

APPLIED CANCER RESEARCH

SUPPLEMENT

Number 2

November 2005

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BIBLIOTECA

APPLIED CANCER RESEARCH

RULES FOR PUBLICATION

The Applied Cancer Research (ACR) is a comprehensive oncology journal that publishes original articles, case reports, historical articles, editorial comments and reviews focusing on each aspect of cancer research, including biology, epidemiology, pathology, diagnosis and treatment (medical, pediatrics, radiation and surgical oncology). Oncology nursing and palliative care articles are also published. Every original article undergoes peer review.

The ACR accepts original article and case reports on the following areas of oncology: molecular biology, genetics, pathology, head and neck, lung, breast, gastrointestinal tract, genitourinary tract, and skin cancers, endocrine tumors, neurology, gynecology, pediatrics, radiotherapy, clinical oncology, ethics, clinical studies, nursing, rehabilitation and support.

Besides the original articles and case reports, other sections of ACR include: editorial, historical article, letters to the editor, and reviews. Editorials are related to published articles in the same journal number or on subject of interest. Letters to the Editor must refer to published articles in the two last journal numbers, always with the right to reply. Experts will be invited to elaborate review articles on subjects of interest.

The decision to publish or not the manuscript is made by the Editorial Council, Associated Editors, and Editor-in-Chief.

The ACR is published by Tecmedd Editor on acid-free paper.

Instructions to the Authors

Manuscripts submitted to publication must be sent to:

Benedito Mauro Rossi, MD, PhD
Editor-in-Chief
Applied Cancer Research
A.C. Camargo Cancer Hospital
Centro de Estudos
Rua Professor Antônio Prudente, 211
01509-010 - São Paulo, Brazil

The manuscripts must follow the recommendations of the International Committee of Medical Journal Editors – Uniform Requirement for Manuscripts Submitted to Biomedical Journals: writing and editing for biomedical publications, available at URL: <http://www.icmje.org>.

Type of Manuscripts

Original article

Case report: maximum of 1000 words, 3 illustrations and 6 citations. The number of authors must be limited to 5. Abstract shall not be included.

Letter to the editor: It must be related to articles published in the 2 last numbers of the Journal and it must have less than 500 words and 3 authors.

Review: only by Journal Editorial Council invitation.

Ethical Considerations on the Research Development and Reporting

According to the International Committee of Medical Journals Editors, author is considered the individual that: (1) has contributed substantially to the conception, data acquisition, analysis, and interpretation; (2) has participated in the manuscript elaboration and has approved the final version.

In cases of a multicentered group study, individuals with direct responsibility for the manuscript must be identified, and they must fulfill the authorship criteria above described. When submitting the manuscript, the corresponding author must indicate the preferred citation and identify all the authors as well as the group name.

Attainment of financial support, data collection or general research group supervision does not justify the authorship. Each author must take on public responsibility for the content.

Acknowledgements

Every individual that has contributed with the research elaboration but do not fulfill the authorship criteria must be listed in the acknowledgement. Financial and material support must also be cited in this section.

Substantially contributing groups to the article,

without justifying authorship must be listed under the title of "clinical investigators" or "participating investigators" and their function has to be described: "revised critically the article", "collect data", "has offered and took care of patients under study".

Patient privacy and confidentiality

Patients have the right to privacy that cannot be infringed without signed informed consent. Identifying information, including name initials and/or hospital registers must not be published, unless essential and with patient signed consent. It is necessary to report that the participants consent was obtained.

Protection to the subjects of the research

The authors must guarantee the procedures were conducted according to the ethical principles, national legislation and Helsinki Declaration (revised in 2000). The research must be approved by the Institution's Ethical Committee where it has been developed.

Requirements for Manuscript Submission

The articles must be preferably written in English (articles in Portuguese will be accepted with a translation fee), submitted in two printed copies containing submission letter, title page, abstract, text, references, figures and tables. The text must be typed in MS Word®, double space, Times New Roman, 12 pt, in A4 (210x297 mm), with 3 cm margin. It must be annexed to the manuscript: CD or floppy disk with the title page, abstract, text, references, figures and tables. Figures must be in TIFF or EPS format.

It is essential to inform in the title page the e-mail address, fax and phone numbers, and complete corresponding address.

Submission letter

The submission letter with the corresponding author signature in it must accompany the manuscript. In this letter, it must be stated that the article has not been previously published or currently submitted to other journal; each author has contributed to the manuscript elaboration and has approved its content; the authors guarantee originality and validity of the article or case report; and there is no financial interest in the publication. Any financial source other than that from the institution must also be specified.

Signature of the copyright transfer form

The formulary must signed by each author after the acceptance confirmation of the article.

Title page

The title page must contain the following information:

Title of the article;
Article brief description with a maximum of 40 words;
Authors' names (complete), institution and titles;
Name of the institution in which the work was developed;
Corresponding author: name, address, phone number, and e-mail address.
Source of financial supports, if exists;
Words total number, excluding abstract, acknowledgements, legends, and references.
Number of figures and tables.

Abstract and Keywords

The abstract must be presented after the front page and must contain the following sections: Objectives, Material and Methods, Results, and Conclusions.

It must be limited to 250 words.

After abstract, it must be listed 3 to 7 keywords from the Medical Subject Heading (MeSH) in the Index Medicus list.

Text body – original article

The article must contain the following section: introduction, material and methods, results, and discussion.

Introduction

Expose the essence of the study and supply only pertinent references, without reviewing extensively the subject. Expose clearly the study purpose.

Material and Methods

Describe clearly the selection of the subjects (patients or animals, including controls). Identify in detail the methods, equipments, and procedures to allow others to reproduce the study.

Supply reference of the method, including statistical tests.

Results

Present the results in a logical sequence in the text, tables and figures. Do not repeat in the text data presented in the tables or figures.

Discussion

Emphasize new and important aspect of the study. Do not repeat in detail data presented in the *Results*. Include in the discussion the implication of the findings and their limitations.

References

The reference model follows the Vancouver Style (available at URL: <http://www.library.uq.edu.au/training/citation/vancouv.html>) and must be presented in separate page after the conclusion. References must be cited in order of appearance in the text and the citation in the text must be *subscribed*.

Personal communication, not published in observations, submitted manuscript should not be cited. Accepted but not published articles can be listed as "in press".

BOOK

The elements that constitute the reference of a book are: author (listed the surname, space, capital initials, without punctuation between initials); title of publication; edition (from the second edition, abbreviate the word "edition" to "ed."); local of publication (the city name, ";", one space); editor (editor's full name, followed by ";", and space); year of publication (after, introduce "." and one space if the number of pages is added).

Cite full name of all authors if six or less. Book with seven or more authors, cite the first six and then add "*et al.*" Include comma and one space between names. After the last author add "." after the last initial.

Example:

Lodish H, Baltimore D, Berk A, Zipursky SL, Matsudaira P, Darnell J. Molecular cell biology. 3rd ed. New York: Scientific American; 1995.

Book chapter: Porter RJ, Meldrum BS. Antiepileptic drugs. In: Katzung BG, editor. Basic and clinical pharmacology. 6th ed. Norwalk: Appleton and Lange; 1995, p.361-80.

ARTICLES

It must be included the following elements: authors name; article title; journal title (abbreviated according to Medline style). The abbreviations list can be found in URL: <http://www.nlm.nih.gov> (do not punctuate the abbreviation and add one space after journal name); year of the publication (";" after without space); volume (add the number in parenthesis, if necessary, followed by ":" without space); initial and final numbers (do not repeat numbers, after add ".").

Example:

Russel FD, Coppel AL, Davenport AP. In vitro enzymatic processing of radiolabelled big ET-1 in human kidney as a food ingredient. *Biochem Pharmacol* 1998;55:697-701.

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Utilize the following format: author ("," after the last author, one space); title ("," followed one space); year (if applicable, add ";"); number of pages in parenthesis ("," one space); available at ("," followed by one space); URL ("," one space); accessed (add month, day, year and ".").

Example:

Health on the net foundation. Health on the net foundation code of conduct (HONcode) for medical and health web sites. Available at: URL: <http://hon.ch/Conduct.html>. Accessed June 30, 1998.

Tables

Tables must be presented after the references in separate pages and consecutively numbered, in Arabic Numerals in order of citation in the text. The table number must be followed by a brief description. Neither horizontal nor vertical lines should be used. All the non-standardized abbreviations must be described at the end of the table. Permission to reproduce any previously published material must be obtained.

Figures and legends

Figures and illustrations must be consecutively pencil numbered in Arabic Numerals in the back of each figure. Legends must be listed in double space and in a page apart. Patient identification must be withdrawn from the figures, except when patient written permission is included.

It is necessary authorization whenever reproducing a previously published figure.

Digital figures will be accepted in TIFF or EPS format, with the minimum resolution of 300 dpi. Drawings must be sent in the same format above but with minimum resolution of 600 dpi. Each figure and digital drawing must be accompanied by two printed copies.

EDITORIAL

The aim of the "São Paulo Research Conferences" (SPRC) is to put together Brazilian and foreign specialists in different subjects of science and culture, to provide students and other interested parties with an overview on the selected field. Accordingly, the SPRC conferences are not addressed to specialists, which are of course also welcome, but rather to students and young investigators of related topics. Our conferences do not compete with professional and learned societies, but are complementary to them.

Three SPRC have already been held, one on "Molecular Medicine", a second on "Mechanisms of Infection/Vaccines", and a third one on "Asthma and the Hygiene Theory".

The program and abstracts from the fourth conference *Cancer Today: from Molecular Biology to Treatment* are now kindly published by *Applied Cancer Research*. The title expresses our purpose to provide a clinical perspective to recent basic discoveries in the field of cell biology and oncology. This is a very challenging field, which has interested a growing number of Brazilian laboratories. Cancer research in Brazil bloomed in the last 20 years (see figure), leading to the establishment of several groups with different expertises in the area, from epidemiology to genomics and therapeutics. The conference serves as a meeting point to these groups and hopefully will foster new collaborations and concerted initiatives in Cancer Research.

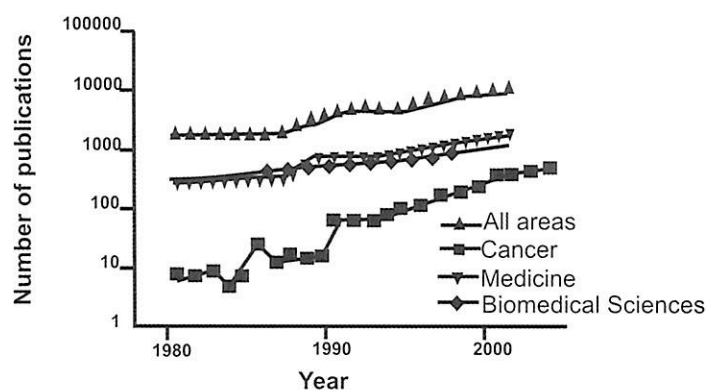


Figure 1 - Brazilian Scientific Production has steadily increased over the last two decades (data from *Ministério da Ciência e Tecnologia* and Institute for Scientific Information). In the early 80's, Cancer Research in Brazil counted with a handful of groups, publishing up to 10 papers/year. Presently, the number of papers on cancer or using tumor models is about 400.

Leading Cancer Research Centers in Brazil, Hospital do Câncer de São Paulo, Instituto Nacional do Câncer (Rio de Janeiro), and the Ludwig Institute for Cancer Research (São Paulo) sponsored this conference held under the auspices of the University of São Paulo. The conference was supported by *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq), *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES), *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP), *Laboratórios de Investigação Médica do Hospital das Clínicas da Faculdade de Medicina da USP* (LIM, HC-FMUSP) and *Division of Oncology, USP Medical School*. We also thank the Brazilian Societies of Pharmacology and Therapeutics (SBFTE), Cell Biology (SBBC) and Immunology (SBI) for their support.

The success of this meeting encourages us for the next conferences, particularly on "The theory of Evolution: principles and impact" which will be held on May 18-20, 2006. We will also proceed with topics of growing interest, including a second conference on "Mechanisms of Infection/Vaccines" in 2006, and another on Cancer, in 2007.

Boris Vargaftig and Roger Chammas
São Paulo Research Conference Organizing Committee

IV SÃO PAULO RESEARCH CONFERENCE

CANCER TODAY: FROM MOLECULAR BIOLOGY TO TREATMENT - PROGRAM

NOVEMBER 10, 2005

9:00-9:30

Keynote lecture

*Chairperson: Gilberto Schwartzmann
Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil*

Contributions of molecular biology to clinical oncology

*Ricardo Brentani
Universidade de São Paulo Medical School and Ludwig Institute for Cancer Research, São Paulo, Brazil*

Session I - Cancer causes: The epidemiologist standpoint

*Discussion leader: José Eluf Neto
Universidade de São Paulo Medical School and Laboratórios de Investigação Médica*

9:30-10:00

Cancer Epidemiology: Triumphs, Pitfalls, and Growing Pains

*Eduardo L F Franco
McGill University, Montreal, Canada*

10:00-10:30

Environmental causes of cancer

*Gulnar Azevedo
Instituto Nacional de Câncer, Rio de Janeiro, Brazil*

10:00-11:00 Coffee break

Session II - Mitotic cell cycle control

*Discussion leader: Hugo A. Armelin
Instituto de Química, Universidade de São Paulo, São Paulo, Brazil*

11:00-11:30

FGF2 and proliferation of tumorigenic cells

*Hugo A. Armelin
Instituto de Química, Universidade de São Paulo, São Paulo, Brazil*

11:30-12:00

Nitric oxide stimulates the Ras-MAPKinases signaling pathway promoting cell cycle progression and proliferation of endothelial cells

*Hugo P. Monteiro
Universidade Federal de São Paulo, São Paulo, Brazil*

12:00-12:30

Cell cycle regulation by NFAT transcription factors

*João Paulo B. Viola
Division of Experimental Medicine, Brazilian National Cancer Institute, Rio de Janeiro, Brazil*

12:30-14:00 Lunch break

Session III - Tyrosine kinases, biology and therapeutic modulation

*Discussion leader: Bernd Groner
Georg Speyer Haus, Germany*

14:00-14:30

ErbB receptors and cancer

*Nancy E. Hynes
Institute for Biomedical Research, Basel, Switzerland*

14:30-15:00

C-kit in hematologic malignancies

*Olivier Hermine
Hopital Necker, France*

15:00-15:30

BRAF mutation in thyroid cancer

*Edna T. Kimura
Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil*

15:30-16:00 Coffee Break

16:00-16:30

BCR-ABL and apoptosis

*Gustavo P. Amarante-Mendes
Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil*

16:30-17:00

Mechanisms of resistance to tyrosine kinase inhibitors

*Sara T. Saad
Universidade Estadual de Campinas -Medical School, Campinas, Brazil*

17:00-17:30

Keynote lecture

*Chairperson: Nancy E. Hynes
Institute for Biomedical Research, Basel, Switzerland
PDGF signaling and kinase inhibitors
Carl H Heldin
Ludwig Institute for Cancer Research, Uppsala, Sweden*

17:30-19:30 Poster Session 1

NOVEMBER 11, 2005

9:00-9:30

Keynote lecture

Chairperson: Carl H Heldin

Ludwig Institute for Cancer Research, Uppsala, Sweden

Interference with crucial signal transduction events in cancer cells as a therapeutic principle

Bernd Groner

Georg Speyer Haus, Germany

Session IV - Tumor Suppressor Networks

Discussion leader: Mari Sogayar

9:30-10:00

Search for new molecular targets for tumor diagnosis and therapy

Mari Sogayar

Instituto de Química, Universidade de São Paulo, São Paulo, Brazil

10:00-10:30

Structural Biology of the Metastable States of the Tumor Suppressor Protein p53: New Targets for the Development of Antineoplastic Drugs

Jerson L. Silva

Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

10:30-11:00 Coffee Break

11:00-11:30

Molecular mechanisms of leukemogenesis

Eduardo Rego

Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brazil

11:30-12:00

Keynote lecture

Chairperson: Boris Vargaftig

Instituto Butantan de Ciências Biomédicas, Universidade de São Paulo São Paulo, Brazil

Mouse cancer modifier loci: human relevance

Tommaso Dragani

Istituto Nazionale Tumori, Milan, Italy

12:00-13:30 Lunch Break and Pfizer Satellite Symposium

Session V - Cell death and survival

Discussion leader: Marcello Barcinsky

Instituto de Ciências Biomédicas, Universidade de São Paulo and Instituto Nacional do Câncer, Rio de Janeiro, Brazil

13:30-14:00

Targeting early events of programmed cell death in the central nervous system

Rafael Linden

Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

14:00-14:30

Apoptin and cancer gene therapy

Mahvash Tavassoli

King's College London, London, United Kingdom

14:30-15:00

Targeted therapy in oncology: Lessons learned from translational studies

Carlos Gil Ferreira

Instituto Nacional do Câncer, Rio de Janeiro, Brazil

15:00-15:30 Coffee Break

Session VI - Tumor as microenvironments

Discussion leader: Helena Bonciani Nader

Universidade Federal de São Paulo, São Paulo, Brazil

15:30-16:00

Bone marrow microenvironment and tumor growth

Radovan Borojevic

Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

16:00-16:30

Prostate tumour cells: role for GPCRs in regulated secretion of lysosomal proteases

Júlio Scharfstein

Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

16:30-17:00

Serine protease inhibitors and cancer

Jair R. Chagas

Universidade de Mogi das Cruzes, São Paulo, Brazil

17:00-17:30

Abnormal gene expression of chronic lymphoproliferative diseases

Marco Antonio Zago

Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brazil.

17:30-19:30 Poster Session II

NOVEMBER 12, 2005

9:00-9:30

Keynote lecture

Chairperson: Roger Chammas

Universidade de São Paulo-Medical School, São Paulo, Brazil

Imag(in)ing the tumor microenvironment and its relation to progression and therapy

Robert Gillies

University of Arizona, USA

Session VII - Tumor Immunology and cancer vaccines

Discussion leader: Adriana Bonomo

Instituto Nacional de Câncer, Rio de Janeiro, Brazil

9:30-10:00

Prevention of cervical cancer in the era of vaccines against HPV

Luisa Lina Villa

Ludwig Institute for Cancer Research, Sao Paulo, Brazil

10:00-10:30

Dendritic cells in cancer immunotherapy

J. Alexandre Barbuto

Instituto de Ciências Biomédicas- Universidade de São Paulo, São Paulo, Brazil

10:30-11:00 Coffee Break

11:00-11:30

Keynote lecture

Chairperson: Luiz Fernando Lima Reis

Ludwig Institute for Cancer Research, São Paulo, Brazil

Chromatin Modifying Enzymes Which Mediate Gene Silencing as New Therapeutic Targets in Cancer

Frank Rauscher III

The Wistar Institute, Philadelphia, USA

11:30-13:30 Lunch Break

Session VIII - Post-genomic cancer biology and clinical oncology

Discussion leader: Guilherme Suarez-Kurtz

Instituto Nacional do Câncer, Rio de Janeiro, Brazil

13:30-14:00

Contribution of proteomics to molecular diagnostics

Samir Hanash

Fred Hutchinson Cancer Research Center, Seattle, USA.

14:00-14:30

Molecular Profile in Cancer Research: from basics to applications

Luiz Fernando Lima Reis

Ludwig Institute for Cancer Research, Sao Paulo, Brazil

14:30-15:00

Intronic non-coding RNAs and cancer

Sergio Verjovski-Almeida

Instituto de Química, Universidade de São Paulo, São Paulo, Brazil.

15:00-15:30

The role of ADAM23 in breast cancer metastasis

Anamaria Aranha Camargo

Ludwig Institute for Cancer Research, Sao Paulo, Brazil.

