq and FAEP

PT.078

The Antiproliferative Effect of Viscum Album on Melanoma

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Aim: to investigate the anti-cancer effect of Viscum album on murine melanoma cell line (Tm5) *in vitro.* **Materials:** Drugs: Mistletoe preparations VA Qu D1, D2 and D4 will be provided by Weleda (Brasil) and will be administered in the in vitro proliferation assay. **Cell Lines:** The aggressively growing Tm5 murine melanoma cells were was cultured in RPMI (pH 6.9) supplemented with 5% FCS (Sigma, St. Louis, MO). **Cell Proliferation:** For cell proliferation studies, $2.5x10^3$ cells were plated in 96-well plates and harvested at 0 and 24hr after VA treatment; their number was estimated using the MTT assay (Sigma, St. Louis, MO) following standard protocols. **Flow citometric Assay:** Tm5 melanoma cells were seeded in plates at a density of 10^6 /well. After 24 h, the cells were either treated with VA (dilution 1/1;1/10 and 1/100) and PBS (control). After 24h incubation, the adhesive cells and suspended cells were harvested, pooled and pelleted. After washing with PBS, cells were treated with 70% ethanol for 1 hour. Then, after again washing with PBS, cells were incubated with staining buffer containing RNase A and propidium iodide (PI) for 1 hour. Cell cycle analyses were carried out with FACS Calibur (Becton Dickinson, USA). **Statistical Analysis:**The Kruskal-Wallis test was performed using GraphPad Prism version 3.03 for Windows (GraphPad, San Diego, CA). The Dunn's test for multiple comparisons was used when significant differences were observed. $p < 0.05$ was taken as statistically significant. **Preliminars Results:** After 24 hours of VA treatment, melanoma cells were examined with MTT and flow cytometry to check the cell cycle status. The MTT results showed a very significant antiproliferative effect of VA D1 and D2 in 0 and 24h ($p<0,001$) but not D4. Similary, the flow citometric analysis result was found in for dilution treatment once the proportion of cells in sub-G1 stage, representing the apoptotic cells, was significant ($p<0,001$) in the VA D1 (all dilution) and D2 (dilution 1/1, 1/10) treated group than in the control group. Viscum album D4 not show this effect. **Conclusion:** We will demonstrate that VA profoundly inhibits melanoma *in vitro*. VA D1 and D2 suppress melanoma cell proliferation and induce cell to undergo apoptosis, suggesting a effective anti-cancer treatment while VA D4 is not effective for melanoma cancer treatment.

PT.079

Apoptpsis Induced by Manganese in Hematopoietic Cell Lines

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Background: Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells, usually driven by an altered ratio of apoptosis to cell division. Tumor cells may acquire resistance to apoptosis either by the expression of anti-apoptotic proteins or by the down-regulation or mutation of pro-apoptotic mediators. Thus, agents that can induce or potentiate apoptosis triggered by chemotherapic agents are extensively investigated. Many metals are known to be cytotoxic and induce cell death. Manganese is one of these metals, however the underlying mechanisms of manganese-induced apoptosis are not well defined.**Objectives:** Delineate the mechanisms of manganese-induced apoptosis and its ability to potentiate chemotherapic drugs already used in the clinic. **Methods:** Apoptosis was analyzed under different conditions and cell lines by several criteria: cell shrinkage; hypodiployd DNA content and phosphatidylserine exposition. **Results:** Our results show that manganese induces apoptosis in Jurkat human T cell leukemia cell line, SKW 6.4 line of EBV-transformed human B cells and HL-60 human promyelocytic leukemia cells in a time and dose-dependent manner. Furthermore, this ability to induce apoptosis is specific to manganese, since treatment with high concentrations of calcium or magnesium does not affect cell viability or cell cycle. HL-60 cells overexpressing the anti-apoptotic proteins Bcl-x_L or Bcr-Abl are more resistent to manganese treatment when compared to control cells. **Conclusion:** Manganese induces apoptosis in different hematopoietic cell lines and the study of its mechanism may lead to new therapeutic alternatives.

CNPq

PT.080

Study of Pro and Anti-Apoptotic Genes to Understand the Resistance of Apoptosis Mediated by bcr-abl

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Background: The chronic myeloid leukemia (CML) is a three-phase myeloproliferative disorder, dependent on the expression of the oncoprotein Bcr-Abl, which is the product of the reciprocal translocation between chromosomes 9 and 22, resulting in the Philadelphia chromosome (Ph). Bcr-Abl protein is the constitutively activated tyrosine kinase responsible for changes occurred in intracellular biochemical cascades, which culminates into hematopoieticic stem cell malignant transformation. It has been observed that the expression of Bcr-Abl is the initiation event in CML, raising the importance of understanding the survival signals triggered by this oncogene. Different altered signals transducers and transcription factors has been associated with the anti-apoptotic phenotype of CML cell, and some of them lead to the expression and/or activation of apoptosis modulators from Bcl-2 family. The elucidation of the molecules involved in the resistant phenotype of Bcr-Abl expressing cells still remains not completely elucidated and studies to investigate the apoptotic machinery involved in this resistance are necessary. **Objectives:** Based on these data, our aim was to analyze the expression of pro and anti-apoptotic genes in two different cells lines named Jurkat and THP1 cells transfected with Bcr-Abl or empty vector. **Methods:** C-DNA from Jurkat and THP1 cells transfected with Bcr-Abl or empty vector was synthesized. The expression of Bcl-W, Bcl-xl, c-FLIP, cIAP-1, FAIM, Mcl-1, Noxa, Survivin and TRAIL genes were analyzed by Real Time RT-PCR. **Results:** Among the molecules studied, c-FLIP, an anti-apoptotic molecule, was expressed in much higher levels in Bcr-Abl-expressing Jurkat cells than the cells that did not express Bcr-Abl. The same was not observed with THP1 and THP1.Bcr-Abl cells. **Conclusions:** Taken together, c-FLIP can be a good candidate for being the main responsible molecule concerning resistance to apoptosis.

Supported: FAPESP, CNPq

PT.081

Insights Into the Localization and Function of the Predicted Highly Conserved Protein KIAA0090

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Background: In a previous work we observed an increase in the mRNA levels of *KIAA0090,* an evolutionarily conserved gene of unknown function, in metastatic melanoma cell lines in comparison to cell lines from earlier tumor stages. **Objectives**: To gain insight into the localization and function of KIAA0090 protein in tumoral and non-tumoral cells. **Methods**: Liposome-mediated transfection, immunocytochemistry, confocal microscopy; Annexin V/propidium iodide staining; antibody production and affinity purification; cell lysates; SDS-PAGE/ Western blotting. **Results**: The cell death induced by KIAA0090/EGFP fusion proteins was shown not to involve cytochrome c release, but it requires caspase activation, since it was partially inhibited by a caspase-3 inhibitor. At low levels EGFP-KIAA has a diffuse cytoplasmic distribution that progressively changes to an association with mitochondria and culminates in perinuclear coaggregation with these organelles. Colocalization with ubiquitin, vimentin and proteasome staining suggests similarity between this aggregate and so-called aggresomes. KIAA-EGFP exhibits localization restricted to the endoplasmic reticulum (ER) and as its expression levels increase, it induces a pronounced expansion of the ER, associated with an increase in the levels of the ER chaperone calreticulin. The endogenous KIAA0090 appears to be predominantly associated with mitochondria in melanoma cells and in lymphoblastic and promyelocytic leukemia cells, with less pronounced, variable levels of staining throughout the cytoplasm and nucleus. Though, we observed a marked difference in the distribution of endogenous KIAA0090 between two paired melanoma cell lines (VGP vs metastatic). A tissue survey showed staining for KIAA0090 in vascular and visceral smooth muscle, skeletal muscle and lymph nodes, among other tissues, in rats. Immunoblots with anti-KIAA0090 revealed polypeptides ranging from 40 to 130 kDa, compatible with EST evidence for alternative transcripts. **Conclusion**: These results and evidence from high throughput approaches support the view that KIAA0090 may be involved in apoptotic/stress pathways and might have a role in tumor progression.

Supported by: FAPESP, CNPq, CAPES, FAEPA.

PT.082

Allo and OrthoTopic Xenograft Model for Human Glioma

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Astrocytomas are the most common intracranial neoplasms, accounting for about 60% of all primary brain tumors. Despite the advances in molecular biology of gliomas, the prognosis continues to be poor, due to a lack of efficient treatment. Therefore, it is urgent to search for *in vitro* and in *vivo* experimental models to allow testing new therapeutical approaches. **Objective**: 1) to analyze the tumorigenic capacity of U87MG cell line in allo and orthotopic xenograft tumors, 2) to analyze cell growth and proliferation capacity after both types of xenograft models. **Method**: U87MG of human malignant astrocytoma was injected in athymic Rowetts rats in subcutaneous and in brain sites. Primary cultures of tumors of both sites were obtained for i*n vitro* studies. The primary culture from subcutaneous tumor was implanted in brain, and its tumorigenic capacity was compared to the parental cell line. **Results**: all animals of the orthotopic xenograft presented motor deficit after 15 days. The brain macroscopical analysis showed tumor masses of 30mm. Histological preparations showed absence of tumor invasiveness, although necrosis and pseudopallisading cells were detected. The growth analysis in agarose suspension showed plate efficiency of 53%, 4%, 18% after 30 days for the parental cell, primary cultures from subcutaneous and intracerebral tumors, respectively. The proliferation analysis corroborates the growth assay results, where parental cell showed higher proliferative activity in relation to primary cultures. Xenograft tumor of parental cell leads to brain tumor formation in 24 days, whereas primary culture cells from subcutaneous tumor implanted in brain formed tumor in 15 days, suggesting an "activation" of the parental cells with a previous passage in the animal. **Conclusion**: A robust animal model for human glioma was established to evaluate future potential therapeutical targets. However, the lack of tumor invasiveness in the actual experiment demands further studies to achieve an ideal model for human malignant glioma.

FAPESP

PT.083

Basal Activity of ERK on B-1 Cells is Important to Increase the Metastatic Potential of B16F10 Melanoma Cells

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B-1 cells are the prevalent linage of B cells in the peritoneal and pleural cavities of mice. Studies in our group demonstrated that co-cultivation of B-1 cells with B16F10 murine melanoma cells increases the metastatic potential of the later. However, the molecular mechanism of how B1 cells mediate this effect has not yet been addressed. The extra-cellular signal-regulated kinase (ERK) signaling has been implicated in the regulation of tumor cell invasion and metastasis in several cancer types. In addition, it has been demonstrated that enhanced ERK signaling correlates with up-regulation of proteins associated with tumor cell metastasis, such as TIMP-1, MMP-9 and CXCR4. Therefore, the aim of this work was to investigate whether B1 cells increase the metastatic potential of B16F10 melanoma cells by modulating the activation of ERK and gene products that are associated with the activation of this kinase. Our results shows that ERK phosphorylation was increased in B16F10 cells after co-cultivation with B-1 cells and this effect was associated with up-regulation of TIMP-1, MMP-9 and CXCR-4 mRNA expression, correlating with the enhanced metastatic potential of B16F10 cells. Inhibition of ERK phosphorylation on B16F10 cells with PD98059 partially prevents this effect. Interestingly, when basal activity of ERK on B-1 cells itself was inhibited, ERK phosphorylation on B16F10 cells remained basal and there was not increase in the metastatic potential of these cells after B-1 cells contact. These results suggest that basal activity of ERK on B1 cells is important to induce a more metastatic phenotype on B16F10 cells.

FAPESP

PT.084

Metastic Melanoma Positively Influences Pregnancy Outcome in a Mouse Model: Could a Deadly Tumor Support Embryo Life?

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Background: The incidence of melanoma is increasing worldwide. It is one of the leading cancers in pregnancy and the most common malignancy to metastasize to placenta and fetus. **Objectives:** Considering the absence of experimental models of melanoma and pregnancy, we proposed a new experimental murine model to study the effects of melanoma on pregnancy and its metastatic process. **Methods:** The optimal dose, which produces tumor growth and allowes animal survival to the end of pregnancy, was obtained after testing different doses of melanoma cells *in vivo*. Two control groups, non-treated (control, C) and PBS-treated (stress control, S), and three different routes of inoculation, intravenous (IV), intraperitoneal (IP) and subcutaneous (SC), were used. All the fetuses and placentas were examined macroscopically and microscopically. **Results:** The results suggest that melanoma is a risk factor for intrauterine growth restriction but does not affect placental weight. When inoculated by the (SC) route, the tumor grew only in the site of implantation. The (IP) route produced peritoneal tumor growth and also ovarian and uterine metastasis in 60% of the cases. The (IV) route produced pulmonary tumors. No placental or fetal metastasis were obtained, regardless the inoculation route. The injection of melanoma cells by any route did not increase the rate of fetal resorptions. Surprisingly, animals in the (IV) group had no resorptions and a significantly increased number of fetuses. **Conclusions:** This finding may indicate that tumor factors released in the host organism to favor tumor survival may also have a pro-gestational action and consequently improve the reproductive performance of these animals.

CAPES

PT.085

Diverse Acute Inflammatory Stimuli Promote *in Vivo* Growth of Melanoma Cells Through the 5-lipOxygenase Pathway

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Introduction and Objectives: Although it is well established that chronic inflammation contributes to development of neoplasia, the role of acute inflammation on tumor development is less understood. An experimental model developed in our laboratory showed that subcutaneous injection of large numbers of apoptotic cells promote tumor growth of subtumorigenic inocula of viable melanoma cells in syngeneic C57BL/6 mice (*Int J Cancer. 114(3): 356-363, 2005*). The aim of this work was to determine if different kinds of inflammatory stimuli are involved in tumor growth promotion, and to elucidate the underlying mechanisms of the observed positive modulation by acute inflammation on melanoma progression *in vivo*. Inflammatory pathways, immune cells, cytokines and chemokines produced in this tumor growth-supportive microenvironment were investigated. **Results:** Both lipopolysaccharide (LPS) and carragenin, but not thioglycolate and zimosan, were able to promote tumor growth of subtumorigenic inocula of viable melanoma cells. Inhibitors of the 5-lipoxygenase pathway (caffeic acid and MK886), but not cicloxygenase-1 and -2 (indomethacin and meloxican), reduced tumor growth in mice injected with subtumorigenic inocula of viable tumor cells and apoptotic cells or LPS. Also, leukotriene B_4, a product of 5-lipoxygenase pathway and an important leukocyte chemoatractant, promoted growth melanoma cells *in vivo,* but not *in vitro*. To confirm LTB_4 role, treatment with LTB_4 etanolamine, a LTB_4 antagonist, was able to reduced tumor growth induced by acute inflammation. In addition, the treatment with eicosapentanenoic acid, an arachidonic acid antagonist and lipid mediators precursor that has a pivotal in promoting inflammation resolution, was also able to inhibit tumor growth induced by apoptotic and viable tumor cells coinjection. **Conclusion:** Arachidonic acid metabolites produced by 5-lipoxygenase pathway activation may induce melanoma progression associated to acute inflammation.

CNPq e Fapesp

PT.086

Effects of Plant Proteinase Inhibitors on Melanoma Models *in Vivo* and *in Vitro*

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Background: *Bauhinia* and *Enterolobium* are *Leguminosae* widely spread in Brazil. *B. bauhinioides* Kallikrein (**BbKI**), Cruzipain (**BbCI**) Inhibitors and *E. contortisiliquum* Trypsin (**EcTI**) Inhibitor isolated from their seeds, are Kunitz peptidase inhibitors. **BbCI** inhibits neutrophil elastase (K_{iapp}5.3 nM), cathepsin G (K_{iapp}160 nM), cathepsin L (K_{iapp}22 nM), cruzain (K_{iapp}0.3 nM) and cruzipain (K_{iapp}1.3 nM), but not clotting enzymes, trypsin

and chymotrypsin. **BbKI** inhibits trypsin (K_{iapp}20 nM), chymotrypsin (K_{iapp}26 nM), plasmin (K_{iapp}330 nM), plasma kallikrein (K_{iapp}2.4 nM) and tissue kallikrein (K_{iapp}200 nM). **EcTI** inhibits trypsin (K_{iapp}0.88 nM), plasmin (K_{iapp}9.36 nM), chymotrypsin (K_{iapp}1.11 nM), plasma kallikrein (K_{iapp}6.15 nM), neutrophil elastase (K_{iapp}55 nM) and also inhibits the activation of MMP-2 and MMP-9. **Objectives:** Investigation of the action of these inhibitors on tumor models dissemination *in vitro*, and also the tumor development *in vivo* using nontumorigenic lineage of pigmented murine melanocytes (**melan-a**) and murine melanoma (**Tm5**). **Results: BbCI** showed more effective inhibitory action on **Tm5** cell proliferation and migration comparing to the effect on **melan-a** despite of its interference on G1 phase **melan-a**. **BbKI** stimulated the G1 phase of both and the inhibition of cell migration was achieved only in concentration higher than that of 6.25 mM. Neither **BbCI** nor **BbKI** affected **melan-a** and **Tm5** cell adhesion on fibronectin, collagen IV and vitronectin. On the other hand, **EcTI** (6.25-12.50 mM) strongly inhibits (80-100%) proliferation and cell cycle of two cell lines. Analyses of tumor growth *in vivo* in C57BL6 mice and daily treatment with inhibitors (2mg/100µl/kg/mice) showed that **BbCI** and **EcTI** decreased the tumor volume (80-90%) while **BbKI** increased it. **Conclusion: EcTI** and **BbCI** inhibited melanoma growth *in vitro* and *in vivo* and their interference on G1 cell cycle phase may be important for the effect demonstrated. **BbKI** does not affect melanoma. The results show that the inhibitory properties of these inhibitors may be useful tools on studies of inflammatory processes and for tumor development investigation.

FAPESP, CNPq and SPDM/FADA, Probal (CAPES/DAAD) MCT/CNPq, DECIT/MS, CT-BIOTECNOLOGY

PT.087

The Disintegrin Domain of Human ADAM9 Ssupports Tumor Cell Adhesion by αvβ5 and α2β1 Integrin Binding

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Background: Members of the ADAM (*A D*isintegrin *A*nd *M*etallopeptidase) protein family are composed by a series of conserved protein domains including a disintegrin domain, which interacts with cell surface integrins, triggering intracellular signaling pathways. Malfunction of this system has been implicated in some diseases such as cancer and metastasis. **Objectives:** To verify the effects of the recombinant disintegrin domain of ADAM9 (ADAM9D) on human fibroblasts (HF) and prostate tumor (DU-145) cell adhesion. **Methods:** The ADAM9D cDNA was isolated by RT-PCR using total RNA from VMM12 human melanoma cells and specific primers based on the human ADAM9 DNA sequence (NM003816). The PCR product was cloned into the pGEX-4T-1 vector which was used to transform *E. coli* AD494(DE3) cells. The synthesis of GST/ADAM9D was induced by IPTG (0.1mM, 4 hours) as confirmed by SDS-PAGE. After purification on a Glutathione-Sepharose 4B resin, the ADAM9D was released from GST by cleavage with thrombin and further purified in a Benzamidine-Sepharose 4B column. For adhesion studies, cells were labeled with 12.5µM CMFDA and incubated ($1x10^5$ cells/well) on ADAM9D-coated wells at 37°C for 30min. After removing unbound cells, the remaining cells were lysed and the fluorescence was read in a fluorimeter with 485-nm excitation and 530-nm emission filters. Inhibition assays were done as described but in the presence of anti-integrin antibodies. HF and DU-145 cells were treated with ADAM9D for 24h (200mM, 37°C, 5%CO_2), incubated with different anti-integrin antibodies and then analyzed by flow cytometry. **Results:** Recombinant ADAM9D was able to induce normal and cancer cell adhesion through binding on αvβ5 and α2β1 integrins on DU-145 and HF cells respectively, as demonstrated by cell adhesion competition assays and by flow cytometry analysis. The ADAM9D significantly decreased the levels of αvβ5 and α2β1 integrins after 24h incubation. The signaling mechanisms triggered after integrin binding are currently being investigated. **Conclusion:** ADAM9D can be used as a tool for investigating the role of ADAMs in carcinogenesis and cancer progression and for the design of selective inhibitors against the growth and survival of cancer cells.

FAPESP, CNPq

PT.088

Tumoral Metastatic Specialization: a Progression to a Pre-Malignant Phenotype Associated with Loss of Adam 23 Expression

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Background: The ADAM family - A Desintegrin And Metalloprotease – comprises 30 known members of cell surface type I transmembrane glycoproteins that can perform two different functions related to

tumorigenesis and metastatic dissemination: cell adhesion and proteolysis. ADAM23 protein lacks a functional metalloprotease domain and has been shown to support integrin–mediated cell adhesion by binding to $\alpha_V\beta_3$-integrin and other cell-surface molecules. We have recently demonstrated that the ADAM23 gene is frequently silenced by promoter hypermethylation in primary breast tumors, being strongly associated with the development of distant metastasis and predicting a worse disease outcome. **Objectives:** These clinical observations prompted us to characterize the biological role of ADAM23 during malignancy progression using different cell lines with a reduced or overexpressed ADAM23 expression levels. **Results:** As a result, functional assays showed that ADAM23 knock-down cells display higher levels of active $\alpha_V\beta_3$ integrin and an increase of two fold in FAK phosphorylation levels, correlating with up to 40% higher haptotactic migratory and adhesive capacities in both vitronectin- and collagen-coated plates when compared with ADAM23$^+$ cells. These effects reflected *in vivo* increasing the metastatic potential of ADAM23 knock-down cells, as analyzed by pulmonary cell arrest. In summary, ADAM23 knock-down maximizes tumor cell motility and enhances avidity for ECM ligants being related to an increase in metastatic ability. Despite these promigratory alterations, ADAM23 knock-down cells displayed a 10-20 fold reduction in proliferation rate *in vitro* and 85% lower tumorigenic competence in immunodeficient mice, when compared to ADAM23$^+$ cells. **Conclusions:** Although these results could be apparently counterintuitive we propose that they strongly suggest that loss of ADAM23 induces a specialized phenotype in tumor cells: cells with reduced levels of ADAM23 become more adjusted to successfully progress in the early steps of metastasis.
Ludwig Institute for Cancer Research and Fapesp

PT.089

Fragmented Laminin-5 Gamma 2 Chain in Canine Mammary Cancer

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Background: Laminin-5, a heterotrimeric glycoprotein consisting of alpha 3, beta 3 and gamma 2 chains, is a component of epithelial basement membranes and functions as a ligand of the alpha 3 beta 1 and alpha 6 beta 4 integrins regulating cell adhesion, migration, and morphogenesis. Laminins also regulate cell motility, growth and differentiation, induce inflammatory response. While other carcinomas exhibit an increased laminin-5 deposition, which has been suggested as an invasion promoting factor, the loss of laminin-5 in canine mammary cancer supports the view that mammary carcinomas do not utilize laminin-5 for invasion. **Objective and Method:** In the present study, we analyzed the expression of laminin-5 gamma 2 subunit (laminin γ-2) in canine mammary neoplasia samples by western blot. **Results:** The adenoma, a benign mammary neoplasm, showed a intact laminin γ-2 (140 kDa), but the malignant samples exhibited a fragmented laminin γ-2, between 36 and 50 kDa. **Conclusion:** The degradation of laminin γ-2 may be linked to loss of laminin-5 in canine mammary cancer and supports the view that these mammary carcinomas do not utilize intact laminin-5 for invasion.
FAPESP, CNPq

PT.090

Investigation of Annexin A1 and Stroma-Tumor Interaction in Head and Neck Cancer

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Background: The importance of stromal cells and the factors that they express during cancer initiation and progression has been highlighted by recent literature. The cellular components of the stroma of epithelial tissues are well-recognized as having a supportive role of carcinogenesis, where the initiating mutations of a tumor originate in the epithelial cells. And the annexin A1 protein has also been strongly implicated in the regulation of the cell cycle. The mechanism of ANXA1 action is poorly understood and its potential to influence gene expression has rarely been addressed. **Objectives:** To investigate possible mechanism by which the annexin A1 and the tumor microenvironment might contribute to genetic instability, we asked whether the annexin A1, the soluble factors found in head and neck carcinoma and cell lines conditioned medium could influence the growth cell and the gene expression. **Methods:** We measured the growth of the Hep-2 cell line (larynx carcinoma) co-cultured in direct contact (with conditioned medium) with oral carcinoma-associated fibroblast and vice versa. Besides, we measured the growth of the Hep-2 cell line co-cultured with annexin A1 and in direct contact with oral carcinoma-associated fibroblast plus annexin A1 and vice versa. Using subtractive hybridization (RaSH), we also characterized the gene expression in

all of these treatments. **Results:** We found that annexin A1 and fibroblast conditioned medium decreased cell line proliferation. Moreover annexin A1 and cell line conditioned medium decreased fibroblasts proliferation yet. By RaSH assay, ten genes are expressed in Hep-2 control and two genes are expressed in Hep-2 co-cultivated with conditioned medium fibroblasts. These genes are involved in inflammatory process, apoptosis, cell proliferation and differentiation. **Conclusion:** The results suggest that the annexin A1 and the stroma-tumor microenvironment might be one of the factor contributing to proliferation in head and neck cancer.
FAPESP, CNPq, CAPES

PT.091

Effects of Proteinase Inhibitor on Fibrosarcoma Proliferation and Adhesion

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Background: Fibrosarcoma is a malignant tumor derived from fibrous connective tissue and characterized by immature proliferating fibroblasts or undifferentiated anaplastic spindle cells. Studies report the relationship between peptidase activity and various biological processes, including cancer progression. In this work we used proteinase inhibitors to investigate metabolism in a tumorigenic cell line. The recombinant protein *B. bauhinioides* Cruzipain Inhibitor (rBbCI), *B. bauhinioides* Kallikrein Inhibitor (rBbKI) and *Enterolobium contortisiliquum* Trypsin Inhibitor (EcTI) isolated from *E. contortisiliquum* seeds, were shown to play a role on enzyme activity. Thus rBbCI inhibits human neutrophil elastase (K_i 1.7 nM), porcine pancreatic elastase (K_i 47 nM), cathepsin L (K_i 9.0 nM) and cruzipain (K_i 0.3 nM). On its turn, rBbKI inhibits trypsin (K_i 28 nM) and plasma kallikrein (K_i 2.0 nM), whereas EcTI inhibit trypsin ($K_{i(app)}$ 0.88 nM), plasmin (K_i 9.36 nM), chymotrypsin (K_i 1.11 nM), plasma kallikrein (K_i 6.15 nM), and human neutrophil elastase (K_i 55.00 nM). Moreover, EcTI inhibits the activation of both MMP-2 and MMP-9. **Objective:** This aim of the present study was to investigate the effects of rBbCI, rBbKI and EcTI on fibrosarcoma cell line L929 adhesion on fibronectin and on cell proliferation after 24 hours, 48 hours and 72 hours of incubation at 37ºC. **Results:** rBbKI inhibited 40% L929 cell proliferation at 12.5 mM and no significant effect was observed with rBbCI and EcTI. The confocal microscopy analyzes indicated that those inhibitors bind to the cell surface, being the effect on cell adhesion mediated by fibronectin markedly affected. While rBbKI strongly inhibited cell adhesion (50% and 70%, respectively, at 6.25 mM and 12.5 mM), rBbCI did not interfere and EcTI increased 40% cell adhesion. **Conclusion:** The kallikrein inhibitor rBbKI displayed anti-proliferative and anti cell-adhesive properties on the fibrosarcoma model.These results indicate that differences of these peptide inhibitors may be useful to investigate the processes of tumor stabilization and progression.
Supported by FAPESP, CNPq and SPDM/FADA, Probal (CAPES/DAAD) MCT/CNPq, DECIT/MS and CT-BIOTECHNOLOGY

PT.092

High Levels of Recombinant Murine Endostatin Secreted by Viable Ovary Chinese Hamster Cells (CHO) Implanted *in vivo* in Immunoisolation Theracyte Device

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Background: Endostatin is a specific endogenous inhibitor of endothelial cell proliferation and tumor growth. Most antiangiogenic therapy studies are based on systemic delivery of the active compound. The Theracyte system is a membrane encapsulation system developed for implantation of cell lines genetically engineered for therapeutic protein delivery *in vivo*. The system prevents host cell entry into the device, allowing the passage of nutrients for the encapsulated cells, keeping them viable for the delivery of the therapeutic protein to the systemic circulation. The ability of encapsulated endostatin-expressing cells to deliver high levels of endostatin to the circulation system of mice was evaluated. . **Objective:**The following work aimed to study *in vivo* the ability of the Theracyte devices containing recombinant CHO cells to secrete high levels of endostatin and to maintain high and constant systemic levels of this antiangiogenic protein **Methods:** Immunoisolation devices were implanted at dorsal region on SCID male mice containing 10^6 CHO cells expressing high levels of endostatin. For another group, the devices were implanted in the mice and the cells were transplanted after wound healing (15 days) in the devices. Histological analysis of the devices were performed. The levels of recombinant endostatin from the serum of the animals were estimated by Immunoenzymatic Assay. **Results:** We detected growing levels of up to 3,7 µg of endostatin/mL of serum from the animals throughout the six weeks duration of the study. In another assay higher and increasing levels

of endostatin were obtained when the devices where implanted concomitantly with the cells. In contrast, animals implanted with the same amount of "free" cells showed higher serum levels (up to 6,7 µg/mL), but all the animals dyed before one month of treatment. Viable cells were detected inside device with two months of the study in histological analysis. **Conclusion:** This study demonstrated that the immunoisolated recombinant cells produce continuously high levels (up to 2,1 µg) of the antiangiogenic protein endostatin in animal serum for 11 weeks, possibiliting their use for treatment of tumoral growth.
Supported by FAPESP and CNPq.

PT.093

Endostatin-Bax: a Novel Fusion Protein with Enhanced Antiangiogenic Activity

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Endostatin, a 20kDa C-terminal fragment of collagen XVIII, is a potent anti-angiogenic protein and inhibitor of tumor growth. The mechanism of antiangiogenesis induced by endostatin is related to the inhibition of cell proliferation and induction of apoptosis of the endothelial cells. In the present study we report the higher activity of a fusion protein composed of two functional domains: endostatin, that presents affinity for activated endothelial cells and a second domain composed by Bax, a short peptide, corresponding to the minimal sequence required to promote apoptosis. This protein should present higher degree of antiangiogênic activity after internalization than the isolated endostatin.**Objective:** The following work aimed to study the antiangiogenic activity of the fusion protein, endostatin-Bax, after internalization by endothelial cells. **Methods:** The fusion protein was obtained using site-specific mutagenesis and expressed in *E. coli* BL21(DE3) as insoluble cytoplasmic inclusion bodies. The refolding was performed by High Hydrostatic Pressure (200MPa). The expressed protein was purified through heparin affinity chromatography. The biological activity of the protein was measured through the inhibition of the proliferation on Human Umbilical Vein Endothelial Cells (HUVEC), and the degree of apoptosis was analyzed on the endothelial cells (C-PAE) by flow citometry. **Results:** The expression of the fusion protein was confirmed by SDS-PAGE and Western blot. The cell proliferation assay demonstrated that the fusion protein, endostatin-bax, present a higher inhibitory effect on HUVEC cells (45%) when compared to the native protein (15%). C-PAE cells which were treated with the fusion protein exhibited a higher degree of apoptosis if compared to the native endostatin (25% and 14,5%, respectively), measured by flow citometry. **Conclusions:** Our results indicate that compared with endostatin alone, the fusion protein, endostatin–Bax, shows enhanced antiangiogenic activity on endothelial cells. This mutant protein could be more efficient in the treatment of mice tumors than the isolated endostatin.
Supported by FAPESP and CNPq

PT.094

Histopathological Characterization of a Syngeneic Orthotopic Murine Bladder Cancer Model

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Objectives: We developed and characterized by histopathology and immunohistochemistry a syngeneic murine bladder tumor model derived from the MB49 tumor cell line. **Methods:** Bladder tumor implantation was achieved by intravesical instillation of 5×10^5 MB49 tumor cells in C57/BL6 mice. A chemical cauterization of the bladder was performed in order to promote intravesical tumor implantation. The bladder wall lesion was accomplished by transurethral instillation of $AgNO_3$. After 15 days, the animals were sacrificed, examined macroscopically for intravesical tumor and bladder weight. Histology and immunohistochemistry were performed using citokeratin 7 (CK7), carcinoembrionic antigen (Dako-CEA), p53 and c-erbB2 oncoprotein (Her2/neu). **Results:** 29 out of 30 animals (96.7%) developed intravesical tumors in a 15-day period. Macroscopically the mean bladder weight was 0,196g (0,069-0,538g), 10 to 15 times the normal bladder weight. The immunohistochemical analysis showed significant membrane expression of CEA and CK7: a similar finding for the human urothelial cancer. We also characterized absence of expression of p53 and anti-Her2/neu in the murine model. **Conclusions:** High tumor take rates were achieved by using a chemical cauterization of the bladder. Although electric cauterization is widely described in the literature for syngeneic orthotopic animal models, the technique described in this study represents an alternative for intravesical bladder tumor implantation. Moreover,

the histopathology and immunohistochemical analysis of the murine bladder tumor model derived from the MB49 cell line showed resemblance to the human infiltrating urothelial carcinoma, allowing clinical inference from experimental immunotherapy testing.
FAPESP

PT.095

Effects of Rapamycin in Gene Expression Profile on ErbB2-Positive/ErbB2-Negative Breast Tissues in Organ Culture

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Background: c-erbB-2/HER-2/neu (ErbB2), a member of epidermal growth factor receptor (ERGF) family, has been found to be amplified or overexpressed in 30% of human breast cancers and correlates with tumor progression and poor prognosis. One of the major pathways triggered by ErbB2 is the PI3K/ AKT kinase pathway of which the mammalian target of rapamycin (mTOR) is a down stream effector. Rapamycin (the prototype of mTOR inhibitors) targets mTOR inhibiting signals for cell cycle progression and growth. **Objective:** The purpose of this study was to analysis rapamycin effects on gene profile in organ cell cultures from ErbB2 positive (ErbB+) or negative (ErbB-) ductal invasive breast cancer. **Methods:** Ten carcinoma samples classified in ErbB+ and ErbB- were selected according to ErbB2 determination. Tissue slices were obtained by the Krumdieck tissue slicer and maintained during 24 hours submerged in supplemented growth medium in the presence or absence of 20 nM Rapamycin. Total RNA was extracted, amplified and subjected to microarray gene expression profiling using the Codelink Human Whole Genome (GE). After normalization, the differentially expressed genes were selected and gene sets ontologically related were identified using the Onto-Tools (http://vortex.cs.wayne.edu/projects.htm) data base. In addition, rapamycin effects on transcriptome and proliferation of a normal luminal breast cell line transfected with ErbB2-cDNA (HB4aC5.2a) were determined. **Results:** In C5.2a several genes of the mTOR pathway were altered with concomitant decrease of cell proliferation. Analysis of genes differentially expressed in ErbB2+, ErbB2- and C5.2a after short exposure to rapamycin treatment indicated that the most perturbed biological processes, were 'cell proliferation', 'metabolism', 'transcription', 'transport', 'phosphorylation', 'proteolysis' and 'signal transduction'. However the number of overlapping genes was small among the three conditions. **Conclusion:** These findings suggest that organ culture can be used to analyze changes in transcriptome but rapamycin-regulated genes may vary even if the cells expressed similar levels of ErbB2.
FAPESP

PT.096

Dual Role of Galectin-3 in Murine Melanoma Growth

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Background: tumors have been described as microenvironments composed not only by malignant cells, but also by endothelial cells, fibroblasts and leukocytes, which can promote tumor growth and angiogenesis. Galectin-3, a b-galactoside binding protein, is expressed by monocytes/ macrophages and others leukocytes. In fact, several lines of evidence suggest that galectin-3 act as a master regulator of the inflammatory response. **Objectives:** based on the fact that the inflammatory infiltrate can promote tumor progression, the proposal of this study was to evaluate if galectin-3, either from tumor or stromal cells, could modulate melanoma growth. **Methods:** Tm1 murine melanoma cell line, which expresses no endogenous galectin-3, was transfected with the galectin-3 gene. Both clones (Tm1 gal-3+ve and gal-3-ve cells) were injected subcutaneously in female C57BL/6 wild-type (WT) and galectin-3 knock-out (KO) mice to tumor engraftment and growth analysis, besides immunohistochemistry and gene expression assays. **Results:** regardless the galectin-3 expression level in the melanoma cell, tumors from galectin-3 KO mice were smaller than those from WT animals, suggesting that galectin-3 expressed by stromal cells promotes tumor growth. Interestingly, both vascular area and the number of functional vessels in animals injected with Tm1 gal-3+ve cells were smaller in WT as well as in KO mice compared to the same animals injected with Tm1 gal-3-ve cells. Gene expression analysis showed that VEGF (vascular endothelial

growth factor) mRNA levels were smaller in WT animals injected with Tm1 gal-3+vê cells compared to those injected with Tm1 gal-3-ve cells, indicating that galectin-3 expressed by tumor cells can act as an anti-angiogenic molecule. According to this, we also observed an increase in Delta-4 (Notch ligand) mRNA levels within tumor microenvironment in KO animals injected with Tm1 gal-3-ve cells. **Conclusion:** the present study suggests that galectin-3 can act either as a pro or anti-tumoral molecule, depending on which type of cell (tumoral or stromal) this lectin is expressed within the tumor microenvironment.
Supported by: FAPESP, CNPq.

PT.097

NFAT-Mediated Regulation of Sarcoma 180 Growth does not Depend on Classical Angiogenesis

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Background: Tumor growth depends not only on tumor cell proliferation but also on an intricated crosstalk between the neoplastic cells and a variety of host cells which form the tumor microenvironment. Molecular signaling pathways involved in tumor growth and composition of tumor microenvironmental cells are still poorly understood. **Objective**: To study the role of calcineurin-NFAT pathways in the regulation of tumor growth. **Methods**: Sarcoma 180 (S180) was transplanted subcutaneously in wild type (wt, B6xSv129) and NFAT deficient (NFATKO) mice. Tumor growth was assessed by daily measurement. Necrotic area, and CD31, CD34, galectin-3 tissue expression were determined by histopathology (HE) and immunohistochemistry, respectively. **Results:** S180 transplanted NFAT deficient mice presented increased mortality when compared to wt ($p<0.05$). On the 4th week post-transplantation, tumor size varied from 9.25±5.67mm^3 versus 15.22 ± 1.04 mm^3 in wt and NFATKO, respectively. On day 11-14, large necrotic areas were observed in both wt and NFATKO (5.32 ± 13.30 mm^3 vs 13,87 ± 25,48mm^3) though necrotic masses were more frequently observed in NFAT deficient mice when compared to their counterparts ($p=0.02$). Mix population of cells including lymphomononuclear cells , neutrophils and non functional capillaries were found within tumors in both groups analyzed. Intriguingly, S180 tumor growth was not followed by classical angiogenesis. Sparse CD31 and CD34 positive cells were present in both groups, but failed to form functional capillary structures. Galectin-3 positive cells, which include predominantly a subset of infiltrating macrophages, were more numerous in tumors transplanted into wt than NFATKO mice ($p<0.01$). **Conclusion:** Our findings suggest that tumor growth and infiltration of tumors by some inflammatory cells which express galectin-3, such as macrophages, might be regulated by NFAT signaling pathway.
CNPq and FAPESP

PT.098

Production of HPV 16 L1 VLP in Human Cell Culture for Basic Studies in Anogenital Cancer

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Background: Viruses' infections play a crucial role in a number of cancers and at least 15 distinct Human Papillomavirus (HPV) types are described to be involved in genital, mouth, throat, and skin cancers. More than 50% of cervical cancers worldwide are attributed to HPV 16. L1 is the HPV major capsid protein with around 55 kDa and it can self-assembly into virus-like particles (VLPs), structurally similar to native HPV virions. VLPs are non-infective particles applied as prophylactic vaccines, but it is possible to insert the double-stranded viral genomic DNA containing about 8,000 base pairs, converting them in infective HPVs. **Objectives:** We are producing virus-like particles of human papillomavirus type 16 L1 major capsid proteins for investigating the mechanisms by which HPV infection cause cancer. **Methods:** Cell cultures of 293T human embryonic kidney cell line were transfected with the DNA constructs encoding for humanized L1 (L1h) protein of HPV 16 sub cloned into the mammalian expression vector pUF3L1h, using GeneJuice® Transfection Reagent (Merck). Primary antibodies against L1, Camvir-1 (BD) and Anti-L1 HPV 16 (Biodesign), and secondary antibody anti-mouse IgG_{2a} conjugated with FITC (BD) were applied in immunofluorescence assays. Western blotting method was used to control protein expression. **Results:** Recombinant L1 was expressed in 293T cells transfected with vector pUF3L1h, in a high efficiency. At least 85% of cells expressed the intracellular L1 protein and

VLPs, as detected with the non-conformational and conformational L1-specific antibodies. **Conclusion:** We are establishing a pattern for an efficient system of recombinant L1 protein expression in mammalian cell cultures. The production of HPV 16 L1 VLPs by transfected 293T cells opens the possibility for new basic studies concerning to HPV-cell interactions and carcinogenesis mechanisms.
Supported by : DKFZ-ATV, Ludwig Institute, CAPES and Butantan Foundation.

PT.099

Lympho-Stimulatory Effect of Monocytes Transfected with Tumor RNA from Breast Tumor

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Background: New methods for the evaluation of specific immunity induction by cancer immunotherapy could be of great benefit, leading to the improvement of strategies like the dendritic cell-based vaccines, which show promising but not totally satisfactory results yet. With this aim, we are studying the ability of monocytes transfected with tumor RNA to mimick tumor cells and, therefore, be recognized *in vitro* by autologous lymphocytes. **Methods:** To establish the most effective conditions, monocytes of healthy donors were transfected passively with tumor RNA in different experimental settings (RNA dose and transfection time). After transfection, monocytes were irradiated (1250 cGy), used as stimulators of autologous lymphocytes (CFSE labeled) for 7 days and cytokine release was measured (IL-4, IL-10 and IFN-□). **Results:** Flow cytometry showed that RNA-transfected monocytes have an increased lympho-stimulatory activity. Lower RNA quantities (0,1 and 0,5 □ g) did not induce proliferation, while higher RNA amounts (1 and 10 μg) caused monocytes to activate higher lymphocyte proliferation. The best time of transfection was 48 hours, instead of 12 or 24 hours. The incubation of monocytes with RNA did not have effects upon the functional phenotype of these cells. The only cytokine released in the lympho-stimulation assay was IFN-□ . **Conclusion:** These data indicate that transfection with tumor RNA may become an effective tool for the evaluation of specific cellular immunity induced in patients after cancer vaccination.
Fapesp

PT.100

Microsatellites in the MHC Region: an Additional Tool for the Selection of Compatible Donor-Recipient Pairs for Transplants of Patients with Bone Marrow Cancer

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Background: Hematopoietic stem cell (HSC) transplantation is an established therapy of hematological malignancies, such as leukemias. *HLA* matching for related or unrelated donors and recipients is considered to be the principal factor influencing the success of HSC transplantation. Due to the difficulty of typing accurately some genes of the HLA region, such as *HLA-B* and *HLA-DRB1*, it is important to find new markers which could bring more information about *HLA* haplotypes. In this scenario, microsatellites in the MHC region are very good candidates due to their high diversity and previous reported linkage disequilibrium (LD) with HLA genes. **Objectives:** Analyze 5 microsatellites in the MHC region (*DS2874, DS273, STR-MICA*, *D6S2810* and *D6S2792*) to evaluate their linkage disequilibrium with *HLA* genes. **Methods:** We selected 200 possible bone marrow donors and the microsatellite typing was made through PCRs, followed by electrophoresis in MegaBACE1000 and sizing with the software Fragment Profiler 1.2 (GE Healthcare). Allele and haplotype frequencies were estimated by maximum likelihood using an expectation-maximization algorithm. **Results:** A high LD was found between alelles of the microsatellites and *HLA* genes. For this analysis we reported only very high values of D' (at least 0.7), including frequent alleles and $P<10^{-2}$. The strongest LD (D'=1.0) was found between *D6S2810*334-HLA-B14, STR-MICA*A5.1-HLA-B08* and *D6S273*151-HLA-B39*. Other important associations were found between *D6S2874*193-HLA-DRB1*1302* (D'=0.87), *D6S2874*193-HLA-DQB1*0201* (D'=0.78), *D6S273*147-HLA-DRB1*0411* (D'=0.9), *D6S2810*328-HLA-B15* (D'=0.8), *D6S2810*338-HLA-B07* (D'=0.8), *D6S2810*358-HLA-B44* (D'=0.92), *STR-MICA*A6-B51* (D'=0,88) and *D6S2792*101-HLAB08* (D'=0.93). **Conclusion:** Microsatellites in the MHC region are strongly associated with *HLA* genes and thus represent a valuable source of markers in the selection of compatible donor-recipient pairs for bone marrow transplantation.
Financial support: CAPES- FUNPAR-UFPR

PT.101

Conditioned Medium From CD83+ Tumor Cells Impairs T Cell Proliferation Induced by Dendritic Cells in Mixed Lymphocyte Reactions – Possible Participation of Soluble CD83 Secreted by Tumor Cells

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Introduction and Objectives: CD83, first described as a molecule present on membrane of human activated dendritic cells (DCs), later has been shown to be also expressed on surface of B and T cells, macrophages, neutrophils and surprisingly on Hodgkin's tumor cells. More recently, we also reported membrane CD83 expression on lung cancer cells and several human tumor cell lines. If, by one hand, block of expression of membrane CD83 on surface of DCs impairs their capacity of activating T cells, on the other hand, addition of recombinant soluble CD83 to co-cultures of T cells and alogeneic DCs, decreased the in vitro proliferation of T cells induced by DCs. Since we have previously shown CD83 expression on tumor cells, we asked if that molecule might be secreted and play an immunosuppressive role, effecting proliferation of T cells induced by DCs, which was the aim of this work. **Material and Methods:** Monocyte-derived DCs were irradiated (25 Gy) and used as stimulators for allogeneic T lymphocytes (previously labeled with CFSE 5mM/mL per $1x10^7$ cells) at a 1:20 ratio. Cells were cultured for 5 days in R-10 plus 10, 20 or 30% of conditioned medium from tumor cells P9 or SK-BR3 previously adsorbed with anti-CD83 or IgG, after which they were stained with antibodies to CD4 and CD8 and analyzed by flow cytometry. **Results:** We observed that there was a dose-dependent inhibitory effect in proliferation when tumor-conditioned medium was added into co-cultures, and that this inhibitory effect was decreased by the treatment of the tumor-conditioned medium with anti-CD83. **Conclusions:** Our results provide evidence that CD83 might be secreted by tumor cells or shed from their membrane to the microenvironment and play an immunosuppressive role, impairing dendritic cells-induced proliferation of T cells.
FAPESP

PT.102

Cytokine Production and Frequency of Dendritic Cells and Macrophages in Human Lung Cancer

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Introduction and Objectives: Monocytes recruited into inflamed tissues may differentiate into DCs and macrophages, a phenomenon that is affected by the tissue microenvironment. Cytokines such as IL-6, IL-10 and M-CSF favor monocytes differentiation toward macrophages and prevent the generation of DCs. On the other hand, GM-CSF, IL-4 and TNF-a act synergistically to generate mature DCs from monocytes. Since DCs play a crucial role in the induction of an effective immune response, a local deviation toward macrophage differentiation could be an efficient mechanism of tumor evasion from immune system. Thus, the aim of this study was to analyze the frequency of DCs' and macrophages' marker-positive cells in the tumor micro-environment of patients with primary lung neoplasia and the production of cytokines by these cells. **Methods and Results**: Patients were analyzed as to the frequency of HLA-DR, CCR7, CD11c, CD14, CD40, CD80, CD83, CD86 and CD123 positive cells in the primary tumor and in non-affected lung tissues by flow cytometry. The production of the cytokines IL-4, IL-6, IL-10, GM-CSF, M-CSF e TNF-a was analyzed by ELISA in the supernatant of lung tissue digests after 48 hours cultures. A lower frequency of phenotypicaly mature DCs (CD14-CD80+CD86+) was found in tumor affected lung. For the other parameters analyzed, no consistently significant differences were observed between the tumor affected and non-affected lung, though in individual patients clear differences were present. **Conclusions:** Our data suggest that the DCs, present within the tumor may be poor Ag-presenting cells due the lack of co-stimulatory molecules and cytokines important for inducing an immune response against the tumor.
FAPESP

PT.103

Immune Response Induced by Recombinant Bacillus Calmette-Guerin Expressing the Antigen S1PT in Bladder Cancer

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Purpose: BCG is regarded as the most successful immunotherapy for superficial bladder cancer to date. However, regularly observed side effects at certain degree of nonresponders remain a major obstacle. Since successful treatment of superficial bladder cancer with BCG requires proper induction of Th1 immunity, we have developed a rBCG-S1PT strain that induced stronger cellular imune response than BCG. This preclinical study was designed to test the potencial of rBCG-S1PT to serve as immunotherapeutic agent for intravesical bladder cancer therapy. **Materials and Methods**: The bladder cancer cell line, MB49, was used in C57/BL6 mice. A chemical cauterization of the bladder was performed in order to promote intravesical tumor implantation. Mice were treated by intravesical instillation with BCG, rBCG-S1PT or PBS twice a week for 4 weeks. After 35 days the bladders were removed and weighted. Th1 (IL-2, IL-12, INOS, INF-γ, TNF-β), and Th2 (IL-5, IL-6, IL-10, TGF-β) cytokine responses in individual mouse bladder were measured by real time quantitative PCR. The spleen proliferation assay was analyzed after PDS protein stimulatory effect. **Results**: In so far BCG as rBCG-S1PT immune therapy showed bladder weight reduction and increase surviving time compared to control group. There were increase of TNF-β, INOS and IL-2 cytokines in BCG treat group, and TNF-β, IFN-γ, IL-12 and IL-10 in rBCG-S1PT group. The spleen proliferation was more evident in BCG group than rBCG-S1PT. **Conclusions**: BCG and rBCG-S1PT therapy both improve outcomes at reducing bladder tumor weight and lengthen surviving course. But the increase of IL-10 cytokine in rBCG-S1PT group could suggest bad prognostic. These results indicate that rBCG could serve as a useful substitute for wild-type BCG in future studies.

FAPESP

PT.105

Allogeneic T Cell Stimulated by Dendritic Cell Pulsed with CD40L+ Leukemia: a Model for Preferential Transduction of Alloreactive Cells with CD20 Transgene

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Background: The use of components of the immune system for the treatment of leukemia represents a promising alternative. The donor lymphocyte infusion is one of such strategies, which have been used with success in the treatment of relapsed chronic myelogenous leukemia, but fails when used to treat relapsed acute lymphoblastic leukemia patients. Furthermore, development of graft versus host disease (GVHD) is a common drawback, demonstrating the need to improve this therapy. **Objectives:** To generate allogeneic leukemia-specific T cells expressing the suicide gene CD20 for the control of potential GVHD. **Methods:** Dendritic cells (DC) were generated by culturing monocytes with IL-4 and GM-CSF for 7 days. DCs were pulsed with apoptotic CD40L+ leukemia blasts and then co-incubated with allogeneic T cells. In this system, the CD40L molecule acts as maturation signal and the apoptotic blasts as a source of leukemia antigen. Lentiviral vectors expressing human CD20 cDNA were produced by transient transfection of 293T cells. Viral vector titer determination, phenotype profile and CFSE-labeled T cell proliferation were determined by flow cytometry. **Results:** DCs expressed a mean of 55% (25%-95%) of the DC marker CD1a after 7 days of culture. After 24 hours of incubation with the CD40L+ leukemia blasts as maturation stimulus, DCs expressed the maturation marker CD83 (34%, range 27-75%). Using the mature DCs we established the kinetics of CFSE-marked T cell proliferation. Proliferation peaked at 5 days of co-culture (CFSE-low T cells> 40%). Viral vectors titers ranged from 1-8x10^6 particles per mL. **Conclusion:** The *in vitro* culture system to generate activated allogeneic T cells was established. The activated status of these T lymphocytes should allow their transduction by lentiviral vectors. We intend to transduce T cells and select the CD20+ population for further characterization of the leukemia-directed and allogeneic responses, as well as the susceptibility of these cells to *in vitro* Rituximab-mediated lysis. Rituximab could function as a suicide inducing drug *in vivo* for these transgenic cells in case the patients develop GVHD.

CNPq–Instituto do Milênio de Terapia Gênica, FAPERJ, FAF-INCa.

PT.106

Therapeutic DNA Vaccine Targeting the HPV Oncoproteins E5, E6 AND E7 Confers Protection in the TC-1 Tumor Cells Challenge Model

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Background: Cervical cancer is the second leading cause of cancer death among women worldwide. The human papilloma virus (HPV) is associated with more

than 99% of the cases of cervical cancer and the type HPV-16 is found in 50-60% of cervical malignances. The HPV oncoproteins (E5, E6 and E7) are the main targets for the development of anti-tumor therapies associated with HPV infection. In our previous studies, we developed a DNA vaccine that encoded the E7 oncoprotein genetically fused to the glycoprotein D (gD) of HSV-1. The vaccine generated E7-specific $CD8^+$ T cell responses and protected mice challenge with cancer expressing the HPV oncoproteins following administration of 4 vaccine doses. **Objectives**: In the present work, we constructed a new version of the DNA vaccine that contains the genes of the oncoproteins E7, E6 and E5 of HPV-16 fused to the HSV-1 gD. This construction was tested in cell culture and in mice to test the expression of the hybrid protein and generation of specific $CD8^+$ T cells responses and protection against tumor development, respectively. **Methods:** To achieve these goals, transfect COS-9 cells were by immunofluorescence to determine the expression of the recombinant protein. C57Bl/6 mice were immunized i.m. with the DNA vaccine (100 mg/dose) and blood cells were used for intracellular cytokine staining assays (IFN-g) challenges with TC-1 tumor cells. **Results**: The expression of the hybrid protein was confirmed by immunofluorescence assays and flow cytometry. The immunization of mice with only one dose of the DNA vaccine pgDE7E6E5 stimulated CD8+ T cell responses, as measured in ICS assays for intracellular IFN-g production, and protect 100% of the animals against tumor development. Additionally, 3 doses of the vaccine this vaccine conferred 70% protection to mice challenged with tumor cells previous to the immunization regimen. One dose of the vaccine was also able to generate memory responses protecting mice challenge with tumor cells 100 days after the vaccine administration. **Conclusion**: All together, these data show that the new version of the DNA vaccine can efficiently generate specific CD8+ T cell response against the HPV-16 E7 oncoprotein and confer both prophylatic and therapeutic protection to tumor development in vaccinated mice.