15:30-16:00

Closing remarks

Gilberto Schwartzmann

Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

the xenobiotics metabolism as well as the genetic susceptibility to cancer, including oral cancer, is being emphasized. Among the most studied susceptibility genes are those of the Cytochrome P-450 family, like *CYP1A1*, and those of the Glutathione S-transferases family (*GSTM1*, *GSTT1* and *GSTP1*), because of their importance in activating and detoxifying carcinogens. These genes are polymorphic in humans and certain allelic variants may be associated to the genetic susceptibility to oral cancer. To examine this, 91 patients with oral cancer and 64 controls, matched by age, sex, ethnicity and smoking habits, and without history of neoplastic disease, were analyzed considering *CYP1A1*/*MspI*, *GSTM1* and *GSTT1* polymorphisms. In the same sample, 72 patients and 60 controls were subsequently analyzed for *CYP1A1*/*IleVal* and *GSTP1* polymorphisms. The molecular analysis was carried out through PCR-RFLP, Multiplex PCR and specific-PCR techniques. Statistical analyses were performed using odds ratios (OR) and confidence intervals (CIs). In the *MspI* polymorphism analysis, we observed that 60% of the patients were wild-type homozygous and 40% were composed of risky individuals (heterozygous and mutant homozygous) and in controls 59% were wild-type homozygous and 41% were risky individuals. The frequencies of the *GSTM1* and *GSTT1* null genotypes were respectively of 40% and 33% in patients and 50% and 33% in the control group. According to these RESULTS, there was no positive or negative association for any of the studied genes: *CYP1A1* (OR=0,96; CI 95%=0,50-1,84), *GSTM1* (OR=0,65; CI 95%=0,34-1,25) and *GSTT1* (OR=1,01; CI 95%=0,51-1,99). There was also no statistically significant association among the variants of the genes *CYP1A1*/*IleVal* (OR=1,06; CI 95% = 0,49 - 2,29) and *GSTP1* (OR=1,40; CI 95% = 0,70 - 2,79). Therefore, based on this sample, we concluded that the polymorphic variants of the *CYP1A1*, *GSTM1*, *GSTT1* and *GSTP1* genes do not influence the genetic susceptibility to oral cancer. Supported by: CAPES/CNPq/Pibic and PRONEX-Fundação Araucária; Centro Odontológico Universitário Norte do Paraná/Uel; ICL/Londrina and Hospital Erasto Gaertner/Curitiba

P-012

A Case Control Study of *GSTM1*, *GSTT1* AND *CYP1A1* Genetic Polymorphisms in Sporadic Breast Cancer

Torresan C,¹ Abuzar CS,¹ Oliveira MMC,¹ Lima R,² Guembarovski RL,³ Ribeiro EMF,¹ Cavalli IJ¹

1 Universidade Federal do Paraná - Departamento de Genética;

2 Hospital Nossa Senhora das Graças - Serviço de Oncologia;

3 Universidade Estadual de Londrina - Departamento de Biologia Geral

Breast cancer (BC) is the main cancer to affect women in Brazil and its incidence is increasing every year. The major known risk factors for female breast cancer are associated with prolonged exposure to increased levels of estrogen and due to this common polymorphisms in genes that affect estrogen levels may be associated with

BC risk. The cytochrome P450A1 (*CYP1A1*) gene and the glutathione S-transferases (GSTs) genes play an important role in the metabolism of environmental carcinogens and of estrogen. OBJECTIVES: We investigated the associations between BC and polymorphisms in genes in the estradiol/estrone pathway (*CYP1A1*, *GSTM1*, *GSTT1*) in a case-control study of 55 BC cases and 55 controls age (± 5 years) and ethnicity-matched. We analyzed common deletion mutations in *GSTM1*/*T1* and the functional *MspI* polymorphism in *CYP1A1*. METHODS: Genotyping analyses were performed by PCR-based methods. The mean values were compared by *t* test. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to estimate relative risks. RESULTS: The mean ages of cases (n= 55) and controls (n= 55) were: 56.55 ± 13.14 ; 53.55 ± 12.92 , respectively ($t=1.2$; $P>0.3$). The mean of time between menarche and menopause was: cases (n=37) and controls (n=25) 35.46 ± 4.72 and 35.4 ± 5.68 , respectively ($t= 0.04$; $P>0.9$); and the mean of oral contraceptive use was: cases (n=22) (8.39 ± 7.09) and controls (n=10) (9.8 ± 9.35) ($t=0.46$; $P>0.6$). ORs were close to 1.0 for all the genes studied: *CYP1A1*/*MspI* (OR=0.91; CI=0.38-2.16), *GSTM1* (OR=0.75; CI=0.35-1.58) and *GSTT1* (OR=1.08; CI=0.49-2.34). The combined effect of *GSTM1*/*GSTT1*-null genotypes was also not significantly different between cases and controls (OR=0.9; CI=0.28-2.91). CONCLUSION: There were no differences in the distribution of *CYP1A1*/*MspI*, *GSTM1* and *GSTT1* genotypes between cases and controls, suggesting that there is no overall risk associated with any of the genotypes studied and BC risk. Larger studies are warranted to confirm these findings and to gain a clear picture of the contribution of these genes to BC. Supported by: UFPR - CNPq

P-013

MTHFR Polymorphisms and Haplotypes and Risk of Acute Lymphoblastic Leukemia: a Meta-analysis

Pereira TV,¹ Franco R,² Rudnicki M³

1 Universidade Federal do Rio Grande do Sul - Departamento de Genética; 2 Laboratório Fleury - Laboratório de

Hemostasia; 3 Universidade de São Paulo - Faculdade de Ciências Farmacêuticas

BACKGROUND: There is evidence supporting a role for folate metabolism in acute lymphoblastic leukemia (ALL). In order to provide a more robust estimate of the effect of the methylenetetrahydrofolate reductase gene (*MTHFR*) variants on the risk of ALL, we performed a meta-analysis to re-evaluate the role of the two most common polymorphisms and haplotypes of *MTHFR* on the risk of ALL. METHODS: All case-control studies investigating an association between the C677T and A1298C polymorphisms and risk of both childhood and adult ALL were included. We applied both fixed and random-effect models to combine odds ratio (OR) and 95% confidence intervals. Breslow-Day test was used to evaluate the homogeneity and both Egger's and Begg's tests were used to assess publication bias. RESULTS: The

meta-analysis of the C677T *MTHFR* polymorphism and risk of ALL included 10 studies with a total of 3275 individuals. No evidence of heterogeneity was observed (Breslow-day test, $P=0.16$). The combined OR under a fixed-effect model showed an association of the 677T allele with a significantly reduced risk of ALL (TT vs CT + CC, Mantel-Haenszel common OR: 0.77; 95% CI: 0.64 to 0.93, $p=0.006$). In relation to the A1298C polymorphism, data from 7 independent studies (2586 individuals) were combined. Significant heterogeneity was detected (Breslow-day test, $P=0.08$). Under a random-effect model the A1298C alone failed to show a significant association with the risk of ALL (CC vs AC + AA, DerSimonian-Laird common OR: 0.80; 95% CI: 0.51 to 1.28, $p = 0.36$). Although haplotypic data revealed consistent results among studies considering TT/AA vs others and CC/AA vs others, these data reached marginal significance only (4 studies pooled: 884 individuals). No evidence of publication bias was detected. CONCLUSIONS: Our findings support the proposal that common genetic variations in the *MTHFR* gene contribute to the risk of ALL presumably by influencing DNA synthesis and/or DNA methylation. More studies with larger samples are warranted in order to define the role of the *MTHFR* haplotypes and the involvement of the C677T polymorphism in specific subtypes of acute leukemia.

P-014

A Case Control Study of *CYP2D6**4 Allelic Variant in Sporadic Breast Cancer

Oliveira MMC, Abuazar CS, Torresan C, Guembarovski RL, Bicalho MG, Wassen R, Lima R, Cavalli IJ, Ribeiro EMF
Universidade Federal do Paraná - Departamento de Genética

Many risk factors are known to modulate the development of breast cancer and it is thought that genetic differences in the metabolism of environmental carcinogens may also be associated with individual variations in susceptibility to breast cancer. The cytochrome P450 (*CYP*) enzymes play a critical role in the oxidative metabolism of a variety of carcinogens, and polymorphisms in genes of this family have been extensively associated with a variety of cancers. The gene *CYP2D6* encodes for debrisoquine hydroxylase, whose substrates include aromatic amines and tobacco nitrosamines. The most frequent inactivating polymorphism in this gene is the *CYP2D6**4 allele, which generates a non-functional gene product. The poor metabolizers phenotype (mutant genotype) are unable to metabolize various substances, while the wild-type homozygous or heterozygous genotypes are termed extensive metabolizers and seem to be associated with an increased risk of cancer susceptibility. OBJECTIVES: evaluate the impact of the *CYP2D6**4 allelic variant on the risk of breast cancer. METHODS: we used data from 80 Caucasian women, obtaining 40 patients and 40 controls. The polymorphism was analyzed using PCR-RFLP assay. Odds ratios and 95% confidence intervals were used to estimate relative risks. RESULTS: The poor

metabolizer phenotype was found in 2.5% of the patients and in 7.5% of the controls. The extensive metabolizer phenotype was presented in 97.5% of the patients and in 92.5% of the controls. The mean age of patients was 57.8 ± 9.4 years and 57.5 ± 9.6 years in controls ($t = 0.98$; $p > 0.30$). Women with the wild-type allele were not at increased risk of breast cancer [OR=3; CI 95%=0,29 -30,92]. CONCLUSION: the findings suggest that the extensive metabolizer phenotype has no association with the increased risk of developing breast cancer. Supported by: UFPR/ CNPq/Pibic e PRONEX-Fundação Araucária

P-015

Insights into a Critical Pathway of Breast Tumorigenesis: the Type 1 Growth Factor Receptor Family (T1GFR)

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OBJECTIVES: T1GFR is a complex family of interacting transmembrane tyrosine kinase receptors [epidermal growth factor receptor (EGFR), c-erbB2 (HER2), c-erbB3 (HER3), and c-erbB4 (HER4)] that compose, with their relatives -ligands in the extracellular space, and second messengers in the cytoplasm- a modular structure of functional proteins. One of the receptors, the c-erbB2, is largely known to be associated with an unfavorable clinical course in breast cancers. Other members are coming to the stage, particularly after the genome and proteome projects. The expression of c-erbB4, for example, has shown an antagonist effect on the clinical influence of c-erbB2. Investigation of ligand-binding specificities has revealed that EGF and betacellulin bind to c- Erb2 and c-erbB3 when expressed together. Because the existence of c-erbB2 ligand has not yet been established, researchers have to presume -and they do it explicitly- that these receptors form heterodimers. The functional complexity of this modular structure is further increased by the discovery that genes of all four receptors and some ligands are subject to alternative splicing. This study reviews the interactions between ligands-receptors, receptors-receptors, and receptors second messengers associated to the T1GFR family for the purpose of modelling a qualitative understanding from which to extract information from databases such as the Cancer Genomic Anatomy Project (CGAP). METHODS: Information from CGAP and serial analysis of gene expression (SAGE) were searched and analysed, particularly those related to signaling pathways. RESULTS Some important information for exploring complex interacting proteins of the T1GFR family exists in the databases but complementary inferences are necessary from microarrays data, particularly for interpreting the differential expression from alternative splicing. CONCLUSIONS: A new understanding of the concept of ligand seems to be necessary, that favours some indistinctiveness between the different agents (ligands, receptors, and second messengers).

P-016**Identification of Differentially Expressed Genes Associated with *ERBB2* Over-expression in Human Mammary Cells in Response to Docetaxel**

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INTRODUCTION: the taxane, docetaxel, which is an antimicrotubulin agent, is currently the most effective chemotherapeutic drug for the treatment of advanced breast cancer. Over-expression of *ERBB2* gene, a member of the transmembrane receptor tyrosine kinase family, occurs in 15-30% of breast tumors and is associated with chemoresistance to a variety of anticancer drugs. **OBJECTIVES AND MATERIAL AND METHODS:** in the present study, aiming to identify genes involved in *ERBB2*-mediated chemoresistance to docetaxel we generated SAGE libraries from two human mammary luminal cell lines expressing different levels of *ERBB2* before and after intensive exposure to docetaxel. **RESULTS:** a total of 307,506 tags were generated from HB4a (81,684 tags; 30,854 unique tags), HB4a treated with docetaxel (79,948; 30,568 unique tags), C5.2 (79,948 tags; 30,568 unique tags) and C5.2 treated with docetaxel (75,159; 31,293 unique tags). Comparisons of the transcriptome of the cell lines before and after docetaxel exposure revealed substantially different expression patterns. The use of SAGE technique allowed us to identify a large number of potential *ERBB2* target genes implicated with a variety of cellular processes including cell proliferation, cell adhesion, apoptosis and cytoskeleton organization. The cell line C5.2, which express high levels of *ERBB2*, showed considerable higher number of ESTs and hypothetical proteins mainly after docetaxel exposure. At present, some of the differentially expressed transcripts are been examined by qPCR analysis in the cell lines and in primary breast tumors. **CONCLUSIONS:** our study provides a comprehensive view of the expression changes induced in human mammary cells expressing different levels of *ERBB2* in response to docetaxel that might contribute to elucidate the mechanisms involved in *ERBB2*-mediated chemoresistance in breast cancer. Supported by: FAPESP

P-017**Wnt Signaling Pathway is Involved with Nephrogenesis and Wilms Tumor**

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INTRODUCTION: WNT signaling pathway (WNT) plays critical roles in the embryonic development and tumorigenesis. Wilms tumor (WT) is an embryonic tumor, which has been long viewed as a prototype of differentiation failure. WT is composed of three histological distinct components representing a different stage of kidney embryonic development - blastemal (BT), stromal (ST) and epithelial (EP). Morphologically, each of them has its own characteristic of aggressiveness and treatment response. **OBJECTIVE:** To analyze gene expression pattern of WNT genes in WT. **METHODOLOGY:** Components were scalpel or laser microdissected from 12 frozen tumors (5 EP, 3 ST and 4 BT), 3 mature non-neoplastic (NK) and 1 pool of fetal kidney (FK). Total RNA were amplified in 2 rounds using T7 based methodology and hybridized in a customized cDNA microarray platform (4,608 human genes), where 54 genes belong to WNT. It was used reference design with dye swap as duplicate. Permutated Student test was used for statistical analysis in order to determine the differentially expressed genes. **RESULTS:** Since the intrinsic characteristic of WT comprises loss of developmental control and gain of tumorigenic potential we compared gene expression profile of WT, NK and FK to classify genes in two different sets: development (set A) and tumorigenesis (set B) related-genes. Set A showed statistically relevant genes with potential *cis*-regulatory elements to PAX-2, a transcription factor involved in nephrogenesis. Moreover, Hierarchical clustering based on set A and set B discriminated NK from the FK and tumor samples and NK and FK from tumor samples, respectively. The 54 WNT genes were analyzed among sample classes and 23 were significantly differentially expressed (set A-12 genes and set B-11 genes). Clusters performed with these genes maintained the behavior of the sets A and B. Analysis of correlation between WNT genes showed that 14 pairs of genes lost correlation and 11 genes had their correlation inverted. **CONCLUSION:** Our results suggest that a disruption of WNT plays an important role in WT arising. Supported by: CAPES and CEPID/FAPESP

P-018**Characterization of Novel c-erbB2 Regulated Genes Using Massively Parallel Signature Sequencing (MPSS)**

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Overexpression of the proto-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 approximately 17,000 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 tags 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 of these fragments using BLAST allowed the identification of 10 transcripts putatively regulated by c-erbB2. Differential expression in the C5.2 cell line of 3 out of the 10 transcripts putatively regulated by c-erbB2 was confirmed by Real-Time PCR. Two of these transcripts also showed differential expression in c-erbB2 positive breast tumors, validating them as novel c-erbB2 regulated genes. Supported by: FAPESP CNPq Ludwig Institute for Cancer Research

P-019

Cell Cycle Regulation by NFAT1 Transcription Factor

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OBJECTIVES: Proteins belonging to the NFAT (Nuclear Factor of Activated T cell) family of transcription factors play a central role in the regulation of transcription of several genes. NFAT activation is regulated by a phosphatase calcineurin, which is inhibited by cyclosporin A (CsA). Several evidences demonstrate the involvement of NFAT proteins in regulation of genes that control cell differentiation, activation, and malignant transformation. Analyses of the NFAT1-deficient mice (NFAT1^{-/-}) showed that its lymphocytes hiperproliferate when compared with wild-type lymphocytes (NFAT1^{+/+}). This phenotype is related to an altered cell cycle control and an overexpression of cyclins A2, B1 and E, suggesting that NFAT1 controls cell cycle progression. Thus, our aim is to evaluate whether NFAT1 transcription factor directly regulates genes involved in cell cycle progression. **METHODS AND RESULTS:** Lymphocytes were purified

from lymph nodes of C57BL/6 mice and then stimulated *in vitro* with PMA plus ionomycin in presence or absence of CsA. Cell cycle analysis demonstrated that CsA inhibits lymphocyte proliferation by arresting cells at G1 phase of the cell cycle. Furthermore, CsA inhibits cyclins A2 and E expression upon anti-CD3 stimulation. Analysis of cyclin A2 promoter by luciferase reporter gene assay unraveled a negative regulatory region located between the positions -490 and -217, which present a binding site for NFAT transcription factor. EMSA analysis showed that NFAT1 protein binds to the -380 region of this promoter. In addition, chromatin immunoprecipitation (ChIP) demonstrated that NFAT1 binds to cyclin A2 *in vivo*. Analysis of a cell line that stably expresses NFAT1 protein (CHO-NFAT1) showed that these cells presented a decrease in cell proliferation, which was related to a delay in the entrance at S phase of the cell cycle and a reduction of the expression of some cyclins genes. Finally, CHO-NFAT1 cells presented a diminished growth when inoculated in BALB/c nude/ nude mice compared with wild-type cells. **CONCLUSION:** Together, these results suggest that NFAT1 transcription factor negatively regulates cyclin gene expression and acts as a repressor of cell cycle progression. Supported by: CNPq, Furnas, Fogarty/NIH, ICGEB, FAF

P-020

Iron and Proliferative Capacity in K562 Cells, a Chronical Myeloid Leukemia Model

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Neoplastic cells are different from normal cells in several aspects, and the high proliferative rate is one of their most important characteristics. The mechanisms that determine such feature are still unclear, once it involves complex cell signaling pathways. Many drugs have been studied regarding their anti-proliferative potential, and the anthracyclines are among the most prominent ones. The K562 cell line is composed of undifferentiated erythroid precursor cells, and it is used as a major chronical myeloid leukemia (CML) cell model. In this work, K562 cells were treated with doxorubicin, aclarubicin, hydroxyurea and dGTP during 7 days, and their morphology and cell cycle were analyzed. We also evaluated differences in gene expression following the treatment with doxorubicin and aclarubicin by differential display (DD-PCR). The K562 cells treated with aclarubicin did not show morphological differences when compared to control cells. Cells treated with doxorubicin, hydroxyurea and dGTP, however, presented increased cell size in comparison to control cells. The cell cycle was affected, resulting in S-phase blockade during the treatment with hydroxyurea and dGTP, G2-phase blockade during the treatment with doxorubicin and a G1 transient blockade was observed during aclarubicin treatment. Differential display showed several differentially expressed genes along

treatments. Interestingly, five sequenced genes were involved in the iron metabolism pathway. Our results show that the drugs used seem to interfere with the iron signaling pathway, as previously described for iron chelating agents acting as antiproliferative drugs. Therefore we propose a crucial role for iron in the cellular proliferation and non-proliferation balance. Further studies in this area will allow the development of more efficient antiproliferative drugs. Supported by: CNPq, CAPES

P-021

Cell Cycle Controle Throughout Melanocyte Transformation Induced by Adhesion Blockade

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BACKGROUND. Several studies demonstrate the importance of DNA methylation in cancer initiation and progression. The mechanisms regulating DNA methyltransferases (DNMTs) expression and activity are still poorly understood, but recent results confer to P53, P21 and PCNA potential DNMT regulatory function. **OBJECTIVE.** We have been interested in studying the involvement of cell cycle components in DNMTs regulation during melanoma carcinogenesis. **METHODS.** An experimental model of cell transformation was developed, where different melanoma cell lines were established after submitting a non-tumorigenic murine melanocyte lineage, melan-a, to sequential substrate adhesion blockade. **RESULTS AND CONCLUSION.** Alterations in DNMTs expression are shown along melan-a transformation. Melan-a cells submitted to sequential deadhesion cycles respond to this stressful condition with progressively reduced P53 levels, and melanoma cell lines do not express this protein even when cultured in adherent conditions. In contrast to melan-a melanocytes, which express p19^{ARF} in nucleoli, the more aggressive cell lines do not express this protein and less aggressive melanoma cell lines have heterogeneous p19^{ARF} expression. Melan-a cells express p19^{ARF} and P53, but melanoma cell lines, which have lost p19^{ARF} expression, show P53 reduced levels and a significant increase in Mdm2 expression. It is possible that p19^{ARF} loss results in higher levels of Mdm2, since this protein causes P53 degradation. A significant reduction in P21 protein was detected in melan-a cells submitted to deadhesion cycles and an augmented expression of PCNA was also observed in melanoma cell lines compared to melan-a cells. P21 compete with DNMT1 for PCNA binding and the P21 decrease observed in melan-a submitted to adhesion impediment cycles may affect DNMT1 activity. Ongoing experiments using chromatin (ChIP) and protein immunoprecipitation might elucidate DNMTs regulation by cell cycle components along melan-a transformation induced by adhesion blockade. Epigenetic events seem to have contributed to acquisition

of a transformed phenotype in this model, and alterations in cell cycle components may participate in DNMTs regulation. Supported by: FAPESP and CNPq.