FAPESP, CNPq

PT.107

Analysis of EGFR Expression, Amplification and Mutation in Astrocytomas

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Objectives: To analyze Epidermal Growth Factor Receptor (EGFR) expression, amplification, and mutation in astrocytomas.

Background: EGFR is a transmembrane protein consisting of an extracellular EGF-binding domain and an intracellular domain with ligand-activated tyrosine kinase activity. Amplification leading to overexpression of EGFR is observed in glioblastoma (GBM), which occurs in ~40% of primary GBM, but rarely in secondary GBM. The majority of the *de novo* GBM overexpress EGFR, and 50-70% of these express EGFRvIII, a deleted form of EGFR. **Methods:** In the present study 95 surgical astrocytoma samples and 10 non-neoplastic brain tissue from epilepsy surgery were studied, including 10 pilocytic astrocytomas (PA); 19 low-grade astrocytomas (LGA); 14 anaplastic astrocytomas (AA); and 52 GBM. EGFRvIII deletion was analyzed by RT-PCR, and also confirmed by real time PCR (RQ-PCR). The relative EGFR expression was studied by RQ-PCR using SYBR Green method, compared to non-neoplastic tissue, normalized for hypoxanthine guanine phosphoribosyl-transferase (*HPRT*) gene. The EGFR amplification was also determined by RQ-PCR relative to the hemoglobin beta gene, described as a single copy gene. Immunohistochemistry was performed to analyze the protein expression in tumor samples. **Results**: The overexpression of EGFR was found in 51 cases (53.7%), corresponding to 50% of GBM, 71.4 of AA, 73.7% of LGA and 10% of PA. The amplification was observed in 16 cases (16.8%) corresponding to 25% of GBM and only one case of PA, LGA and AA. The EGFRvIII deletion was found exclusively in GBM cases (18/52, 27.3%). Moreover, the EGFR cytoplasmic accumulation was detected in 51 cases (53.7%), corresponding to 53.8% of GBM, 50% of AA, 68.4% of LGA and 40% of PA. **Conclusion**: High frequency of EGFR overexpression, amplification and presence of EGFRvIII deletion were observed among high-grade astrocytomas, mostly in GBM. These findings will be useful to select patient eligible for immunogenic therapy with specific antibody against this receptor.

CNPq, FAPESP, Instituto Ludwig de Pesquisa em Câncer

PT.108

Cancer-Testis (CT) Antigen Expression in Medulloblastoma

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Medulloblastoma is the most common infantile malignant tumor of the central nervous system requiring harmful therapy and nevertheless carrying a poor prognosis. Due to their presence in various cancers and their limited expression in normal tissues, CT antigens are ideal vaccine targets for tumor immunotherapy. CT antigens such as MAGE and NY-ESO-1 have already been employed in clinical trials in various malignancies but little is known about their presence in medulloblastoma. Here, we analyzed 25 medulloblastomas for the expression of a panel of CT antigens by RT-PCR and immunohistochemistry. mRNA expression was as follows: GAGE (16/25; 64%), MAGE-A3/6 (14/25; 56%), SYCP1 (11/25; 44%), SLCO6A1 (8/25; 32%), MAGE-C1 (7/25; 28%), MAGE-C2 (7/25; 28%), MAGE-A4 (6/25; 24%), NY-ESO-1 (5/25; 20%), MAGE-A1 (4/25; 16%). TPTE (0/25); 24/25 (96%) cases were positive for at least one CT antigen. However, there was little CT antigen expression on a protein level. Monoclonal antibody E978 (NY-ESO-1) was negative in all cases, MA454 (MAGE-A1), 57B (MAGE-A4), M3H67 (MAGE-A3/6), CT10#5 (MAGE-C2) and #23 (GAGE) were each positive in 1/25 cases; highest incidence was seen with CT7-33 (MAGE-C1) showing - albeit heterogenous - immunostaining in 3/25 cases. This discrepancy between mRNA and protein expression in medulloblastoma has not been observed in other tumors. Due to the lack of knowledge regarding the biology of CT antigens, no explanation for this discordance can be given as yet. However, the low protein expression casts doubt on the assumption that CT antigens are useful targets for vaccine-based therapies in medulloblastomas and requires further analyses.

Fapesp e Ludwig Institute for Cancer Research

PT.109

Immunostaining of E-Cadherin and β-Catenin in Oral Epithelial Dysplasia, Carcinoma *in situ* and Squamous Cell Carcinoma

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Background: Several immunocytochemical studies have shown that loss or reduction of cadherin-catenin complex mediated adhesion is an important step in the development of many epithelial carcinomas. The changes in expression of E-cadherin and β-catenin suggest that disruption of the complex is a late event associated with invasion. **Objective:** The purpose of this study was to investigate the expression of the E-cadherin and β-catenin in mild, moderated and severe dysplasias, carcinoma *in situ* and primary oral squamous cell carcinomas. **Methods:** Seven samples of oral mucosa had been selected presenting mild epithelial dysplasia; moderate dyplasia; severe dysplasia; carcinoma *in situ*; well differentiated carcinomas; moderately differentiated carcinomas and poorly differentiated oral squamous cell carcinomas of the Anatomic Patology Service of the Antônio Pedro University Hospital / UFF and the National Institute of the Cancer (INCa). The immunostaining technique was carried through for antibodies anti-E-cadherin (1:150; NOVOCASTRA) and anti-β-catenin (1:250; NOVOCASTRA), followed of a semi-quantitatively analysis of the slides by three independent observers. The immunostaining had been characterized as (0) absent, (1+) weak, (2+) moderate e (3+) strong for the intensity of membranous and cytoplasmatic immunostaining and the distribution has been classificated as focal or diffuse. **Results:** E-cadherin in epithelial dysplasias showed strong and diffuse membranous staining with different intensity. Reduction of the membranous staining and diffuse cytoplasmatic staining were observed in carcinoma *in situ*. In carcinomas reduced membranous and cytoplasmatic staining were observed for cadherin and catenina. The worst degree of differentiation was associated with lower membranous and higher cytoplasmatic staining. β-catenin showed strong membranous and focal cytoplasmatic staining in epithelial dysplasias.Moderated membranous and focal cytoplasmatic staining were present in carcinoma *in situ* and well differentiated carcinoma. In moderately differentiated oral squamous cell carcinomas, diffuse cytoplasmatic immunostaining with focal membranous staining was observed. In the poorly differentiated oral squamous cell carcinomas there was loss of membranous and cytoplasmatic staining for the antibodies. **Conclusion:** These results demonstrate that the increase of dysplasia and/or loss of differentiation in epithelium, showed the reduction of the membranous and increase of cytoplasmatic staining of these proteins. It confirms the participation of this complex in the integrity of the oral epithelium, being able to function as amarker of progression of oral squamous cell carcinomas.

CNPq, CAPES

PT.110

Analysis of Gene Expression and DNA Methylation of Biomarkers Candidates for Head and Neck Tumors

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Background: Head and neck squamous cell carcinoma (HNSCC) is one of the most common malignancies in humans. The heterogeneous biological behavior of these tumors and high recurrence and low survival rates of these patients require further efforts to understand the pathogenesis of the disease and to identify better tumor markers and therapeutic targets. **Objectives:** To identify genes deferentially methylated in HNSCC and to validate them as novel biomarkers for diagnosis and prognosis of this disease. **Methods:** RaSH and cDNA microarray analysis were used to identify genes silenced by DNA methylation in 4 different head and neck tumor cell lines and that were induced upon treatment with the demethylating agent 5-aza-2´-deoxycytidine. Real time PCR was used to validate gene induction upon treatment and bissulfite genomic sequencing was used to evaluate DNA Methylation pattern in the promoter region of selected genes. **Results:** Of the 181 genes identified by RaSH and microarray analysis as induced upon 5-aza treatment, 35 were selected for validation by Real Time PCR and induction was confirmed for 12, 8, 11 and 2 genes in the UM-SCC-14A, UM-SCC-17A, FaDu and UM-SCC-38 cell lines, respectively. Methylation pattern of the promoter region of the CDK7, CRABP2, GOLT1B, BTAF1, CLTC and ATP5L genes were then analysed by bissulfite sequencing. The CRABP2 gene was the only gene presenting a significant reduction at DNA methylation level after 5-aza treatment. **Conclusion:** Our results suggest that CRABP2 gene is aberrantly methylated in head and neck tumors and could be used as a molecular marker in HNSCC tumors. CRABP2 hypermethylation analysis in primary tumors is currently underway.
Financial support: FAPESP

PT.111

Novel Recombinant Peptides for Prostate Cancer Diagnostics

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Background: Prostate cancer is the most common cancer observed in men in North America and Europe, and the second cause of male death in Brazil, but when detected in initial phases it can easily be treated. The identification of new antigens or specific genes may provide new biomarkers and novel tools for the development of specific treatment modalities. The Phage Display technology has been capable of selecting peptides with several purposes, such as the antigens mapping that are recognized by antibodies, with possible use as tumor markers. This methodology consists of successive cycles of selection, washing, elution, and amplification of filamentous bacteriophages, which express random peptide sequences that recognize diverse molecules, mainly the immunoglobulins. **Objectives**: The aim was the identification of biological targets, mimotopes, by Phage Display that are highly reactive against purified immunoglobulins G that were bound to protein extracts of prostate tumors. **Methods:** Three cycles of selection was performed and 200 clones were sequenced, which had generated 12 distinct peptide sequences. The selected peptides were submitted to Phage ELISA assays for analyses of reactivity against a panel of sera from 10 patients of each group: CaP, BPH, and controls. **Results:** Six of the selected recombinant peptides presented high sensitivity (50-80%), specificity (80-100%) and accuracy (65-85%); however, specific combinations of three peptides have increased the accuracy in up to 95%. **Conclusion:** These peptides present great potential as biomarkers for prostate cancer diagnostics in the peripheral blood, much higher than that observed for the current marker, PSA, but further tests are necessary to validate these amazing results.
CNPq, FAPEMIG, CAPES, UFU.

PT.112

Involvement of b-Catenin Gene in WNT Pathway in Medulloblastoma: Molecular and Immunohistochemical Analysis

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Involvement of b-catenin gene in WNT pathway in medulloblastoma: molecular and immunohistochemical analysis. Silva R, Oba-Shinjo SM, Wakamatsu A, Matushita H, Rosemberg S, Marie SKN. Laboratório de Biolçogia Molecular – Lim 15 Departamento de Neurologia-Faculdade de medicina da USP

Introduction: Medulloblastoma is a malignant invasive embryonal tumor of the cerebellum with a preferential manifestation in children. b-catenin gene (*CTNNB1*) is localized at chromosome 3p22-p21.3 and codifies a cytoplasmatic protein of 92 kDa, involved in cellular adhesion and signal transduction during embriogenesis and tissue morphogenesis. Studies have demonstrated the presence of b-catenin in the nucleus of tumoral cells, an unexpected finding because it is a protein involved in cellular adhesion and its normal localization is at the cellular membrane. **Objective**:The aims of this study were to analyze in medulloblastomas: *CTNNB1* gene mutations, the protein b-catenin accumulation, as well as to correlate both findings between them and with the histological type and to analyze the relative expression levels of genes involved in the WNT pathway (*CTNNB1, AXIN1*, *WNT1* and *APC*). **Methods**: DNA samples were extracted from 67 cases of medulloblastoma. Alterations of *CTNNB1* exon 3 were analyzed by polymerase chain reaction (PCR) and direct sequencing. The expression of the protein b-catenin was assessed by immunohistochemistry (IHC). The relative expression analyses of *CTNNB1*, *AXIN1*, *WNT1* and *APC* were determined by quantitative real time PCR (RQ-PCR) in 31 medulloblastoma samples. **Results**: The frequency of *CTNBB1* exon 3 mutations in the *CTNNB1* was 15%. We identified six *missense* heterozygous mutations in ten cases. It was observed b-catenin immunoreactivity in the nucleus in 36.4% of all cases. No correlation between histological type and IHQ qualitative and semi-quantitative. Also, there was no correlation between histological type and mutations. No difference in the expression levels of the genes *CTNNB1*, *AXIN1* and *APC* were observed in medulloblastomas in relation to normal cerebellum. In the analysis of relative expression and the histological classification, only APC presented significant difference between classic and desmoplastic type. There was no difference of the relative expression levels of any gene with the patient's age. The presence of *CTNNB1* mutations did not affect the relative expression of *CTNNB1* and *APC*. On the other hand, *AXIN1* presented a higher relative expression in the cases with mutation. APC expression level was lower when b-catenin nuclear accumulation was higher. **Conclusion:** Our data indicate that other proteins of WNT pathway can be involved in b-catenin accumulation in medulloblastoma cells, as well as the involvement of other different pathways.

Capes; FAPESP

PT.113

P53 and Survivin CO-Expression Suggests a Correlation With Poor Prognosis Features in Chronic Myeloid Leukemia

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Background: p53 protein has an important role in the regulation of Survivin expression, an anti-apoptotic protein related to poor prognosis in different types of cancer. TP53 gene mutations are correlated with Survivin overexpression. In this context, the study of the co-expression of these proteins in chronic myeloid leukemia (CML) might offers prognostics information for this disease. **Objectives**: To verify the expression of Survivin and p53 in CML samples and correlate it with Sokal score and clinical previous treatment. **Methods:** Forty-nine samples were analyzed. The expression of p53 protein was verified by flow cytometry while Survivin expression was analyzed by Western blot. **Results:** Forty-one out of 49 samples (83,7%) were positive for Survivin expression while 9 samples (18,4%) were positive for p53 protein. Survivin and p53 co-expression was verified in 6 samples (12,2%): 5 samples were obtained from patients with intermediate Sokal score and treated previously. Five out of 6 samples from patients showing negative expression for both proteins presented a low Sokal score and were not previously treated. **Conclusions:** The co-expression of Survivin and p53 was demonstrated in patients with poor prognostic suggesting an unfavorable phenotype for disease evolution. In contrast, the absence of the expression for both proteins was demonstrated in low Sokal score and untreated patients, probably suggesting a better prognosis for CML patients.

Swiss Bridge Foundation, CAPES, FINEP.

PT.114

Autoantibodies Against Osteopontin are Potencial Serum Markers for Early Prostate Cancer

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Osteopontin (OPN) is a secreted glysosylated phosphoprotein that has been implicated in many physiological and pathological processes *in vitro* and *in vivo*. This protein is overexpressed in prostate cancer (PCa) cells and contributes to the progression of the disease. Autoantibodies against tumor associated antigens has been a great promise for the early detection of cancer, monitoring cancer progression, guiding individualized therapeutic interventions, and identification of novel therapeutic targets. In the present work, we evaluated if OPN does evokes a humoral immune response in PCa patients and whether anti-OPN antibodies could be used as a potential serum marker for this disease. The frequence of anti-OPN antibodies were significantly higher in PCa (62%) and BPH (33%) plasma samples as compared to HD controls (10%). When evaluating anti-OPN reactivity levels as potential serum markers for PCa patients, we obtained 62%, 81%, 66% and 78% values respectively for sensitivity, specificity, positive and negative predictive values (PPV and NPV). The fact that anti-OPN antibodies were also detected in a high proportion of PCa patients which presented Gleason Score lower than 6 (57%), PSA values less than 10 ng/ml (67%) and pT2 organ confined disease (70%), suggesting that anti-OPN antibodies could be used as an early serum marker for PCa. To our knowledge this is the first description of OPN as a tumor autoantigen and one of the most reactive individual autoantigen described to date. This data support its inclusion in a multiplex of tumor antigens in order to perform antibody profiling in PCa and probably in other malignancies overexpressing OPN. Swiss Bridge Foudation, FAPERJ, PRONEX-Rio, CNPQ, Fundação Ary Frauzino, Ministério da Sáude

PT.115

Increased Expression of *SPARC* (Osteonectin) is Frequently Observed in Primary Breast Tumors and its Expression is Modulated by EGF (Epidermal Growth Factor) in Human Mammary Cell Lines

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The *SPARC* (secreted protein, acidic, cysteine-rich) gene encodes a 32kDa calcium binding protein, also named osteonectin and BM40, which belongs to the group of matricellular proteins that interact with cell-surface receptors, growth factors and the ECM (extracellular matrix) components. SPARC plays a role in tissue remodeling, cell migration, angiogenesis, embryonic development and tumorigenesis. Altered expression of *SPARC* has been reported in many types of tumors including breast cancer, but examining a limited number of cases. In the present study, SPARC protein expression was evaluated by immunohistochemintry (IHC) on tissue microarrays (TMA) containing 699 primary breast tumors. Eighty-one percent (521/641) of the cases analyzed showed positive SPARC protein expression. Significant associations were found between SPARC protein expression and advanced clinical stage (p=0.006), higher nuclear grade (p=0.004), presence of estrogen receptor (p<0.001) and *ERBB2* over-expression (p=0.011). However we failed to find any significant associations between SPARC protein expression with the overall survival (p=0.074) or disease-free survival (p=0.366) of the patients. We also investigated the effects of EGF on SPARC transcript regulation in three breast cell lines expressing different levels of ERBB2. The Hb4a cells, which express basal levels of erbb-2, showed time-dependent SPARC down-regulation upon EGF treatment with a maximum decreased observed after 24 hours. The C5.2 cells, which express high levels of erbb-2, displayed 2-fold decreased in SPARC expression after EGF exposure for 2h. SPARC transcripts expression was undetected in the breast cancer cell line SKBR3 that over-express erbb-2. In conclusion, SPARC is frequently over-expressed in breast tumors and might influence tumor development and progression. Our results also indicate that *SPARC* transcripts are modulated by EGF, however further studies are required to determine whether this modulation is mediated by erbb-2 . FAPESP and CNPq

PT.116

PAWR/PAR-4 Expression is Modulated by 17beta-Estradiol And IGF-I in Breast Cancer Cells

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PAWR (PRKC, apoptosis, WT1, regulator gene), also known as *Par-4*, is located on chromosome 12q21 and was first identified in prostate cells undergoing apoptosis in response to exogenous stimuli. *PAWR* encodes a protein that sensitizes cells to diverse apoptotic stimuli. *PAWR* knockout mice exhibit prostatic neoplasia, and show an increased frequency of estrogen-inducible tumors in the endometrium, whereas overexpression of *PAWR* in prostate cancer xenografts results in apoptosis and tumor growth inhibition. PC12 cells exposed to an ischemic insult showed increased apoptotic cell death and *PAWR* expression, and treatment with IGF-I inhibited both the apoptosis and the increase in *PAWR* mRNA expression. Therefore, our goal was to investigate the effects of 17α-estradiol (E2) and insulin-like growth factor I (IGF-I) on *PAWR* mRNA expression in MCF-7 breast cancer cell line. MCF-7 cells were grown in striped serum for 48 h and treated with 17α-estradiol 10 nM or IGF-I 12.5 nM for 2, 6 and 24 h. To evaluate which pathways are involved in E2 and IGF-I modulation of *PAWR* expression, cells were treated with 1mM of fulvestrant (ICI-182.780), 30μM of LY294002 or 30μM of SB202190 for 1 hour prior to the hormones treatment. The relative levels of *PAWR* mRNA expression were determined by real-time RT-PCR. The results were expressed in *n*-fold differences in gene expression of the target gene relative to the expression of the *GAPDH* gene, used as a reference gene, and to the cells maintained in 10% FBS, used as the calibrator sample. MCF-7 cells maintained in striped serum showed 3-fold increase in *PAWR* mRNA expression compared to cells maintained in 10% FBS. Treatment with E2 for 24 hours inhibited *PAWR* expression (2-fold reduction, compared to cells maintained in striped serum). Administration of the specific anti-estrogen, ICI-182.780, abolished this effect and resulted in 5.5-fold increase in *PAWR* mRNA expression. Cells treated with IGF-I for 24 hours showed 3.5-fold reduction in the transcript expression, compared to cells maintained in striped serum, while pre-treatment with the PI-3K inhibitor, LY294002, the p38MAPK inhibitor, SB202190, or with ICI-182.780 abolished this effect, resulting in 6.5, 2.5 and 1.8-fold increase in *PAWR* expression, respectively (compared to cells treated with IGF-I alone). These findings suggest that *PAWR* mRNA expression is modulated by E2 via ER activation and by IGF-I through the activation of PI-3K, p38MAPK and ER pathways. They also indicate that there might be a cross-talk between ER and IGF-IR on the modulation of *PAWR* mRNA expression in MCF-7 breast cancer cell line.

FAPESP and CNPq.

PT.117

Expression of MicroRNAs During Progression of Human Melanoma

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Introduction: MicroRNAs (miRNAs) are a class of naturally occurring small non-coding RNAs that control gene expression by targeting mRNAs for translational repression or degradation. Recent studies indicate that many miRNAs are aberrantly expressed in various human cancers. However, there is no report concerning the expression of miRNAs during tumor progression. **Objectives**: Here, we investigated the expression of 16 microRNAs in melanocytic cells isolated from different stages (radial, vertical and metastatic) of human melanoma progression. **Method**: The expression of mature miRNAs was evaluated by using stem-loop RT followed by TaqMan real-time PCR analysis. MiRNAs of normal melanocytes were used as control. **Results**: Our results showed that *miR-21, miR-29a, miR-29b-2, miR-146, miR-200a, miR-200b* e *miR*-213 are over-expressed (≥ 3-fold) whereas *miR-129.1, miR-194* e *miR-205* are down-regulated (≥ 5-fold) during tumor progression. We also found that *miR-96, miR-145, miR-182, miR-199a e miR-219* are over-expressed (> 600-fold) only in radial stage. *MiR-215* expression had no change during tumor progression. **Conclusion**: This study is the first demonstration of an aberrant expression of miRNAs during tumor progression, suggesting that they could be involved in the melanoma development. However, the correlation between miRNAs expression and their effects on targets mRNAs of proto-oncogenes and tumor suppressor genes is still not fully understood. It is noteworthy that *miR-21* has been described to down-regulate the tumor suppressor gene TPM1 in breast cancer cells. Thus, additional work is required to elucidate the role played by miRNAs in human melanoma progression.

FAPESP and CNPq

PT.118

NDRG4 IS Epigenetically Silenced in Breast Tumors

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Background: Genetic and epigenetic abnormalities associated with cancer can be explored for the development of more specific and sensitive diagnostic and prognostic tools. Hypermethylation in promoter region is one of the most widely studied epigenetic alterations and has been associated to gene silencing. Aberrant methylation pattern has been used in the characterization of cancer-related genes as well as in the identification of new tumor markers. **Objectives:** Identify genes silenced by DNA methylation in breast tumor cell lines and evaluate their potential as molecular markers in breast cancer. **Methods:** The demethylating agent 5-aza-2′-deoxycytidine (5Aza-dC) was used to re-induce the expression of genes silenced by DNA methylation in three breast tumor cell lines (MCF7, MDA-MB-231 and MDA-MB-435). After treatment, cDNA microarray analysis (4,800 genes) was used to identify epigenetically inactivated genes induced upon treatment. Induction of gene expression was further validated by Real-Time PCR and the methylation pattern of the promoter region of validated candidates was determined by bissulfite sequencing. Methylation specific PCR (MSP) was then used to analyze the methylation status of selected candidates in 83 breast tumor samples and to establish associations between DNA methylation status and clinico-pathological parameters. **Results:** Using this approach, we identified NDRG4 as an epigenetically silenced gene in breast tumors. Expression of NDRG4 was induced by 4.6, 7.5 and 15.8 times after treatment in the MCF-7, MDA-MB-231 and MDA-MB-435 cell lines respectively. As expected, DNA methylation analysis of NDRG4 promoter region revealed a significant decrease in the DNA methylation levels in all three cell lines after 5Aza-dC treatment. NDRG4 hypermethylation was also detected by MSP in 5 out of 83 invasive breast carcinomas. NDRG4 hypermethylation was associated with the number of positive lymph nodes (p=0.001) and p53 protein over-expression (p=0.001). NDRG4 hypermethylation also seems to be associated with a lower distant metastasis-free survival (60% versus 84,2%, p=0,167) in breast cancer patients, although the difference between groups was not statistically significant. **Perspectives:** We are now using immunohistochemistry to validate the methylation analysis at the protein level and to increase the number of breast tumor samples using TMA.

Ludwig Institute for Cancer Research/Hospital A. C. Camargo - FAP

PT.119

Global DNA Methylation Analysis in Breast Tumors Using Promoter Microarray

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Aberrant DNA methylation of CpG sites located in promoter regions is among the earliest and most frequent epigenetic alterations in tumors and is emerging as an important molecular marker for disease diagnosis and prognosis. As a further step to understand the role of DNA methylation in tumorigenesis and to improve cancer diagnosis and prognosis, abnormally methylated sequences occurring in the tumor genome need to be identified. In this work we used a microarray-based strategy to identify differentially methylated genes between lymph node negative breast cancer patients that did and did not developed distant metastasis after surgery. Human RefSeq promoter sequences were retrieved from GenBank and 12800 fragments amplified by PCR were spotted onto glass slides resulting in a promoter microarray. Genomic DNA from tumors were digested with the methylation sensitive enzyme *Hpa*II and genomic DNA from a reference cell line (Hb4a) was digested with methylation insensitive enzyme *Msp*I. Oligonucleotide linkers specific for these enzymes were ligated to the digested fragments and PCR using linker-specific primer was used to amplify tumor and reference digested DNAs. PCR fragments from tumors and reference were then labeled with Cy5 and Cy3, respectively, and hybridized to the microarray. Using this strategy we were able to identify 207 genes that were differentially methylated between node negative patients that did and did not develop distant metastasis. Ten genes found to be hypermethylated in node negative patients with metastasis were selected for gene expression analysis by Real-Time PCR in 14

breast tumor cell lines. Three out of four genes tested so far we found to be down-regulated by at least 2 fold in 30% of the tumors cell lines. The methylation status of the promoter region of down-regulated genes will be determined by sodium bisulphite conversion of the genomic DNA followed by sequencing in tumors samples. In the future, validated genes could be used as molecular marker for disease management.
This work is supported by Ludwig Institute for Cancer Research, Sydney Kimmel Cancer Center, FAPESP and CNPq.

PT.120

First Proteomics Analisys of Tissues from Penile Cancer with HPV Infection

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Background: Penis cancer is a rare illness affecting mainly in the fourth and fifth decades of life. The incidence in Brazil of this invasive carcinoma represents 2.1% of the malignant neoplasias in men; however in certain underdeveloped regions this rate reaches 17%, disclosing a serious public health problem. HPV (Human Papiloma Virus) infection is a risk factor for developing penis cancer and prevalence was nearly 50% in these neoplasias from Brazilian patients, with oncogenic HPV16 and 18 prevailing in malignant carcinomas. The treatment is still a matter of controversy until the present moment definition of the best therapy approach was not established. Beyond the surgical treatment, some protocols using radiotherapy have been tested without improving the survival rate. Proteomics investigation of penile cancer can help to develop new methods for early diagnose and/or follow-up of the disease and to create new drugs. **Objectives:** Standardization of 2D Gel for analysis of proteins differentially expressed between patients tissues of penile cancer and the healthy´s group. **Methods:** We collected 18 samples from tissues of patients and normal men. Samples were classified according to differentiation and as HPV negative or positive. HPV typing was done by PCR using specifics primers. Tissue was lysed and supernatant was saved. Protein quantification was done by Lowry-Peterson method. 1D SDS Gel was done to observe the integrity of proteins in the different groups. Before proteins were separated in a 2D Gel, focalization was performed using a strip of pH 3-10, 13 cm long (Amersham Bioscience) on the Multiphor™ (GE Health) Platform. **Results and Conclusion:** The first pool was composed of 6 tumors and normal tissues, separately. It was observed on 2D Gel differences between protein expression of healthy donors and patients. The next step will be the analysis of proteins different expressed and their identification.
INCA / Proteoma-Rio

PT.121

Molecular Analysis of the FHIT Gene in Patients With Brest Cancer

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Background: The fragile histidine triad (FHIT) gene, which spans the most common fragile site, FRA3B, at chromosome 3p14.2, is a tumor suppressor gene involved in the carcinogenesis of breast cancer. Deletions in the FHIT gene have been observed in preneoplastic lesions, suggesting that FHIT deletions could be an early event in breast carcinogesis and that protein level is correlated with disease prognosis. **Objectives:** Detect the loss of heterozygosity (LOH) of the FHIT gene in patients with breast cancer and study their association with the clinical pathological characteristics of the breast cancer. **Methods:** Breast tissue and peripheral blood specimens were obtained from 20 patients diagnosed with breast cancer and 11 patients with benign breast disease. DNA samples were analyzed with PCR primers which amplify microsatellite markers within the FHIT gene: D3S4260 (intron 4) and D3S1234 (intron 5). LOH was detected by the comparison of the allele's intensity among normal and tumoral DNA of a given patient. **Results:** No alterations were observed in the site of D3S4260 microsatellite marker in patients with breast cancer, likewise, in patients with benign breast diseases. However, the analysis of D3S1234 microsatellite marker evidenced a loss of heterozygosity in 35% of patients with malign alterations, whereas in those with benign diseases the LOH occurred in only 18% of cases. **Conclusion:** LOH in the FHIT gene is an early event in the development of breast cancer. Inactivation of a unique allele is a factor for the decrease of Fhit protein expression. Nevertheless, another gene alterations undetectable by LOH analysis, such as point mutation or methylation, can also contribute to the abnormality

of Fhit protein level.
FAPEMIG, CAPES, FUNEPU-UFTM

PT.122

PRAME Expression is Associated with Down-Regulation of TRAIL in Chronic Myeloid Leukemia

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The tumor antigen PRAME (preferentially expressed antigen of melanoma) is frequently overexpressed in a wide variety of malignant diseases. It was recently shown that PRAME can function as a repressor of retinoic acid receptor and this transcriptional repression depends on the formation of a complex with the polycomb group EZH2 (enhancer of zeste homolog 2). To test whether these mechanisms play an important role in Chronic Myeloid Leukemia (CML) physiopathology, we analyzed the expression of *prame*, *ezh2* and *trail* (gene regulated by retinoic acid signaling) in 31 patients in different phases, 9 in cytogenetic remission post-imatinib and 10 healthy individuals. We also investigated in Bcr-Abl$^+$ cell lines (K562, KCL22 and LAMA-84) and in HL60 transfected or not with Bcr-Abl untreated or treated with imatinib 10micro molar for 8 hours. Bcr-Abl$^+$ cell lines presented higher *prame* and *ezh2* expression and lower *trail* expression than Bcr-Abl$^-$ cells. Imatinib treatment reduces *ezh2* expression to normal levels in HL-60.Bcr-Abl and K562 cells but do not interfere with *prame* and *trail* mRNA levels. CML patients showed a significant higher prame expression in the advanced phases of the disease with a significant down-regulation after complete cytogenetic remission (CCR), no differences were found in *ezh2* levels. Furthermore, there was a significant reduction in *trail* levels in the advanced phases of the disease with a significant up-regulation after CCR. We also found a significant correlation between *prame* and *trail* expression in CML patients. In conclusion, the above mechanisms seem to play an important role in the pathogeneses of the CML by the down-regulation of trail, an inducer of the extrinsic pathway of apoptosis.
FAPESP, CNPq

PT.124

Bone Deposition, Bone Resorption and Osteosarcoma

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Background: Bone deposition and bone resorption constitute the bone remodeling process. In the normal bone there is a balance between bone deposition and resorption. Imbalance in any one of these ways can have serious consequences, contributing to genesis and progression of the bone diseases, including bone tumors. Some bone tumors, such as osteosarcoma, stimulate focal bone deposition. Osteosarcoma (OS) is the most common primary bone tumor in children and young adults and despite the dramatic advances in OS treatment, patient survival reached a plateau. **Objective:** The aim of this study was to analyze the expression profiles of the bone remodeling regulator genes, on OS samples and to relate them with the OS clinical aspects. **Methods:** We used real time PCR to analyze the mRNA levels of the *TRAP* (Tartrate-Resistant Acid Phosphatase), *CSF1* (Colony Stimulating Factor-1), *BMP-7* (Bone Morphogenetic Protein 7), *COL11A2* (Collagen, type XI, Alpha 2) and *PTPRZ1* (Protein Tyrosine Phosphatases Zeta 1) genes, on 30 biopsy tumor samples of the OS patients and correlated with clinical and pathological data. **Results:** *TRAP*, *BMP-7*, *COL11A2* and *PTPRZ1* were identified as over-expressed genes in OS ($p<0.0001$). In particular, *BMP-7* gene was over-expressed in 23 of 30 (77%) samples and patients with OS showing high levels of expression of *BMP-7* in biopsy samples revealed better overall survival ($p= 0.0019$) and event-free survival ($p= 0.0001$). **Conclusion:** Currently, one of the proposed mechanisms of OS progression involves the tumoral secretion of remodeling bone compounds. *BMP-7* plays a central role in the regulation the remodeling bone, stimulating growth and differentiation of osteoblasts. OS patients with over-expressed *BMP-7* gene could have tumors with possibility of cell differentiation, lower rate growth and consequently better survival.
Supported by: GRAACC and FAPESP (2004/12150-8)

PT.125

CCR2 Gene Expression Analysis in Children with Acute Lymphoblastic Leukemia

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Background: The identification and characterization of leukemia-microenvironment interactions and the elucidation of their impact in Acute Lymphoblastic Leukemia (ALL) development should provide novel targets for therapeutic intervention. Chemokines play a central role in leukocyte physiology by controlling basal and inflammatory trafficking. CCL2 activates the receptor CCR2 in cell surface and may contribute to angiogenesis, acting directly in the endothelial cells and indirectly, through chemotactic effect and activation of monocytes. Little is known about CCL2 and its expression and function in ALL. **Objectives:** The aim of this study was to analyze the expression level of CCR2 in ALL patients and its association with clinical/ biological characteristics. **Methods:** The cDNA of 106 ALL patients at diagnosis were analyzed by qualitative PCR to CCR2A and CCR2B transcript isoforms and by quantitative PCR to CCR2B, the latter using SYBR Green dye. Absolute expression values were detected by using a standard curve obtained by serial dilution of plasmidial DNA cloned with CCR2B or ABL genes. The number of CCR2B transcripts was normalized by the number of ABL transcripts (NCN value). Bone marrows (BM) from seven normal individuals were analyzed as control group. **Results:** Ten patients were positive to CCR2A and 64 to CCR2B by qualitative PCR. The ALL patients CCR2B NCN values had 0.0110 as median and this was used as a threshold to distinguish between high and low expression level patients. The expression of CCR2B in normal BM samples was approximately 35 times higher than the patient's expression (median of 0.3986 versus 0.0110). ALL cells that expressed at least one myeloid marker (CD13, CD15 and CD33) had an increased expression of CCR2B ($p = 0.02$). No association could be found between CCR2 expression and either clinical/biological features or patient outcome. **Conclusion:** Expression of CCR2B by ALL cells is associated to myeloid markers. Although no association was found between CCR2B expression by ALL cells and patient outcome, future studies are needed to investigate the role of this chemokine in ALL-stroma interaction.

CNPq and FAPESP

PT.126

Repetitive DNA in Cutaneous Malignant Melanoma

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Background: Further biological understanding of melanoma (CMM) is highly desirable since the incidence of this malignancy has increased in the last years. Genetic instability is often involved in malignant processes and can be easily detected by PCR-based methods like RAPD (*Random Amplified Polymorphic* DNA). The single primer used in this procedure amplifies anonymous DNA sequences with different sizes creating a *fingerprint* pattern of bands. **Objectives:** To compare DNA *fingerprints* of different histopathological degrees CMM with normal tissues or leukocytes, in search of particular bands, which could be involved with this disease. **Methods:** DNA of 12 CMM samples and controls were extracted by current protocols and screened by PCR amplification with 13 RAPD primers (*Operon Technol.*), at suitable annealing temperatures. Statistical analysis was done with GraphPad Prism 5.0 program. Selected bands were cloned and sequenced to verify possible open reading frames. **Results:** All samples (100 %) showed altered patterns regarding the respective controls. RAPD primers OPA 4, 7, 11, 13, OPB 1 and 19 showed changes in position, but not in number of bands. Increased number of bands was found in tumor samples with OPA 3 and 17, whereas OPA 5, 14, and OPB 11 showed decreased numbers. CMM samples analyzed with OPA 2 exhibited significant reduction in the number of bands ($p=0,0133$), as already observed in a previous study. Some bands (1550, 900 and 500 bp), absent in most tumor samples, were selected from the patterns, eluted, cloned, sequenced and submitted to GeneBank data in order to detect possible genes related to tumor genesis. Preliminary results showed 99 % similarity of the 5'-end of the 500 bp band, absent in three tumor samples (CMM I, III and V), with an EST (*Expressed Sequence Tag*) sequence to be further analyzed. **Conclusion:** Genome instability of CMM samples was confirmed using the RAPD technique. Cloned and sequenced DNA fragments picked from those patterns revealed alteration of specific sequences involved with some CMMs. These and other DNA sequences to be examined may add to the biology of CMM and also constitute tumor markers, to support medical diagnosis and therapeutical conducts.

CAPES and FAPESP

PT.127

Effects of 17b-Estradiol and ICI 182 780 on *SPG-7* and *SPRED2* Gene Expression in MCF-7 Breast Cancer Cells

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In a previous study, using cDNA microarray we identified the transcrips of *SPG-7* (spastic paraplegia 7) and *SPRED2* (sprouty-related, EVH1 domain containing 2) genes as differentially expressed in breast tumors regarding estrogen receptor (ER) positivity. *SPG-7* encodes a mitocondrial metalloprotease named paraplegin, which is associated with the susceptibility to hereditary spastic paraplegia. *SPRED2* encodes a member of Sprouty family that regulates growth factor-induced activation of MAP kinase cascade. We analyzed the promoter region of these genes for estrogen response elements (EREs) and both, *SPG-7* and *SPRED2* showed potential to be regulated by estrogen receptor. The aim of the present study was to investigate the effects of 17b-estradiol (E2) on *SPG-7* and *SPRED2* mRNA expression in MCF-7 breast cancer cells using quantitative real time PCR (qPCR). Before the treatments with 17b-estradiol and the anti-estrogen ICI 182 780 (ICI), the cells were cultured in media without phenol red, containing 5% of charcoal-stripped FCS (ST) for 48hours. After hormone deprivation, the cells were incubated with E2 10nM or ICI 1mM or E2 (10 nM) plus ICI (1m) for 2, 6 and 24 h. The cells maintained in media without phenol red and 5% ST were used as controls. MCF-7 cells maintained in charcoal stripped serum showed increased expression levels of *SPG-7* transcripts (2-fold) compared to cells cultured in 5% FBS. The *SPG-7* mRNA levels were only partially up-regulated after E2 treatment for 2 h and returned to the control levels after 24h of treatment. However, MCF-7 cells exposed to ICI for 2 hours showed decreased levels of SPG-7 transcripts, this effect was not observed for ICI treatments for 6 or 24h. In contrast, the estrogen-deprivation, E2 or ICI treatment had no effect on the level of the *SPRED2* transcripts. The same set of experiments was used previously in our laboratory to describe for the first time the up-regulation in a time dependent manner of JDP1 transcripts by E2. The results presented here suggest that 17b-estradiol has no effect on the transcriptional regulation of *SPG-7* or *SPRED2* genes. Further experiments will be carried out to investigate the possible regulation of these genes by growth factors in cross-talk with estrogen receptor.
Supported by FAPESP

PT.128

Identification and Validation of Differentially Expressed Proteins in Oral Squamous Cell Carcinoma

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Background: Squamous cell carcinoma (SCC) is one of the most common cancers of the oral cavity encompassing at least 92.8% of all oral malignancies. It is a universal aggressive disease in the population of smoking and drinking. Despite improved diagnostic and therapeutic methods over the 20 last years, this tumor is still characterized by a high rate of mortality. Proteomic technologies provide tools for rapid screening of a large number of potential biomarkers in malignant cells and their results may help to understand the mechanisms involved in the oral carcinogenic process. **Objectives and Methods:** In order to identify changes in protein expression, oral tumor samples and apparently normal surgical margins were examined by two-dimensional electrophoresis, differential in-gel electrophoresis (DIGE), western blot and mass spectrometry. **Results:** Qualitative and quantitative variations between tumor and normal cells were detected including upregulation of calgranulin B, galectin 7 and heat shock protein 27 and downregulation of creatine kinase, myosin light chain 1, myosin regulatory light chain 2, myosin alkali light chain 3 and tropomyosin 2 in tumors. One member of cytokeratin family also showed alteration in expression as validated by western blot, consistent with proteomic results. Their functions range from cell signaling, inflammatory response, protein folding and proliferation to apoptosis, signal transduction, transcription regulation and cell motility and adhesion. **Conclusion:** These proteins have been shown to have altered expression in OSCC and in other tumors and may be used in future studies of carcinogenesis or as diagnostic markers and therapeutic targets for OSCC.
FAPESP, CAPES, CNPq

PT.129

ADAM23 Promoter Hypermethylation as a Molecular Marker to Detect Metastatic Cells in Sentinel Lymph Nodes of Breast Cancer

Patients

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Recently our group demonstrated that the ADAM23 gene is frequently silenced by promoter hypermethylation in advanced stage breast tumors. In order to evaluate the potential of using ADAM23 hypermethylation (HM) as a molecular marker to detect metastatic cells in the sentinel lymph nodes (SLN) of breast cancer patients, we here evaluated by Methylation Specific PCR the methylation pattern of two CpG islands (referred as region 1 and 2) located at the promoter region of the ADAM23 gene in primary breast carcinomas and SLN of the same patient. Initially we evaluated the sensibility of our assay by analyzing ADAM23 HM in the SLN from 12 patients considered lymph node positive (N1) for metastatic cells by histo-pathological analysis and whose primary tumors presented ADAM23 HM. The presence of ADAM23 HM in at least one of the two regions was detected in the SLN of all patients, ensuring the sensibility of our assay. Next we verified the specificity of our assay by analyzing the SLN of 3 N1 patients whose primary tumors did not show ADAM23 HM in none of the two regions and in lymph nodes of patients with a negative diagnosis of lymphoma. Unexpectedly, ADAM23 HM at region 1 was detected in the SLN of one of the 3 patients, suggesting that metastatic cells can acquire ADAM23 HM after leaving the primary site. Finally, we evaluated the presence of ADAM23 HM in SLN from 23 patients considered lymph node negative (N0) for metastatic cells by histo-pathological analysis and whose primary tumors presented ADAM23 HM. The presence of ADAM23 HM at region 1 was detected in the SLN of 20 out of the 23 patients, suggesting the presence of metastatic cells not detected by histo-pathological analysis. Interestingly, we could not detect ADAM23 HM at region 2 in the SLN of none of these patients, despite the fact that the paired primary tumor presented ADAM23 HM at this region. These results show that the presence of ADAM23 HM at region 1, but not region 2, can be used to detect metastatic cells in the SLN of breast cancer patients, offering a more sensitive and specific assay as compared to conventional histo-pathological analysis.

LICR, FAPESP and CNPq

PT.130

Clinical Analysis of the Anomalous Phenotype Membrane (m) HLADRα/Ii(+) ,HLADRβ(-) NFkB(+) in Ovarian Cancer (OVCA)

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Background: OVCA accounts for 3% of female malignancies; nonetheless, it is the fourth cause of cancer-related deaths among women. As cancer occurs in immunocompetent patients the immunoediting hypothesis had emerged. In OVCA, this had been verified in cells sustaining significant (m)HLADRα/Ii(+), silenced HLADRβ, suppressed mature HLADR, then evading immune system. It has been shown that Ii can interact with NFkB, an anti-apoptotic molecule prone to contribute to drug resistance in some cancer types. **Objectives:** Assess clinical relevance of the (m)HLADRα/Ii(+),hlRdrβ(-),NFkB(+) phenotype as a prognostic and therapeutic biomarker of OVCA. **Methods:** Based on our primary OVCA tumor bank, 2mm samples were taken from archival paraffin-embedded blocks to generate tissue array platforms, assayed by immunohistochemistry (HLADRα, HLADRβ, Ii, NFkB monoclonal antibodies), and further correlated to cases clinical data (Consent Term signed, confidential). **Results:** We had confirmed the OVCA-specific phenotype (m)HLADRα/Ii(+),HLADRβ(-) previously observed; cases were also NFkB(+). (m) HLADRα/Ii,HLADRβ(-)Ii/NFkB(+) proteomic pattern was detected in 50% of OVCA studied. Clinical data correlation had revealed that while 40% of the tumors were diagnosed in such an advanced stage that the patient died before the establishment of chemotherapy, 40% of them were platin (PL) resistant; the other 20% are currently under PL therapy for less than 6 months and will be followed up. The OVCA-phenotype correlated with 57% of obits, 60% of highly metastatic tumors, and with moderated or low differentiation degree. Normal, adenomas and borderline tumors of the ovary were negative for the phenotype studied. **Conclusion:** Our data suggest that the analysis of the (m)HLADRα / Ii,HLADRβ
(-),NFkB(+) phenotype should be introduced in the OVCA diagnostic routine as prognostic and PL-responsiveness biomarker, aiming to avoid the recurrence of the disease with PL-resistant aggressive clones, then rationalizing chemotherapy regimens while evaluating precisely the aggressiveness of the OVCA diagnosed.

PT.131

Aberrant Expression of Genes Involved in Placenta in Human Astrocytomas

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Background: There are many similarities between embryo implantation and the growth of cancer cells, including expression or repression of specific molecules, acquisition of a rich blood supply, evasion of immune system and increased proliferation. All these processes are common to placental and astrocytomas, the most frequent brain tumors. **Objective:** to search for molecular markers with increased expression in placenta and astrocytomas. **Methods:** Highly expressed genes in placenta and in different kind of tumors selected from MPSS data were compared to microarray and SAGE data of astrocytomas and non-neoplastic brain tissues. The selection criteria were localization (preferentially the cellular membrane), function related to tumorigenisis and low expression in normal tissues. The expression levels of selected genes were validated by quantitative Real Time PCR in 10 non-neoplastic brain samples and 20 glioblastoma (GBM) samples and in 18 different normal tissues. Those with high expression in GBMs relative to controls and with low expression in normal tissues were analyzed in grade I to grade IV astrocytoma samples. The relative expression data were correlated with the overall survival of patients by Kaplan-Meier analysis. **Results:** We selected 12 genes, which expression were higher in GBMs relative to control samples. Among them, four genes, *TNFRSF12A*, *CNN3 MAPKAP1* and *PMP22*, presented low expression levels in normal tissues, and also high expression in all malignant grades of astrocytomas. *CNN3* and *PMP22* mRNA levels showed association with overall survival in GBM patients: those with higher relative expression of *CNN3* had a tendency of longer overall survival, while these with lower relative expression of *PMP22* had a statistically significant longer overall survival. **Conclusion:** High expressed genes in placenta may revealed important genes involved in the tumorigenesis of astrocytomas. This selection strategy, based on public available database as well as on our microarray data, have allowed the selection of potential prognostic markers and therapeutic targets.

Fapesp and Institute Ludwig for Cancer Research

PT.132

Estrogen Receptor α E β (ERα & ERβ) Relative Abundance (RA) Expression in Breast Cancer (BC): Evaluation of a Novel Prognostic and Hormone-Therapy (HT) Biomarker Profile

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Background: HT with estrogen and its analogs is a major strategy against menopause symptoms. Conversely, ER(+) BC are subjected to HT with ER blockers as tamoxifen. The discovery of ERα & ERβ revealed that ubiquitous Erα (+) BC had high curative index, while prevalent ERβ(+) BC were poor prognosis tumors. This is mainly due to their specificities, so that, for instance, like tamoxifen is an ERa-antagonist, it acts as an ERb-partial agonist. So, HT failure could be associated to an inappropriate diagnosis evaluation of ER expression in BC. **Objectives:** Establish the clinical relevance of the ERα & ERβ RA expression as a BC diagnosis tool. **Methods:** From 2,400 BC cases, we assessed 227 clinical reports and gathered 49 patients that met the inclusion criteria (≥ 40yo, primary BC, signed Consent Term), of them 9 had been submitted to previous menopause-hormonal therapy (mTH). We generated tissue array platforms, assayed by immunohistochemistry (IHC) (ERα & ERβ specific antibodies). **Results:** IHC analysis of BC samples revealed that the ERα & ERβ RA expression was: ERβ/ERα>1 (65.8%), ERβ/ERα<1 (2.6%), ERβ/ERα=1 (7.9%), ERβ,ERα(-) (23.7%). Among the poor prognosis cases (ERβ/ERα>1), 32% presented significant membrane (m) expression of ERβ. **Conclusion:** Herein we show that the majority of BC cases describes the ERβ/ERα>1 phenotype, including mTH patients, some with impressive (m) ERβ expression. Albeit we still have to elucidate the role of (m)ERβ, others had verified (m)ERα in BC cells coupled to PI3K/AKT pathway, demonstrating that estrogen might elicit non-nuclear actions. Then, (m) ERβ BC would be expected to have high incidence of metastasis, relapses, and obits, as TH would not prevent disease progression. We believe that our study may introduce novel perspective in the diagnosis and treatment of BC. We present a simple and accurate BC diagnosis tool aiming to contribute to the efficient control of BC, as it rationalize HT, hence subsiding Public Health to control BC, the first cause of female cancer-related deaths worldwide.

PT.133

Mitochondrial DNA Copy Number and Expression of Mitochondrial Transcriptional Factors in Different Grades of Astrocytomas

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Background: Warburg effect was first described by the author in 1920, as an increased glycolysis in tumours even in the presence of oxygen, and then named aerobic glycolysis. Our oligonucleotide microarray analysis of diffusely infiltrative astrocytomas (grade II to IV astrocytoma WHO classification) have demonstrated an overexpression of genes coding for proteins of glycolytic pathways whereas an underexpression of genes coding for oxidative phosphorylation (OXPHOS) when compared to non-neoplastic brain tissue collected from epilepsy surgery. Considering the contribuition of two distinct genomes, nuclear and mitochondrial, encoding for OXPHOS genes, we have checked the composition of the mitochondrial genome (mtDNA) in these astrocytomas. Surprinsingly, we have found a marked decrease in the copies of mtDNA in paralell to the increase of malignancy, being mostly depleted in glioblastoma (GBM). Human mtDNA is present at extremely high levels (10^3-10^4 copies per cell) and this quantity maintenance depends mainly on nuclear-encoded factors, such as mitochondrial transcription factors A (*TFAM*), B1 (*TFB1M*) and B2 (*TFB2M*). **Objective**: To analize the expression levels of *TFAM*, *TFB1M* and *TFB2M* and to correlate these results with the mtDNA copy number in GBM. **Method**: The mtDNA copy number and the expression of *TFAM*, *TFB1M* and *TFB2M* gene were analyzed by quantitative Real Time PCR by Sybr green method, in 20 GBMs and 10 non-neoplastic brain tissues. **Results**: The relative copy number of mtDNA is reduced in grade I astrocytomas (0.27), grade II (0.36), grade III (0.44), and GBM (0.10, $p<0.001$), when compared to non-neoplasic tissue. Concerning the relative expression of TFB1M and TFB2M, both showed hipoexpression with significant p values (p=0,0001 ; p=0,0015, respectively) compared to non-neoplastic ones. The Pearson correlation showed statistical significance ($p<0.05$) between mtDNA copy number and TFB2M transcript levels, as well as between TFAM and TFB1M, TFB2M transcript levels. **Conclusion**: The present results suggest that the depletion of mtDNA might be explained by low expression of mitochondrial factors B1 and B2 in GBM.

FAPESP and CNPq

PT.134

Expression of the Interferon Receptors and 2´5´ Oligoadenylate Synthetase in Cervical Intraepitelial Neoplasia

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Introduction: Interferons are potent cytokines that performing various biological functions by stimulating cells to activate a series of interferon-stimulated genes that encode proteins with antiviral activity, and demonstrated antiproliferative and immunodulatory activities. The interferons interact with its receptor on the surface of cells (IFNAR), which are widely expressed in tumor cells and the immune system. The synthesis of proteins involved in intracellular resistance against viruses includes the production of 2´5´OAS. This enzyme binds with high affinity to an RNase L and endoribonuclease that catalyzes the cleavage of single mRNA and rRNA tapes leading to the inhibition of viral proteins. **Objectives:** The present study evaluated by RT-PCR the expression of interferon alpha receptor (IFNAR) and the 2´5´OAS enzyme in cells from injury high squamous intraepitelial lesions (HSIL) and low squamous intraepithelial lesions (LSIL) induced by papilloma virus human (HPV). **Methods**: Biopsy of uterine cervix was collected with injury of high and low degree, (HSIL) (n = 28) and controls (LSIL) (n = 17) without injury or infection with HPV. Levels of mRNA of the IFNAR and 2´5´OAS were determined by RT-PCR. The cDNA was submitted to amplification with primers specific for IFNAR2 and 2´5´OAS, after the transcript. The PCR fragments were revealed in the polyacrylamide gel. **Results**: The expression levels of IFNAR2 was larger in the samples controls (8/17) than in the patients with lesions (2 / 28 p=0.018), the enzyme expression levels 2'5'OAS was larger in patients with lesions (8/28), than in the samples control (2/17). **Conclusion:** Our results show that the samples with lesions induced by HPV had a low expression of the receptor. Recent studies indicate that different viruses are involved with mechanisms to counter the mechanisms of protection mediated by interferon. In addition, a number of virus-encoded antagonists of the IFN system are produced; the action of the interferon system that are encoded by viruses that influence in the expression of the receptors of IFN-a/b. The system 2´5´AOS is suggested that has a role with performance antiviral as effect of IFNs-a/b, our results confirm these data from the literature showing a broad activation of

the enzyme in patients with lesions. The reduction of the receptor may be linked to a mechanism of virus evasion or adaptation of the tumor cells to inhibit the immune system.
Financial support - FAPEMIG

PT.135

Intraoperative Autologous Blood Recovery in Prostate Cancer Surgery: *IN VIVO* Validation Using *GSTP1* Promoter Hypermethylation as a Tumour Marker

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Background: Despite of blood loss**,** intraoperative autologous blood recovery (IABR) during radical retropubic prostatectomy (RRP) is rarely performed due to the potential contamination of tumour cells. Different studies are reported showing that safety is proved only for similar survival between allogeneic and autologous transfusion to oncology patients without standardization. Our previous study assessed this procedure in experimental conditions by using a non specific gene. *GSTP-1* promoter hypermethylation is a specific and sensitive molecular marker for prostate cancer, since it is present in more than 90% of prostate tumours and can be readily detected with PCR-based techniques. Using such tumour marker, we demonstrated that tumour cells could be removed from IABR using leucodepletion filters followed by irradiation. **Methods**: We performed IABR in radical retropubic prostatectomy without reinfusion. Fifty patients with *GSTP-1* promoter hypermethylation in their primary prostate tumours were included in the analysis. Peripheral blood samples were collected during anaesthetic induction and recovered blood was collected throughout the surgery and then submitted to washing, leukoreduction and irradiation. Samples were analysed stepwise for the presence of *GSTP-1* promoter hypermethylation using Real Time methylation specific PCR. **Results:** Positive hypermethylation was found in recovered blood (2 samples), recovered and washed blood (3 samples) and recovered washed and filtered blood (2 samples). After filtration and irradiation of the recovered blood, *GSTP-1* promoter hypermethylation could not be detected in any of the cases analyzed, denoting the absence of viable tumour cells. **Conclusion:** Our results indicate that the risk of disseminating tumour cells during IABR in prostate cancer surgery can be eliminated by blood filtration followed by irradiation using a specific molecular marker for prostate cancer.