P-022

Functional Roles of FGF-Receptors in Mouse Cell Lines

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BACKGROUND. FGF2 and FGF-Receptors (FGFRs) comprise a signaling system involved in the control of a multitude of fundamental biological processes, ranging from cell proliferation to differentiation, tumorigenesis, and programmed cell death, in developing and adult tissues. In vertebrates, FGFRs are single spanning transmembrane proteins harboring Tyr kinase activity, which are encoded by 4 highly related genes. In addition, alternative splicing generates a diversity of FGFR isoforms, whose expression depends on cell type. **OBJECTIVE.** Our goal is to elucidate the functional roles of FGFRs in cell proliferation, tumor growth and programmed cell death of mouse cell lines (Y-1 adrenocortical cells and Balb 3T3 fibroblasts). **METHODS.** a) By PCR amplification and DNA sequencing we are characterizing the FGFR isoforms. b) To knockdown FGFR by RNAi, we designed specific double strand oligonucleotides (inverted double repeats) and inserted them in a pSUPER vector. c) pSUPER constructions were transfected in Y-1 cells to derive stable clonal sublines. **RESULTS.** a) Y-1 tumor cells display the following FGFR isoforms: FGFR1IIIc, FGFR2IIIc, FGFR3IIIc and FGFR4. b) Y-1-FR3, a FGF2 resistant low tumorigenic subline, shows a different pattern of FGFRs. c) Stable Y-1 sublines showing reduced levels of FGFR2 and FGFR3 are still sensitive to FGF2 cell death induction. **Conclusion.** In Y-1 adrenocortical cells, tumorigenicity and cell death induced by FGF2 seem to depend on the repertoire of FGFRs. Reduced levels of FGFR2 and FGFR3 are not sufficient to render Y-1 cells resistant to FGF2. Supported by: CNPq, FAPESP

P-023

Apoptosis, Proliferation and Cell Cycle Analysis in the hCG-PML-RAR α Transgenic Mouse Model

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α Acute promyelocytic leukemia (APL) is associated with the t(15;17) which generates the PML-RAR α fusion gene. The encoded PML-RAR α oncoprotein physically interacts with native PML impairing its function. PML is a potent inhibitor of proliferation and is involved in apoptosis regulation. Transgenic mice (TM) hCG-PML-RAR α develop a form of leukemia similar to human

APL after a long period of latency, suggesting that PML-RAR α expression is necessary but not sufficient to leukemogenesis. We have analyzed TM of different ages without hematological abnormalities, and characterized the distribution of cells in the phases of cell cycle, cell proliferation and resistance to apoptosis *in vivo*. Cell cycle was analyzed in bone marrow (BM) cells stained with propidium iodide (PI) and analyzed by flow cytometry (FC). In TM younger than 9m (n=12), there was no significant difference in cell cycle distribution compared to wild-type (WT) controls. In contrast, in TM older than 9m (n=15) the % of BM cells in S phase was significantly lower (TM=14,84 $\pm$ 3,39%; WT=18,26 $\pm$ 3,55%; $p=0,005$) and was associated with increase in the % of cells in G0/G1 (TM=81,69 $\pm$ 3,79%; WT=78,14 $\pm$ 3,70%; $p=0,001$). The proliferation was tested *in vivo* by injecting bromodeoxyuridine i.p. and assessing its incorporation by BM cells after 2h. Compared to WT controls, there was a lower % of proliferating cells in TM older than 9m (19,48 $\pm$ 7,81 versus 23,20 $\pm$ 10,80% in WT; $p=0,06$). Apoptosis was induced by γ -irradiation, and after 24h BM cells were obtained and stained with PI. The % of apoptotic cells was determined by quantifying sub-G0 peak by FC. BM cells from TM older than 9m, but not from younger mice, were resistant to apoptosis. In this age group, γ -irradiation induced a 2,24 $\pm$ 0,81-fold increase in the % of apoptotic cells, whereas in WT controls this increase was of 4,06 $\pm$ 3,01-fold ($p=0,018$). Our results showed resistance to apoptosis and a block of the transition G1/S in the pre-leukemic phase of hCG-PML-RAR α . TM older than 9m. Therefore deregulation of cell cycle is a late event during APL genesis and may be associated with additional mutagenic events. Supported by: FAPESP

P-024

Isolation and Analysis of Fibroblast Growth Factor-2 (FGF2)/ Receptors (FGFR'S) Protein Complexes in Mouse Cell Lines

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BACKGROUND. FGF2 (Fibroblast Growth Factor 2), a mitogenic factor, binds membrane tyrosine kinase receptors (FGFRs), activates an intricate signaling network and is internalized. However, the role of internalized FGF2/FGFR and its interactions with intracellular proteins are still obscure. **OBJECTIVE.** Our goal is to elucidate the internalization process and to the components of FGF2/FGFR protein complexes. **METHODS.** To probe into the process of FGF2 internalization, we are producing a) recombinant-FGF2 tagged with the IgG-binding domain of Protein A, aiming to, first, binding to FGFR and, second, isolating protein complexes of FGF2-protA/FGFR in mouse cell lines (Y1 adrenal cells and Balb 3T3 fibroblasts). FGF2-protA was cloned in a *E.coli*-vector with a HisTag-domain, allowing affinity purification in FPLC by two steps via, respectively, HisTag and ProtA. Y1 cells are

being stimulated with purified FGF2-protA, assayed for signal triggering and lysed for affinity purification of FGF2-protA/FGFR protein complexes. b) Rec FGF2-EGFP to follow intracellular traffic of FGF2/FGFR protein complexes by confocal microscopy. **RESULTS.** a) FGF2- fusion proteins display mitogenic and cell death activities, mimicking well recombinant FGF2; b) FGF2-ProtA interacts with several FGFRs. **CONCLUSION.** FGF2 fusion proteins are biologically active allowing to uncover the composition and kinetics of internalization of FGF2/FGFRs proteins complexes. Supported by: FAPESP and CNPq

P-025

WNT Pathway and APC Methylation in Leukemic Cells

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OBJECTIVES Promoter hypermethylation plays an important role in the silencing of tumor suppressor genes (TSGs) in many cancers, including certain hematological malignancies. Each tumor type seems to have its own distinct pattern of methylation, a condition that, in computer modelling, adds complexity to the drawing of hypotheses about how the system works. Abstractions, usually required to infer general patterns of behavior for signal transduction and metabolic pathways, have to be made from a perspective that integrates knowledge from different kinds of tumor. This study reviews some relevant aspects that deserve attention for modelling the Wnt pathway in leukemia. It starts from accounts made in the 2001s about the prevalence of APC methylation in gastrointestinal tumors and its inexistence for leukemia; and looks forward to a clarification of a putative APC methylation status in leukemia. **METHODS** Contrasting with laboratory work, this study squares with an experimental design-choice experiments methodology. Data are "mined" from some Cancer Genomic Anatomy Project (CGAP) databases and from recent literature that review and/or provide evidence for the methylation of CpG islands within TSGs. **RESULTS** Mutation is the most common and primary cause of APC inactivation in colorectal tumors but gastric tumors, that usually do not harbor APC mutations, can disrupt the APC/Beta-catenin pathway through APC hypermethylation. Distinctions have been made between genetic and epigenetic controls of the signaling Wnt pathway, and between degrees of methylation of colon *versus* gastric cancers. In the early 2004s, studies of methylation in hematological malignancies started to include the APC TSG in their profile, categorizing it within the hallmark "tissue invasion and metastasis", by reference to the role of APC in cancerous gastric epithelia, and not within the "insensitivity to anti-growth signals" as suggested by the Hanahan & Weinberg-model (Cell, v.100, 57, 2000). This interpretation started to change in the early 2005s when the promoter of the APC gene in samples of adult T-cell leukemia/lymphoma (ATL) was shown to be

hypermethylated; while none of normal samples were. Also, the silencing of the APC promoter was recognized to allow ATL cells to receive uncontrolled signals. Some data about the Wnt pathway already exist in CGAP databases, including two-dimension arrays, but inferences about the methylation status of APC in leukemia are still limited. **CONCLUSIONS** Results suggest that, for modelling purposes, findings about genetic and epigenetic alterations in genes previously restricted to models of both familial and sporadic tumors (such as the APC) can be extended to account for leukemia in a more comprehensive kind of representation. "Insensitivity to anti-growth signals" arises as a putative hallmark, allowing for a more abstract and accurate specification of the Wnt pathway. Supported by: PIBIC/CNPq/LNRCC/FINEP

P-026

Immunosuppressive Cyclosporin A Modulates Tumor Promoter Genes in Human Lymphocytes

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The treatment of autoimmune diseases involves the administration of immunosuppressive drugs (ISD) to attenuate patient immune response. Cyclosporin A (CsA) is a potent ISD that has been used for the treatment of various disease conditions. The molecular mechanism of CsA has been investigated in T cells, and it has been shown to involve the intracellular calcineurin pathway. However, studies have been demonstrated that CsA treated patients presented higher risk to cancer. Since the mechanism of ISD to promote cancer is still unclear, we are characterizing the gene expression profile of human peripheral blood lymphocytes in the presence (or not) of CsA. For this, we use glass-slide microarrays composed by 4,500 cDNA target sequences from the IMAGE Consortium which are hybridized with Cy-3 or Cy-5 labeled cDNA complex probes originated from in vitro CsA-treated or control cells. The hybridization images are quantified by using the Spot Finder software, normalized and the numerical values analyzed by bioinformatics programs such as AROMA, LIMMA, KTH and Bioconductor. For data mining we used the SOURCE database. Our results showed several genes differentially modulated after in vitro treatment of lymphocytes with CsA. Among the induced genes, we identified those involved in cell cycle regulation (TRRAP), apoptosis (PRKDC, MAEA, TIA1), cell proliferation (FEN1, BIN1) and response to DNA damage stimulus (PRKDC, FEN1). Up regulation of FEN1, TRRAP, and BIN1 genes have been also found in lung, gastric and pancreatic cancer.

Moreover, TRRAP gene that was up regulated is considered an oncogene involved in pancreatic cancer suggesting that CsA treatment induces genes related to carcinogenesis. Among the repressed genes, we emphasize the BTG2 involved with DNA repair and evidences indicate that this gene has tumor suppressive properties. It was shown that BTG2 is one of the primary targets of p53. In addition, it has been suggested that BTG2 down-regulation play a role in renal cancer development. These results shown that at the same time CsA is an ISD it can modulate genes involved with tumorigenesis. Supported by: FAPESP (01/13996-0, 99/12135-9), CNPq, CAPES and Pró-Reitoria de Pesquisa da USP

P-027

The DU-145 Human Prostate Carcinoma Cell Line Exhibits a Temperature Sensitive Mutant P53 Phenotype

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OBJECTIVES: The p53 protein is frequently mutated in human cancers (Science, 252: 49-53, 1991). The vast majority of missense mutations occur in the core domain of p53 which mediates DNA binding (Genes and Development, 7:2565-74, 1993). The human prostate carcinoma cell line DU-145 is heterozygous for p53, carrying two different mutations within the DNA binding site of p53, with one allele mutated at codon 223^{Leu} and the second at codon 274^{Phe} (Cancer Research, 63: 2905-12, 2003). The mutation at codon 223 was shown to be temperature sensitive (TS) through a gal-4 based assay in yeast (Journal of Biological Chemistry, 279(1):348-55, 2004). Here we describe molecular and functional assays which confirm the TS phenotype of the endogenous mutant p53(223) allele in the DU-145 lineage. **METHODS:** DU-145 cells were transduced with reporter vectors encoding EGFP driven by a p53 responsive promoter or a constitutive promoter and were then cultivated at 32 or 37°C. EGFP expression was evaluated by flow cytometry. PC-3 cells (human prostate carcinoma, p53-null) were transduced with retroviral vectors encoding either the p53(223) or p53(274) mutant alleles (kindly provided by Andrei Gudkov, Lerner Research Institute, Cleveland, OH, USA) and TS activity was assessed as described above. In the PC-3 model of TS p53 activity as well as DU-145, Northern and Western blots were used to analyze expression of a p53 target gene. The cell cycle profile of these cells was ascertained by propidium iodide staining followed by flow cytometry. **RESULTS:** Reporter assays confirmed that DU-145 cells do indeed exhibit a TS p53 phenotype since p53-responsive reporter activity was increased at the permissive temperature. Using PC-3 cells, we identified the p53(223) allele, derived from DU-145, as being responsible for the TS activity. Interestingly, expression of the p53 target gene p21Waf1 was induced by the p53(223) allele in PC-3 cells, but not in DU-145, at 32°C. No significant cell cycle alterations were observed with

respect to the p53 allele or cell type examined at either the restrictive or permissive temperatures. **CONCLUSION:** The prostate carcinoma DU-145 cell line exhibits a TS phenotype due a p53 mutation at codon 223. This mutant also causes a TS phenotype when expressed in the PC-3 cell line. Interestingly, the cellular context may influence which p53 target genes will be activated by this allele at the permissive temperature. The TS phenotype of the DU-145 cell line may prove to be a useful tool for study of the p53 pathway. Supported by: FAPESP

P-028

Cell Cycle Control of Tumor Cells: an Unsuspected Biological Function of FGF2: Death Cell Induction as a Restraining Mechanism of Oncogene Induced Proliferation

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BACKGROUND: We have previously reported that FGF2 is a mitogen for the mouse Y1 adrenocortical tumor cell line because in G0/G1-arrested cells, FGF2 triggers the following sequential events: Erk1/2 activation (5min); c-Fos and c-Myc induction (2-4h); cyclin D1 induction by 5h and DNA synthesis initiation by 8h. **OBJECTIVE:** To report a new and unsuspected FGF2 effect: Y1 tumor cells treated with FGF2 lose viability beyond S phase initiation and do not reach mitosis. However, non tumorigenic Y1 sublines are resistant to FGF2. In addition, Y1 sublines selected for resistance to FGF2 display low tumorigenicity. **METHODS:** cell cycle and cell proliferation were analysed by BrdU labeling, FACS and cell counting in hemocytometer. Cell death was estimated by clonogenic assays. Tumorigenicity was monitored by subcutaneous injections of cells into Balb/c Nude. **RESULTS:** a) The cell death induced by FGF2 depend on the chronic high levels of Ki-Ras-GTP found in Y1 cells *in vitro*; b) FGF2-resistant clonal sublines selected from Y1 population display low tumorigenicity in spite of high basal levels of Ki-Ras-GTP; c) Y1 sublines that have spontaneously lost amplified c-k-ras sequences are FGF2-resistant and non-tumorigenic. **CONCLUSION:** These results led us to hypothesize that FGF2 exerts an unsuspected biological function activating a tumor suppressor response to restrain oncogene driven tumorigenesis. Supported by: CNPq, FAPESP

P-029

Replacement of p16, p21 or Both Tumor Suppressor Genes Altered Growth of Transduced and Non-transduced Neighboring Cells

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INTRODUCTION: A key element of cell cycle control is the negative regulation of CDK activity by p16 and p21, resulting in reduced phosphorylation of the Rb protein. Tumor cells often lose expression of p16 (due to methylation of the promoter or deletion of the gene) and p21 (due to loss of p53 activity), permitting accelerated proliferation. We propose that the simultaneous replacement of both p16 and p21 will result in superior tumor cell inhibition. **OBJECTIVE:** We set out to establish a multigenic gene transfer system that would offer a selectable marker capable of indicating transduction and monitoring the kinetics of cell proliferation. To this end, we constructed the pCLXSG retroviral platform that employs EGFP, instead of antibiotic resistance, as the selectable marker. **MATERIAL AND METHODS:** The pCL retroviral system was first modified by insertion of the EGFP cDNA under the control of the internal SV40 promoter, establishing the pCLXSG system. We then built monocistronic vectors p16 or p21 pCLp16SG and pCLp21SG and the polycistronic vector pCLp16IRESp21SG. Growth curves of transduced (EGFP+) and non-transduced (EGFP-) cells were determined by flow cytometry. **RESULTS:** Using statistical analysis of the proliferative tendencies of transduced cells (U251, PC3, H358, HT1080), we confirmed our expectation that the multicistronic transfer of p16 plus p21 was superior in growth inhibition. Unexpectedly, we observed that transfer of p16 resulted in *increased* proliferation of non-transduced neighboring cells in the case of HT1080. For H358, both p16 and p21 gene transfer resulted in the *inhibition* of non-transduced neighboring cells. **CONCLUSIONS:** Use of EGFP permitted analyses of gene function at the cellular level, identifying both transduced and non-transduced cells. Our assays revealed that simultaneous transfer of the p16 plus p21 cDNA's resulted in enhanced inhibition of cell proliferation as compared to either of the single suppressor genes. Interestingly, after introduction of tumor suppressor genes we found that proliferation of non-transduced neighboring cells could be altered as well. Supported by: FAPESP

P-030

Cloning and Functional Analysis of the NRP/B Gene Associated with the Tumor to Normal Phenotypic Reversion in RAT ST1 Glioma Cell Line

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INTRODUCTION: Malignant gliomas are aggressive, highly invasive and neurologically destructive tumors. A comprehensive view of the molecular mechanisms underlying their tumor biology

is important to understand their molecular basis, identify molecular markers and to develop specific anti-tumor therapeutic protocols. To study the anti-tumor activity of glucocorticoid hormones (GC), we have been using the ST1 cell line, a C6 rat glioma cell variant that is hyper responsive to GC, going from a fully transformed and tumorigenic phenotype to a normal one, both *in vitro* and *in vivo*. In order to isolate genes, which are associated with this phenotypic reversion, subtracted cDNA libraries were generated by Suppression Subtractive Hybridization (SSH). One of the isolated genes is similar to the human NRP/B, being detected as a transcript of ~5.5kb that is induced upon a 5h hydrocortisone (Hy) treatment. OBJECTIVE: Cloning and perform functional analysis of r-NRP/B. RESULTS: The full-length cDNA for r-NRP/B was obtained by RT-PCR using specifically designed primers (GenBank™ accession n° AY669396). The coding sequence was cloned in the sense orientation into a mammalian expression vector namely pLPCX (Clontech). The ST1 cell line was infected with the retroviral pLPCX/NRP/B construct and, in parallel, with the empty pLPCX vector, to generate mixed cell populations. Over expression of NRP/B was confirmed by Northern and Western blot analysis. No difference was observed with respect to growth in monolayer, in the presence and in the absence of Hy (100ng/ml) of the NRP/B infected ST1 cell population when compared to ST1 cell infected with empty vector, but there was a significant reduction of colony cell formation in agarose suspension assay. *In vivo* experiments, to evaluate the tumorigenic potential, suggest that this gene is somehow involved with the tumor formation capacity, inhibiting cellular growth. CONCLUSION: The results obtained point to a critical role played by this NRP/B gene in the molecular mechanisms underlying cell growth control and the anti-tumor activity of glucocorticoids and may contribute for improved in drug design and gene therapy of gliomas. Supported by: FAPESP, CNPq, FINEP and PRP-USP

P-031

Assessment of the *In Vivo* Expression of the pCLPG Retroviral Vector in an Animal Model of Bone Marrow Transplantation

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OBJECTIVES: We have shown that the pCLPG retroviral system (which utilizes p53 as a transcription factor to drive viral expression) offers superior transgene expression in cell culture. Our current efforts include the assessment of pCLPG expression *in vivo*, a scenario which would rely on endogenous p53 to drive viral expression. For this, we established an animal model of bone marrow transplantation (including standardized

dose of irradiation, cell collection and cultivation methods). We are using this model system to observe transduction and long-term expression of the pCLPG retroviral vector system. METHODS: pCLGFP and pCLPGGFP viruses were produced using amphotropic, ecotropic or VSVg (pseudotyped) packaging vectors. The VSVg envelope permits viral concentration using ultracentrifugation; maintenance of the cells in culture with IMDM and different concentrations of cytokines and growth factors; transduction of bone marrow cells with the retroviral vector in the presence of Retronectin; irradiation of the recipient animals (myeloablation); bone marrow transplant with transduced cells; FACS analysis of EGFP expression in cultured and transplanted cells. RESULTS: In this work we confirmed that the combination of virus pseudotyped with VSVg and transduction in the presence of Retronectin yielded the highest rate of transduction observed in cultured bone marrow cells. We show that our transplantation technique is reliable and we currently have animals surviving several months post-transplant. Currently, transplantation of transduced bone marrow cells in primary recipients is being performed. CONCLUSIONS: With this study, we established a protocol for transplant and transduction of bone marrow cells with our new retroviral vector. We expect to observe long-term, high-level expression of the pCLPGGFP vector, but silencing of the parental pCLGFP virus. Using p53 to drive viral expression may be advantageous since p53 activity can be induced, leading to on-demand transgene activity. This model system and the pCLPG virus may prove to be powerful tools for the development of gene therapy strategies for hematopoietic diseases. Supported by: FAPESP, CNPq