PT.136

RAB1A and *LAMB3* MRNA Expression in Tongue Squamous Cell Carcinoma

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Background: The prognosis of tongue squamous cell carcinoma (SCC) patients could be better defined by molecular markers. Two possible candidates, as shown by Differential Display RT-PCR, are *RAB1A* (member RAS oncogene family) and *LAMB3* (laminin, beta 3). *RAB1A* plays a role in membrane vesicles trafficking and *LAMB3* is a mediator of environment signals to the cell. **Objectives:** Determinate the *RAB1A* and *LAMB3* mRNA expression and correlate these findings with clinicopathological parameters in tongue SCC. **Methods:** By using real time PCR, we determined the expression of these genes in 34 fragments of primary tumor and adjacent mucosa of tongue SCC patients. **Results:** No difference was found in the expression of either *RAB1A* or *LAMB3* in matched adjacent mucosa and primary tumor (P = 0.75 and P = 0.27 respectively, paired Wilcoxon test). We also could not find any association between clinicopathological parameters and *RAB1A* or *LAMB3* mRNA expression. *RAB1A* negative (-) patients (expression £ tumor median relative expression) presented a shorter overall survival (median survival (MS): 18.77 months, n = 14) than positive (+) patients (expression > tumor median relative expression; MS: not reached, n = 13, *P* = 0.01, Log Rank). Besides, *RAB1A* - patients presented a shorter disease free survival (MS: 8.83 months, n = 14), than *RAB1A* + patients (MS: not reached, n = 13, *P* = 0.02, Log Rank), as shown by Kaplan Meier survival curve. We did not find any correlation with *LAMB3* mRNA and overall survival or free disease survival. **Conclusion:** *RAB1A* is a candidate prognostic marker in tongue SCC, membrane remodeling is the possible mechanism to be investigated.
Fapesp: 02/01738-9

PT.137

TF and asHTF mRNA Expression in Larynx Squamous Cell Carcinoma

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Background: The prognosis of larynx squamous cell carcinoma (SCC) patients (pts) could be better defined by molecular markers. Two possible candidates are Tissue factor (TF) and splicing alternative tissue factor (asHTF). The TF and its soluble isoform, asHTF, seems to play roles in the blood coagulation and intracellular signaling pathways. Therefore, these genes have been involved in angiogenese, proliferation and metastases process. **Objectives:** Determinate TF and asHTF mRNA expression and correlate these findings with clinical-pathogical parameters in larynx SCC. **Patients and Methods:** The mRNA expression of these genes was determined in primary tumor and adjacent mucosa samples of 36 pts by Real time PCR. The data were correlated with clinical-pathological parameters. **Results:** When we compared the expression of TF and asHTF between primary tumor and matched adjacent mucosa, no significant correlations were found (TF: P=0.158; asHTF: P=0.272 - paired Wilcoxon test). Also, we could not find any correlation between TF and asHTF mRNA expression and pathological parameters (Lymph node status: P=0.224 and 0.310; Tumor size: P=0.225 and 0.414, Differentiation status: P=0.269 and 0.417, TF and asHTF mRNA expression, respectively). Next, pts were classified as TF or asHTF negative (expression ≤) or TF or asHTF positive (expression >) according to tumor median relative expression. By Kaplan Meier survival curves, no significant differences in overall (TF: P= 0.763; asHTF: P=0.640; Log-rank test) or disease free (TF: P= 0.846; asHTF: P=0.692) median survival of categorized pts were found. **Conclusion:** Our data indicate that these genes are not prognostic markers in the population analyzed.
FAPESP 06/53755-5

PT.138

The Isopeptidase USP2a and Fatty Acid Synthase (FAS) Expression in oral Squamous Cell Carcinoma: Clinicopathological Findings

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Background:: Deubiquitinating enzymes or isopeptidases can prevent the destruction of protein substrates through deubiquitination prior to proteasomal degradation. The ubiquitin-specific protease 2a (USP2a) plays a critical role in prostate cancer cell survival through fatty acid synthase (FAS) stabilization. **Objectives**: This study investigates whether gene expression of FAS and USP2a are correlated with the clinicopathological characteristics of oral squamous cell carcinoma (OSCC). **Methods:** Were included in this study 41 patients with OSCC from the Hospital do Cancer A.C. Camargo, São Paulo, Brazil. Clinical and treatment data were obtained from the medical records and all histolopathologic diagnosis were reviewed. OSCC was microdissected using laser capture microdissection and one round of linear mRNA amplification was done based on template switch and T7-driven amplification. USP2a and FAS expression was analyzed by Real Time RT_PCR and compared to normal morphologically tissue. **Results:** Our study showed a strong positive correlation between FAS and USP2a expression in the OSCC samples. Microscopic characteristics as clinical stage and lymphatic embolization were associated with FAS and USP2a expression in Real Time RT_PCR (p=0.050 and 0.049 respectively for FAS) and (p=0.039 and p=0.050 for USP2a) Additionally, FAS and USP2a was significantly associated (p=0.0001). **Conclusion:** These data strongly suggest that high-level expression of FAS in OSCC is linked with USP2a expression and both involved with tumor progression.
FAPESP

PT.139

MBNL2 and MARCKSL1 Differential Gene Expression in Human Gliomas

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Background: The most common tumors of the Central Nervous System are the infiltrative gliomas of astrocytic origin, which are classified by the

WHO in grades I, II, III and IV, according to their histopathology and clinical outcome. The lower-grade (I and II) tumors can progress to high-grade (III and IV) astrocytomas, grade IV (glioblastoma multiforme) being the most infiltrative and fatal of all gliomas. Previous work from our laboratory revealed that the MBNL2 and MARCKSL1 genes are differentially expressed in rat glioma cell lines upon treatment with glucocorticoids, which are commonly used in chemotherapy of these tumors. The muscleblind like 2 (MBNL2) protein displays an RNA-binding activity, playing a role in RNA-dependent protein localization and alternative splicing. The myristoylated alanine-rich C-kinase substrate like 1 (MARCKSL1) protein, also known as MLP1/MRP, has been implicated in membrane-cytoskeletal rearrangement during cell attachment and spreading, secretion and phagocytosis processes. **Objectives:** The MBNL2 and MARCKSL1 genes are involved in important cell processes and may be functionally altered during glioma tumor progression and invasiveness, therefore, this study aims at investigating the role of these genes in glioma progression by quantifying their expression in normal human glial tissue versus clinical glioma samples of different histological grades. **Methods:** Quantitative RT-PCR (qPCR) was used to evaluate the relative expression levels of the MBNL2 and MARCKSL1 genes, with specifically designed primers and the hHMBS gene as an endogenous control. Reactions were carried out in triplicates with non-tumoral (n=7) and glioma grade I, III and IV (n=10) and II (n=12) samples. **Results:** A significant difference was found for MARCKSL1 expression between non-tumoral and grade II ($P<0.01$) or grade III samples ($P<0.05$) (ANOVA test). Also, a slight decrease in MARCKSL1 expression was observed in grade IV, whereas MBNL2 expression was increased in high-grade gliomas. **Conclusion :** The gene expression profiles suggest that MARCKSL1 may be downregulated in glioma progression, whereas MBNL2 seems to be overexpressed in high-grade gliomas. In conclusion, these genes may represent new therapeutic targets for human gliomas and deserve to be further investigated.

FAPESP, CNPq & FINEP

PT.140

Apoptosis Protein Expression in Laryngeal Carcinoma Associated with a Low Frequancy of HPV Infection

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Background: In Brazil, laryngeal malignant lesions represent 2% of all cancers, corresponding to around 3.000 deaths annually. Human Papillomavirus (HPV) has been considered a potential risk factor to laryngeal carcinoma. Some apoptosis proteins like galectin 3 could be altered in carcinogenesis. **Objective:** The aim of the study was evaluated galectin 3 expression HPV-related to promote laryngeal carcinogenesis and metastasis. **Methods:** Sixty-five patients were diagnosed as laryngeal malignant neoplasia and stratified in three subgroups: Out of 65 patients, 10 were in situ carcinoma, 27 invasive carcinoma without metastasis and 28 with metastasis. Twenty-eight of cervical lymph nodes rising from metastatic lesions were also evaluated. Nested PCR method was performed in order to detect and type HPV DNA. Immunohistochemistry method evaluated galectin 3 expression. **Results:** HPV was detected in 7(10, 8%) out of 65 patients, which 1(16.7%) were laryngeal in situ carcinoma, 2(33.3%) invasive carcinoma without metastasis and 4(16.7%) invasive carcinoma with metastasis. Over expression of galectin 3 was observed in 2/10 in situ carcinomas, 21/28 without metastasis, 24/28 with metastasis and 26/28 of lymph nodes. Low and high galectin 3 expression was verified in, respectively in 1 (14.3%) and 6 (85.7%) out of 7 laryngeal tumors harboring HPV .**Conclusion:** High expression of galectin 3 was observed in invasive laryngeal tumors with a significance incidence in lymph nodes, suggesting galectin 3 could be a good marker to metastasis. Despite of HPV infection or not , galectin 3 expression doesn´t have any influence in this lesions.

CAPES, CNPq, FAEPA, FAPESP

PT.141

Expression OF BCL-2, BCL-X and Bax in Oral Squamous Cell Carcinoma and its Relationship with Clinicopathological Features

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Background: Oral squamous cell carcinoma (OSSC)

has a remarkable incidence in Brazilian population and a fairly onerous prognosis. Defects in the regulation of apoptosis contribute to the pathogenesis and progression of cancer. Bcl-2 is an apoptosis inhibiting protein and its association with a favorable outcome is controversial. Different members of Bcl-2 family may promote or inhibit apoptosis. **Objectives:** Assessment of correlations among proteins involved in apoptosis-Bcl-2, Bcl-X and Bax -as well as relationships of these proteins with selected clinicopathological features and survival in OSSC. **Methods:** The expression of the proteins was evaluated using immunohistochemical method in paraffin-embedded specimens of 53 primary OSCC patients treated surgically at a single institution in 1999. All slides were reviewed to apply WHO grading system and Histologic Risk Assessment (HRA). Quantitative computer-assisted analysis was performed on digital images to evaluate the expression of the antibodies. Descriptive statistics were calculated and survival was analyzed using Kaplan-Meier method followed by Cox proportional hazard model. **Results:** Disease-specific 5-year survival (DSS) was 61% (56% overall, and 51% disease-free). Kaplan-Meier analysis defined initial pathologic stage, negative nodes status, presence of lymphocityic response, and Bax expression as being associated with both better overall and DSS. Tongue tumors, worst pattern of invasion, lack of lymphocityic response, perineural invasion, poorly differentiated tumours, and low Bcl-X expression were associated with worst disease-free survival. Cox regression set pathologic stage as an independent prognostic factor for overall and disease-specific survival, while lymphocityic response, perineural invasion and poorly differentiated tumours were predictors of disease-free survival. Bax emerged as an independent marker of favorable disease-specific survival. **Conclusion:** Pathologic stage should be more widely applied in OSCC. Histopathological parameters are useful to predict survival and Bax expression was a marker for good prognosis.
CNPq, CAPES

PT.142

A1, Survivin and c-FLIP Anti-Apoptotic Genes Overexpression in Head and Neck Squamous Cell Carcinoma (HNSCC) Patients

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Background: Head and neck cancers are the 6th most common human neoplasm in the developed world. In Brazil this tumor represent the 8th most common type (INCA, 2006). More than 90% of this cancer type is of squamous origin and common sites include hypopharynx, larynx, oral cavity, nasopharynx, oropharynx, paranasal sinus, nasal cavity, parathyroid and salivary glands. The cause of head and neck squamous cell carcinoma (HNSCC) is multifactorial and, despite recent advances in treatment, the long-term survival rate has remained at 50% with high rates of associated mortality. Late presentation of lesions, lack of suitable markers for early detection and failure of available chemotherapy response in advanced lesions contribute to a poor outcome of HNSCC. In addition, little is known about the molecular mechanisms underlying this type of cancer. Abnormalities in the control of programmed cell death (apoptosis) play an important role in tumorigenesis and this regulation involves a number of anti-apoptotic genes. **Objectives:** We evaluate the expression of anti-apoptotic genes and the resistance to *in vitro* apoptogenic stimulus in oral cavity HNSCC patient samples. **Methods:** Cells isolated from HNSCC tumor and adjacent tissues (normal cells) were used for cDNA synthesis and for apoptogenic stimulus assays. Expression of A1, Survivin and c-FLIP anti-apoptotic genes from HNSCC patients was analyzed and compared to the adjacent tissue from the same patient, by Real Time RT-PCR. Cells were exposed to actinmicin-D, ara-C, etoposide and CHX, for 48 hours and the apoptotic cells were detected by DNA fragmentation analysis. **Results and conclusions:** Overexpression of A1, Survivin and c-FLIP was observed in tumor cells when compared to the adjacent tissue in all patients. Tumor cells treated with apoptogenic stimulus *in vitro* showed increased in the resistance of death with all drugs used. Taken together, these results can correlate the overexpression of anti-apoptogenic genes with increased resistance to chemotherapeutic drugs in HNSCC patients.
Supported by: FAPESP and CNPq.

PT.143

Identication of genes Candidate to Predict Adverse Outcome in Wilms Tumor

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Background: Wilms Tumor (WT) is an embryonic renal cancer composed by three histological components: blastema (BL), epithelia and stroma. Nowadays, with the current therapeutic approach, more than 80% of the WT can be cured and the actual efforts are toward to intensify treatment for patients with poor outcome and reduce therapeutic interventions in the better outcome group. BL is the cellular element with higher clinical impact and differences in gene expression between resistant (RB) and sensitive blastema (SB) may allow the identification of genes able to predict the outcome in WT. **Objectives:** To identify genes or group of genes able to select patients at time of diagnosis for risk-adapted therapy. **Methods:** Twenty-six WT samples, being 13 RB and 13 SB were received from Children Oncology Group. Samples were manual dissected in order to have an enrichment of BL. Total RNA was amplified by a T7-driven methodology and aRNA was hybridized in a customized cDNA platform containing 4,608 human genes. **Results**: Gene expression comparison of RB and SB revealed 69 differentially expressed genes ($p<0.01$), whose fold-change ranged from -2.02 to 1.74. Non-supervised hierarchical clustering based on the expression of the 69 genes discriminated 92.3% of SB from RB. In this set of genes, an exhaustive search for trios was performed and previously selected 20 trios. A detailed analysis of these trios was carried out and one of them, composed by SORCS2, CASP4 and KIAA0913, could precisely separate both, RB and SB groups of samples. We also looked for functional modules that might discriminate RB and SB. To do that, the entire array was classified according to STKE database and non-supervised hierarchical clustering based on expression profile of group of genes belonged to each functional module was performed. Cytoskeleton was the most promissory group of genes, discriminating quite well both classes of samples. **Conclusion:** As expected, high similarity in terms of molecular characterization was observed between RB and SB. However, our data revealed some genes that seem to play key role in progression of Wilms tumor and need to be deeper investigated.
CEPID and FAPESP

PT.144

Alternative Splicing in Head and Neck Tumor

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Background: Alternative splicing is an important molecular mechanism that increases transcriptome and proteome complexity and allows a gene to code for different transcripts and therefore for different proteins with related or even new functions. Abnormal splicing has been linked to the etiology of many diseases, including cancer. Therefore, understanding how alternative splicing contributes to tumorigenesis may lead to the development of new ways to control and treat human diseases. **Objectives and Methods:** In the present study, we have focused on identifying new alternative splicing variants in head and neck squamous cells carcinomas (HNSCC) by using *in silico* and quantitative PCR analysis. After clustering ORESTEs generated from head and neck and thyroid tissues and aligning to the draft human genome sequence (Reis et al, Cancer Res 65:1693,2005), potential new splicing isoforms were subjected to manual curation. In addition, the expression of putative new variants of *MPV17*, *ACTN1*, *CEP192*, *BAT3* and *TRIP6* genes were investigated by real time PCR. These genes are implicated in the metabolism of reactive oxygen species, responses to genotoxic stress, cell adhesion and motility. **Results:** Both alternative and constitutive isoforms of *MPV17* gene, and constitutive *ACTN1* and *TRIP6* isoforms showed higher expression levels in metastatic HNSCCs when compared with the matched normal samples. Otherwise, in non-metastatic tumors, the alternative isoform of *ACTN1* e *CEP192* genes exhibited low expression and the constitutive *BAT3* variant was apparently upregulated. **Conclusions:** Cancer mutations in splice sites and regulatory elements may disrupt a tumor suppressor function or affect the expression of constitutive and alternative variants, contributing to tumor development and progression. Therefore, the analysis of alternative splicing may provide valuable opportunities to define gene-expression signatures of tumors and to draw conclusions on the neoplastic process.
Financial Support: FAPESP, Ludwig Institute, CNPq, CAPES.

PT.145

Comparison of Clinicopathological Features of Oral Squamous Cell Carcinoma in Patients with and Without Recurrence

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Background: Recurrence is one of the main causes of treatment failure after definitive therapy of oral

squamous cell carcinoma (OSCC), contributing significantly for the relative low survival rates of this neoplasia. **Objectives:** The aim of this study was to determine the failure patterns of OSCC and investigate the clinical and histopathological factors involved in different types of recurrence of OSCC, comparing the groups with and without recurrence. **Methods**: The records of all the patients that underwent surgery, regardless of adjuvant radiotherapy, for resectable primary OSCC treated at a single institution during the year of 1999 were identified. Patients with positive surgical margins who were not submitted to neck dissection and had less than 12 months follow-up were excluded. The demographics, site, clinical and pathologic stage, pathologic grading (WHO and Histologic Risk Assessment), treatment, and survival data were collected and statistically analyzed in an attempt to identify factors associated with loco-regional control. Descriptive statistics were calculated for each variable and survival was calculated using the Kaplan-Meier method. For purposes of comparison, patients were divided into 2 groups: those with recurrence (R) (n = 25) and without recurrence (NR) (n = 28). **Results:** Of 53 patients identified, 25 (47,2%) presented a recurrence, 6 (24%) of them showed a further episode of relapse and only two (8%) a third relapse. Distribution according to sex showed a higher percentage of women in R group (28%) when compared to the NR group (11%). Adjuvant radiotherapy was more frequent in NR (71%) group, than R group (56%). Fisher's exact test showed that poorly differentiated tumours (p=0,007), patterns of invasion 4 and 5 (p=0,016) and tongue carcinomas (p=0,02) were associated with R group. The 5-year disease-specific survival rate was 92% for NR and 30% for R group (p <0,0001 log-rank test). **Conclusion:** Clinicopathological features seem to be involved in recurrence for OSCC and should be taken into account for treatment and follow-up of the patients.
CNPq

PT.146

Vimentin and Pancytokeratin Expression in Poorly Differentiated Oral Squamous Cell Carcinomas

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Background: Spindle cell carcinoma (SpCC) is a rare microscopic type of cancer of the mouth and oropharynx thought to arise from squamous cell carcinoma (SCC). It carries a worse prognosis, presenting loss of epithelial characteristics, acquisition of mesenchymal phenotypes, and increased propensity for invasion and metastasis. Hence, SpCC usually shows downregulation of epithelial differentiation markers and progressive acquisition of mesenquimal markers. Poorly differentiated (PD) SCC could represent a previous stage in this spectrum. **Objectives:** This study purpose was to assess immunohistochemical expression of pancytokeratin and vimentin in PD oral SCC in an attempt to seek its common features with SpCC and to investigate the possible correlation of PD oral SCC with survival. **Methods:** The records of 94 OSCC patients submitted to surgery in a single institution during the year of 1999 were identified. 14 of these tumors were classified as poorly differentiated according to WHO histological grading and submitted to immunohistochemical analysis for pancytoceratin and vimentin. Patients with positive surgical margins who were not submitted to neck dissection and had less than 12 months follow-up were excluded and survival analyzes was performed in 53 cases (7 poorly differentiated) using the Kaplan-Meier method. **Results:** Cytokeratin positivity was higher in epithelial areas than in PD areas, whereas vimentin was only focally positive in different areas of the tumor. All 14 cases expressed both markers. Of 7 cases in which survival analysis was done, 6 presented a relapse and 3 died from the cancer. No association of PDSCC with disease-specific survival was found using Kaplan-Meier method (p=0,1818 log-rank test). However, PDSCC was identified as having a statistically significant effect on disease-free survival (p=0,0007 log-rank test). **Conclusions:** The results suggest that immunohistochemical expression of pancytokeratin and vimentin alone in oral SCC is not enough to classify it as SpCC subtype. Clinicopathological features and percentage of positivity are fundamental to define this entity. Patients with PDSCC should be carefully followed to evaluate relapses.
Universidade Federal Fluminense (UFF), Instituto Nacional de Câncer (INCA) Financial support:
CNPq, CAPES

PT.147

Association of the Who Grading System Proposal and the Histologic Risk Assessment with Oral Squamous Cell Carcinoma Prognostic Factors

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Background: TNM System has been used for many decades in an attempt to predict the clinical behavior and therapy for oral squamous cell carcinomas (OSCC). Studies demonstrate that histopathologic grading systems can be important as predictive factors. **Methods:** This study reviews the profile of patients diagnosed with OSCC in INCa during the year of 1999 and analyses the association of its prognostic factors with the results of WHO grading system and Histologic Risk Assessment (HRA). Data collected included: sex, age, tumor site, clinical and pathologic stage, nodes involvement, recurrence and death. Survival analysis was performed through Kaplan-Meier method and log rank test. Differences were considered significant for p value ≤ 0,05. Statistical program for Social Sciences (SPSS) computer software was used for all analysis. **Results:** The sample was composed of 44 patients, 86% were men and 65.9% of them were 60 to 70 years old. Floor of the mouth and tongue were responsible for 61.2% of the cases. In the clinic and histopathologic analysis 63.6% of the patients were in stage II and III. 79.5% had been moderately differentiated (OMS) and 56.8% of intermediate risk (HRA). Metastasis for lymph nodes occurred in 38,6% of the cases, 40.9% of the sample presented recurrence and 72.2% of these were moderately differentiated. In HRA, 41.6% of those in high risk (HRA) recurred. The well and moderately differentiated presented 66.2% of lymph nodes involvement, as well as 66,6% of those in intermediate and low risk (AHR). Amongst the deaths, 86.4% were of moderately and 59.1% of intermediate and low risk. **Conclusion:** The histopathologic grading systems had not shown significant association with the prognostic factors analyzed, probably due to the limited sample size or to the trend of the cases to be grouped in the intermediate category.

CNPq, Capes, FAPERJ

PT.148

Comparative Study of Cleaved Caspase 3 Expression in Oral Hyperplastic, Potentially Cancerous and Carcinoma Lesions

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Background: Mutations and molecular alterations avoiding physiological apoptosis are related to oral carcinogenesis. Caspase 3 is considered the main effector caspase of the apoptosis cascade and its expression has being studied in many malignant neoplasia. **Objectives:** Evaluation of apoptosis, through cleaved caspase 3 detection, in hyperplastic, potentially cancerous and both lower lip and intraoral carcinoma lesions. **Methods:** 20 paraffin-embedded specimens were selected from each lesion group: intraoral and labial fibrous inflammatory hyperplasia, actinic cheilitis, oral leukoplakias with and without epithelial dysplasia and intraoral/lower lip squamous cell carcinoma, thus making a total of 120 specimens. One section of each sample was used for immunohistochemical staining with anti-cleaved caspase 3 antibody. Five high magnification fields (20x) were captured for each positive case and immunohistochemical results were analyzed both descriptive and quantitatively with Image Pro Plus 4.5 software.The apoptotic area index (AAI) were obtained through adding positive area and then dividing by total area of evaluated epithelium. **Results:** AAI was correlated with behavior of studied lesions. Oral squamous cell carcinomas presented AAI greater than other lesions and premalignant lesions had AAI higher than hyperplasias, although this was no statistically significant. Positive cells in hyperplastic lesions were rarely detected. Carcinomas of the lower lip, which are characterized by slower evolution and low recidive rate, had lower AAI than intra-oral samples. Actinic cheilitis without evidence of epithelial dysplasia presented AAI higher than epithelial dysplastic lesions. Inversely, all leukoplakia samples with epithelial dysplasia were positive for cleaved caspase 3. **Conclusion:** Apoptosis ocurrs in different proportions, according with lesion type and etiological factors. More studies are needed in order to determine its application as prognostic factor.