P-032

A Multigenic Tumor Suppressor Virus for Cancer Gene Therapy

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INTRODUCTION: Cancer is the product of accumulated mutations where the combined functional loss of p16 and p53 is the most common event in carcinomas. Considering that p16 and p53 act in different, but intertwined, pathways of cell cycle control, the simultaneous replacement of multiple suppressor genes of distinct cell cycle control pathways may have an enhanced effect. Recently, the demand for polycistronic vectors has intensified in response to the discovery of functional interactions between multiple genes. Polycistronic vectors are a practical solution when the strategy requires the simultaneous co-expression of various genes in order to obtain a combined and/or synergistic effect. Cancer is an example of a multigenic disease that could benefit from the development of polycistronic vectors. For these reasons, we are

investigating the effect of the simultaneous replacement of p16 and p53 in tumor cells using bicistronic virus. **OBJECTIVES:** Our goal is the *in vivo* validation of the cooperation between p16/p53 as an efficient cancer gene therapy strategy. **MATERIAL AND METHODS:** We have constructed and produced the bicistronic adenoviral Adp16IRESp53 and the monocistronic Adp16 and Adp53 as well as the reporters AdeGFP and AdLacZ. For *in vitro* characterization and functional assays we are applying Western blot, RT-PCR, cell cycle analysis by FACS-PI, Annexin-apoptosis and beta-Gal senescence. Using the H358 xenographic tumor model for preclinical *in vivo* validation, our work includes standardization of a protocol for *in situ* administration to assess dose, temporal-spatial transduction and biodistribution of the viral agents AdeGFP and AdLacZ. In our therapeutic model, application of Adp16, Adp53 and Adp16IRESp53 will be compared. **RESULTS:** *In vitro* characterization of the Adp16, Adp53 and Adp16IRESp16 viruses confirmed transgene expression and functional effect. *In situ* transduction of the H358 tumors with the AdLacZ virus yielded significant reporter gene activity. **CONCLUSIONS:** Considering that alteration of p16 and p53 are present in practically 100% of human tumors, our results support the notion that Adp16IRESp53 would be a powerful drug for cancer gene therapy. Supported by: FAPESP; CAPES

P-033

Expression Pattern and Methylation Analysis of ADAM 12 and ADAM 33 Genes in Breast Tumors

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The ADAMs (A Desintegrin And Metalloprotease domain) comprise a family of membrane-anchored cell surface proteins with a common structural organization. Members of this family play important role in proteolysis and cell-cell and cell-matrix interactions. The loss of cell adhesion and increased secretion of proteases that degrade extracellular matrix are crucial steps in tumor progression. As a further contribution to investigate the role of ADAM family members in cancer, we have evaluated the mRNA expression level and methylation status of the promoter region of 8 different ADAM family members (ADAMs 8, 10, 12, 15, 17, 19, 22 and 33) in normal breast tissue and in breast tumor cell lines. Semi-quantitative RT-PCR followed by Southern blot and densitometry showed that ADAMs 12 and 33 were down-regulated in 80% and 95% of the breast tumor cell lines respectively. Methylation status of ADAM12 and 33 promoter regions was then evaluated by bisulfite treatment followed by DNA sequencing. A positive correlation between the presence of DNA methylation

in the promoter region and the absence of ADAM 12 and 33 mRNA expression was observed. Finally we evaluate the methylation status of ADAM12 and 33 promoter regions by Methylation Specific PCR (MSP) in 108 breast invasive ductal primary tumors. We have detected ADAM12 promoter hypermethylation in 27.9% of the cases and ADAM33 promoter hypermethylation in 55.04% of the cases. Hypermethylation in both promoter regions was significantly associated with tumor stage ($p=0.005$ and $p=0.017$ respectively). In addition, hypermethylation of ADAM 12 promoter region was significantly associated with tumor size ($p=0.028$) and number of positive lymph nodes ($p=0.038$). However no association was found between ADAM 12 and 33 hypermethylation and disease-free and overall survival. These results suggest that DNA methylation has an important role in regulating the expression pattern of ADAM12 and 33 and that alterations in normal DNA methylation patterns can be associated with tumorigenesis. Supported by: FAPESP, CNPq, Ludwig Institute for Cancer Research

P-034

Construction and Characterization of AdPG: An Adenoviral Vector with p53-Driven Expression

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OBJECTIVES: The work of our laboratory includes the construction of viral vectors with strategic promoter/transgene combinations. Previous success with the pCLPG retroviral system, which employs p53 to both drive viral expression and suppress tumor growth, indicates that this promoter/transgene combination may be a powerful anti-tumor strategy (Virology, 321: 165-172, 2004; Cancer Gene Therapy, in press, 2005). In order to make a more potent gene therapy vehicle, we have transferred this technology to an adenoviral vector. **METHODS:** A chimeric promoter was used in the construction of AdPG. First, the components of the enhancer-like PG sequence (Kern et al, 1992), adenoviral tata-box E1G (Xavier-Neto et al, 1998) and the β -globine intron (Schambach et al, 2000) were cloned and assayed in reporter constructs to verify the best combination, originating the PGTx β promoter. The PGTx β cassette was inserted in a promoterless adenoviral vector, creating the AdPG platform. An experimental vector, AdPGLuc, encoding luciferase as a reporter gene was constructed and assayed in DU-145 prostate cancer cells, which harbor a temperature sensitive mutant of p53 (Bajgelman et al, manuscript in preparation). At 37°C, the p53 protein takes on the mutant conformation and is unable to drive transcription. At 32°C, the p53 protein assumes the wild-type conformation. **RESULTS:** With plasmid-based vectors, we show that the PG element can be used as a promoter of transgene expression, but specificity for p53 was compromised. Addition of the Tx element improved specificity, but reduced transgene

expression. The complete chimeric promoter arrangement, PGT α β , which includes an intronic region, yields p53-specific, high-level transgene expression. The AdPGLuc vector offers p53-dependent expression. Transient transfection of the plasmid form of this vector in DU-145 cells yielded an 80-fold increase in luciferase activity at the permissive as compared to the restrictive temperatures. Strikingly, DU-145 cells transduced with the AdPGT α β -Luc virus had a 475-fold increase of Luc activity at 32°C as compared to 37°C. CONCLUSIONS: We have shown that the chimeric promoter PGT α β achieved the best performance in reporter constructs. The experimental adenovirus vector AdPG-Luc was successfully constructed and we have confirmed that expression from this vector is driven by p53. The AdPG system is reliable and will be further developed as a new platform for *in vivo* gene transfer. Supported by: FAPESP

P-035

Strategic Promoter/Transgene Combinations for Cancer Gene Therapy

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OBJECTIVE: We set out to create strategic combinations of promoter and transgene activities in order to improve the efficacy of cancer gene therapy. Typically, gene transfer vectors treat the transgene and promoter as independent units. Here, we have synergized the promoter and transgene characteristics in order to improve expression as well as transgene activity. METHODS: We constructed the pCLPG retroviral system (Virology, 321(2): 165-172, 2004) by inserting 13 copies of a p53-responsive sequence, the PG element (Science, 256:827-830, 1992), in the promoter of the pCL retrovirus (J Virol, 70(8):5701-5705, 1996). We then inserted the p53 cDNA within the pCLPG virus, establishing an expression cassette where p53 is employed to drive its own expression. These vectors were tested in a variety of reporter and biological assays. RESULTS: We have shown that expression of the newly established pCLPG system is indeed driven by wild-type p53 and that expression could surpass that of the parental system by 7-fold. When employed to drive expression of p53, the pCLPG system establishes a positive feedback regulatory loop. Tumor cell inhibition and induction of apoptosis were superior with the pCLPGp53 virus compared to the parental pCLp53 vector. Interestingly, the activity of p53 was markedly improved in the pCLPG system, yet expression was comparable to that of the pCL system in a prostate cancer model. CONCLUSIONS: We have established a novel gene transfer strategy, that of combining promoter and transgene activities in order to increase efficacy. The pCLPG system utilized p53 for two functions: 1) drive viral expression, and 2) act as a tumor suppressor. Since p53 function was improved, yet

expression was equal to the parental system, we believe that the pCLPG system may selectively express an activated form of the p53 protein. Supported by: FAPESP

P-036

A Lentiviral Vector with Expression Limited to Proliferating Cells for Cancer Gene Therapy

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OBJECTIVE: Our goal was to create a recombinant lentiviral gene transfer vector with a strategic promoter that would limit expression to dividing cells, including tumor cells. Such a vector would permit transgene expression specifically in cells that offer an active form of the well known transcription factor and indicator of cell proliferation, E2F. Specifically, the E2F-1 gene promoter is activated during the G1-S transition and is induced by members of the E2F family. METHODS: We inserted the luciferase reporter gene into the FUW lentiviral vector (Science, 295: 868-872, 2002), creating the FULW vector (our positive control), where luciferase expression is controlled by the ubiquitin gene promoter. We then isolated the promoter of the E2F-1 gene by PCR amplification and inserted it in place of the ubiquitin promoter, rendering expression from the FE2FLW vector under the control of the cell-cycle dependent E2F-1 promoter. A negative control vector lacking an active promoter, FLW, was also constructed. RESULTS: Lentiviral supernatant was used to transduce rat cardiomyoblasts (diploid, primary cells), NIH3T3 (immortalized mouse fibroblasts) and HT1080 (human fibrosarcoma, transformed cells). After serum starvation, we observed that reporter activity was markedly reduced only from the FE2FLW vector in all cell types. In contrast, expression from the control vectors remained constant. Similar RESULTS were obtained using the cytostatic drug doxorubicin (HT1080) or contact inhibition (NIH3T3). Cell cycle alterations were confirmed by propidium iodide staining followed by flow cytometry. We are currently testing an inducible expression system to verify the specific role of E2F family members in the induction of the FE2FLW vector. CONCLUSIONS: In these experiments, treatments that inhibited the cell cycle also inhibited expression specifically from the FE2FLW lentiviral vector. We propose that this vector may be further developed for cancer gene therapy, an application that would benefit from tumor-specific transgene expression. Alternatively, the FE2FLW vector may serve as an interesting marker of cell proliferation, especially where modifications of the Rb-E2F pathway are involved. Supported by: FAPESP

P-037

RECK Gene Expression During Cell Cycle and Glioma Progression: Correlation with C-Myc Expression and Prognosis

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INTRODUCTION: The *RECK* gene is widely expressed in normal human tissues, but is downregulated in both tumor cell lines and in oncogenic transformed fibroblasts. *RECK* suppresses both tumor invasion and metastasis by negatively regulating matrix metalloproteinases. **METHODS AND RESULTS:** We have shown that *RECK* transcript levels are upregulated in quiescent mouse fibroblast when compared to serum-stimulated cells, showing an inverse correlation with *c-myc* expression during cell proliferation. NIH-3T3 cells transfected with the pCXN2-*RECK* vector (Pop-hRECK) display a lower ability to grow in medium containing high serum concentration in comparison with control transfectant cells (Pop-pCXN2) or with the parental NIH-3T3 cell line, showing significantly higher doubling time and lower saturation densities. Analysis of the G0/G1 to S transition in Pop-hRECK cells showed that entry into the S phase is significantly inhibited in comparison to the parental NIH-3T3 cell line and the control transfectant cell line, indicating that inhibition of *RECK* expression is an important step for cell cycle progression. The molecular mechanisms underlying the action of *RECK* during cell cycle progression occurs, at least in part, through activation of the p21 CDK inhibitor, that promotes a delay in Rb phosphorylation. On the other hand, a correlation has been found between the abundance of *RECK* expression in tumor samples and the better prognostic of the cancer patients. Cytochemical analysis showed that expression of the *RECK* gene was regulated during glioma progression, being undetected in the most malignant phenotype endpoint, the multiform glioblastoma. **CONCLUSION:** Better understanding of *RECK* gene regulation may contribute to uncover the mechanisms of cell cycle and tumor progression and to develop new strategies for cancer therapy. Supported by: FAPESP, CNPq, FINEP and PRP-USP

P-038

Strategic Combination of pCLPG-Mediated Transfer of the p53 and p19ARF Tumor Suppressors for Gene Therapy of Melanoma

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OBJECTIVES: Investigate the combined anti-tumor

effect of p53 and its functional partner, p19^{ARF}, when transferred to melanoma cells using the pCLPG retroviral system (Virology, 321: 165-172, 2004; Cancer Gene Therapy, in press, 2005), which utilizes p53 as a transcription factor to drive viral expression. When this vector encodes p53, a positive feedback expression mechanism is established. With this work, we begin our investigation of the possible role of p19^{ARF} in cooperating with p53 to activate expression of the pCLPG system and inhibit melanoma cell proliferation. **MATERIALS AND METHODS:** The cDNA's for p53 or p19^{ARF} were inserted individually in the novel pCLPG vector, forming monocistronic constructs. We also created a bicistronic arrangement of these two cDNA's coupled by an IRES element. To assess the role of p53 and p19^{ARF} in controlling viral expression, these viruses were used to transduce the B16 (p53 wt) mouse melanoma cell line, total RNA was isolated from transduced cells with or without treatment with the genotoxic, p53-activating chemotherapeutic agent doxorubicin (dox) and used as template in a semi-quantitative RT-PCR assay. We analyzed a variety of transcripts, including viral genes (human p53 and NeoR), endogenous p53 target genes, p21^{WAF1} and MDM-2, and the mouse β -actin transcript as an internal control. Biologic assays, such as colony formation, have also been performed. **RESULTS:** We confirmed that expression of the pCLPG system was dependent on p53 activation, as evidenced by induction with dox. We observed that transduction with the pCLPGp19^{ARF} virus resulted in superior expression from the pCLPG system, better even than that seen with the pCLPGp53 vector, with or without dox treatment. The expression of p53 target genes, p21^{WAF1} and MDM-2, increased with dox treatment as expected, but was markedly increased in the presence of pCLPGp19^{ARF}. Surprisingly, the bicistronic vector, pCLPGp19^{ARF}p53, yielded decreased levels of viral and target gene expression. **CONCLUSIONS:** We have made an initial observation that p19^{ARF} can complement endogenous p53 to bring about elevated levels of expression of the pCLPG system as well as cellular targets of p53. The combination of p19^{ARF} and p53 encoded by the bicistronic pCLPG vector was not beneficial for viral expression in this assay and we are currently investigating the cause of this discrepancy. Supported by: FAPESP

P-039

Proliferation. Differentiation and Apoptosis: Involment of Cellular Prion Protein

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Prion diseases are transmissible fatal neurodegenerative disorders associated with the conversion of the cellular prion protein, PrP^c, to its infectious isoform, PrP^{sc}. The physiological functions of PrP^c are under intensive investigation. We described that the PrP^c binds STI1 (Stress Inducible Protein 1) and

this interaction rescue neuronal cells from apoptosis (EMBO J. 21:3307, 2002). Herein, the role of PrPc-STI1 interaction in astrocyte proliferation and differentiation, as well as intracellular signaling involved in these events were studied. Embryonic cerebral cortex primary cultures (E17) obtained from wild type (*Prnp*^{+/+}) and PrPc gene ablated (*Prnp*^{0/0}) mice were performed. After 5 days, sub confluent astrocytes were treated with recombinant STI1 (0.05mM) for 24 hours and their proliferation evaluated by tritiated thymidine incorporation for more 24hours. We showed that STI1 inhibited *Prnp*^{+/+} astrocyte proliferation by $67 \pm 8\%$ ($p < 0.01$) and *Prnp*^{0/0} cells by $60 \pm 12\%$ ($p < 0.01$) (compared to untreated cells), indicating that this event was independent on PrPc expression. The STI1 effect on astrocyte proliferation was reversed by a PKC inhibitor (KT5720) while no effect was observed when PKA, PI3K or MAPK blockers were applied. On the other hand, when *Prnp*^{+/+} confluent astrocytes (15 days in culture) were treated with STI1 (0.5mM), closely to 100% of the cells differentiate from a flat to a process-bearing morphology, while no alteration was observed in *Prnp*^{0/0} ones. This morphological change was repressed by a MAPK inhibitor (U0126) but not by PKC, PKA or PI3K blockers. In order to analyze survival, astrocytes were pre-treated with STI1 (1.0mM) and the cell death was induced by Staurosporine, an apoptosis inducer. The cell death, detected as a condensed and pyknotic profile (propidium iodide staining), in PrPc null astrocytes was higher than in wild-type cells. STI1 rescued wild-type astrocytes from apoptosis, an effect that was reversed by a PKA inhibitor (KT5720). Our data indicate that STI1 is able to mediate a PrPc independent astrocyte proliferation through PKC pathway and a PrPc dependent astrocyte differentiation and survival through MAPK and PKA pathways, respectively. Supported by: FAPESP

P-040

Evaluation of Cytotoxicity Mediated by Lapachones Derived Compounds

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OBJECTIVE: Lapachones (LAP) are the main representative of naphthoquinones, compounds originally isolated from a tree (*Tabebuia* sp). These compounds are interesting because they induce apoptosis in cancer cells but not in normal cells. Distinct mechanisms of cell death are related to the cytotoxicity activity by LAP derived compounds as well as topo I/II inhibition. The increase of reactive oxygen species (ROS) was observed. In this study we evaluate the viability of cancer cells after treatment with various LAP derived compounds. **MATERIAL AND METHODS:** Hep2C and Hela cells were obtained from FIOCRUZ -RJ. Cells were grown as a monolayer culture in DMEM supplemented

with 10% fetal bovine serum (FBS). The pure compounds were obtained from Chemistry Institute (UFF), dissolved in DMSO and used as a solution at 50 mM concentration. The cells were plated at 5×10^6 cells/well in 96 wells plate. After a 24 hr preincubation period, LAP dilutions were added in quadruplicate. The experiments were repeated 2 times. The evaluation of mitochondrial function (MTT) and the tripan blue permeability was performed to assay cellular viability. **RESULTS:** We found Hep2C viability decreased with Norb-LAP(57%) and Epoxy Norb-LAP(35,2%), using MTT test. Additionally, Hela cells viability decreased with Epoxy a-LAP(64,19%) and Epoxy b-LAP(86,43%). But on the other hand, using tripan blue test, Hep2C cells viability decreased with Norb-LAP(19,45%) and Epoxy Norb-LAP show high cytotoxicity. Furthermore, Hela cells show high cytotoxicity with Epoxy Norb-LAP and Epoxy a-LAP show apparently no cytotoxicity. **CONCLUSION:** Among LAP tested, Epoxy Norb-LAP showed high cytotoxicity. Furthermore, LAP cytotoxicity will be tested under different conditions (concentration and incubation time), and cytotoxicity mechanisms by Epoxy Norb-LAP will be studied subsequently through topo I/II evaluation and ROS generation.

P-041

Construction of an Inducible System for Retrovirus Production

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OBJETIVES: Retroviruses are widely used as gene transfer tools. Cytotoxic and cytostatic genes, like tumor suppressor genes encoded by the retroviral vector, or envelope genes encoded by packaging vectors, are expressed in the packaging cells and may halt their growth and, in turn, prejudice virus production. Therefore, we introduce a novel inducible system, which allows the establishment of producer cell lines for retrovirus encoding cytotoxic and cytostatic genes. The proposal of the inducible system is based on the utilization of a temperature sensitive (TS) mutant of p53 as a transcription factor to drive expression of the production system. All plasmid vectors that encode retroviral genome and packaging sequences have a chimeric promoter fused with the PG element, a p53-responsive sequence. In this way, the transcription of the retroviral genome and the packaging genome is controlled by the conformation of the p53TS product. **METHODS:** Viral vector and packaging vectors were constructed cloning genes and sequences from vectors pCL (Journal of Virology, 70:5701-05, 1996), pCLPG (Virology, 321(2):165-72, 2004), pCMV-gag-pol (PNAS, 93(21):11400-6, 1996) and pCMV-G (PNAS, 90(17), 8033-7, 1993). Northern Blot assays were performed with all the inducible vectors at permissive and non permissive temperature (32 and 37°C) to confirm the transcriptional

control of the temperature sensitive inducible promoters. Functional assay using reporter genes luciferase (Luc) and enhanced green fluorescent protein (EGFP) were performed to verify the transcriptional control of the chimeric promoters of inducible vectors. The inducible vectors encoding reporter genes and packaging genes were employed to produce virus. Virus preparations were harvested and tittered. RESULTS: The inducible vectors and TSp53 were assayed to confirm their function. Using EGFP or Luc as a reporter of inducible promoter activity, we observed a 7-fold increase in reporter activity in cells expressing TSp53 and after shifting the cultivation temperature from 37 to 32°C. With Northern blot assays, we have shown an increase of Luc, gag-pol and VSVg transcript levels when cells transfected with these components of the inducible virus production system were cultivated at the permissive temperature (32°C) in the presence of TSp53. Utilizing these inducible vectors and TSp53 for virus production, we show virus accumulation in the culture supernatant in a temperature dependent manner. Conclusion: We have developed a new TS inducible system to modulate retrovirus production. At 37°C, the p53 takes on the mutant conformation and is unable to drive transcription, thus protecting the producer cells from the toxic effect of encoded gene. At 32°C, the p53 assumes the wild-type conformation and is able to induce transcription from the chimeric promoter, thus initiating virus production. Supported by: FAPESP

P-042

Nitric Oxide Donors, Nitrosyl Ruthenium Complexes, Support Either Cell Death or Survival in Different Cancer Cell Lines

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INTRODUCTION: The nitrosyl ruthenium complexes 4-Pic: $\text{cis}[\text{Ru}(\text{bpy})_2(4\text{-pic})(\text{NO}_2)]$ and Py: $\text{cis}[\text{Ru}(\text{bpy})_2(\text{py})(\text{NO}_2)]$ are intracellular nitric oxide donors. Both of them are found within the cells in the NO_2 -bound form. By the action of intracellular reductases, NO_2 is reduced releasing the NO^0 , by the following reaction: $\text{NO}_2 + e^- \rightarrow \text{NO}^0$. OBJECTIVES: Characterize the effect of these intracellular NO donors on cell viability in different cancer cell lines. We show here the RESULTS obtained for B16 F10 (a metastatic melanoma cell line); melan-a (a non tumorigenic melanocyte cell line); a group of human melanoma cell lines, including a radial growth phase-like cell line (WM1552), and a pair of cell lines, the WM278 (vertical growth phase) and WM1617 (metastatic line). The leukemic cells used were a myeloblastic cell line (HL 60) and the lymphoblast T cells (Jurkat). METHODS: Cell culture and MTT assay (to

assess cell viability). RESULTS: The RESULTS were different according to the cell type. Both drugs were very effective in killing the leukemic cells, HL60 and Jurkat (almost 90% of cell death). Similar RESULTS were obtained for Melan-a (38% of cell death at the same dose), whereas the metastatic B16-F10 revealed rather an increase of 60% in the cell survival rate. For the human melanoma cells WM1617 and WM278 were resistant to the drugs while the non-tumorigenic WM1552 was sensitive (60% of cell death on 1mM). CONCLUSIONS: Our RESULTS are compatible with previous findings for the same or related cell lines exposed to NO, therefore the nitrosyl ruthenium complexes tested here were proved to be effective as NO donors, and most importantly, the differential effects obtained among the cell lines tested suggests that the effects on cell viability are exclusively dependent on the released NO and not due to secondary toxic effect. In addition, the RESULTS suggest that NO response may serve as a marker of tumor progression in melanoma cells and also prompt us to direct efforts towards uncovering the molecular pathways that mediate cell survival or death in response to NO in the different cell lines. Supported by: FAPESP, CNPq, Faepa

P-043

Increased Expression of PAWR is Associated with Breast Cancer Progression

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INTRODUCTION: the PAWR gene (PKC apoptosis WT1 regulator; also named PAR-4, prostate apoptosis response-4) encodes a 38 kDa protein that induces increased sensitivity to apoptosis in most cancer cells, especially in hormone independent cancer cell lines, including breast cancer cells. However, its clinical significance in breast cancer remains to be established. OBJECTIVES: in this study, our goal was to determine the expression pattern and prognostic value of PAWR in primary breast tumors. MATERIAL AND METHODS We first evaluated the PAWR mRNA expression in the breast cancer cells MCF-7 in the absence and presence of 17 β -estradiol and in 74 primary breast tumors using quantitative real time PCR (qPCR). PAWR protein expression was further evaluated by immunohistochemistry (IHC) using Tissue Microarrays (TMA) containing 699 primary invasive breast tumors and the RESULTS were correlated with patients' clinicopathological characteristics and survival. RESULTS: MCF-7 cells maintained in charcoal stripped serum showed increased expression levels of PAWR mRNA (2-fold), which was down-regulated by the presence of 17 β -estradiol 10nM for 24h. Down-regulation of the PAWR transcripts was observed in 20 out of 74 tumors analyzed (27.4%; more than 2-fold decrease) and up-regulation in 22 tumor (30.1%; more than 2-fold

increase). High levels of *PAWR* transcripts were significantly associated with the presence of lymph node metastasis ($p=0.01$). Fifty-two percent (331/640) of the analyzable cases showed positive *PAWR* protein expression. Significant associations were found between *PAWR* protein expression and advanced clinical stage ($p=0.0001$), lower nuclear grade ($p=0.0001$) and the presence of estrogen receptor ($p=0.0001$). In addition, *PAWR* protein expression was directly associated with shorter disease free and overall survival of the patients. **CONCLUSIONS:** our RESULTS suggest that down-regulation of *PAWR* expression might be involved in the regulation of cell proliferation and survival by estrogens and provide evidence that increased *PAWR* protein expression play a role in breast cancer progression and could serve as useful prognostic marker of the disease outcome. Supported by: FAPESP

P-044

Myosin-VA Fragment Induces Spontaneous Apoptosis in Different Cancer Cell Lines

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INTRODUCTION: Myosin-V is a molecular motor that uses the multifunctional dynein light chain-2 protein (DLC2) as light chain, which interacts with the Bcl2-modifying factor (Bmf). This protein complex appears to play a role in sequestering Bmf on the actin cytoskeleton, under conditions that promote cell survival and may have a central role in mediating *anoikis*. Ongoing research in our laboratory has previously shown that a myosin-Va fragment (MVaf), which corresponds to the binding domain of the DLC2, induces a dramatic decrease in the viability of the B16-F10 murine melanoma cell line. This result was unexpected, as metastatic melanoma cells are well known for their highly therapeutic resistance. **OBJECTIVES:** (1) To evaluate the capacity of MVaf to induce cell death in tumorous cell lines in comparison with the effect observed in B16-F10. (2) To analyse the morphological and molecular characteristics of the putative apoptotic response. **METHODS:** Cell lines tested were transfected with the plasmidial construct pEGFP-C1/MVaf or the pEGFP-C1 as control. Viability was assessed by counting the transfected cells over a period of 6 days after transfection. The blebbing characteristics were analysed by fluorescence microscopy and scanning electron microscopy (SEM) assays in transfected B16-F10 melanoma cells plated on Cellocate coverslips. The target cells were localized by means of the grid on the Cellocate. **RESULTS:** A 40-fold decrease in cell viability was observed for MVaf transfected B16-F10 cells, in comparison with EGFP controls. Decrease of 13 or 8-fold was obtained, respectively, for S91-Cloudman melanoma cells or HeLa carcinoma cells. The images acquired by SEM showed B16 cells with high amount of membrane blebbings and

other surface alterations. **CONCLUSIONS:** These results indicate that MVaf is a potent inducer of cell death in the cell lines analysed. The intense blebbing observed reinforced that this fragment leads to morphological changes that are typical of apoptotic cell death. Our results point towards the possibility of using MVaf as molecular therapeutic tool able to decrease or even eliminate tumoral cells. Supported by: CAPES, CNPq, FAEPA and FAPESP

P-045

Genomic Approach to Understand the Mechanisms Involved in BCR-ABL Mediated Resistance to Apoptosis

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INTRODUCTION: Bcr-Abl is an oncoprotein involved in malignant transformation and resistance to apoptosis, processes that are not fully understood. **AIMS:** To investigate the impact of enforced expression of Bcr-Abl on global gene expression and apoptotic machinery. **METHODS:** Ectopic expression of Bcr-Abl was obtained using retroviral vectors. Apoptosis was evaluated against different stimuli by flow cytometry (annexinV/PI and DNA content). Expression of apoptotic genes was examined by RT-PCR and Western-blot. Global gene expression of wild-type and Bcr-Abl⁺ cells was performed with oligonucleotide microarrays. Differentially expressed genes were identified based on log-ratios consistently above/below intensity-dependent cutoff curves obtained by applying HTself method to self-self data. **RESULTS:** Microarray analysis of Bcr-Abl transfectants identified 465 common genes that were overexpressed and 70 underexpressed in cells that became resistant to apoptosis. The upregulated genes are mainly related to cell motility, communication, growth, death, signal transduction and metabolism. Among them, genes involved in apoptosis and immune system regulation such as *faim*, *caspase-9*, *nfat*, and *prame* were found. Most of the underexpressed genes are related to signal transduction pathways (*tyrobp*, *calcineurin A beta*, and *traf-3*). Bcr-Abl expression conferred resistance to death-inducers in HL-60 and HeLa cells, but not in SKW6.4 cells. Protein levels of Bcl-x_L, Mcl-1 and Flip were higher in HL-60/Bcr-Abl than in HL-60. These effects were not observed in HeLa.Bcr-Abl and SKW6.4.Bcr-Abl. In contrast, pro-apoptotic protein Bid was reduced in HL-60/Bcr-Abl and SKW6.4/Bcr-Abl. In sum, ectopic expression of Bcr-Abl is capable of protecting HL-60 and HeLa, but not SKW6.4 cells from

apoptosis, suggesting that the cellular context is also important to Bcr-Abl cell malignant transformation and apoptosis resistance. The differentially expressed genes identified deserve further investigation in order to evaluate their potential as therapeutic targets or prognostic markers for CML. Supported by: FAPESP, CNPq and SBIBAE.

P-046

Expression of Survivin Gene and Its Splice Variants Survivin-DeltaEx3 and Survivin-2B versus p53 Expression in Astrocytic Tumors

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INTRODUCTION: Survivin (SVV) is an antiapoptotic gene that is expressed in a cell cycle-dependent fashion. SVV expression is upregulated in most human cancers, but is undetectable in nonproliferating normal adult tissues. The presence of SVV is correlated with reduced apoptotic index, abbreviated overall survival, unfavorable prognosis, and accelerated rates of recurrences. Recently, two functionally divergent splice variants of SVV were identified. The variant SVV-DeltaEx3 has antiapoptotic potential while the variant SVV-2B exhibits a loss of antiapoptotic potential and may be a natural antagonist of SVV. SVV acts at the interface between apoptosis and cell cycle regulation. SVV expression is repressed by the p53 tumor suppressor gene and induced by the E2F transcription factor. SVV is a substrate for p34CDC2-cyclin B kinase and also binds to CDK4. Astrocytic tumors are the most common tumors of the CNS with molecular defects in p53 and Rb cell cycle control pathways. **OBJECTIVES:** We aimed to investigate the expression of SVV and its splice variants in astrocytic tumors in parallel with p53 and p21 expression in order to make correlations with patient prognosis and possible treatment strategy. **MATERIAL AND METHODS:** We examined SVV, p53 and p21 mRNA expression in seven astrocytic tumors by RT-PCR. **RESULTS:** All cases tested expressed the dominant SVV transcript and the variant SVV-DeltaEx3 (though at subtle different levels) while the expression of the variant SVV-2B was lost in all samples, except in the control. Also, all samples expressed p53 mRNA, but the sequence has not yet been confirmed. **CONCLUSIONS:** In renal cell and gastric carcinomas the loss of SVV-2B expression was found in the later stage of cancer development. On the other hand, expression of SVV-2B is predominant in neuroblastomas with good prognosis. Our results suggest that the expression of SVV splice variants, especially the loss of the non-antiapoptotic SVV-2B variant may start to be considered as an important prognostic factor for astrocytic tumor cases. Supported by: FAPESP

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P-047

Cellular Prion Protein and Its Partner as Apoptosis Regulators

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Cellular prion protein (PrP^c) is a ubiquitous glycosylphosphatidylinositol (GPI)-anchored protein involved in several biological roles such as protection against oxidative insults, neuronal adhesion, cell differentiation and protection against apoptosis. Strong evidence for a neuroprotective PrP^c function was derived from our description of a PrP^c ligand, which was later identified as the Stress Inducible Protein 1 (STI1), a co-chaperone protein that modulates Hsp70 and Hsp90 activities. We showed that the interaction of PrP^c with either STI1 or a peptide mimicking the PrP^c-binding domain of STI1 prevented programmed cell death of retinal cells through cAMP-dependent protein kinase A (PKA). Herein, we examined the signaling pathways and functional consequences of the PrP^c interaction with STI1 in hippocampal neurons treated with an apoptosis inducer. STI1 was able to rescue those cells from apoptosis through cAMP-dependent protein kinase A (PKA). Furthermore, the neuroprotective effect was found to be dependent on PrP^c-STI1 interaction, since that a STI1 deletion mutant to PrP^c binding site had no effect. The results presented here support a significant role for PrP^c as a response mediator in neuroprotection. Finally, these molecules may represent new targets for therapeutic intervention in apoptosis related diseases. Supported by FAPESP

P-048

Evaluation of Cytotoxicity Activity of Compounds Derived from Marine Brown Algae

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OBJECTIVE: Cancer is the second death cause in Brazil and, estimated incidence and mortality in Brazilian population are 402.190 and 126.960 until the end of this year (data from INCA). In spite of a large number of available chemotherapeutic agents the medical needs is still largely unmet. The oceans have big potential for being the source of novel molecules. Compounds isolated from marine organisms are usually part of toxic defense mechanisms, what make them ideal candidates for pharmacological investigation. Despite of this, the different types of complex and unusual chemical

substances produced by these organisms can be expected to produce new anticancer drugs. These are some of the reasons why drugs based on nature, mainly based in marine life, are being used as templates or direct treatments for cancer and infective diseases. The purpose of this research is to evaluate the cytotoxicity activity of compounds purified from order Dictyotales (marine brown algae). MATERIAL AND METHODS: Cytotoxicity activity was evaluated using tumor cells, Hela and HEP-2. The cells were maintained with DMEM culture medium supplemented with 10% calf serum bovine, pH 7.4, at 37°C in 5% CO₂. It was seeded 2,0x10⁶ cells into 24 wells microtiter culture plate. The extracts were diluted in DMSO and tested at a concentration of 50mM, in exception of the dolastane diterpene (diluted in water at 10mg/ml). The cell viability, made in the presence and absence of the drug, was evaluated by two different tests: exclusion of Tripa Blue, described by Philips (1973), and [³H] Timidina incorporation. RESULTS: Of nine drugs tested, the dolastane and the atomaric acid, have the biggest cytotoxicity activity, approximately 60% of cell viability. CONCLUSION: In the future, another method for determination of the cytotoxicity activity of these compounds will be used (MTT) with different concentrations and incubation times. The drugs with anticancer activity confirmed will be evaluated about their action mechanisms.

P-049

Ganglioside G_{D3} Modulates Chemotherapy-Induced Cell Death *In Vitro*

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OBJECTIVES: Gangliosides are a family of sialic acid-containing glycosphingolipids mainly located in the outer leaflet of the plasma membrane. The monosialoganglioside G_{M3} is expressed in melanocytes and upon transformation, melanoma cells accumulate disialogangliosides like G_{D3} and derivatives. Gangliosides are involved in many biological processes and G_{D3} could either stimulate cell growth or promote cell death. The underlying mechanisms for this dual function remain unknown. Here we have analyzed how G_{D3} interferes with cell death-induced by chemotherapeutic agents used in melanoma treatment. METHODS AND RESULTS: The murine melanocyte cell line, melan-a, was used as a model for the expression of G_{D3} synthase gene, which converts G_{M3} into G_{D3}. Like the parental cell line, the transfected clones required phorbol esters (PMA) to grow. In the absence of PMA, both G_{D3}⁻ and G_{D3}⁺ cells were growth-arrested in G₀/G₁ phases within 3 days but G_{D3}⁻ cells progressed to cell death after 6 days, suggesting that G_{D3} modulates survival cells. We next examined whether G_{D3} could modulate cell death. G_{D3}⁺ cells were more sensitive to staurosporine-, vinblastin- and to cisplatin-induced cell death.

Otherwise, G_{D3} conferred resistance to temozolamide-induced cell death. In this case, G_{D3}⁺ cells were growth-arrested in G₂/M phases within 3 days and tended to accumulate in a polyploid population. Next we used glucosylceramide synthase inhibitors, like PPPP (phenyl-palmitoylamino-pirrolidino-propanol) or d-PDMP (d-phenyl-decanoylamino-morpholino-propanol), which rendered cells G_{M3}⁻/G_{D3}⁻. We showed that these cells were more sensitive to vinblastin-induced cell death. However, these cells seemed to be more resistant to cisplatin-induced cell death while there were no differences to temozolamide treatment. CONCLUSIONS: These data suggest that G_{D3} augments or confers resistance to cell death, depending on which chemotherapeutic agent is used. In this way, we can associate a specific drug with glucosylceramide synthase inhibitors to augment death of G_{D3}⁺ cells and sensitizes melanoma cells. So, our RESULTS suggest that gangliosides could be a target for melanoma treatment and G_{D3} may be considered a predictive marker for chemosensitivity/chemoresistance in melanomas. Supported by: FAPESP and CAPES

P-050

Molecular Characterization of Chemo Sensitive and Chemo Resistant Wilms' Tumor Blastemal Type Using SAGE Libraries

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INTRODUCTION: Wilms' Tumor (WT), the most common childhood renal cancer, is an embryonal tumor composed by blastemal, stromal and epithelial histological components, which are correlated with differences in tumor behavior. Nowadays, as more than 80% of the WT can be cured, the current treatment protocols aim to decrease therapy aggressiveness for patients undergoing good prognosis and to adjust the most severe treatment for the smaller group (20%) of poor responders. The blastemal component (BC) is the most suitable for therapeutic response studies: although highly aggressive, it usually presents great sensibility to chemotherapy (CT) leading to good prognosis. However, in some cases, it is found to be resistant to CT, being associated to most therapeutic failures and bad prognosis. Thus, the identification of chemo-sensitive (CS) and chemo-resistant (CR) tumors becomes of great importance for an early implementation of a more individualized therapeutics. OBJECTIVE: to identify differences in gene expression profile from CS and CR BCWT using SAGE analysis. METHODS: Two SAGE

libraries were constructed from microdissected BCWT tissues originated from tumors characterized as CS and CR. Data from a second CR BCWT library, constructed by another group, were added to the comparative analysis. RESULTS: it was generated 64,365 tags from CS and 62,317 tags from CR libraries, corresponding to 19,095 and 20,310 clusters, respectively. After a bioinformatics and gene function analysis, we select 11 over-expressed (GNG2, GNA13, CRABP2, GAS6, RNF130, IGFBP4, SPARC, MAT2A, RAP1B, HSPA8 and SERPINH1) and 3 under-expressed genes (MCM7, FSCN1 and EI24) in CR tumors. These genes are involved in different biologic processes that could be related to CT response as apoptosis, cytoskeleton maintenance, DNA replication and repair, cell signaling and responsiveness to Retinoic Acid. Supported by: FAPESP

P-051

10-Oxa-Octadecylphosphocoline Promotes Apoptosis and Inhibits Proliferation in Acute Myeloid Leukemia Cells

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The alkylphospholipids (APL) are drugs that interfere with the phosphatidyl-inositol 3 kinase pathway which is constitutively activated in the majority of acute myeloid leukemia (AML) cases. Edelfosine and miltefosine are typical members of this class and both have a well documented anti-neoplastic activity. 10-oxa-octadecylphosphocoline (OPC) is an APL recently described but without a known action in AML. The drug was synthesized in accord with a previous report and its structure confirmed by nuclear magnetic resonance spectroscopy. AML cells K562 and NB4 in exponential growth were incubated with the drug (1, 10 and 100µM). After 24h, the cells were pulsed with bromodeoxyuridine (BrdU) a substance that is incorporated in newly synthesized DNA in place of thymidine. After this step the cells were fixed, permeabilized and treated with DNase in order to expose the BrdU antigenic sites. BrdU incorporation was measured by direct immunofluorescence using an APC conjugated anti-BrdU monoclonal antibody. A total DNA dye (7-AAD) was used to quantify the cell cycle phases and the apoptotic cells (sub G0). The experiment was carried out in a BD-FACScalibur flow cytometer and analyzed by FlowJo software (TreeStar inc). The OPC 100µM decreases proliferation (BrdU positive cells) in K562 (43,22 to 17,44%). NB4 cells had proliferation reduced in the 10mM to 2,76% and in the 100µM to 0,24%; the basal proliferation was 51%. In the 100µM dose OPC increases apoptotic cells in K562 (4,77 to 12,47%) and in NB4 (5,33 to 24,77%). The anti-proliferative effect was caused by a G2/M transition block observed by an increased in the G2 cells in K562 (14 to 45%) and in NB4 (10 to 52%). We conclude that OPC is an anti-leukemic agent with a potential clinical benefit.