Programa de Pós-graduação em Patologia - Universidade Federal Fluminense

PT.149

Expression and Functional Analyses of M*aternal Embryonic Leucine Zipper Kinase* (*MELK*) in Human Astrocytomas

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Background: Expression of *maternal embryonic leucine zipper kinase* (*MELK*), a member of the AMP serine/threonine kinase family, is frequently elevated in cancer relative to

normal tissues and exhibited multiple features consistent with the potential utility of this gene as an anticancer target and could involved in the mitosis process. Yet unknown about the expression profile of *MELK* in diferent grades of adult human astrocytomas and its involment in the tumorigenesis. **Methods:** We used cDNA from 10 non-neoplastic and 93 tumor samples (12 pilocytic astrocytomas, 13 grade II astrocytomas, 15 grade III astrocytomas and 53 glioblastomas-GBM). The relative expression of *MELK* was analysed by *Quantitative real time RT-PCR (QT-PCR)* using SYBR Green method, compared to non-neoplastic tissues. We also investigated mechanisms of *MELK* over-expression by gene amplification analysis and sequencing promoter region CpG island and the biological consequences of *MELK* downregulation for tumor cell proliferation, anchorage independent growth, motility and apoptosis in malignant astrocytoma cell lines. **Results:** We found the highest expression of *MELK* in GBM when compared to non-neoplastic, pilocytic, grade II and grade III astrocytomas (p=0.0005; 0.0005; 0.0016 and 0.0046, respectively). We found neither gene promoter hypomethylation nor gene amplification to be a factor in *MELK* expression but we demonstrated that *MELK* knockdown in malignant astrocytoma cell lines caused a growth-inhibitory response as determined by the MTT cell proliferation and anchorage-independent growth assays but no effect was observed in cell migration. We also show a modest but significant increase in both pro-caspase-3 expression and in propidium iodide/annexin-V-stained cells indicating that *MELK* may have a role in inhibiting apoptosis. **Conclusion:** Our results suggested that *MELK* may have important implication in the tumorigenesis of astrocytomas and therefore constitute a novel therapeutic target for the management of this disease.

FAPESP and Ludwig Institute for Cancer Research

PT.150

Molecular and Functional Study of Two Potential Protein Markers in Oral Cancer

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The identification of gene and protein expression profiles in tumors and in their normal counterparts may lead to the discovery of new markers that can distinguish normal from neoplastic cells, and may provide new molecular targets for chemoprevention and treatment. Actually, we are studying two protein markers in HNSCC: SET and HNRNPK which showed different expression in some tumors. The SET protein has been showed as an inhibitor of histone acetyltransferase, inhibits active demethylation of DNA, integrating DNA methylation and transcriptional silencing. HNRNPK is an important regulator of transcription and one of targets is c-myc gene. There are many results suggesting these proteins as potential targets in cancer and other pathologies. Our proposal was obtain the recombinant proteins for structural and molecular analysis and develop an assay to verify the protein expression. The expression in *E. coli* and purification has been obtained successfully in enough quantities for circular dicroism, EMSA assay and detection system. The pure recombinant human proteins have been used to production of antibodies and to generate antigen ELISA plates. We expressed and purified the HNRPK protein isoform a and b, and evaluated the binding to pyrimidine rich strech (oligo DNA) using EMSA assays. Our data showed similar binding protein-DNA and the CD spectra of both proteins proved that the recombinants proteins are structured and functional. We estimated the composition of secondary structure of total SET and HNRPNK proteins. The ELISA plates have been prepared and the determination of these two proteins performed. Actually, the system is working and we will use it to test biological samples. The development of new strategies for evaluation of tumor proteins in routine is very important and constitutes a new way for us. Also, our work is the first investigation to demonstrate secondary composition of both proteins and similar binding of hnRNPK isoforms in ssDNA. This approach follows new insights to discovery of new therapeutic targets and drugs in cancer.

FAPESP, CNPq, Rede Proteômica do RJ

PT.151

Identification of Supposedly Involved Genes With Tumoral Invasion of breast Cancer by Rash Methodology

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Background: Breast cancer occurs at high frequency in world-wide female population. Metastasis is the main cause of death and invasion of tumor cells into adjacent tissues means the first step to its establishment. Identification of genes involved in early stages of this process may contribute to its better understanding, and may be tested as molecular markers of invasion and prognosis. **Aim:** Identify differentially expressed genes between matched ductal carcinoma *in situ* (DCIS) and invasive (IDC) that might participate in the first step of metastasis, which is tumor invasion. **Methods:** Breast cancer samples were laser capture microdissected and the extracted RNA was amplified with 1 round using T7 based methodology. Then subtractive libraries of cDNA were confectioned (RaSH-*Rapid Subtraction Hybridization*) **Results:** After few alterations in RaSH original protocol to use amplified RNA, it was generated 4 subtractive libraries from two cases of matched samples (two IDC-tester and two DCIS-tester libraries). It was identified 443 specific genes among the 4 libraries. Only 7 specific genes had similar behavior between the two cases witch were submitted to validation of differential expression by qRT-PCR, and 5 of them presented reproducible experimental data and only FN1 (3,1 up-regulation in IDC) and METTL3 (3,25 up-regulation in DCIS) were confirmed. To aim a large scale differential expression confirmation using an independent group of samples a customized microarray platform was designed. From 443 specific genes 414 were selected to be immobilized. The platform was hybridized with amplified RNA from laser microdissected cells from an independent group of 6 matched DCIS-IDC samples. Comparative analysis between the 6 DCIS and 6 IDC samples identified 5 differentially expressed genes witch agree with RaSH results. An individual analysis of 6 matched samples (pairwise comparison) identified 114 differentially expressed genes (1.5 fold) which 15 of them were found in at least 2 pairwise comparisons. After using the independent group of samples to analyse ten candidate genes (7 especific genes with similar behavior between the two libraries, 2 genes from comparative analyses and 1 gene from pairwise comparision) by qRT-PCR- it became possible to select 4 candidate genes: KRT19, METTL3, TFF1 and TRIP6 (2.0 fold; at least in two samples) **Conclusion:** This study identified candidate genes that may present key role in tumor invasion mechanism in breast ductal carcinoma being potential candidates to be tested as molecular markers of invasion and/or prognosis.
CNPq

PT.152

Altered Gene Expression of Adhesion Molecules and Their Role in the Malignant Transformation of Nevi

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Cutaneous melanoma arises from the malignant transformation of melanocytes. Although melanoma is curable by surgery when diagnosis occurs during its early stages, the prognosis of advanced melanoma is poor due to the high metastatic potential and failure to clinical treatment. A sequence of steps for tumor progression has been proposed: commom nevi, dysplastic nevi, RGP (radial growth phase) and VGP (vertical growth phase) primary melanomas, and metastatic melanomas. Nevi are benign lesions composed by melanocytes with focal proliferation, organized in nests and temporally restricted in their growth.They are both precursors and markers for melanoma. Thus, by establishing the molecular profile of nevi, and comparing it to those of melanomas, we may be able to both improve our knowledge on the biology of nevi and identify genes involved in the early steps of tumor progression. We performed a systematic analysis of genes expressed by 23 nevi samples (intradermal and compound) and 18 melanoma samples using cDNA microarray technology. Our results showed substantial differences in global gene expression between nevi and melanomas, as samples were precisely grouped by non-supervised clustering methods. Moreover, we identified 510 molecular classifiers able to distinguish samples

with 100% efficiency. Thirteen functional modules showed alterations with statistical significance between nevi and melanoma. Among them were the modules corresponding to genes involved with intercellular junction, cell communication and cell adhesion, highlighting the importance of cellular communication of melanocytes in their microenvironment. Several genes from these modules were further studied. Desmocollin 3 and Claudin 4 were investigated by RT-PCR, and were downregulated in neoplastic lesions. Kalikrein 7 and members of the claudin family (claudin 7 and 11) had decreased expression at the protein level, as observed using immunohistochemistry. In summary, our data pointed to molecular differences between benign nevus and primary melanoma, particularly in modules of cell adhesion and cell communication that could help in the better understanding of the biology of nevi and melanomas.

CNPq and CEPID/FAPESP

PT.153

Search for Tissue-Associated Molecular Markers in Non-Characterized Transcribed Regions of the Human Genome

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The Human Cancer Genome Project (LICR/FAPESP) generated sequence data from 1,190,044 cDNA's fragments through ORESTES methodology, from RNAs extracts from 24 different tissues [1, 2]. About 30% of the sequences produced in this Project did not align to cDNA sequences (ESTs) generated by other projects kept in public databases. Focusing on the transcriptional activity of the human genome and supposing that part of these ESTs could represent new human transcripts, a pipeline was developed in the Bioinformatics Laboratory from the A. C. Camargo Hospital in order to maximize the chances of *in silico* identification of these new transcripts [3]. Taking the data generated by this pipeline, we constructed a cDNA microarray containing 4,355 ORESTES sequences with high probability of representing new transcripts. This array was hybridized against 56 cDNAs from normal or tumoral tissues from different topographic regions. The data generated was analyzed by several mathematic and statistical tools, using strict criteria to eliminate possible false-positive results. At the end of the analyses, we identified, at least, 3,194 sequences that might represent new human transcripts [4]. Today the idea of the human genome being pervasively transcribed and the non-coding regions playing an important role in the regulation of the human genome is rising [5, 6]. Supposing that part of the 3,194 sequences that might represent new human transcripts could represent non-coding RNAs and products of alternative splicing and because of the value of these type of sequences like molecular marker for diagnosis, prognosis and for cancer treatment, we propose a new computational analysis of the sequences that compound the array used in the first work emphasizing their classification as non-coding or splice variant as well as analysis of differential expression of themselves, searching for expression variation of transcripts tissue-associated, tumor-associated, and tumor/tissue-associated, and posterior biological validation in normal or tumor tissue samples through real-time PCR. [1] Brentani H, Caballero OL, Camargo AA, *et al*. The generation and utilization of a cancer-oriented representation of the human transcriptome by using expressed sequence tags. Proc Natl Acad Sci USA 2003; 100:13418-23. [2] Camargo AA, Samaia HP, Dias-Neto E, *et al*. The contribution of 700,000 ORF sequence tags to the definition of the human transcriptome. Proc Natl Acad Sci USA 2001; 98:12103-8. Erratum in: Proc Natl Acad Sci USA 2004; 101:414. Melo, M [corrected to Melo, MB]. [3] Fonseca RS. Avaliação das Oreste no Match geradas pelo Projeto Genoma Humano do Câncer (LICR/FAPESP-HCGP). São Paulo; 2005. [Dissertação de Mestrado-Fundação Antônio Prudente]. [4] Mello, BP. Identificação de novos transcritos humanos através da exploração racional do banco de dados do projeto genoma do câncer humano (hcgp). São Paulo; 2007. [Dissertação de Mestrado-Fundação Antônio Prudente]. [5] ENCODE Project Consortium. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature. 2007 Jun 14;447(7146):799-816. [6] Mendes Soares LM, Valcarcel J. The expanding transcriptome: the genome as the ´Book of Sand´. EMBO J. 2006 Mar 8;25(5):923-31. Epub 2006 Mar 2.

FAPESP, CNPq

PT.154

High-Throughput Identification and Gene Expression Analysis of Novel Antisense Transcripts Using MPSS

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Background: Natural Antisense Transcripts (NATs) are RNA messages containing sequences that are complementary to other endogenous RNAs. Due to the diverse mechanisms in which NATs can affect the expression of eukaryotic genes, it is not surprising that changes in antisense transcription could lead to abnormal gene expression patterns and in this way contribute to the development of pathological states, such as cancer. **Objective:** Our aim is to identify, validate and evaluate the gene expression pattern of new NATs present in the human genome using information generated by Massively Parallel Signature Sequencing (MPSS). **Results:** Using computational tools, a total of 5.580.158 unique virtual MPSS tags mapped once in the genomic sequence were identified. This set of virtual tags was then compared to a list of unique experimental tags observed in 41 MPSS libraries generated from different human tissues. A total of 340,829 unique MPSS tags, present in both the virtual and the experimental datasets, were used to identify tags that mapped on the opposite strand of both intron and exons of known human transcripts. Excluding NATs represented by other transcribed sequences such as mRNA or ESTs, we identified 4308 new NATs exclusively represented by MPSS tags. We then used the GLGI-MPSS technique to convert 96 antisense MPSS tags into their corresponding 3′ cDNA fragments. We were able to generate 27 specific 3′ cDNA fragments presenting the expected alignment and orientation. Based on the annotation of the corresponding sense transcripts, 9 of these 27 NATs were selected for experimental validation using strand specific RT-PCR. The existence of 8 of these novel NATs was confirmed and their expression patterns are currently under evaluation. **Conclusions:** The strategy used in this project allowed the identification of 4308 novel NATs exclusively represented by MPSS tags.

Financial Support: FAPESP, CNPq and Ludwig Institute for Cancer Research

PT.155

Utilization of Expressed Sequence Data fot the Identification and Characterization of Novel Cancer/testis Antigens

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Cancer/testis (CT) antigens are a subgroup of tumor antigens with a restricted expression in normal testis and in different types of tumors. These antigens are capable of eliciting humoral and cellular immune response in cancer patients and due to their restricted expression pattern, they are considered promising candidates for cancer immunotherapy. Our goal consisted on the identification of novel CT antigen candidates using an *in silico* expression analysis followed by experimental validation of the expression pattern using RT-PCR and evaluation of humoral immune response against the candidates in cancer patients using immunoblotting. Expressed sequences (mRNA and ESTs) were aligned against the human genome sequence allowing the clustering of the expressed sequences derived from a same gene. Considering the tissue origin of the expressed sequences in a cluster it was possible to define an *in silico* expression pattern for each gene and to select clusters composed of ESTs derived from testis and/or tumors. After computational selection the expression pattern of CT candidates was validated by RT-PCR using panels of cDNAs from 21 normal tissues, 17 tumor cell lines and 160 tumor samples. A total of 1255 candidates was identified and 70 of them were selected for experimental validation. We were able to identify 5 CT antigens candidates frequently expressed in different types of tumors (18-65%). Three of the 5 CT candidates have a full-length sequence available and these were chosen to evaluate the presence of humoral response in cancer patients. The respective recombinant proteins were expressed in a bacterial system and antibodies against these proteins were detected in plasma samples from cancer patients and 30 plasmas from healthy blood donors to evaluate its potential for cancer immunotherapy. Moreover we performed RACE experiments to characterize the other two candidates that did not present a full-length mRNA sequence available in public databases. Taken together these results showed that the *in silico* strategy used in this work was efficient in the identification of novel CT antigens.

FAPESP and Ludwig Institute for Cancer Research

PT.156

Introdution of Comparative Genomic Hybridization (CGH) Technique Using cDNA Microarray

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Deletions and amplifications through the genome frequently contribute to alterations in the expression of tumour- suppressor genes and oncogenes and is recognized as a key event during oncogenesis. CGH and, more recently, CGH arrays is providing a great deal of information to access genome wide variations as a function of tumor as well as tumor biology. To establish this technology in our lab, we performed CGH array using genomic DNA from Head and Neck tumors and reference genomic DNA. Differentially labeled DNA was co-hybridized to a cDNA microarray containing approximately 5.000 human genes. Following hybridization, we scanned the microarray to produce a pseudocolour image. The objective of this study is standardizing the CGH technique that goes from the DNA labeling through statistical analysis. DNA digestion was performed with two restriction enzymes, DpnII and EcoRI. The direct labeling protocol uses the fluorophores Cy3 and Cy5 and the hybridization protocol reveal data that exceeds our quality control. We are now performing hybridizations with a series of 20 DNAs derived from head and neck tumors and our goal is to correlate genomic alterations with gene expression levels.
CNPq- Pibic

PT.157

ASPM Expression in Medulloblastoma

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Background: Medulloblastomas are the most common malignant tumors of central nervous system in the childhood. The incidence is about 19-20% between children youngest than 16 years old, and with peak of incidence among 2 and 7 years old. The 5-year survival rates are around 50-60%. Despite its sensibility to no-specific therapeutic like chemotherapy and radiotherapy, the treatment is very aggressive and frequently results in neurological development and growth deficit, and endocrine dysfunction. Hence, new treatment approaches are needed such as molecular targeted therapies. In our study, we validate *ASPM* (abnormal spindle-like microcephaly associated) gene expression as this gene was over-expressed in microarrays investigations. Studies in Glioblastoma demonstrated that *ASPM* gene was over-expressed when compared to normal brain and *ASPM* inhibition by siRNA-mediated inhibits tumor cell proliferation and neural stem cell proliferation, supporting *ASPM* gene as a potential molecular target in Glioblastoma. **Objective:** The aim of our study was to study *ASPM* expression in 12 Medulloblastoma tumor fragments and one normal brain sample as control. **Methods:** *ASPM* mRNA was measured by quantitative real-time RT-PCR. **Results:** All samples over-expressed *ASPM* gene. **Conclusion:** This result suggest that *ASPM* overexpression could be related with brain tumor development and that as first propose for Glioblastoma, in Meduloblastoma *ASPM* could be a good molecular target for therapies.
Supported by: GRAACC/CAPES/FAPESP n° 2004/12133-6

PT.158

Chromosomal Alterations Detection in Primary Tumors and Their Corresponding Sentinel Lymph Node Metastasis Using CGH

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Background: The sentinel lymph node (SLN) is the first node to harbor malignant cells in breast tumors with metastasis, and its positivity is an indicator for axillary lymph node dissection. Genetic alterations in the SLN metastatic lesions may represent the initial genetic events that occur early in the metastatic process, before distant metastasis takes place. Therefore, genetic studies comparing changes in primary breast tumors (PT) and corresponding SLN metastatic lesions are important and can contribute to elucidate the molecular mechanisms of axillary lymph node metastasis development. **Objectives:** To evaluate and differentiate chromosomal alterations present in paired samples of PT and SLN metastatic lesions using CGH. **Methods:** Paraffin embedded sections from 20 patients with PT and SLN metastatic lesions were microdissected for CGH analysis. Tumor and normal reference DNAs were labeled by nick translation and twenty carefully selected metaphase spreads were analyzed from each sample. Gain and amplification was defined when the copy number ratio of tumor DNA/reference DNA were >1.25 and >1.50 respectively, while loss and deletion were considered when the ratios were < 0.75 and < 0.5, respectively. **Results:** In 15 of the 20 cases

there was at least one chromosomal change common to both lesions. In the remaining 5 pairs of lesions the chromosomal alterations differed completely between them. The most frequent changes present in both lesions analyzed were gains of chromosomes 19, 16, 1p32~pter, 20, 17, 12q23~qter, 1q22~pter, 1q22~qter, 18p and losses of chromosomes 13q13~32, 6q13~q23, 2q22~q34. **Conclusion:** In most of the sites where non-random chromosomal alterations were identified, important genes with relevance to breast cancer metastasis are located. Additional studies in matched samples are necessary in order to establish whether distinct groups exist and whether these groups are associated with distinct prognosis, which ultimately could allow patients to receive an individualized cancer treatment.
CNPq, Capes, UFPR

PT.159

Identification of Putative Genes Involved in the Transition from in Situ to Invasive Ductal Carcinoma of the Breast by Combining Laser Capture Microdissection and Microarray

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Introduction: Metastasis is the main cause of death and invasiveness is the first step for its establishment. Therefore, the identification of molecular markers characterizing the transition from in situ (DCIS) to invasive ductal carcinoma (IDC) matched-pair lesions can reveal candidates genes to be tested as early prognostic markers. **Objective:** To characterize the molecular aspects of the transition of DCIS to IDC and identify candidate genes able to predict for the risk of invasion. **Material and Methodos:** Sixteen matched-pair DCIS and IDC and 4 non-neoplasic breast samples were laser capture microdissected in order to obtain homogenous population of epithelial cells from both types of lesions and non-neoplasic tissue. RNA was extract and amplified in two rounds using the T7-based methodology. cDNA microarray was performed using a customized cDNA platform containing 4,608 elements that represent different human genes using reference design with dye swap. Fluorescent intensities of Cy5 and Cy3 channels on each slide were subjected to spot filtering and normalization. Comparison between matched-pair of DCIS and IDC was performed by Pairwise Student test and was considered differentially expressed when the expression level difference was at least 1.5 fold and pvalue<0.05. **Results:** Our results showed a high molecular similarity between DCIS and IDC obtaining only 33 differentially expressed genes with a maximum fold-change of 2.5. Hierarchical clustering based on this set of genes could not separate the histological types, confirming the molecular similarity between the 2 morphologically distinct lesions. Three genes were selected and quantitative RT_PCR was performed confirming the result for one of them. Lumican regulates collagen fibril organization, epithelial cell migration and tissue repair and showed up-regulation in IDC, strongly suggesting its involvement in invasion process. **Conclusion:** Our results lie on the identification of a candidate gene that may be promising molecular marker to predict progression of DCIS to invasive disease.
Supported by: CAPES and CEPID/FAPESP

PT.160

Identification of Intronic RNA Expression in CD34+ Cells of Patients with Myelodysplatic Syndrome by RNA Microarray Analysis

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Myelodysplatic syndromes (MDS) are a group of clonal hematological disorders characterized by ineffective hematopoiesis with morphological evidence of marrow cell dysplasia resulting in peripheral blood cytopenia. Microarray technology has permitted a refined high-throughput mapping of the transcriptional activity in the human genome. RNAs transcribed from intronic regions of genes are involved in a number of processes related to post-transcriptional control of gene expression, and in the regulation of exon-skipping and intron retention. The characterization of intronic transcripts in progenitor cells of MDS patients could be an important strategic to understand the gene expression regulation in this disease. We conducted a pilot study in CD34+ cells of 4 MDS-RARS patients and 4 healthy individuals. Gene expression analysis was performed using a 44k intron-exon oligoarray custom-designed by the group of Verjorvski-Almeida and collaborators and printed by Agilent Technologies. This oligoarray includes probes for protein-coding genes, for sense and antisense

strands of totally intronic noncoding (TIN) and for partially intronic nonconding (PIN) RNAs. CD34+ cells were isolated from bone marrow samples using MACS magnetic columns. The integrity of total extracted RNA was confirmed with the Agilent Bioanalyzer 2100. We amplified 300ng of each total RNA using the Agilent Low RNA Input Fluorescent Linear Amplification Kit PLUS, two-Color and samples were hybridized using the Gene Expression Hybridization Kit (Agilent) and then scanned on a GenePIX 4000B Scanner (Molecular Devices). Data extraction was performed with Agilent Feature Extraction Software 9.5. Each transcript was considered in the analysis only when the intensity was significantly above the average background in all samples. Data were normalized among the samples by quantil using Spotfire DecisionSite® for Microarray Analysis. To identifying genes differentially expressed between MDS-RARS and healthy individuals, we applied the SAM (Significance Analysis of Microarray) approach using as parameters: two-class unpaired response, t-statistic, 1,000 permutations, delta value = 1.89 and FDR <0.001%. After using SAM, a fold change > 2 filter was applied. We identified 139 differentially expressed genes (67 up-regulated and 72 down-regulated), of which 33 were TIN and PIN transcripts (21 up-regulated and 12 down-regulated). These intronic transcripts were grouped according to the main role of the corresponding protein-coding genes transcribed from the same *loci*: gene transcription (ZNF76, CC2D1A, ASXL1, TOP2B, NR4A3 and NR4A2); immune response (CTSH, CTSS, IFI30, NPY, SERPINA1 and PAG1); growth factor and receptor (RP5-1022P6.2, PRG4 and FGFR1OP2); adhesion (PPP1R15A and FN1); cell differentiation (B3GNT5, RALGPS1, C16orf67 and C5orf13); cell cycle and apoptosis (CYFIP2, PPP1R15A, DDX3X and NASP) and cellular trafficking (WIPI1, ICA1 and SLC11A2). These results demonstrated that 22% of the total amount of differentially expressed genes corresponds to TIN and PIN transcripts in CD34+ cells of MDS-RARS patients, suggesting that intronic transcripts can play an important role during the development of myelodysplastic syndrome.
Supported by FAPESP and CNPq.

PT.161

Selection of Endogenous Control Genes for Analysis of Gene Expression in Human Breast Cancer Samples and Cultured Fibroblasts from Lymphnodes

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Background: Selection of appropriate internal reaction control genes is a fundamental step to obtain reproducible results in RT-PCR assays. These housekeeping genes should not vary in the specific investigated cells or tissues, neither be affected by experimental conditions, however, the choice of best reference gene may not be the same among different tissues and cell lines. Thus, identification of the most stable reference genes among samples may allow the calculation of a normalization factor leading to more accurate expression results. **Objective**: Our aim was to identify suitable control genes for real time RT-PCR in fibroblasts obtained from involved and uninvolved lymph nodes from breast cancer patients maintained in cell culture and breast cancer tumor samples. **Methods**: Expression of six frequently used housekeeping genes, including ACTB, GAPDH, RPLP0, GUSB, PPIA and TFRC was assessed in 20 lymph nodes derived fibroblasts and 26 breast cancer tissue samples. After total RNA extraction Real time RT- PCR assay was conducted using SyberGreen system. **Results**: The expression stability of control genes on the basis of non-normalized expression levels was calculated using the by GeNorm application for Microsoft Excel. This measure relies on the principle that the expression ratio of two ideal internal control genes is Identical in all samples, regardless of the experimental condition or cell type. Stability measure was determined for 6 housekeeping genes on 20 fibroblasts samples and 26 breast tumor samples: ACTB (1.08-1.86); GAPDH (1.27-1.57); RPLP0 (1.10-1.74); GUSB (1.26-2.15); PPIA (1.14-2.23); and TFRC (1.54-1.97). **Conclusion:** The three best control genes for normalization of gene expression were GUS, PPIA and RPLP0 for lymph node fibroblasts and GAPDH, RPLP0 and ACTB for human breast cancer tissue. RPLP0 (RIBOSOMAL PHOSPHOPROTEIN, LARGE, P0 RIBOSOMAL PHOSPHOPROTEIN, LARGE, P0) seems to be a good choice as a reference constitutive gene as its expression presents less variation among samples.
FAPESP

PT.162

Differential Gene Expression Analysis in Penile Carcinoma

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Background: Penile cancer is a rare malignancy affecting more than 400 men per year in the UK. There is a substantial geographical variation in the prevalence and incidence of this disease. Studies in Europe and USA show incidence of 0.1–0.9 men per 100 000, while up to 19 men per 100 000 occurs in Asia, Africa, and South America. Yet, Brazil presents a higher incidence. General socioeconomic factors and access to health-care systems might contribute to the discrepancies in this incidence. Studies on the aetiopathogenesis of this cancer have focused on its association with the HPV; however, there have also been several studies on the occurrence of genetic and molecular changes. Without treatment, patients with penile cancer usually die within 2 years after diagnosis of the primary lesion, because of uncontrollable locoregional disease or from distant metastases. **Objectives:** This study aims to evaluate the differential gene expression between penile carcinoma and normal penile tissue. **Material and Methods:** Samples of penile carcinoma and normal tissues was obtained at Tumor Bank of Barretos Cancer Hospital. After RNA extraction and cDNA synthesis the samples were submitted to Rapid hybridization Subtraction (RaSH) methodology for subtractive libraries elaboration**. Results and Discussion:** The RaSH subtractive libraries revels the presence of 57 genes differentially expressed between the samples: 30 tumor samples and 27 control tissues. Among the genes differentially expressed in penile tumor we emphasize the pre-B-cell colony enhancing factor 1 (PBEF1) and the KIAA1033 "no match gene". The PBEF1 gene encodes the adipokine protein that is localized on the bloodstream and has various functions, including the promotion of vascular smooth muscle cell maturation and inhibition of neutrophil apoptosis. These results suggest that these genes could be correlated with the inhibition of apoptosis mechanisms that contribute for disease evolution. **Conclusion:** The putative molecular markers found in this work may help to find the basis for a molecular comprehension of penile cancer, thus improving diagnosis, treatment and outcome for patients with this carcinoma.