P-052

Antineoplastic Effect of Rapamycin is Potentiated by Inhibition of IRS-1 Signaling in Prostate Cancer Lacking PTEN

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Proper activation of the phosphatidylinositol (PI) kinase-Akt pathway is critical for the prevention of tumorigenesis. Many recent studies have characterized a negative feedback loop in which components of the downstream branch of this pathway, composed of the mammalian target of rapamycin (mTOR) and ribosomal S6 kinase, block further activation of the pathway through inhibition of insulin receptor substrate function (IRS). However, the potential of IRS-1 antisense oligodeoxynucleotide (ASO) as an inhibitor of proliferation during rapamycin treatment has not been examined. In the present study, we show that IRS-1 ASO and rapamycin cooperatively antagonize the activation of mTOR in vivo and act synergistically in tumor suppression in prostate cancer xenograft. IRS-1 ASO also reduces the activation of the PI 3-kinase pathway, which rapamycin alone reduces the activation of mTOR, while activation of the MAPK pathway. Combined treatment with rapamycin and IRS-1 ASO leads to a quantitative inhibition of molecular signaling through the PI 3-kinase and MAPK pathway. In accordance with these results, the proliferation inhibition of PC-3 xenografts after treatment with IRS-1 ASO and rapamycin was more pronounced than when each one was administered alone. Our data demonstrate that combined inhibition of both IRS-1 and mTOR signaling can limit the growth of PC xenografts. This, in turn, has important therapeutic implications by showing that the combination of treatment with agents that concomitantly block IRS and mTOR could be beneficial. Supported by: FAPESP, CNPq

P-053

Lipid Bodies Are Reservoir of Cyclooxygenase-2 and Sites of Newly Formed Prostaglandin-E₂ in Colon Cancer Cells

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OBJECTIVES: Colorectal carcinoma is the leading cause of death by cancer at every developed country. In practice, 98% from the cases of rough bowel cancer are

adenocarcinomas. Various studies suggest that COX-2 play an important role in carcinogenesis of colon. Moreover, nonsteroidal anti-inflammatory drugs (NSAIDs) are used as chemopreventive agents for carcinogenesis of colon. Accumulating evidence suggest that the inflammatory process and COX-2 represent a central role in colorectal carcinogenesis and the lipid bodies are sites involved in the production the eicosanoids during the inflammatory process. Thus, the OBJECTIVE of this study was to evaluate the involvement of lipid bodies in the development of colon carcinoma. METHODS AND RESULTS: Our results demonstrated a drastic increase in the number of lipid bodies in a human colon adenocarcinoma cell line (CACO-2) when compared to a non-transformed intestinal epithelial cell line (IEC). Furthermore, CACO-2 cells present an increased expression of COX-2 when compared to IEC cells. Interestingly, most COX-2 immunoreactivity was localized in the lipid bodies. Treatment with aspirin significantly reduced the number of lipid bodies in CACO-2 cells within 1 and 24 hours. Analysis of adenocarcinoma of colon from patients demonstrated that number of lipid bodies in the tumor tissues is increased when compared with a normal tissue from the same patient. CONCLUSION: Together, our results demonstrated that adenocarcinoma of colon present an increased number of lipid bodies that is correlated with the overexpression of COX-2, and the lipid bodies could be the major source of eicosanoids related to the pathogenesis of colon cancer. Supported by: FAF, CNPq and FURNAS

P-054

Role of Secretory Phospholipases A₂ In IL-6 and VEGF Production by Mast Cells

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Mast cells are central to several inflammatory processes, especially those of allergic origin. Mast cell role can also be extended to cancer, they are localized at the border of most aggressive tumors and may modulate the growth and invasiveness of cancerous tissue. The pro-cancer activities are at least in part mediated by cytokine production by mast cells, in particular IL-6 and VEGF. Secretory phospholipases A₂ (sPLA₂) may participate in this process through several different mechanism that lead to mast cell activation. AIM: To determine the role of endogenous secretory phospholipases A₂ in IL-6 and VEGF production by murine bone marrow-derived mast cells (BMMCs). METHODS: Bone marrow cells were cultured in RPMI (20% FBS) plus IL-3 (10U/ml). >From 4 and up to 12 weeks, >95% of the cells were mast cells as judged by cell morphology and toluidine blue staining. BMMCs were activated with 100 ng/ml SCF, 5 ng/ml IL-1b, and 10U/ml IL-10 for 8 h. To evaluate the role of sPLA₂ in IL-6 and VEGF production cells were pre-treated with: i) two selective sPLA₂ inhibitors LY 311727 and 12-epi-Scalaradial; ii) heparinase, what prevents sPLA₂ interaction with membrane proteoglycans; iii) the

mannose receptor agonist, p-amino-phenyl-mannopyranoside-BSA (pAMP-BSA), that mimics sPLA₂ receptor activation; iv) WEB 2170, a PAF receptor antagonist. To identify if a particular sPLA₂ enzyme was involved in cytokine production by mast cells we evaluated BMMCs derived from group V gene-disrupted mice and wild type littermates. RESULTS: IL-6 and VEGF generation stimulated by SCF + IL-1b + IL-10 were dose dependently inhibited by Scalaradial. LY311727 and heparinase did not modify the cytokine production stimulated by cytokines. pAMP-BSA fail to directly induce significant levels of IL-6 production by BMMCs. Meanwhile, treatment with WEB 2170 only inhibited VEGF generation, indicating a role for PAF. Group V sPLA₂ null BMMC produced up to 45% less IL-6 after SCF + IL-1b + IL-10 activation when compared to wild type controls. CONCLUSION: sPLA₂ are involved in IL-6 and VEGF production by BMMCs. The mechanism of action seems to be dependent on PAF generation at least for VEGF production. Other sPLA₂-derived mediators such as lysophospholipids are currently under investigation. Supported by: PROFIX/CNPq, FURNAS

P-055

Formation of Spheroids in Mammary Adenocarcinoma MCF-7 Cells

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In the biology of tumor cells it is important to obtain the attainment of systems which can be related to the *in vivo* cell to cell interaction. In this aspect, 3D cell cultures have advantages over those in monolayer. OBJECTIVE: The aim of this work is the characterization of breast tumor cell culture growth in spheroids, in comparison to the monolayers. MATERIAL AND METHODS: The methodologic approach consisted in inhibiting cell adhesion to substrate in order to obtain spheroids. Changes in morphology during the culture development were first analyzed with a phase inverted microscope. During the development of the cultures, the cells were submitted to immunofluorescent assays, using antibodies against the cytoskeleton elements, cell-junction and apoptotic markers (M30). The cells were, then, analyzed with a laser scanning confocal microscope. RESULTS AND CONCLUSIONS: In early cultures the cells were clustered in small compact bodies, the spheroids. Mitotic figures were frequent in these structures. The spheroids grew until the 72nd day. The spheroids became tubular and branched. From 30th day on, the central part of the spheroids started to become hollow (lumen-like). Concomitantly, the M30 positive cells appeared mainly next to this region. Older spheroids presented a peripheral cylindrical cell layer laying down a actin rich substrate. The positivity for laminin was observed on the external surface of the cells. The typical organization of cytoskeleton elements found in monolayer cell culture was modified in spheroids. The location of the gap junction connexin 43 showed a slight expression in the monolayer, but it had not

presented communication channels in cell membrane until the superexpression of the connexin 43 fused to GFP led to the formation of communication channels. Therefore, this system reveals itself sufficiently promising when considering the studies of cell-cell interactions, being also able to contribute to advances in the knowledge of tumoral biology. Supported by: Secretaria da Educação, CNPq and FAPESP

P-056

Transient Expression of Galectin-3 Interferes with the Kinetics of Melanoma Engraftment

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Galectin-3, a b-galactoside binding protein, is an important mediator of many biological process such as inflammatory responses and angiogenesis. In addition, galectin-3 gene expression is altered in different types of malignancies and some studies suggest that this protein is important for tumor invasion and metastasis. OBJECTIVES: to address a possible role of galectin-3 in melanoma progression, we evaluate the growth of melanoma cells expressing or not galectin-3 in C57BL/6 mice. Moreover, we also analyzed the microenvironment of these tumors by immunohistochemistry. Experimental Procedures: galectin-3 positive and negative melanoma cells were injected either in the footpad (histopathological analyses of the tumor microenvironment) or subcutaneously (tumor growth analysis) in C57BL/6 mice (10 animals/group). After 16 days, subcutaneous tumors were excised and processed for immunohistochemistry and gene expression analyses. RESULTS: immunohistochemical analyses of engrafted melanomas suggested an increase in the number of inflammatory cells in the tumor microenvironment derived from galectin-3⁺ melanoma cells as compared to adequate controls. In addition, both necrotic and caspase-3 reactive areas were less abundant in animals injected with galectin-3⁺ cells. We also observed an increase in the expression levels of important angiogenic genes in these tumors, suggesting that the transient expression of galectin-3 by tumor cells favoured melanoma progression. CONCLUSIONS: We have set a model for studying how galectin-3 modifies the tumor microenvironment through recruitment of inflammatory cells. This model will be useful to exploit the putative roles of galectin-3 in different phases of tumor progression. Supported by: FAPESP, CNPq.

P-057

Acute Inflammation Favors Melanoma Tumor Progression *In Vivo*

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BACKGROUND. Although it is well established that chronic inflammation contributes to development of neoplasia, the role of acute inflammation on tumor development is less studied. An experimental model developed in our laboratory showed that subcutaneous injection of either large numbers of apoptotic cells or LPS promote tumor growth of subcutaneous melanoma cells in syngeneic C57BL/6 mice. OBJECTIVE. The aim of this work is to elucidate the underlying mechanisms of 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. METHODS AND RESULTS. No differences in tumor growth were observed in nude mice submitted to the coinjection protocol (apoptotic cells and viable tumor cells), indicating that T cells are not involved in this process. In wild type C57BL6 mice, an intense inflammatory infiltrate composed mainly by neutrophils and macrophages is observed at the coinjection site during the first 48 h. Newly formed vessels and a reduced inflammatory infiltrate were observed 72 h after injection. Increasing levels of TNF were observed in sera 24 and 72 h after coinjection, and TNF knockout mice presented increased tumor growth compared to wild type counterparts ($p < 0.05$). Inhibitors of 5-lipoxygenase (caffeic acid and MK886), but not cyclooxygenase-1 and -2 (indomethacin and meloxicam), reduced tumor growth in mice injected with subcutaneous melanoma cells or LPS. Also, leukotriene B₄, a product of 5-lipoxygenase pathway and an important leukocyte chemoattractant, promoted growth of melanoma cells *in vivo*, but not *in vitro*. CONCLUSIONS. Our data point to the participation of neutrophils and macrophages and the involvement of 5-lipoxygenase inflammatory pathway in tumor promotion induced by acute inflammatory stimuli, and suggest the participation of TNF as an important mediator. Supported by: CNPq and CAPES

P-058

Role of Cytosolic PLA₂ and kinase Signaling Pathways on PGE₂ Generation by a Colon Cancer Cell Line

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COX-2-derived PGE₂ controls every aspect of colon cancer development, modulating from cell proliferation and resistance to apoptosis to angiogenesis, invasion, and metastasis. The first step in generation of eicosanoids, such as PGE₂, is the release of arachidonic acid (AA) esterified to phospholipids, what is performed by one or more phospholipases (PL)A₂. Cytosolic (c)PLA₂- α , a.k.a. group IVA, is the most important PLA₂ for AA release and eicosanoid synthesis, however other PLA₂s, in particular low molecular weight secretory (s)PLA₂s, can also participate in AA release for eicosanoid synthesis. OBJECTIVE: To investigate the role of different PLA₂s in

supplying AA for COX-2-dependent PGE₂ generation. We also investigated the signaling pathways that lead to PGE₂ generation. **METHODS:** Human colon cancer cell line Caco-2 was activated in complete DMEM medium with addition of NaCl (100 mM) to emulate the colon hyperosmotic environment. AA release, PGE₂ and VEGF generation were analyzed in the cell culture supernatants, while, COX-2 expression, cPLA₂ and kinases phosphorylation were analyzed on the cell lysates by western blotting. Pharmacological inhibitors of cPLA₂ (ATK), calcium-independent (i)PLA₂ (BEL), sPLA₂ (Scalaradial), COX-2 (NS-398), p38 (SB202190), JNK (SP600125), MEK 1/2 (U0126) were added 15-30 min before Caco-2 activation. **RESULTS:** Hyperosmotic stress induced marked, AA release, COX-2 expression and PGE₂ generation within 24 h of activation. sPLA₂ (scalaradial) and iPLA₂ (BEL) inhibitors did not modify, while COX-2 (NS-398) and cPLA₂ (ATK) inhibitors each completely inhibited PGE₂ generation. ATK also abolished stimulated AA release, but did not modify COX-2 expression. All three kinases inhibited AA release and completely abolished PGE₂ generation, however only inhibition of MEK 1/2 reduced COX-2 induction. **CONCLUSION:** Our results indicate that PGE₂ generation by Caco-2 cells exposed to hyperosmotic stress depends on cPLA₂ and COX-2 activity. Kinases participate in PGE₂ generation, however MEK 1/2 kinase is involved in cPLA₂ activation and COX-2 induction, while p38 and JNK only modulate cPLA₂ activity. Supported by: PROFIX/CNPq, Edital Universal 2004/CNPq, Primeiros Projetos/FAPERJ, FURNAS

P-059

Snake Venom Disintegrins as Tools for Detecting and Studying Integrins

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INTRODUCTION: Integrins are heterodimeric cell surface receptors that interact with extracellular matrix proteins and are critical for biological processes such as angiogenesis and cancer. Integrins usually bind to the RGD motif present in their natural ligands. This motif is also present in disintegrins, small integrin antagonists from viper venoms. Among disintegrins, eristostatin (Er) exclusively binds to αIIbβ3 platelet receptor, which is also present in melanoma cells during metastasis; while echistatin (Ec) additionally binds to αvβ3 and α5β1 integrins, which are involved in angiogenesis. **OBJECTIVE:** To produce molecular tools for the study/detection of integrins based on Ec and Er disintegrins. **METHODS:** Ec and Er genes were synthesized by PCR and produced in fusion with 6xHis and alkaline phosphatase (APv), respectively. Ec was tested in a platelet aggregation assay and in a direct binding assay

to M21 human melanoma cells. The binding of Er-APv to platelets and cells was assayed by a one-step dot blot where we used the APv portion as a reporter enzyme. **RESULTS:** Ec inhibited ADP-induced platelet aggregation with an IC₅₀ of 65 nM. However, when attached, Ec exerted an agonist effect, promoting cell binding through αvβ3 integrin as detected by a competition assay with LM609 monoclonal antibody. Both activities were RGD dependent as checked with an Ec-RGE mutant. Er-APv preserved its selectivity towards platelets and αIIbβ3 integrin transfected cells. When tested with Glanzmann thrombasthenia (GT) platelets, characterized by a defective expression of αIIbβ3 integrin, Er-APv was able to clearly discriminate the most common types of GT from normal samples. Just samples from heterozygous patients, whom are clinically asymptomatic and 2 other rare GT cases were not clearly differentiated. These samples will be further assayed by flow cytometry using a new EGFP-Er construction. **CONCLUSION:** These results point out the importance of disintegrins as alternative tools for prognosis/diagnosis of integrin-dependent disorders or simply to study integrin function. Supported by: FAPESP.

P-060

Higher Expression and Activity of Metalloproteinases in Association with HPV Presence in Human Cervical Carcinomas Cell Lines

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Cervical cancer, one of the most common cancers among women, is caused by certain types of human papillomavirus (HPV). MMPs -2, -9, and -14 activities are required for basement membrane degradation in cervical carcinoma. RECK gene is a tumor and metastasis suppressor gene that inhibits MMPs activity. **OBJECTIVES:** We evaluated the role of MMPs and their inhibitors RECK and TIMP-2, in human invasive carcinoma cell lines. **MATERIAL AND METHODS:** SiHa and CaSki (HPV16-positive) and C33A (HPV-negative) cell lines were cultured for five days either onto type-I collagen gel, MATRIGEL® and plastic, in order to recreate the tridimensional growth and to evaluate MMPs, RECK and TIMP-2 expression using Real-Time PCR. Gelatinolytic activities of MMP-2 and -9 were analyzed by zymography. **RESULTS:** HPV positive cell lines showed expression of MMP-2, -14 and TIMP-2 higher than the HPV negative one. MMP-9 and RECK are expressed in both HPV negative and positive cell lines in very low levels. Surprisingly, higher RECK expression

levels were detected in association with higher MMP-2 and -9 activities in CaSki cells, which contain more HPV 16 genome copies than SiHa cells. Extracellular matrix substrate does not induce differential expression of these genes. On the other hand, we found that type-I collagen gel but not MATRIGEL®, can positively modulate MMP-2 and -9 activities for all cell lines. CONCLUSIONS: Our results suggest that: i) the presence of HPV is related to increased expression of MMPs -2, -14 and TIMP-2, ii) in uncoated surfaces the MMP2 activity is higher for HPV positive cells than HPV negative cells. Supported by: FAPESP, CNPq, FINEP and PRP-USP

P-061

Maspin is Physically and Functionally Associated with Integrins Regulating Cell Adhesion in Mammary Epithelial Cells

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INTRODUCTION. Maspin (SERPINB5) is a tumor suppressor gene belonging to the family of serpins (serine protease inhibitors). Maspin is expressed in many tissues such as the mammary gland, prostate, skin, stomach and uterus and is down regulated in carcinoma cells. Maspin has several biological activities including inhibition of tumor growth, cell migration and invasion, inhibition of angiogenesis, and induction of apoptosis. Biochemical analyses revealed, however, that maspin does not act as a protease inhibitor. The objective of this study is to investigate the molecular mechanism underlying maspin tumor suppressor activity. METHODS: model-MCF-10A cells (non-transformed human mammary epithelial cell line). We used cell adhesion assays, RNA interference, confocal microscopy and immunoprecipitation. RESULTS: We report that maspin acts on the cell surface increasing cell adhesion. Addition of recombinant maspin rapidly increased MCF-10A cell adhesion to the endogenously deposited matrix, and conversely both an anti-maspin antibody and maspin knockdown by RNA interference resulted in decreased cell adhesion. Mutation analyses revealed that a region of 86 amino acids located between aa139 and aa225 was responsible for maspin effect on adhesion. Function-blocking antibodies against b1, b4 and a2 integrins prevented maspin-dependent increase in cell adhesion, and co-immunoprecipitation assay demonstrated the association of maspin with b1 integrin, indicating that maspin is functionally and physically associated with integrins. In addition, maspin increased cell avidity for the substratum, and it is associated with detergent-insoluble cortical cytoskeleton elements. In conclusion, maspin is part of the supramolecular structure of the adhesion plaque and it modulates cell adhesion via a physical and functional link with integrins. Supported by: N. Cella - Americas Fellowship provided by the NICHD/NIH (Bethesda, MD). M.Zhang - DOD grant (DAMD17-02-1-0294) and a NIH grant (CA79736).