FAPESP

PT.163

Identification of Splicing Variants Associated to Breast Tumor Through Microarray and qRT-PCR Technology

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Background: Current analyses have shown that alternative mRNA splicing (AS) appears in at least 60% of human genes and contribute to the wide diversity of transcripts and increase of proteomic complexity. The importance of genetic diversity generated by AS in cancer resides in the possibility to correlate specific variants with clinical information in order to identify tumor markers. **Objective:** To identify splicing variants associated to breast cancer. **Methods:** 270 tumor-associated exons were selected by a computational analysis. Exons were immobilized on a nylon membrane and hybridized with 27 tumor and 5 non-neoplastic breast tissues. T-student test was applied in order to determine differentially expressed exons ($p<0.05$). Fold-change higher than 3 was established as criterion for technical validation by quantitative RT-PCR using the same sample set. For identifying the variants as over expressed, for those confirmed by real time RT-PCR considering fold-change higher than 3, a set of primers was designed for evaluating the expression of the variants that do not contain the exon. For that forward primer was designed in the exon-exon junction and the reverse one was designed into the common exon for both variants. Very stringent expression control was adopted in order to avoid unspecific amplification. **Results:** 63 exons were selected by microarray experiments. From them, 16 were selected for validation by qRT-PCR. By using stringent criteria (fold≥ 3), 3 were confirmed through qRT-PCR as over expressed in breast tumor, such as TRIM37, tripartite motif-containing 37, MK-STYX, a map kinase phosphatase-like protein and BRRN1, barren homolog (Drosophila). At the moment, for 2 of them (TRIM37 and MK-STYX) the variants that does not contain the exon were also evaluated by qRT-PCR showing over expression in breast tumor as well. In spite of both have been over expressed in tumor samples, the balance between the variants was significantly different, suggesting that they are indeed tumor associated variants. These candidates will be analyzed in an independent set of samples and correlated with clinical information.

CEPID/ FAPESP

PT.164

The Actin Cytoskeleton Regulating Genes WASF2, Plexin C1 and Semaphorin 6A are Downregulated in Glioblastoma

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Background: Gliomas are the most common type of tumors in the central nervous system. These tumors are usually lethal because of their infiltrating properties and tendency to undergo malignant transformation, features that turn treatment or surgical interventions inefficient. Recently obtained progress with high throughput analysis has shown that molecular characterization is essential to better understand tumor biology, generate more precise diagnosis and provide subsides for development of new therapies. **Objective:** In order to find new genes associated to glioma invasiveness, we exploited a set of 1,500 genes previously identified as potentially different expressed in multiforme glioblastoma (GBM), the most aggressive type of glioma. **Methods:** We submitted this set of genes to metabolic pathway analysis using the WebGestalt software and manually inspected literature information for the genes belonging to all classes related with cell motility. Using this *in silico* approach, we selected for further expression analyses 9 genes, related to cell adhesion and motility control, not yet associated with glioma. Expression levels of these genes were analyzed in 23 GBMs and 10 non-neoplasic white matter samples using real time PCR **Results:** Six of the analyzed genes, ROCK1, NAP125, NFAT, EPHB, NCK1 and NCK2, did not present significant differences in RNA expression between tumor and control groups. Three of them, WASF2 (-2,8X), PLXNC1 (-2,2X) and SEMA6A (-4,5X), presented significant lower mRNA levels in tumor tissue when compared to normal white matter. **Conclusion:** These data indicates that WASF2, PLXNC1 and specially SEMA6A, which presented more accentuated difference, are possibly related to infiltrating properties of GBMs.

Financial Support: FAPESP, CNPq and FAEPA.

PT.165

Identification of Splicing Variants Through Construction of Alternative Splicing Enriched cDNA Libraries

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Background: Alternative Splicing (AS) is the phenomenon by which one single gene produces different transcripts becoming a major contributor to protein diversity. Alterations in AS have been related to human diseases, including cancer. Cancer associated transcripts are potential molecular markers, contributing to the development of more accurate diagnosis and prognosis methods. **Objectives:** This study aims to identify splicing variants associated to breast cancer by the construction of an AS enriched cDNA library. **Methods**: One round of amplified RNA obtained from 1 mg total RNA of a human breast cell-line and a pool of invasive breast ductal carcinoma were used for double-strand cDNA synthesis. The dscDNA was denaturated/renaturated inducing formation of heteroduplexes comprehending 2 AS transcripts from the same gene. Next, to 2 consecutive cleavages with *Exonuclease* VII and the restriction endonuclease *Dpn*II was performed. Heteroduplexes were then recovered by biotinilated random oligos and magnetic streptavidin particles and coupled to linkers for PCR reactions, followed by cloning and sequencing. **Results:** A total of 2,140 sequences from both libraries were generated, 2,083 were aligned against the human genome, resulting in 288 consensus of which 107 (37%) presented introns and were aligned against mRNA, EST and prediction databases. From the 107 AS events, 18 (16,8%) were validated *in silico* by databases, 16 (14,9%) were confirmed by gene predictions and 73 (68,3%) were considered as putative novels AS events. Eighteen events were selected for RT_PCR validation of which 5 have already been confirmed (the remaining are still on progress). The expression pattern of 2 of these events was analyzed through qRT-PCR in an independent group of samples. One of them, a novel transcript of the bile-acid-beta-glucosidase gene, showed no amplification for normal samples, suggesting preferential expression in tumoral tissues. We also analyzed 2 distinct variants of the ribosomal gene RPS2. In spite of both variants being less expressed in tumor than normal samples, the balance between them was significantly different.

Support: FAPESP Fundação de Amparo a Pesquisa do Estado de São Paulo

PT.166

Proteomic Approach to Identify Changes in Protein Expression Associated with the Action of BCR-ABL

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Background: Chronic myeloid leukemia is a hematopoietic stem cell disorder caused by the expression of Bcr-Abl fusion protein which results from the Philadelphia translocation. The constitutive tyrosine kinase activity of Bcr-Abl promotes malignant transformation by activating signaling pathways that stimulate uncontrolled cell proliferation, abnormal cell adhesion and resistance to many apoptotic stimuli, including various anticancer agents. It is well known that Bcr-Abl regulates diverse signaling pathways as Ras, PI3-kinase, Jak-STAT and NF-κB, however the precise functions of these signal molecules in leukemic transformation are not fully understood. **Aims:** To investigate the impact of Bcr-Abl on cellular global protein expression profile and identify novel cellular targets of this oncoprotein. **Methods:** Total protein extracts of HL-60 and HL-60.Bcr-Abl cell lines were separated by two-dimensional gel electrophoresis and the 2-D gels were compared to determine differentially expressed proteins possibly directly involved in Bcr-Abl signaling. Changes in spot intensity were deemed significant where the normalized volume altered greater than 1.5-fold between samples. All the selected spots were tryptically digested and protein identification was performed by MALDI-TOF mass spectrometry and peptide mass fingerprinting. **Results:** After Coomassie blue staining, 592 and 532 spots were observed on the gels for HL-60 and HL-60.Bcr-Abl cells, respectively. Global analysis revealed 267 common spots that could be visualized in HL-60 and HL-60.Bcr-Abl gels. From these common spots, 93 were increased and 83 were downregulated as a result of Bcr-Abl expression. Some of the spots represented the same protein. The upregulated proteins are mainly related to cell motility, communication, growth, death, signal transduction and metabolism. Among them we could identify vimentin, alpha- and beta-tubulin, histone-binding protein RBBP4, mannose-6-phosphate receptor-binding protein 1, SET protein, serpin B12, peroxiredoxin-2 and -6 and others. Most of the underexpressed proteins are also associated with signal transduction pathways, cellular motility, adhesion, communication, growth, response to stress and metabolism. Among them we could observe L-plastin, lamin –B1 and B2, actin, 3-phosphoglycerate dehydrogenase, RuvB-like 2 and others. **Conclusion:** In order to evaluate the potential of the differentially expressed proteins identified in this study as therapeutic targets or prognostic markers for CML they will be further investigated.

FAPESP and CNPq

PT.167

Genes Constaining 5' Region CpG Islands are Eneriched Amongst Genes Overexpressed in Primary Melanomas in Comparison to Melanocytes, Nevus and Metastasis

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Introduction: CpG islands (CGI) within the 5' region of many genes has important role on gene expression control. Methylation of the cytosine of this dinucleotide can result in transcriptional silencing, and deregulated methylation is frequently associated to tumorigenesis. **Objective:** Compare the proportion of CGI-containing genes among differentially expressed gene sets, as an indirect measure of epigenetic influence on melanoma progression. **Methods:** Sets of differentially expressed genes in melanoma progression were constructed based on data from: two subtractive suppression libraries, generated in our laboratory using an RGP vs metastatic cell lines; SAGE libraries from vertical growth phase and metastatic tumors (Oncogene 23:2264,2004); a microarray study containing samples from melanocytes, benign nevi and malignant melanomas (~90% primary tumors) (ClinCancerRes 11:7234,2005). Then, using a locally developed program that compare the genomic position of all CpG islands predicted by the software CpGCluster (BMCBioinf 7: 446,2006) with the genomic position of the 5' region of all RefSeqs, we obtained the number of CGI-containing genes for any sets of genes **Results:** Analysis of the gene sets constructed based on SSH and SAGE libraries revealed a significantly (p-value < 0.01) higher proportion of CGI-containing genes within the set overexpressed in primary tumors in comparison to metastasis than in the reverse pattern set. In the microarray data, we observed higher proportion of CGI-containing genes within the set overexpressed in melanoma in comparison to nevi than in the reverse pattern set; and also within the set overexpressed in nevi in comparison to melanocytes than in the reverse pattern set. In addition, we observed a positive correlation between the increase in the number CGI-containing genes and overexpression of *HSP90AA1*, a gene whose product seems to be involved with SMYD3

in the protection against DNA methylation. **Conclusion:** Our data suggest that an event of hypomethylation of gene promoters is taking place in melanoma primary tumors and that members from the HSP90 family might be involved in the establishment or maintenance of this methylation status.
Supported by: CNPq, FAPESP, CAPES, FAEPA

PT.168

Comparative Genomics for the Investigation of Expression Pattern of Splicing Factors and Tumor Associated Splicing Variants

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Alternative splicing allows individual genes to express multiple mRNAs that may encode proteins with diverse and antagonistic functions. Recent studies have suggested that alternative splicing of human genes is the rule and not the exception, and may play an important functional role in human cancers. Alternatively spliced transcripts may be has a great potential to serve as cancer biomarkers for clinical purposes. Spliceosome is the accurate and dynamic splicing machinery constituted by ribonucleoproteins, undergo assembly stages and conformational changes during the splicing reaction. In this study we intend to analyze Massively Parallel Signature Sequencing (MPSS) data in humans and mouse to investigate the expression pattern of splicing factors that might cause the appearance of such tumor associated splicing variants. We visually inspected 254 proteins and manually curated 132 of them. Virtual tags were assigned to 132 curated orthologous genes, encoding spliceosomal proteins in the genomes of human and mouse. The expression pattern of the curated orthologous genes was assessed by analyzing their virtual tags counts in normal and tumor, 51 human and 81 mouse MPSS libraries. Firstly we investigated a significant Pearson product-moment correlation coefficient (r>0.5) between the expression gene of splicing factors in system nervous, gland tissues, sex-specific tissues, and other organs from human and mouse MPSS libraries. The initial approach showed that, the system nervous and sex-specific tissues had the most divergent pattern of expression of splicing factors genes. The unusual patterns of expression seen in the system nervous for splicing factors may contribute to the high level of alternative splicing in this tissue group. With the comparative investigation between the levels of alternative splicing in human and the 132 curated splicing factors genes, we found the overexpression of candidate genes in tumor MPSS libraries. Furthermore, we corroborate *in silico* relevant gene overexpression of some splicing factors that are believed to be involved in oncogenesis pathways. This correlation between the expression of splicing factors and the presence of specific patterns of alternative splicing in human and mouse is also crucial to improve our understanding of the regulatory mechanisms involved in alternative splicing and to elucidate the carcinogenic potential of the alternatively-produced mRNA species.
Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP

PT.169

Direct Infusion of Gamma-Linolenic Acid Into C6 RAT Gliomas Implanted *IN Vivo* Alters the mRNA Expression of Proteins Involved in Angiogenesis, Cell Cycle Control and Invasion

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Introduction: Gamma-linolenic acid (GLA) is an inhibitor of tumour cell proliferation in both *in vitro* and *in vivo* conditions. **Objectives**: The aim of the present study was to identify specific targets of GLA action by analyzing the mRNA expression profiles of proteins of importance for glioma proliferation and invasion. Immunohistochemical analysis of angiogenesis-related proteins was performed by light microscopy. **Methods**: GLA was infused into the tumour bed with Alzet osmotic pumps over a 14 day period at a concentration of 5mM and 0.5µl/hr. **Results**: GLA decreased the expression of E2F1, p16, p53, p65, bax, Ku70, Ku80, PPARα, ERK1, nm23b, matrix metalloprotease 2, prostaglandin receptors EP1, EP2 and EP3, vascular endothelial growth factor A (VEGFA), VEGF receptor Flt1 and cyclooxygenase 2 (COX2). No changes were found for cyclin D1, c-myc, pRb, p21, p27, bcl2, nm23a, tenascin C and prostaglandin receptor EP4. GLA decreased the protein expression of VEGF and its receptor Flt1, but not Flk1. **Conclusions**: The infusion of GLA caused marked changes in mRNA expression of proteins critical to glioma progression and induced a visible reduction in tumour size, with apoptosis identified by TEM. These findings increase our knowledge of the action of gamma-linolenic acid in gliomas and show its effects on both cell cycle and angiogenesis-related pathways, both of which are clear targets for cancer chemotherapy. These data lend further support to the proposed use of gamma-linolenic acid as an adjuvant therapy in the treatment of patients with malignant gliomas.
Financial Support: FAPESP, CNPq

PT.170

Antittumoral Effect of a Synthetic Phenolate Analogue of Natural Product Obtained from Brazilian Propolis in B16F10 Melanoma

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Background: Melanoma is a type of skin cancer least common but also the most dangerous. Is responsible for 75% of the deaths by skin cancer in world, and in the majority of the cases, it produces metastasis and exhibit resistance to conventional treatment. The biggest difficulty of its treatment must it the existing interactions between the tumors cells that if spread and the homeostatic mechanisms that disappear. The DM-1 is a synthetic phenolate analogue of natural product from Brazilian propolis and presents same biological properties including its powerful antitumoral and antioxidant activities. This compound, a sodium phenolate pentadienone, can be easily synthesized by condensation. **Objectives:** We compared cytotoxicity and antiproliferative effects of DM-1 phenolate *in vitro* by treatment of B16F10 melanoma cells, fibroblast and *in vivo* by treatment of mice bearing melanoma. **Methods:** Normal fibroblasts and tumor cells (B16F10) were treated with DM-1 at concentrations ranging from 50 to 0,000095 mg/mL using saline as diluent. The cellular viability was determined by MTT colorimetric assay of the calculated concentration inhibitory (IC_{50}). The treated mice received the compound intraperitoneal (ip) and endovenous (ev) route following 14 days. **Results:** The obtained DM-1 IC_{50} value was 29.6 µg/mL to B16F10 cells and don't were observed antiproliferative effects in normal cells. The treatment by intraperitoneal route increased in 40% the survival rate and by endovenous route the increase was of 37.5% compared with the control group. It was observed a reduction of 84% of the tumors burden in treated groups when compared to the untreated in the endovenous route and of 54% for the intraperitoneal. **Conclusion:** DM-1 was able to induce cytotoxic effect in B16F10 melanoma cells. This effect was not observed toward normal fibroblasts what suggests specificity and selectivity of DM-1 action. Treatment of mice bearing melanoma tumor with DM-1 showed antitumoral effects as well as increased the survival rates demonstrate efficacy of the compound as a potent antineoplasic agent.

FAPESP

PT.171

Glutathione S-Transferase PI (GSTP1) Expression is Associated with Osteosarcoma Chemosensitivity

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Background: Osteosarcoma (OS) is a malignant bone tumor that presents high incidence in children and adolescents. Glutathione S-transferase pi (*GSTP1*) is an enzyme responsible for detoxification of reactive chemical species and plays an important role in anticancer drugs and carcinogens metabolisms. *GSTP1* transcriptional silencing due to CpG island hypermethylation has been related to malignant transformation. On the other hand, *GSTP1* overexpression in several tumors has been associated with increased resistance to chemotherapeutic agents as cisplatin and doxorrubicin that are used in osteosarcoma treatment. **Objectives:** We investigated *GSTP1* gene expression in 41 osteosarcoma specimens and 2 normal bones to evaluate the association with therapeutic response. We also analyzed methylation profile of *GSTP1* promoter region in 9 osteosarcoma specimens. **Methods:** Quantitative real time PCR was carried out using the SYBR®Green system. The investigation of *GSTP1* promoter region hypermethylation was performed through Quantitative Methylation Specific PCR (Q-MSP). **Results:** The quantitative analysis showed *GSTP1* overexpression (P=0.0002) and none of selected samples presented *GSTP1* promoter region methylation. In addition, *GSTP1* overexpression was associated with poor therapeutic response of patients (P=0.020). The overall survival of osteosarcoma patients with different *GSTP1* expression levels revealed a high survival rate from those with normal expression (P=0.036). **Conclusion:** *GSTP1* overexpression is associated with poor therapeutic response of osteosarcoma patients. In this sense, *GSTP1* probably plays a critical role in the complex mechanism of osteosarcoma chemosensitivity, since high *GSTP1* expression decreases osteosarcoma sensitivity to cisplatin and doxorrubicin. These findings confirm the need to new therapeutic approachs that seek for *GSTP1* specific inhibitors to improve the effect of chemotherapeutic agents already used.

Supported by: GRAACC, FAPESP 04/12150-8 and 04/12146-0

PT.172

Serial Transplatation of Transduced Bone Marrow Cells as an *IN VIVO* Assay of Viral Vector Expression

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Background: Retroviruses are often used as gene transfer tools in laboratory and clinical protocols. Our group has developed a new retroviral system, called pCLPG, with the hopes of achieving dynamic and sustained expression of the transgene *in vivo*. Here we present the initial steps in testing this possibility. **Methods:** A C57BL6 mouse model of bone marrow cell (BMC) transplantation was established where the recipient animals were first treated with irradiation, 8.5 Gy. Immediately after, the animals were injected i.v. with $4x10^6$ BMC (previously cultivated in the presence of IL3, IL6 and SCF, with or without transduction). Serial transplantation was performed where donor BMC was isolated from previously transplanted mice and injected in irradiated recipients. Virus-mediated expression of eGFP was monitored in the peripheral blood of recipients of transduced BMC by flow cytometry. Real Time-PCR analysis of genomic DNA recovered from these cells was used to determine the number of provirus per cell as well as the degree of chimerism in the transplanted animals. **Results and Conclusions:** Serial transplantation of non-transduced BMC was successfully performed. After 124 days, the primary recipients were sacrificed and BMC used for the secondary transplant. These animals were maintained for 60 days before collection of BMC for the final transplant. The tertiary transplant group was maintained for 180 (n=2) or 367 (n=1) days. Hematological alterations were observed in the tertiary recipients, with an increase in monocytes, but a decrease in lymphocytes, possibly due to the accumulated age of the BMC (total of 390 days). Serial transplantation of transduced BMC has also been performed successfully, though viral expression *in vivo* was not detected. Preliminary assays suggest that expression may be detectable only when the transduction efficiency of the BMC passes a critical threshold. In this study, we demonstrated that our animal model of serial transplantation was successfully established, though *in vivo* analysis of viral expression is ongoing.

Supported by: FAPESP, CNPq, Millennium Institute - Gene Therapy Network

PT.173

Inhibition of Murine Melanoma Metastasis by the Plant Enterolobium *Contortisiliquum* Trypsin Inhibitor (EcTI)

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Background: Melanoma arises from the malignant transformation of pigment-producing cells (melanocytes), and this process results from complex interactions between genetic and environmental factors. Melanoma is responsible for 75% of the deaths by skin cancer, fundamentally by the metastasis, mainly pulmonary. Studies demonstrate the influence of proteinases on the initiation and progression of melanoma and many investigations attempt to investigate the effect of inhibitors on tumor. **EcTI** is a plant inhibitor that blocks trypsin (K_{iapp}0.88 nM), plasmin (K_{iapp}9.36 nM), chymotrypsin (K_{iapp}1.11 nM), plasma kallikrein (K_{iapp}6.15 nM), neutrophil elastase (K_{iapp}55 nM) and also the activation of MMP-2 and MMP-9. **Objectives:** Animal models that emulate melanoma cancer allow analysis of the mechanisms of action of anti-melanoma cancer agents. The aim of this work is to study the *in vivo* response of EcTI on metastasis development. .**Results:** Female mice C57BL10 (4-8 weeks) were inoculated with Tm5 murine melanoma cell line. Tm5 ($5.0x10^5$ cells/100uL/ mice) were inoculated intravenously in tail vein and the groups were daily treated with intraperitoneal injection of EcTI (4.0mg/Kg/500µl) or saline. After 18-20 days the animals were sacrificed and the tumor examined. The results showed that **EcTI** significantly increased tumor apoptosis with no significant change in p53 protein expression. A significantly decrease (80-90%) in number of lung metastases in **EcTI** treated group was observed. Preliminary results from confocal microscopy analyses indicated that **EcTI** binds to cells surface. **Conclusion:** The inhibitory properties of **EcTI** may be used as a tool in studies of the control of proteinase activation in the melanoma metastasis and consequently, in tumor progression.

Supported by FAPESP, CNPq and SPDM/FADA, Probal (CAPES/DAAD) MCT/CNPq, DECIT/MS and CT-BIOTECNOLOGY

PT.174

Combination of Temozolomide and the Gastrin-Releasing Peptide Receptor Antagonist, RC-

3095, Reduces Glioma Gro with in Rats

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Background: Malignant gliomas have a dismal prognosis despite multi-modality treatments like neurosurgical resection, radiation therapy and chemotherapy. Despite glioblastoma multiforme (GMB) represents only 2% of all adult cancers, the median survival time of patients is less than 2 years, mainly due to its high level of invasion. Recent studies demonstrated that gastrin-releasing peptide (GRP) and its receptors play a role in the development of a variety of cancers including gliomas. This receptor was shown to be over-expressed on GMB and its function is related with cellular grown. **Objectives:** The purpose of this work was to evaluate the synergism between temozolomide (TMZ), a DNA alkylanting agent, with RC-3095, a selective antagonist of the GRP receptor. **Methods:** *In vitro* – MTT assays were performed in order to assess cell viability. Sub-G1 population, as an indication of apoptosis, was evaluated by flow citometry using Propidium Iodide . *In vivo* - C6 glioma cells were implanted by sterotaxic surgery into the striatum of rats and 10 days later, the animals received the drugs intraperitonially during one week. Tumors were evaluated by Hematoxilin-Eosin staining. **Results:** Cell proliferation was reduced in all treatments (TMZ, RC-3095 and TMZ + RC-3095) with the combination of TMZ and RC-3095 being the most effective treatment. On *in vivo* experiments, the control group displayed the biggest tumors [8.8 ±1.6 mm^3], whereas RC-3095 reduced the tumor size, with the strongest effect seen with 0.3mg/kg [1.8±0.5 mm^3]. The combined therapy produced the most expressively reduction in the tumor size [0.7±0.6 mm^3], greater than with either treatment alone. **Conclusion:** The group treated with both drugs produced the biggest reduction in *in vitro* and *in vivo* glioma growth. Although indicating that the combination of TMZ with RC-3095 is favorable in reducing tumor growth, it is not clear if the effect is additive or synergistical. These results are in line with current clinical oncology in which the combination of drugs acting on different pathways normally produces the best results. Supported by: CNPq, PROPESQ, PIBIC/CNPq, ICI-RS.

PT.175

Phosphoethanolanime Synthetic Inhibition of Melanoma B16F10 Growth and Dissemination

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Introduction: The synthetic phosphoethanolamine is an artificially phosphorilated lipofilic molecule obtained through the process of molecular latention by the Analytical Chemistry group of USP/São Carlos. It differs from current molecules because of the absorption level (approximately 90%), and it presents possible anti-inflammatory and pro-apoptotic properties. **Objective:** Evaluate *in vitro* and *in vivo* antitumoral effect of phosphoethanolamine in culture melanoma B16F10 and the treatment of melanoma B16F10 tumor bearing mice. **Methodology:** Groups of 40 Balb-c mice weighting ± 20g were kept in Scientific Division of Butantan Institute. The cytotoxic activity was determinated in tumor cells by MTT colorimetric method, with several different molar concentrations of the drugs. The inhibition concentration rate was determined (IC50%), and its toxicity was also evalueted in normal cells (lymphocytes), through cellular proliferation assays. After 14 days of tumor implantation, dorsal tumor bearing mice were treated with 1.6, 3.3, 6.6, and 9.9□g of phosphoethanolamine (i.p) and saline control group.The mice were treated daily for 14 days and killed after 20 days and analysed the parameters antitumor: volume, number of metastasis nodes and histophatological analysis. Histological samples were submeted a cytochemical test Verloff (evaluation of endothelial vases), and Picrosirius (collagen Type-I). **Results:** Our results show that pharmaceutical formulation induced significant *in vitro* cytotoxic effects on melanoma cells (IC50% 1.7 mg/mL), without showing important effects in normal cells proliferative response. We observed an significant (p=0.0067) tumor reduction volume in all treated mice (2, 9 ± 1, 4 mm^3) in compared to the control group (13,8 ± 6,6 mm^3), as well as the inhibition of tumor dissemination. Histological analyses of treated melanoma tumors presented significant necrosis areas and decreased cellular mass with degeneration cells. Also showed decreased density blood vessel, with extensive hemorrhagic areas. **Conclusions:** The synthetic phosphoethanolamine induces dose dependent *in vitro* cytotoxiciyt , and significant reduction of tumor growth and dissemination of melanoma.
Fapesp

PT.176

Effect of Laser in Treatment of Mice Bearing Melanoma B16F10

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Objectives: Low level lasers are healing lasers that quickly balance and restore the body and cells to a state of homeostasis. Healing lasers work on a cellular level, delivering electrons and photons directly to the cells where they help to produce ATP, stimulate immune responses, alleviate inflammation, ease edema and quickly eradicate pain. The use of low-level laser therapy has become more popular for use in the treatment of several conditions; however, the effects of laser irradiation on tumor cells are very contradictory because of its effects on cellular proliferation. The increasing use of lasers for the removal of pigmented skin lesions has led to a growing risk of erroneously treated malignant melanocytic tumors. **Methods and Results:** The present study B16F10 melanoma cells it was cultivated using RPMI-1640 medium containing 10% fetal calf serum. Mice were injected with $5x10^4$ melanoma tumor cells in dorsal region, after 10 days. Mice bearing dorsal melanoma tumor was irradiated during three days with a 660nm diode laser (InGa-AlP), 50mW power radiation, with energy densities of 3 and 21J/cm^2. The treatment of mice bearing melanoma induced a significant increase in the tumoral volume on the group irradiated with 21J/cm^2 (1.38cm^3) when compared to the group irradiated with 3J/cm^2 (0.51cm^3). The group irradiated with 21J/cm^2 showed an increase in the peritumoral vascularization. **Conclusion:** Histologically, in the tumors irradiated with 21J/cm^2 showed figures of cellular division, cells in apoptosis and intense peri and intratumoral inflammatory infiltration mononuclear leucocytes. The results obtained evidenciated that all parameters employed in this study were effective to increase of burden tumoral, that signalize a less invasive procedure in tumor in the near future.