P-062

Expression of Fusion Proteins: Endostatin-Pro-Apoptotic Peptides with Anti-Angiogenic Activity

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Endostatin, a 20 kDa COOH-terminal fragment of collagen XVIII, has been shown to act as an anti-angiogenic agent that specifically inhibits proliferation of endothelial cells and growth of various primary tumors. In the present study we report the expression of proteins composed of two functional domains: endostatin, that presents affinity for activated endothelial cells, used to guide the fusion protein and allow its internalization by the targeted cells, and a second domain composed by a short peptide (16aminoacids), corresponding to the minimal sequence of BH3 domains of pro-apoptotic proteins required to promote apoptosis. These proteins should be more efficient in the treatment of mice tumors than the isolated endostatin. OBJECTIVE: To express fusion proteins that have endostatin advantages of internalization by activated endothelial tumor cells and higher degree of apoptosis induction after internalization. METHODS: The molecular modelling of the fusion proteins was developed using the program Deep View\Swiss-PdbViewer. The pro-apoptotic peptides used in this study were Bax and Bak. The insertion of sequences coding the minimal sequence of the BH3 domain of pro-apoptotic peptides Bax and Bak into the endostatin gene was performed using the site-specific mutagenesis (Kunkel). The fusion proteins, endo-Bax and endo-Bak, were cloned into expression vectors for their periplasmatic or cytoplasmatic expression. The proteins were expressed in *E. coli*. BL21(DE3). The expression was analyzed by SDS-PAGE and Western blot assay. RESULTS: The hypothetical three-dimensional model shows the pro-apoptotic peptide Bax as an α helix in the endostatin C-Terminal region. Mutations of the vectors presenting the apoptotic domains endo-Bax and endo-Bak was confirmed by DNA sequencing. These vectors were transformed in *E. coli*. BL21(DE3) for expression. Western blot analysis confirmed the expression of the fusion proteins, endostatin-Bax and endostatin-Bak. CONCLUSIONS: The fusion proteins, endostatin-Bax and endostatin-Bak were successfully obtained and their expression were confirmed by Western blot analysis. Supported by: FAPESP and CNPq

P-063

Downregulation of Reck-Tumor and Metastasis Suppressor Gene in Glioma Invasiveness

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INTRODUCTION: Invasive behavior is the hallmark of gliomas, causing the failure of surgery, radiation and chemotherapy. Matrix metalloproteinases (MMPs) are essential for ECM remodeling and invasion. RECK protein regulates three members of the MMPs family: MMP-2, MMP-9 and MT1-MMP. Materials and METHODS: A172 and T98G, respectively, non-invasive and invasive human glioblastoma cell lines, were cultured onto uncoated or type I collagen gel-coated surface and maintained for up to 7 days, to allow establishment of the invasive process. Collagen substrate causes decreased growth rates and morphological alterations correlated with the invasive phenotype. RESULTS: Electronic transmission microscopy of T98G cells revealed podosomes, which are typically found in cells in the process of crossing tissue boundaries, since they constitute sites of ECM degradation. Real time PCR revealed higher RECK mRNA expression in A172 cells, when compared to T98G cells and, also, in samples obtained from cultures where the invasive process was fully established. Interestingly, the collagen substrate increases RECK expression in A172 cells and the same tendency is displayed by T98G cells. MMPs-2 and -9 displayed higher levels of expression and activity in T98G cells, and their activities are also upregulated by collagen. CONCLUSIONS: We suggest that: 1) RECK downregulation is critical for the invasion process displayed by T98G cells; 2) type 1 collagen can modulate RECK expression in glioblastoma cell lines. Since a positive correlation between RECK expression and patients survival has been noted in several types of tumors, our RESULTS may contribute to elucidate the complex mechanisms of malignant gliomas invasive process. Supported by: FAPESP, CNPq, PRP-USP and FINEP

P-064

Interaction of Insularin, a Snake Venom Disintegrin, with $\alpha_{\text{IIb}}\beta_3$ and $\alpha_v\beta_3$ Integrins

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INTRODUCTION: Disintegrins represent a family of low molecular weight proteins occurring in venoms of various vipers. They bind with a high affinity to

numerous integrins and are potent inhibitors of platelet aggregation and cell adhesion. We are studying the role of insularin (INS), a disintegrin from the venom of *Bothrops insularis* venom, able to inhibit platelet aggregation and binding of endothelial cells. MATERIAL AND METHODS: To obtain the native INS, *B. insularis* venom was fractionated in reverse phase C18 HPLC. The fraction 3 was analysed by SDS-PAGE and Western Blotting. The cDNA coding for INS was selected from a *B. insularis* venom gland cDNA library, in the clone GH063pro. The cDNA was PCR amplified and cloned into the pGEX-4T-1 vector and expressed in fusion with GST. The native or recombinant INS were evaluated for the inhibition platelet aggregation and binding to endothelial cells. RESULTS: We detected a disintegrin, INS, present in fraction 3. This fraction inhibited 100% of ADP-induced platelet aggregation and showed capacity to binding to endothelial cells. The INS showed a single band when analyzed by SDS-PAGE, which was also recognized by anti-disintegrin antibodies. The N-terminal of this band was sequenced and presents sequences that are highly conserved in disintegrins. The GST-INS (recombinant insularin) inhibited platelet aggregation and the binding of tEND endothelial cells to vitronectin at concentrations of 0,1 mg/mL. The GST control did not present activity in both tests. These RESULTS suggest that GST-INS interacts with $\alpha_{\text{IIb}}\beta_3$ and $\alpha_v\beta_3$ integrins. In the present work we showed the characterization of a novel disintegrin from *B. insularis* venom, through HPLC fractionation combined with recombinant DNA technology. CONCLUSIONS: The GST-INS with preserved biological activity can be used as a molecular tool and as starting point for the development of therapeutic agents for thrombosis and/or angiogenesis. Supported by: FAPESP

P-065

Blood Coagulation Proteins in Cancer: Molecular Mechanisms for the Highly Procoagulant Pattern of C6 Glioma Cells

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INTRODUCTION: A correlation between cancer and blood procoagulant states is well known. Thrombotic vascular occlusion is a frequent finding in human glioblastoma, the most aggressive glioma resistant to therapeutic interventions. This pattern seems to be directly correlated with the procoagulant action. Rat C6 glioma cell line is morphologically similar to glioblastoma multiforme and has been widely used in models of tumor growth and invasion. MATERIAL AND METHODS: The presence of tissue factor (TF) and phosphatidylserine (PS) on C6 cells was investigated by flow cytometric analyses. The assembly of the blood procoagulant complexes extrinsic tenase, intrinsic tenase and prothrombinase on these cells was studied using enzymatic assays employing purified proteins. RESULTS: C6 cells produced a significant decrease in the

recalcification time of rat plasma. This observation was consistent with the presence of TF, a 46-kDa transmembrane protein. TF functions as a receptor for the enzyme factor VIIa (FVIIa) with subsequent formation of the extrinsic tenase complex. This complex converts factor X (FX) into FXa triggering a zymogen activation cascade that results in blood coagulation. TF was identified by flow cytometric and functional (FXa formation in the presence of cells and FVIIa) assays. Alternatively, conversion of FX into FXa was also observed in the presence of C6 cells, FIXa and FVIIIa. This effect was both cell- and FVIIIa-dependent, being consistent with formation of the intrinsic tenase complex. C6 cells also promoted formation of the prothrombinase complex (FXa/FVa), which efficiently activates prothrombin into thrombin. It is known that formation of both intrinsic tenase and prothrombinase complexes are dependent on PS exposure. Therefore, annexin V, which blocks PS binding sites, inhibited FX and prothrombin conversion by their respective C6-assembled activating complexes. **CONCLUSION:** C6 glioma cells display a highly procoagulant pattern as a result of both TF exposure and the presence PS at the outer leaflet of their membranes. Therefore, this animal cell line may be used to explore new aspects concerning the role of blood coagulation proteins in tumor biology. Supported by: CAPES, CNPq, FAF, Faperj, FECD and FUJB.

P-066

Expression of Matrix Metalloproteinases, their Tissue Inhibitors and Membrane-Anchored Inhibitor RECK in Breast Cancer Progression

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Breast cancer is among the most common tumors affecting women. Like most solid tumors, metastatic disease rather than the primary tumor itself is responsible for death. The metastatic process involves a complex cascade of events, including the organized breakdown of extracellular matrix by matrix metalloproteinases (MMPs). The activity of these proteases is tightly regulated by specific inhibitors known as tissue inhibitors of MMPs (TIMPs). Consistent with their role in tumor progression, high levels of a number of MMPs have been shown to correlate with poor prognosis in human cancers. On the other hand, TIMPs are multifunctional molecules with high levels of TIMP1 and TIMP2 having been shown to predict adverse prognosis and correlate with aggressiveness in several different human cancers, including breast cancer. The tumor and metastasis suppressor gene RECK

encodes a membrane-associated MMP regulator protein that is able to suppress tumor invasion and metastasis by negatively regulating MMPs involved in carcinogenesis, namely: MMP2, MMP9 and MT1-MMP. In order to mimic the invasion process, a panel of five human breast cancer cell lines displaying different degrees of invasiveness and metastatic potential were cultured onto uncoated or Matrigel™-coated dishes for different periods of time. Real Time PCR and Zymography assays revealed a correlation between high levels of MMP2, MMP9 and MT1-MMP expression and activity with invasive and metastatic capacities. Conversely, the levels of TIMP1, TIMP2 and RECK expression are also directly associated with aggressiveness. These RESULTS suggest that high expression levels of MMPs and their inhibitors are correlated with invasiveness and metastatic potential of human breast carcinoma cell lines. Furthermore, our RESULTS suggest that the unique membrane-associated MMP regulator RECK acts as a multifunctional protein that may be involved in cell activities leading to tumor progression. Assessment of these MMPs and MMP inhibitors in tissue specimens from breast cancer patients have been contributing for better understanding of the physiological role(s) played by these molecules in regulation of tumor progression. Supported by: FAPESP, CNPq, FINEP and PRP-USP

P-067

Diabetes and Cancer: the Effect of Hyperglycemia in Metastatic Development

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Diabetes and cancer link was suggested by epidemiological findings that point to a higher risk of tumor development and worst prognosis in diabetic patients. Hyperglycemia due to diabetes can modify many processes in cell physiology that may result in altered proliferation and differentiation, apoptosis inhibition, oxidative unbalance and altered gene expression leading to tumor development and metastasis. **OBJECTIVE:** To investigate the effect of diabetes in melanoma pulmonary metastases formation and analyze the potential role of hyperglycemia. **METHODS:** Diabetes was chemically induced by i.v. Alloxan injection (65 mg/kg) in C57Bl/6 mice. Two weeks after diabetes induction, confirmed by blood glucose level above 300 mg/dl, 2 x 10⁵ murine melanoma B16F10 cells were inoculated i.v.. Metastatic capacity is evaluated by counting pulmonary nodules 2 weeks after melanoma injection. Insulin NPH (15 or 45 UI/kg) was injected s.c. twice a day starting 3 days after Alloxan injection and for the remainder of the experiment. To investigate the effect of hyperglycemia on B16F10 cells, cells were cultured in DMEM medium (BFS 10%) at normal (100mg/dl) or high (450mg/dl) glucose levels.

The role of oxidative stress was assessed by treatment with antioxidant butylated hydroxyanisole (BHA, 25-400 μ M) or catalase (1000-3000 U/ml). RESULTS: Diabetic animals had a marked increase in pulmonary nodules (287.1/animal) when compared to normal mice (12.4/animal). Insulin treatment with 15 or 45 UI/kg was able to reduce nodule formation to 200 and 74 nodules/animal, respectively. Insulin effect on metastases was paralleled by reduction in hyperglycemia. Melanoma cells exposed to high glucose medium had a higher proliferation rate *in vitro* when compared to cells cultured in medium containing 100 mg/dl of glucose. The increased proliferation in high glucose medium was inhibited dose dependently by BHA and catalase and was unrelated to differences in medium osmolarity. Conclusion: Diabetic state increases the metastatic capacity of B16F10 cells through a mechanism at least partially dependent on hyperglycemia-induced increased reactive oxygen species generation. Supported by: FAPERJ, CNPq, FURNAS.

P-068

Evaluation of Chemotactic and Phagocytic Functions of Mononuclear Cells from Cervical Cancer Patients

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OBJECTIVE: To evaluate the chemotactic and phagocytic functions of mononuclear cells from patients with cervical neoplastic lesions at different disease stage. METHODS and RESULTS: We prospectively investigated 13 healthy control women and 16 patients with cervical neoplastic lesions. Patients were further divided in Noninvasive or Microinvasive Group (n=8, cervical intraepithelial neoplasia grade III or microinvasive carcinoma) or Invasive Group (n=8, invasive carcinoma). No differences in the *in vitro* migratory responses towards the chemotactic stimuli (fMLP, MCP-1 and RANTES, 10⁻⁷ M), compared to random migration, were observed in purified circulating mononuclear cells from controls and patients, in a microchemotaxis chamber assay. However, considering tumor stage, the number (median and range) of migrated mononuclear cells (minus alleatory migration) toward MCP-1 was significantly increased in Invasive Group (4.2, 0.4-42.8) compared to Microinvasive Group (1.35, 0.1-4.4). Phagocytic assay carried out with plasma-opsonized zymosan as phagocytic particle did not show differences in the phagocytic index between controls (32.2 $\pm$ 3.3) and patients (31.3 $\pm$ 4.1) or patients from Microinvasive (34.1 $\pm$ 6.4, n=8) and Invasive (27.5 $\pm$ 4.4, n=6) groups. Conclusion: Patients with cervical cancer in invasive stages presented increased mononuclear cell migration, which may be due to the release of chemokines in the local tumor microenvironment. Supported by: FAPEMIG

P-069

Cellular Prion Protein Involvement in Migration

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Cellular migration is the key event in the spread of tumor cells, what makes essential to comprehend fully this process. The cellular prion protein (PrPc) is an extracellular GPI anchored protein involved in many biological functions, such as protection against oxidative stress, cell adhesion, neuritogenesis and cell signaling. Its interaction to extracellular matrix proteins, like laminin and vitronectin, suggests a role in adhesion and migration. Herein, we show that those interactions mediate neurite extension, a process that resembles migrating cells. PrPc also presents accumulation in motile structures: lamellipodia of migrating cells and the growth cones in neurons. It was also worthwhile to notice in migrating fibroblasts, that PrPc expression pattern is suggestive of focal adhesions. Those cell-substrate adhesion sites fulfill mechanical and sensing functions that involve reversible anchorage of the actin cytoskeleton to the extracellular matrix during migration. Also, the cell location of such PrPc-containing focal adhesions is suggestive of focal points rather than focal plaques, which indicate a role in migration rather than in adhesion. These data suggest that PrPc takes part in a general process of cell motility, a major feature in metastasis. Supported by: FAPESP

P-070

Evidence for a Protective Role of Galectin-3 in Metabolic Stress

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Galectin-3 is thought to be involved in mitochondrial homeostasis, preventing the formation of reactive oxygen species (ROS). If this is true, galectin-3 null cells are likely less protected from the genotoxic attack of ROS. OBJECTIVE: To evaluate this hypothesis, we have exposed established mesenchymal-derived cell lines from both wild type and galectin-3 null embryos (EMC+/+ and EMC-/-) to metabolic stress. METHODS AND RESULTS: Embryonic mesenchymal cells (EMCs) expressed large amounts of galectin-3, as detected by western blot. While it was possible to establish cell lines from galectin-3 null embryos (3T3 protocol), cells from wild type embryos progressively lost their ability to divide after a few consecutive generations. Wild type cells assumed a senescent phenotype and could be maintained in culture up to the 20th generation. Interestingly, when plated in low glucose, both EMC+/+ and EMC-/- cells were immortalized. Upon

immortalization, cells maintained galectin-3 expression (3T3+/+). We next compared how immortalized cells (3T3+/+ and 3T3-/-) react to oxidative stress, as exposure to different concentrations of sodium pyruvate (10, 50 and 100mM). At the lower concentration of pyruvate, cells behaved essentially the same. However, proliferation of both 3T3+/+ and 3T3-/- cells was severely impaired when cells were exposed to 100mM pyruvate. Furthermore, at higher concentrations of pyruvate, 3T3-/- cell death rate was higher. Mitochondrial ROS levels were analyzed by loading cells with dihydrorhodamine 123 (DHR123). When exposed to the same metabolic stress (pyruvate treatment), 3T3-/- produced significantly more ROS than 3T3+/+ cells, as measured by flow cytometry. Accordingly, single cell electrophoresis in alkaline conditions after 72 hours treatment with pyruvate (50 and 100mM) indicated that 3T3-/- cells were more sensitive to the genotoxic effects of metabolic oxidative stress. Conclusion: Taken together these data suggest that galectin-3 may play a protective role to metabolic stress, thus providing a possible explanation for the increased rate of immortalization of galectin-3 null cells. Supported by: FAPESP/CAPEs

P-071

ARHGAP21 Gene Expression is Modulated by reactive oxygen species in myeloid cell line

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INTRODUCTION: Reactive oxygen species (ROS) are generated *in vivo* under a variety of physiological and pathological conditions. Cell death may be linked to different events, such as oxidative stress in which the production of ROS plays a pivotal role in this process. RhoGTPases mediate many aspects of cell biology including proliferation, apoptosis/survival, adhesion and events related to actin cytoskeleton dynamics. In the last years, an increasing amount of evidence indicates that several members of the Rho family are important players in tumor biology and several human diseases. Rho-GTPase activating proteins (Rho-GAPs) are negative regulators of RhoGTPase signaling pathways. We identified a novel human gene, termed ARHGAP21, which codes for a 1957-aminoacid RhoGAP containing in addition to a RhoGAP domain, a PH and PDZ domain. We demonstrated that this gene is upregulated during myeloid and erythroid differentiation, suggesting a potential role for this RhoGAP in regulating cell differentiation through a gradual inactivation of GTPase cascades. **MATERIALS AND METHODS:** Myeloid cell line (KG1 cells) were grown to subconfluence and treated with *tert*-butylhydroperoxide (t-BHP) in two different concentrations (50 and 100 microM) during 2h.

Measurement of reactive oxygen species production (superoxide anions and hydrogen peroxide) using specific ROS probes and detection of cell death (Annexin V/Propidium Iodide) were performed by FACS analysis. Real Time PCR assays were used to analyze the differential expression of ARHGAP21. **RESULTS:** ARHGAP21 transcript levels were upregulated in myeloid cell line after t-BHP treatment, accompanied by ROS production. **CONCLUSION:** It is possible that the modulation of ARHGAP21 expression by ROS could be involved in the regulation of different cellular process, as cell death, that could be important for the development and progression of different types of tumors. Supported by: FAPESP and CNPq

P-072

Extracellular Galectin-3 Destabilizes Adhesion Plaques by Interacting With B1 Integrin

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OBJETIVES: Expression of galectin-3, a β galactoside binding lectin, is associated with sarcoma tumor progression and metastasis. Galectin-3 is secreted to the extracellular space and may play a role in cell-matrix interactions in a carbohydrate dependent way. In this study we determined the role of secreted galectin-3 in fibroblast-laminin interaction. **METHODS AND RESULTS:** We have established cell lines from methylcholantrene-induced sarcomas from both wild type and galectin-3 null mice. In this system, galectin-3 null cells were less migratory than control cells in laminin. When galectin-3 was transiently expressed in galectin-3 null sarcoma cells, it inhibited cell adhesion to laminin and stimulated the migratory response to laminin. The addition of exogenous galectin-3 also enhanced the migratory capacity of Σ 12 cells in a carbohydrate dependent fashion. Galectin-3 was found in focal contacts of Σ 12 cells where it may interact with many glycoproteins containing polylectosamines in the cell surface. Here we showed that β 1 integrins are among them. Exogenously added galectin-3 led to a decrease in phosphorylated-FAK in lamellipodia and increased the recruitment of Shp-2 phosphatase of migrating cells. The effect of galectin-3 in migration was not dependent on the activation of the p21 activated kinase (PAK). Wortmannin inhibited the increased migration elicited by galectin-3, suggesting the involvement of the PI3-kinase signaling in the galectin-3 pathway. **CONCLUSIONS:** We propose that extracellular galectin-3 bound β 1 integrins and disrupted the focal adhesion plaque, thus favoring cell migration. Supported by: FAPESP, CAPES

P-073

Bone Marrow Micrometastasis is Not Related to Lymph Node Involvement in Breast Cancer

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To establish metastases, breast cancer (BC) cells disseminate from the primary site via lymphatic or haematogenous routes reaching lymph nodes (LN) and other secondary sites. Although the presence of axillary LN involvement predicts the development of distant metastases, 20–30% of the BC patients (pt) free of axillary LN metastases also develop disease at distant sites. This data indicates that haematogenous dissemination may occur independent of LN involvement. The presence of micrometastasis in the bone marrow (BM) was recently shown to be a prognostic marker in BC, associated to a higher death risk. Cytokeratin 19 (K19) expression, a specific epithelial marker, indicates BM invasion. OBJECTIVES: The aim of our study was to investigate micrometastases (by K19 expression) in BM samples of BC patients without distant metastasis by Nested-reverse transcriptase-polymerase chain reaction (RT-PCR) and evaluate its correlation to LN involvement and other tumor characteristics. METHODS: BC pt were accrued at Instituto Brasileiro de Controle do Câncer and 10 ml of BM aspirates were collected, mononuclear fraction was separated by density gradient centrifugation and Nested RT-PCR using specific primers to detect K expression was carried out. Initial experiments were capable to detect one tumor cell diluted in one million lymphocytes. RESULTS: Sixty three patients with invasive carcinomas mainly ductal histology (86,5%) and clinical stage II (82,4%) were included. BM micrometastases were detected in 47,5% of them. An association between the presence of epithelial cells in BM with LN metastasis, tumor size, tumor grade, as well as menopausal status was not detected. However, an association between the presence of epithelial cells in BM with absence of estrogen receptor expression in the primary tumor ($p=0,034$) was observed. CONCLUSIONS: The presence of micrometastasis in BM of BC patients may be associated with negative estrogen receptor, a poor prognosis marker. An association between BM and LN involvement was not found in BC pt clinical stages I-III. Supported by: FAPESP and CAPES.