FUNDAP

PT.177

Hyaluronic Acid Determination in Cranyopharingiomas Patients During Alpha Interferon (IFN-α) Intratumoral Chemoherapy

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Introduction: Craniopharyngioma is the most common of childhood neoplasms in the hypothalamic-hypophyseal region and most common non-glial primary intracranial tumor. Although craniopharyngiomas have a benign nature, they have a malignant clinic evolution. Many therapeutics modalities have been tried to eliminate the tumor or at least to reduce the tumor size to minimize the secondary effects. Cavalheiro et al. (2005) described that the use of alpha Interferon (IFN-α) as an intracystic chemotherapy was able to reduce tumor size and Ierardi et al. (2007) showed the increase of soluble FAS Ligand in the intratumoral liquid during the treatment suggesting the induction of apoptosis through the FAS pathway. Many studies suggest a link between hialuronic acid (HA) and apoptosis, but there are no studies showing the relationship of HA variation induced by IFN-α treatment. **Objectives:** The aim of this study was to verify the HA concentration in the intratumoral liquid of the patients during the IFN-α treatment and correlate it with the tumor size reduction. **Materials and Methods:** Samples of tumor fluid taken in different time points of the IFN-α treatment from nine patients of the Pediatric Oncology Institute (IOP/ GRAACC) of the UNIFESP – Brazil were used to determined the HA concentration. The HA concentration was determined as described by Martins et al. (2003). The tumor size before and after the treatment were monitored by magnetic resonance imaging (MRI) and computed tomography (CT). **Results and Conclusions:** The HA concentration was markedly reduced during the IFN-α injections. Concomitantly, we observed the tumor size reduction in all patients. Our data showed that the IFN-α was able to reduce the HA levels while reduce the craniopharyngioma size. This study open a new window to investigate mechanisms involved with craniopharyngiomas therapy. GRAACC/ FAPESP(#2006/05791-2)

PT.178

Drug Resistance Genes Expression Profile in Acute Leukemias

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Background: One of the most important cause of failure in the treatment of children with acute leukemia is intrinsic or acquired resistance to structurally and functionally unrelated chemotherapeutics. Several cellular mechanisms can give rise to multidrug resistance. Best studied is the transmembrane protein-mediated efflux of cytotoxic compounds that leads to decreased cellular drug accumulation and toxicity. **Objective:** The aim of this study was to evaluate and compare the profile of expression of drug resistance genes in childhood ALL and AML and to correlate their expression levels with age, diagnosis WBC count, risk group, immunophenotype, induction treatment response and event-free survival (EFS) in childhood ALL. **Methods:** The resistance genes expression profile at diagnosis was evaluated by real-time quantitative PCR, in 143 consecutive bone marrow aspirates samples from children with ALL, treated according the GBTLI-99 protocol from August 2002 to December 2004 and 19 children with AML. The association between the analyzed variables and gene expression levels was evaluated using the Mann-Whitney test, EFS by Kaplan-Meier and log-rank test and multivariate analysis by Cox regression model. Overexpression was defined as values higher than the median. **Results:** Gene expression levels were higher in AML patients when compared to ALL to genes MDR1 (p<0,0001), MRP3 (p:0,002) and LRP (p<0,0001). No differences were observed to MRP1 (p:0,95) and BCRP (p:0,66). The 4-years EFS of the overall ALL group was 76,3%. Overexpression of BCRP gene was related to a significant higher 4-years EFS (84,3% vs 68,6%, p: 0,02) with no significance in multivariate analysis. No differences on EFS was observed to MDR1, MRP1, MRP3 and LRP genes (p:0,66, p:0,20, p:0,67 and p:0,47 respectively) or number of genes co-overexpressed (p:0,98). MRP1 was associated with CALLA negative immunophenotype (p:0,004) and BCRP with survival (p:0,02). **Conclusions:** AML patients presented significant higher expression levels to MDR1, MRP3 and LRP genes when compared to those with ALL. These findings could explain in part the higher drug resistance found in AML. Only BCRP gene overexpression was associated with survival in ALL, suggesting a little participation of the other analyzed genes in the treatment failure in childhood ALL.
FAPESP

PT.179

A Synthetic Phenolate Analogue of Natural Product Obtained from Brazilian Propolis Induces Cell Cycle Arrest and Apoptosis in B16F10 Melanoma

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Background: Systemic therapy for melanoma, both as adjuvant therapy and for treatment for disseminated disease, remains unsatisfactory. Few chemotherapeutic agents have demonstrated antitumoral activity against melanoma. The DM-1 is a synthetic phenolate analogue of natural product from Brazilian propolis and presents similar chemical structure of curcumin with same biological properties including its powerful antitumoral and antioxidant activities. **Objectives:** We investigated the role of DM-1 on the growth, metastasis inhibition and cell cycle phases distribution of B16F10 melanoma after allografting in C57BL/6J mice. **Methods:** C57BL/6J mice bearing of dorsal melanoma were divided in three groups: I- control group, II- DM-1 ev. and III- DM-1 ip. The mice were treated with DM-1 for 14 days. After treatment the animals were sacrificed, necropsies and its organs and tumors analyzed with HE. Samples from tumor cells from all groups were collected and analyzed by flow cytometry using propidium iodide methodology. The results were expressed as average values of percentage of cells in each phase of the cell cycle: apoptosis, G0/G1, S and G2/M. **Results:** Analysis of the cell cycle in all groups resulted in the following averages: Apoptosis: Control=7,9±2.3; DM-1 ev.=17,2±5.0; DM-1 ip.=24,8±6,5; G0/G1: Control=29,2±9.9; DM-1 ev.=19,7±3.5; DM-1 ip.=13,8±4.6. The histopathological analysis in mice of the control group exhibited tumor and metastasis

with intense cell proliferation. On the other hand, tests groups which received treatment with DM-1 ev and DM-1 ip, exhibited few morphologic alterations at liver and lung. **Conclusion:** The analysis of the cell cycle showed significant increase in the rate of cells in apoptosis phase and reduction of quiescent cells (G0/G1) which suggests that this compound could act by direct or indirect stimulus of this programmed cell death process. The mice which received treatment with DM-1, intraperitoneal or endovenous, did not present important tissue alterations or tissue toxicity evidences by being treated with DM-1 reveals the high selectivity and specificity in tumor cells. FAPESP

PT.180

Use of NMR to Address Acute Lymphoblastic Leukemia Resistance to Chemotherapy

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Background: Early response to therapy has consistently shown independent prognostic significance in pediatric Acute Lymphoblastic Leukemia (ALL) and can be attributed primarily to the intrinsic resistance/sensitivity of leukemic lymphoblasts to chemotherapy. A metabolomic approach, combining Nuclear Resonance Spectrometry (NMR) and multivariate statistic analysis, can be an important tool for early identification of chemoterapics resistant patients. In this work, we utilized L-asparaginase (ASP), an effective drug that causes depletion of asparagine, an amino acid that is poorly synthesized by leukemic cells. **Objectives:** To standardize the methodology for the detection of ALL resistance/sensitivity to ASP by NMR analysis. **Methods:** In vitro studies were initiated with the ASP-resistant and -sensitive ALL cell lines, 697 and B15, respectively. Cells were cultured in RPMI1640 plus 10% FBS, supplemented or not with ASP (3.6 IU/ml). After 6 hours of incubation, cells were pelleted by centrifugation, washed with PBS and extracted in ice-cold 6% perchloric acid. Culture media were kept for NMR analysis. Lyophilized cell extracts were dissolved in 600 ml of D_2O and then analyzed by NMR. ^1H spectra of samples were acquired using a Varian NMR spectrometer with the resonance frequency of 500 MHz. Quantitative analyses were done using the software Chenomx NMR Suite 4.6 (Chenomx Inc., Edmonton, Canada). **Results:** As expected, ASP treatment caused complete depletion of asparagine and glutamine from culture media. Glutamate and aspartate levels were 5-fold and 2-fold higher in ASP-treated samples, respectively, for both cell lines. Surprisingly, the medium of ASP-treated B15 cells presented twice the amount of acetate as compared with all other samples. In the B15 cell extracts the alanine concentrations were almost undetectable, in contrast to 697 samples. **Conclusion** Asparagine, glutamine, glutamate, aspartate, acetate and alanine are interconnected by complex metabolic pathways. Ongoing studies will help elucidate putative resistance mechanism of leukemic cells to ASP. Our data suggest that acetate and alanine may serve as surrogate markers for ALL resistance/sensitivity to ASP. CNPq

PT.181

Evaluation of Systemic Action of *PFAFFIA GLOMERATA* in the Chemically Inducted Carcinogenesis on the Skin of Hairless Mice

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Background: The search for new synthesized substances from plants can be mostly explained mainly by the intensive research on finding a cure for cancer. The investigation of more efficient and less aggressive antineoplasic agents has stimulated several studies using medicinal plants. The plants of the *Pfaffia* family have been described in the literature as having antineoplasic properties. However these studies do not show the mechanism of action of these plants in the carcinogenesis process. **Objectives:** The goal of this study was to evaluate the effect of systemic administration of the *Pfaffia glomerata* in the chemical carcinogenesis model on the skin of hairless mice using immunohistochemistry for cellular proliferation (PCNA), angiogenesis (VEGF) and apoptosis (bcl-2 and bax). **Methods:** For that we have used 32 hairless mice, female, aged 5 weeks, distributed in control group (C) and experimental groups (E1, E2 e E3). The experimental groups E1, E2 e E3 received systemic administration of the *Pfaffia glomerata* in 200, 400 and 1000 mg/Kg doses, respectively, by oral means, during 15 weeks. Group C received only filtered water. Two weeks after the beginning of the *Pfaffia glomerata* administration, the animals were submitted to chemical carcinogenesis induced by the 0,5% DMBA brushed in the dorsal region. At the 15th week skin biopsy was made and histopathological and immunohistochemical evaluation conducted. **Results:** When comparing C and E groups, the Kruskal-Wallis statistical analysis

has not shown any statistically significant difference for malignancy grading (p=0,8598). The Spearman correlation test showed significant relation for VEGF (p=0,0111) and PCNA (p=0,0296), and no significance for bcl-2 (p=0,1454) and bax (p=0,1319). **Conclusion:** We therefore concluded that the treatment using 200, 400 and 1000mg/Kg of *Pfaffia glomerata* in such an experimental model has not shown antineoplasic action.
CAPES, FUNDUNESP

PT.182

Antimetastatic Activity of the Fatty Acid Synthase Inhibitor Orlistat in a Mouse Melanoma Model

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Background: Fatty acid synthase (FAS) is the enzyme responsible for the endogenous synthesis of the saturated fatty acid palmitate from acetyl-CoA and malonyl-CoA. In contrast to most normal cells, malignant cells depend on FAS activity for growth and survival. FAS is overexpressed in a variety of human cancers including cutaneous melanoma, in which its levels of expression are associated with a poor prognosis and depth of invasion. **Objectives:** The aim of this study was to verify the effect of the FAS inhibitor Orlistat on the metastatic process in a mouse model of melanoma metastasis. **Methods:** B16-F10 cells injected in the peritoneal cavity of C57BL/6 mice metastasize to the mediastinal lymph nodes. Forty-eight hours after cell injections, mice were treated with Orlistat (240 mg/kg/day) and sacrificed 12-15 days later. Samples of the primary tumors were collected and immediately frozen in liquid nitrogen. The lungs, liver, thymus, brain, as well as the primary tumors and metastatic lymph nodes were processed for histological examination. Cell cycle and apoptosis were analyzed by flow cytometry for B16-F10 cell populations treated with Orlistat or siRNA specific for FAS. **Results:** We observed that mice treated with Orlistat exhibited a 52% reduction in the number of mediastinal lymph node metastases, in comparison with the controls. The incubation of B16-F10 cells with Orlistat for 36 hours or siRNA for FAS for 48 hours enhanced the G0-G1 population and dramatically reduced the number of cells in the S phase. In addition, B16-F10 cells treated with Orlistat or siRNA for FAS showed significantly more apoptosis than the control. **Conclusion:** These results suggest that FAS activity is essential for B16-F10 melanoma cell proliferation and survival while its inactivation by Orlistat significantly reduces their metastatic spread. The chemical inhibition of FAS activity could have a potential benefit in association with the current chemotherapy for melanoma.
Supported by FAPESP (Proc. 05/52631-8; 04/13903-0)

PT.183

Continuous *In Vivo* Delivery of Endostatin From Encapsulated Engineered Cells for Tumor Therapy in Mice

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Background: Endostatin, a 20 kDa COOH-terminal fragment of collagen-XVIII, has been shown to act as an antiangiogenic agent, which specifically inhibits the proliferation of endothelial cells and growth of various primary tumors. We have already observed that treatment using "free" transfected murine fibroblast cells, which present a continuous production of this protein, can be an effective therapeutic approach for the treatment of tumors although the presence of tumors in the local of transplantation of the "free" treatment cells, indicate that they are tumorigenic. Therapeutic-expressing cells transplanted in mice, secreting continuously the antiangiogenic protein can be protected from rejection by a semi-permeable immunoisolation system (TheraCyte®). Delivery of endostatin within the systemic circulation with the possibility of withdraw of the device containing the recombinant cells at the finish of the treatment or in a case of an undesirable effect would be a good option for an antiangiogenic therapy. **Objective:** The following work aimed to study the effect of continuous delivery of endostatin by encapsulated cells in the growth of tumors in mice. **Methods:** Ehrlich tumor cells ($4x10^6$) and B16F10 ($1x10^6$) were injected into the left footpad of C3H and C57Bl/6 mice respectively. The treatments began when the tumors reached the thickness of 0,5mm by transplantation of 10^7 LM endostatin-expressing "free" cells, cells injected into an already implanted device (20 days before) or cells injected into the devices which were then implanted s.c. in the animals. The tumor growth curve was determined by measuring the thickness of tumors twice a week and the values were deducted from the thickness of the pads without tumor. **Results:** After 14 days we observed a delayed growth of the tumors of the Ehrlich treated group (44,5%) and

melanoma (53%) compared with the controls and using the device we did not observe the presence of this local tumor and the treatment showed a 38% delay of the Ehrlich and 39% of B16F10 tumors. **Conclusion:** This result indicate that the treatment is efficient due to the continuous liberation of endostatin by transfected cells.

Capes and Fapesp

PT.184

Comparative Study of Synthetic Phosphoetanolamine and Taxol in Treatment of Melanoma B16F10

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Background: The synthetic phosphoethanolamine (PEA-S) is a molecule that presents diverse antitumors activities, pro-apoptotics and antinflamatories. The products of the biotransformation of this composition are related with the maintenance of the mitochondrial transmembranic potential (Deltpsi-m), and with the inhibition of the capacity of cellular proliferation and changes of the transmembrane potential. **Methods:** Mice weighting 20g, were submitted the subcutaneous dorsal implantation of B16F10 cells $5x10^4$ cells/mL. After 14º of the tumor implantation, the mice were treated with 0,0468 and 0,0117 μM PEA-S and 3,75 μM of Taxol for the intraperitoneal route. After 14 days of treatment the mice were necropsies and the tumors cells analyzed by flow cytometry. The phase of the cellular cycle was analyzed in Mod-fit Software. The histological analyses were obtained of the primary tumors and metastasis nodes by the methods cytological (HE) and cytochemical , Verhoeff (blood vessels) and Picrosirius (collagen type I). **Results:** The comparative analyses of treatments showed that PEA-S significantly decrease the proliferative capacity and induced cellular death with enhanced therapeutic effectiveness that the Taxol, however the mice treated with PEA-S, had not presented the collateral effect caused by the chemotherapy. The histological and histochemical analyses of the tumors treated with the PEA-S when compared with the control group presented significant increased necrosis area, density cellular and increased the population with present degeneration DNA and apoptotic. The cytochemical analyses showed deposition of collagen-I and reduction angiogenesis. **Conclusion:** Our results showed that the PEA-S presents potent therapeutic effect compared with o Taxol. The treatment of mice bearing B16F10 melanoma cells with PEA-S increased dead population in tumor, possibly for apoptosis. The PEA-S is a powerfull inhibitor of proliferative capacity of melanoma cells.

FUNDAP e FAPESP

PT.185

Towards the Identification of TNF and Melphalan Molecular Targets in Melanoma

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Background: ILP (Isolated Limb Perfusion) with melphalan (MEL) is an alternative approach in the treatment of non resectable locally advanced cutaneous melanomas of extremity, sparing the limb from amputation. ILP is a locoregional procedure to deliver high doses of drugs to an extremity with minimal systemic and mild local toxicity. The association with the Tumor Necrosis Factor (TNF) improves total complete response rates from 40-50% to 70-90%. The molecular mechanisms underlining this synergistic effect are poorly understood. **Objective:** To determine the gene expression profile of melanoma and its alterations after the treatment with TNF, MEL and TNF+MEL. **Methods:** 6-8 weeks-old male C57BL6 mice were injected subcutaneously, on both flanks, with $5,0x10^5$ of B16F10 melanoma cells. Animals bearing tumors of $1,0cm^2$ (n=9-10 mice/group) received an intravenous injection of TNF(0,05μg/Kg), MEL(12mg/Kg), TNF+MEL or saline (control). One of the tumors was surgically removed 3h after for RNA extraction and histology. Gene expression profile was determined by microarray strategy using a biochip containing 16.128 oligonucleotides. **Results:** The administration of melphalan led to slower tumor growth rate ($p<0,001$), effect that was not modified by the TNF co-administration. Nevertheless, tumors treated with TNF alone or in combination with MEL presented more necrosis ($p=0,017$ and $p=0,014$, respectively) and less mitosis ($p=0,001$). Less mitosis was also observed in response to MEL alone. Three-hour-treatment with MEL or TNF did not affect vascular density. From

the comparison between the gene expression profile by (ANOVA) we identified 118 genes differentially expressed with p<0,05. Among the differentially expressed elements are genes coding for proteins involved in cell adhesion (Pecam1, Tcfe3, Dlg5), apoptosis (Bcl11l, Tia1, Camk2d), signaling pathways (Arhgef6, Flt-1, Pard3), regulation of transcription (Tcfe3, Ifi202b, Irak1bp1, Fabp4,Trp73, Trim24), cell cycle (Cdc7, Tacc2), drug metabolism (Akr1b7), metastatic potential (Trpm1, Mib2) and many other diverse biological functions.
FAPESP

PT.186

Propolis Extract Inhibitis Proliferation and Metastasis in Melanoma B16F10

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Background: Propolis is a chemically complex resinous substance that honeybees produce by mixing their own waxes with resins collected from plant sources. There are reports that propolis has antitumoral, antibacterial, analgesic, anti-inflammatory, antioxidative, immunomodulatory, antiproliferative. Melanoma is the main skin-related letal disease and the incidence and mortality from melanoma are increasing all over the word. The therapeutic effects of propolis and its components claimed by folk medicine have raised the interest in the understanding of the biological properties of this substance related to its chemical composition. **Objectives:** The aim of the present work was to evaluate the antiproliferative and metastasis inhibition activity of red propolis ethanolic extract in mice bearing B16F10 melanoma. **Methods:** B16F10 melanoma cells were maintained as monolayer in plastic culture flasks in RPMI-1640 medium supplemented with 10% fetal bovine serum and antibiotics. Groups of ten C57BL/6J mice were injected with $5x10^4$ cells of B16F10 murine melanoma subcutaneously. Tumor growth was measured and observed weekly and tumor volume calculated. After 10 days the animals bearing dorsal tumors were treated daily by intraperioneal administration. The animals were weighted and the tumor area and volume were measured for 14 days. **Results:** Our results indicated significant reduction in the tumor mass (control group 3.5g ± 0.09 and propolis treated 0.41g ± 0.007) and volume in treated animals in comparison with control group which presented caquexia and increased mortality. **Conclusion:** Intraperitoneal administration of propolis ethanolic extract reduced malignant B16F10 melanoma proliferation and tumor burden and decreased metastasis suggesting a novel antitumor treatment.
FAPESP

PT.187

Evaluation of the Antitumoral Activity of Red Propolis in Association With a Protease in Melanoma

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Background: Propolis is a resinous substance collected by honey bees from different parts of plants. Its chemical composition is complex and varies with geographical origin. A wide range biological properties such as antimicrobial, immunomodulatory, anti-inflammatory, antioxidative, antiviral, antitumor have been described. Recently red propolis from the Brazilian northeast has become a subject of increasing interest for scientists. **Objectives:** The aim of the present work was to evaluate the antitumoral activity of Brazilian red propolis (PV) in melanoma B16F10. **Methods:** Balb/c mice (n=40), 30 days old were inoculated with B16F10 melanoma cells ($5x10^4$) in the dorsal region. After the 12th day implantation were treated during 20 days by epicutaneous application of red propolis gel. The experimental groups were: (I) treated with gel containing a protease, (II) gel and red propolis (0.5%) and (III) gel and red propolis (2.5%). Volume, area, metastasis number and survival rate were evaluated. Phases of the cell cycle (apoptosis, G0/G1, S and G2/M), determined by flow cytometric, histological analysis of the primary tumors and metastasis were analyzed by HE. Cytochemical analysis for the quantification of collagens type I (Sirius-red) and endothelium cells (Verhoeff) were determined in dorsal tumor and metastasis. **Results:** Results showed that the treatment with the formula in gel reduced significantly the area (2.5%PV- 62% and 0.5%PV- 68%) and the tumor volume (2.5%PV-54% and 0.5%PV-67%) when compared with control group. Histopathological aspects after treatment with PV 0.5% showed pigmented dorsal tumors, with rare proliferated cell, necrosis and intratumoral focus of hemorrhage, PV 2.5% treated group dorsal tumors

presented enhanced of necrosis with out hemorrhage. The treatment of PV induced significant reduction of metastasis number, dependent of concentration 2.5% in 75% and 0.5% in 50%. Conclusion: The treatment of melanoma B16F10 with the gel and associated protease of the red propolis applied epicutaneously inhibited the growth and the dissemination of the melanoma cells. Financial support: FAPESP

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Study for Preferential Transduction of Alloreactive T Lymphocytes With a Suicide Gene System for Control of Graft-Versus-Host Disease

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Background: One of major causes of mortality and morbidity after allogeneic hematopoietic precursors transplantation is the occurrence of graft-versus-host-disease (GVHD). GVHD occurs due to donor T lymphocytes recognition of alloantigens expressed in host tissues. The elimination of T cells from the graft prevents the development of GVHD but is also associated with an increase in the relapse rates of leukemia. To control the effects of established GVHD, we propose the use of retroviral vectors to introduce a suicide gene specifically in donor alloreactive T cells, allowing their efficient elimination once GVHD develops. In order to do that, we will take advantage of the fact that those cells can be activated and proliferate *in vitro* in response to host cells and of the properties of retroviral vectors to transduce only cells that are proliferating or activated by the time of infection. **Objectives:** Introduce a suicide gene on alloreactive T cells to prevent GVHD development. **Methods:** As a GVHD model, we perform transplantations of bone marrow and T cells from C57Bl/6 into F1 (C57Bl/6+BALB/c) animals. Donor T cells were stimulated with bone marrow derived dendritic cells (BMDC) or irradiated splenocytes cells from host origin. The proliferation rate was determined by H^3 incorporation or CFSE dilution. **Results:** Donor T cells have a better proliferation response when stimulated *in vitro* with BMDC if compared to irradiated allogeneic spleen stimulation. By stimulating with different proportions of BMDC we determined the best DC/T cell ratio (1/10) for lymphocytes stimulation, with a proliferation peak between days 3 and 5, indicating this as the most appropriated moment for retroviral transduction. **Conclusions:** We have determined the key parameters for *in vitro* alloreactive T cells stimulation. Our next steps will be to perform the transduction of these cells and characterize activation markers and alloreactive response of the transduced population. We intend to use the transduced and control non-transduced cells in the mouse model of GVHD for evaluation of GVHD incidence.

Financial Support : CNPq - Instituto do Milênio Terapia Gênica, FAPERJ, FAF-INCa

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A Mouse Model of Lymphoma-Induced Paralysis due to Spinal Cord Compression and Paravertebral Muscle Degeneration

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Non-Hodgkin´s lymphoma includes more than 20 types of disease that display related symptoms. Experimental models to unravel the physiopathology of this disease and to study the immune response against lymphoma are needed. To this aim, we developed a murine model where C57BL/6 mice were iv injected with different numbers of RMA.egfp (RE) cells and the survival curves, disease and tissue colonization were observed. The disease observed was an ascending, progressive and irreversible paralysis of the tail and posterior legs. It started at day 15 after injection of ^{3}1x10^4 cells and the death occur between days 20-50. Numbers of £1x10^3 RE cells do not induce paralysis, but when re-challenged with 1x10^6 RE cells, the mice were more resistant to paralysis and death. By flow cytometry, we observed that RE cells colonize mainly the bone marrow. Histological, the tumor cells are highly infiltrative, invading liver, spleen, bone marrow and muscle of the vertebral column. Although the cells did not invade the meninges of the spinal cord, the accumulation of tumor cells in the epidural space compresses the central and peripheral nervous systems, contributing with the paralysis. Neither of these events was observed when a mutant cell line-RMA-S-egfp-was injected. The last cell line displays a different pattern of tissue colonization and induces ascites instead of paralysis. In conclusion, we have a mouse model of lymphoma that can be used to a) test novel anticancer drugs; b) study the immune

response against lymphoma; c) unravel the molecular basis of lymphoma-induced paralysis.
Fapesp, Capes, CNPq

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Philadelphia Chromosome in Peripheral Blood Cells from Chronic Myeloid Leukemia Patients Treated With Imatinib

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Background:: The involvement of t(9;22) Philadelphia (Ph) and its gene expression product, BCR-ABL tyrosine kinase oncogene in the pathogenesis of Chronic Myeloid Leukemia (CML) is known. Chemotherapy has inducing presently remissions; however, cytogenetic and hematologic remission in peripheral blood cells can be controversial, when imatinib therapy is used. **Objetive:** To investigate the presence of Ph Chromosome in peripheral blood leukocytes of six chronic myeloid leukemia patients from Cancer Hospital of Cascavel – UOPPECAN, who were receiving chemotherapy with imatinib mesilate . **Methods:** After approval from the Human Ethics Committee of Faculdade Assis Gurgacz, peripheral blood were collected from six patients with clinical and hematological diagnostic of CML. Metaphase chromosomes and G banding preparation were done following National Institutes of Health protocol. **Results:** Five patients presented Ph Chromosome after karyotype analysis. Of these, 100% showed only t(9;22) abnormalities, and 80% took the drug for more than 12 months. Hematological response was displayed in 66,6% of them. **Conclusion:** Imatinib mesilate therapy has been related to variable rates of hematologic and cytogenetic remission. Even though the hematologic remission has been achieved, the impact of the imatinib therapy on cytogenetic remission is promising.