P-074

Free Radicals Modulate DNA Methylation During Melanocyte Transformation Induced by Adhesion Blockade

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BACKGROUND. A model of melanoma carcinogenesis was developed in our laboratory, where different melanoma cell lines were obtained as result of sequential cycles of anchorage-independent growth of a non-tumorigenic cell line, 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 elucidating the participation of DNA methylation, an important epigenetic mechanism, in melan-a transformation and its relation to oxidative and/or nitrosative stress resulting from blockade of cell-substrate interactions. 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 compared to adherent melan-a cells. Intracellular concentrations of O_2^- and H_2O_2 increase progressively during melan-a carcinogenesis. In addition, substrate adhesion impediment leads to a decrease in homocysteine concentration, which could lead to an alteration in DNA methylation. 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 for 24h and gradual genomic hypomethylation in 2C, 3C, 4C and melanoma cell lines. An increment in DNMT1 and 3a in non-adherent melan-a and a progressive decrease in DNMT3b mRNA were also observed in melanoma cells and in non-adherent melan-a cells. The antioxidant N-acetylcysteine, and the NO sintase inhibitor, L-NAME, were capable of preventing the increase in global DNA methylation induced by melan-a adhesion blockade, suggesting that stress resulting from adhesion impediment can modulate DNA methylation. CONCLUSIONS. The presence of non-tumorigenic cells in a new microenvironment might result in intracellular stress that could modify the expression of some genes, by altering their methylation status, contributing to the acquisition of a gene expression profile favorable to cell transformation. Supported by: FAPESP and CNPq

P-075

Gene Expression Modulated by DNA Methylation During Melanoma Carcinogenesis

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OBJECTIVE. Recent studies have shown that gene transcription is largely regulated by chromatin organization and that chemical modifications of DNA

molecules and histones have an important role in malignant transformation and tumor progression. The aim of this work was identify specific genes whose expression are regulated by promoter hyper or hypomethylation throughout melanoma carcinogenesis. METHODS. Different melanoma cell lines (4C3, 4C11, Tm1, Tm5) were established after submitting melan-a cells to 5 sequential substrate anchorage impediment cycles. Melan-a cell sublines submitted to 2, 3 and 4 deadhesion cycles (2C, 3C and 4C) were also established, representing distinct phases of tumor progression. RESULTS. Tm5 melanoma treated *in vitro* with 5-aza-2-deoxycytidine (5AZAdC) and Tricostatin A (TSA) showed a significant reduction in tumor development *in vivo*. Expression of p19^{ARF} was lost in melanoma cells, and reversed by *in vitro* treatment with 5AZAdC/TSA. Analysis of p19^{ARF} promoter methylation in Tm5 cells by CpG island sequencing showed hypermethylation in this region but an intermediate methylation status in suspended melan-a cells compared to parental cells. TIMP1, recently associated with anoikis-inhibition, has a very low expression level in melan-a cells, which is augmented during deadhesion. A higher level of expression is observed for 2C and 4C cells, which is maintained in the tumorigenic cell lines. After 5AZAdC treatment, increased levels of TIMP1 mRNA were observed in melan-a cells, suggesting that TIMP1 levels are regulated by promoter hypermethylation in melan-a, which is reversed during the carcinogenic process. CONCLUSIONS. These results show that a perturbation of methylation profile occurs along the 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, as demonstrated for p19^{ARF} and TIMP1. Supported by: FAPESP and CAPES

P-076

Blood Coagulation Proteins in Cancer: Increased Expression of Tissue Factor and PAR-1 in Esophageal Cancer

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INTRODUCTION: A correlation between cancer and blood procoagulant states has been well documented. Tissue factor (TF) is a transmembrane 46-kDa protein that initiates blood coagulation when bound to factor VIIa (FVIIa). Aberrant TF expression in tumor cells contributes to the prothrombotic state in cancer patients. However, recent studies indicate that TF participate in tumor growth, metastasis and angiogenesis through a pathway that is independent of blood coagulation. Also, a family of G protein-coupled receptors known as protease-activated receptors (PARs) has been implicated in tumor biology. These receptors may be activated by

blood coagulation proteases including thrombin, FVIIa and FXa. MATERIAL AND METHODS: In this study we compared the expression of TF, PAR-1 and PAR-2 between normal and tumor tissue samples obtained from patients with esophageal cancer. Tissue samples were obtained from 16 patients submitted to esophagectomy or endoscopy at HUPE-UERJ (Rio de Janeiro Brazil) or at Gastrocentro, Hospital das Clínicas, UNICAMP (Campinas, Brazil). Tumor samples and the corresponding normal mucosae were obtained and the diagnosis was confirmed by histopathological analysis of adjacent tissues. Total RNA was extracted and further analyzed by reverse transcriptase(RT)-PCR. RESULTS: Our results showed that expression of mRNA for TF and PAR-1 was significantly higher in tumor samples. On the other hand, the levels of mRNA expression for PAR-2 were statistically similar in normal and tumor samples. Previous studies demonstrated that PAR-mediated cell signalling is involved in interleukin-8 (IL-8) expression by a variety of cells. We further analyzed the IL-8 expression in tumor and normal samples. RT-PCR showed an increased expression of mRNA for IL-8 in tumor samples. CONCLUSION: Our data indicate that TF and PAR-1 might play an important role in esophageal cancer. Supported by: CAPES, CNPq, FAF, Faperj, FECD and FUJB.

P-077

Breast Cancer Response to Doxorubicin in Cultured Tissue Slices from *Cannis Familiaris*

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Doxorubicin (DOX) is one of the mainstay drugs in the treatment of breast cancer (BC), however, there are no markers with a predictive value for a patient's response to chemotherapy. We have previously analyzed the gene profile from tumors obtained from BC patients (pt) submitted to neoadjuvant chemotherapy based in DOX and among the differentially expressed genes between responsive and non-responsive pt were PBEF and PRSS11. Pre-B cell colony-enhancing factor (PBEF), promotes growth of B cell precursors and functions as an inhibitor of apoptosis. PRSS11, serine protease 11, or insulin-like growth factor (IGF) binding protein 5 protease, may influence the activity of IGF pathway. OBJECTIVE: To analyze whether this data could be reproduced in another model which preserves similarities to *in vivo* model: BC slice tissue culture *in vitro*, obtained from another species, *C. familiaris*. CAUSISTIC AND METHODS: Thirteen patients (*C. familiares*), median age 11 years, treated at Hospital Veterinário da UMESp were evaluated, and an informed consent was provided by the owners. BC samples were collected during mastectomy, sliced and cultured in the

presence or absence (control) of DOX 1 μ M or 2 μ M for 24h. Cell counting to evaluate response was performed in hematoxylin-eosin stained paraffin-embedded tissue. Responsive samples were considered those with a reduction >10% in cell count as compared to untreated samples. Gene expression was evaluated by real-time RT-PCR using *C. familiaris* specific primers to detect PBEF and PRSS11. RESULTS: Among tissue samples treated with DOX 1, 8 were considered responsive and 5 non-responsive. No differences in PBEF and PRSS11 expression were detected between samples considered responsive or non-responsive. Conclusion: Slice tissue culture is an interesting model as it structurally reproduces an in vivo model, preserving stromal-epithelial interactions. DOX was active in reducing malignant epithelial cells in tissue slices. Differences between species may occur, however, we believe a larger number of samples may help to define PBEF and PRSS11 expression value in detecting response to DOX. Supported by: Fundação Faculdade de Medicina

P-078

Galectin-3 Gene Is Silenced by Epigenetic Mechanisms along Melanoma Progression

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INTRODUCTION: Galectin-3 is a polylactosamine binding animal lectin, shown to be involved in tumor progression and metastasis. Most epithelial cancers seem to lose galectin-3 expression in advanced stages. In this study, we have used a melanoma murine model to investigate the mechanisms for galectin-3 gene silencing. This model system consists of a non-tumorigenic melanocyte-derived cell line (melan-a) and its tumorigenic counterparts (Tm1 and Tm5). **METHODS AND RESULTS:** we examined by Northern blot analyses the mRNA expression of the galectin-3 in this model. We found that galectin-3 mRNA was only present by melan-a cells. Analysis of the promoter region of galectin-3 demonstrated the presence of a high CG content, several SP1 binding sites and lacks TATA consensus. Regarding its genomic organization, the galectin-3 gene shares similarities with *INK4a/Arf* gene that is frequently silenced by hypermethylation along melanoma progression. Bissulfite sequencing of the promoter of galectin-3 demonstrated that the galectin-3 promoter CpG island was totally unmethylated in melan-a cells. However, dense methylation was seen in Tm1 and Tm5 cells. Furthermore, promoter methylation was correlated with lack of expression of galectin-3 mRNA and galectin-3 protein in the tumorigenic cells. Treatment of Tm1 cells with 10 μ M of 5-aza-2-deoxycytidine, a DNA methyltransferase inhibitor, induced de novo the expression of galectin-3. Curiously, the treatment of Tm1 cells with high concentrations of

the histone-deacetylase inhibitor trichostatin A (100 and 200 nM) reverted the effect of 5-aza-2-deoxycytidine. **CONCLUSIONS:** These results suggests that the methylation status control the expression of galectin-3 gene along the malignant transformation of these melanoma cells and indicate that galectin-3 expression is controlled by epigenetic mechanisms. Supported by: FAPESP

P-079

Heparanase Expression in the Mononuclear Fraction of Blood Samples from Breast Cancer Patients

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Heparanase is an endo-beta-glucuronidase that cleavages intradisaccharide heparan sulfate linkage, and has been involved with degradation of cellular surface and extracellular matrix heparan sulfate proteoglycans (Cohen I.R., et al. 1994). Increased expression of heparanase is involved with tumor development and metastasis (Hullet M.D. et al. 1999; Vlodavsky I. et al. 2000). The objectives of our study were to determine heparanase expression in breast cancer patients compared with healthy women, to correlate heparanase expression with metastasis and type of treatment. By immunocytochemistry we analyzed heparanase expression at mononuclear fraction of peripheral blood samples from 30 breast cancer patients and also 20 healthy women and finally we investigated possible mechanisms of heparanase activation at mononuclear fraction of peripheral blood samples. We used semi-quantitative RT-PCR technique to analyze heparanase expression at mononuclear fraction of blood samples from patients and healthy women and immunocytochemistry and immunohistochemistry using polyclonal anti-heparanase antibody HPA2 (Santa Cruz), developed with peroxidase. The statistics analysis was done using a package program SPSS version 13.0 (Illinois, USA). Heparanase was expressed at mononuclear fraction of breast cancer patients (133.44 $\pm$ 53.44), while healthy women did not express this enzyme. In our studies we did not find a significant correlation between heparanase expression and histologic type of tumor, as well as the stage of the disease. However, we found a direct correlation between the increase of heparanase expression and metastasis incidence ($p=0.027$). We also observed an extremely significant correlation between a decrease of heparanase expression after surgery ($p=0.002$). Concerning the type of treatment we observed that patients submitted with

tamoxifen treatment compared with other treatment chemotherapy and/or radiotherapy, there was a decrease at heparanase expression ($p=0.04$). Immunocytochemistry analysis performed had shown that breast cancer's lymphocytes presented intense labeled with polyclonal anti-heparanase antibody (80%), while healthy women's lymphocytes had labeled 10%. Breast cancer patients' plasma and serum, as well as MCF-7 cells were able to stimulated heparanase expression in lymphocytes of healthy women. We also observed that conditioned medium of MCF-7 cells, just after co-culture with mononuclear fraction of healthy women, was able to stimulated heparanase expression lymphocytes of another healthy women. Therefore, the microenvironment, stromal cells from extracellular matrix can determine the liberation of factor by tumor cell that will stimulate heparanase expression by lymphocytes, and heparanase will promote extracellular matrix remodeling that should define different characteristics as adhesion, migration and also other phenomenon that contribute by the development of the tumor as well as angiogenesis. The microenvironment can contribute to define the control and development of the tumor and extracellular matrix remodeling. Each day increase the theories that the microenvironment and the extracellular matrix were signaling for the control of tumor cellular functions and this is just one more experimental model that proves this hypothesis. Supported by: FAPESP, CAPES, CNPq, NEPAS.

P-080

Loss of ADAM23, a Putative New Metastasis Suppressor Gene, Leads to Enhanced Migration of the MDA-MB-435 Breast Cancer Cell Line Possibly by Modulating the α 5 β 3 Integrin Activation

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ADAM23 is a member of the ADAM protein family, unique among cell surface proteins in displaying both potential adhesive and protease activities. ADAM23 interacts specifically with the α 5 β 3 integrin in an RGD-independent manner [1]. However, ADAM23 lacks a conserved metalloprotease domain, which suggests its major enrollment in cell adhesion processes. In neurons, it appears to be localized to sites of intercellular contact, probably mediating cell-cell interactions within the mammalian central nervous system [2]. Cell-cell and/or cell-matrix interactions are known to play a crucial role in tumor progression. We have recently demonstrated that the ADAM23 gene is frequently

silenced by promoter hypermethylation in primary breast tumors of more advanced grades, suggesting that ADAM23 could be downregulated during breast cancer progression [3]. Methylation-specific PCR analysis of the ADAM23 gene, in a group of 109 surgically resected ductal invasive breast tumors revealed that hypermethylation is observed in 34% of the cases, being strongly associated with the development of distant metastasis and predicting a worse metastasis-free survival rate (Costa FF *et al*, submitted). RNA interference (RNAi) has emerged as a powerful tool to silence gene expression through targeted degradation of messenger RNA [4]. In the present study, ADAM23 ablation by RNAi was used to elucidate ADAM23 possible role in tumor formation and metastasis. The human MDA-MB-435 breast cancer cell line was stably transfected with a pAVU6+27-based vector [5] targeting a 19 nucleotides sequence specifically homologous to the human ADAM23 gene. Several cell clones showing significant reduction of ADAM23 mRNA and protein levels were obtained, as assessed by RT-PCR and Western-blot. We have evaluated adhesion and migration of two of these clones, in comparison to the parental cell line, using the α 5 β 3/vitronectin receptor/ligand model. Our results show an enhanced migratory capacity of MDA-MB-435 in the absence of ADAM23. Cell adhesion to vitronectin was facilitated by loss of ADAM23 and higher affinity of ADAM23^{-/-} clones to vitronectin is shown to involve α 5 β 3 integrin. Since the levels of α 5 β 3 were the same despite ADAM23 expression, we investigated the activation status of such integrin, using the AP5 monoclonal antibody [6]. Our preliminary results suggest that the absence of ADAM23 favors integrin α 5 β 3 activation. We are presently testing in vivo the metastatic potential of ADAM23^{-/-} clones in pulmonary colonization assay. Taken together, our results indicate that loss of ADAM23 enhances the MDA-MB-435 migratory behaviour and metastatic potential, possibly by modulating the activation of α 5 β 3. ADAM23 can thus be considered a new metastasis suppressor gene. [1] Cal S *et al* (2000) *Mol Biol Cell* 11:1457-69; [2] Goldsmith AP *et al* (2004) *J Neurosci Res* 78:647-58; [3] Costa FF *et al* (2004) *Oncogene* 23:1481-8; [4] Hannon GJ (2002) *Nature* 418:244-51; [5] Paul CP *et al* (2002) *Nat Biotechnol* 20:505-8; [6] Honda S *et al* (1995) *J Biol Chem* 270:11947-54. Supported by: FAPESP, CNPq, FINEP and the Ludwig Institute for Cancer Research

P-081

Cancer/Testis Antigens are Frequently Expressed in Advanced Stage Multiple Myeloma Cases and Can Be Possible Targets to Immunotherapy

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INTRODUCTION: Multiple myeloma (MM) is a malignancy of plasma cells and, despite current therapies including high dose chemotherapy followed by stem cell transplantation, virtually all patients will relapse and evolve to death. Cancer/testis antigens (CTs) are tumor-specific molecules that can be used as target to immunotherapy. Recently, Jungbluth and collaborators (Blood 2005;106(1):167) demonstrated that CT7 and MAGE-A3/6 are frequently expressed in advanced stage MM patients and that higher levels of CT7 and MAGE-A3/6 proteins also correlate with elevated plasma-cell proliferation index. These findings suggest a possible pathogenic role of such proteins in MM and also show that they could be attractive targets for immunotherapy. **OBJECTIVES:** To analyze global expression of MAGE-A1, MAGE-A2, MAGE-A3/6, MAGE-A4, BAGE-1, CT7, GAGE family, LAGE-1, PRAME, SP17 in MM patients. **METHODS:** We studied the expression of CTs in 10 bone marrow samples from MM patients at diagnosis. 90% of patients presented stage III MM. Total RNA was extracted using Trizol reagent (Invitrogen). After cDNA synthesis, the expression of CTs was evaluated by RT-PCR. **RESULTS:** The most frequently expressed CTs were: MAGE-A3/6 and SP17 100% of cases; CT7 in 80%; PRAME in 70% and GAGE family in 70%. The less frequently expressed CTs were: MAGE-A4 (0%), MAGE-A2 (20%), LAGE-1 (30%) and BAGE-1 (30%). MAGE-A1 gene was expressed in 50% of samples. Curiously, the only stage II MM patient studied at this moment expressed exclusively MAGE-A3/6 and SP17 genes. Conversely, two patients with high number of CTs-positive expression were negative for MAGE-A4/PRAME and MAGE-A4/MAGE-A2. **CONCLUSION:** Our results are still preliminary but demonstrate the high frequency of expression of CTs MAGE-A3/6, SP17, CT7, PRAME and GAGE family in stage III MM patients. These findings corroborate the hypothesis that such CTs, beside possible pathogenic role in MM, are suitable targets for immunotherapy. Supported by: FAPESP

P-082

Identification of Novel Cancer/Testis Antigens by Database Mining and mRNA Expression Analysis

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Cancer/testis (CT) antigens are a subgroup of tumor antigens with a restricted expression in normal testis and in different histological types of tumors. These antigens are considered promising candidates for cancer immunotherapy and the identification of new CT antigens is essential for the development of polyvalent cancer vaccines designed to overcome tumor heterogeneity and antigen loss. Immunological

techniques such as T-lymphocyte epitope cloning and SEREX were initially used to identify new CT antigens. However, due to their restricted expression pattern, non-immunological techniques relying on differential mRNA expression have also been successfully used for the characterization of CT antigens. In the current study, we mined the Ludwig Transcriptome Database in order to identify in silico transcripts expressed exclusively in normal testis and/or tumors. After computational selection, the expression pattern of CT antigen candidates was validated by RT-PCR using panels of cDNAs from 21 normal tissues and 17 tumor cell lines. A total of 1.255 candidates was identified and 93 of them were selected for experimental validation. Seven candidates were expressed exclusively in testis among the 21 normal tissues analyzed and the expression of five of them was also detected in several tumor cell lines. Expression analysis of these 5 CT candidates was carried out in 160 samples derived from 9 different types of tumors to validate them as novel CT antigens based on their expression pattern. Three of the five CT candidates have a full-length sequence available and these were initially chosen to evaluate the presence of humoral response in cancer patients by immunoblotting. Supported by: FAPESP and Ludwig Institute for Cancer Research

P-083

Interfering in Myosin-VA Function Inhibits Migration, Invasion and Adhesion in Murine Melanoma Cell Lines

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INTRODUCTION: Melanoma is an aggressive type of cancer that accounts for most skin tumor-related deaths. This elevated mortality is related to the high capacity of melanoma cells to migrate to distant sites (metastasis). Therefore, uncovering the molecular pathways associated with migration in melanoma cells may lead to novel alternative therapies. **OBJECTIVES:** To investigate the possible involvement of myosin-Va in cell migration, invasion and adhesion, in melanoma cells, using a myosin-Va mutant cell line (S91-Cloudman, *dilute viral*) and a wild type (B16-F10) as models. **METHODS:** Migration was quantified by: (1) Measuring the migrated distances of target cells in Eppendorf Cellocate coverslips $\frac{3}{4}$ images were acquired over a time course of 72 h and measurements were done every 12 h relative to the zero time point (48 h after plating and 24 h after transfection); (2) By scratch assays on 35 mm plates $\frac{3}{4}$ migrated distance from the edge of the scratch was measured at 4, 8, 12, 24 and 48 h after scratching. Cell invasion was tested in transwell chambers coated with matrigel, supplemented with 3T3-fibroblast conditioned medium plus α -melanocyte stimulating hormone and 3-isobutyl-1-methylxanthine in the bottom chamber. Adhesion was analyzed on untreated 96-well plates coated with fibronectin. The influence of the dominant

negative myosin-Va tail domain (dnMVaT) and the full-length myosin-Va (MVaFL) EGFP fusion constructs was tested in both cell lines by transient transfection. EGFP alone was expressed as a control. RESULTS: B16-F10 cells showed a higher rate of migration, invasion and adhesion than S91 cells. Expression of dnMVaT inhibited all three functions in B16 cells but did not affect S91 cells. On the other hand, expression of MVaFL in S91 cells partially restored all of these functions, while it had little effect in B16 cells. Additional experiments are in progress to analyze the morphological changes and the distribution of myosin-Va and associated proteins during adhesion and migration. CONCLUSIONS: These RESULTS indicate that myosin-Va plays a role in melanoma cell adhesion, migration and invasion, but further studies need to be done to clarify the pathway involved. Supported by: CNPq, FAPESP, CAPES and FAEPa

P-084

HLA Class I and II Expression in the HPV-Associated Cervical Cancer and Premalignant Lesions

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OBJECTIVES: The aim of this study was to evaluate the expression of HLA-I and II proteins in 139 samples of HPV associated cervical cancer and precursor lesions grade I to III, and to characterize the inflammatory infiltrating cells. METHODS: Immunohistochemistry was applied to tissue sections using generic antibodies for HLA-I and II, and CD3+ and CD8+ specific antibodies. HPV detection was performed by PCR with MY09/11 or GP5+/GP6+ generic primers followed by SSO to identify up to 40 different HPV types. RESULTS: Our results show that down regulation of HLA-I antigen expression occurs in about 70% of the HPV associated invasive tumors. In some cases we detected these molecules in the cytoplasm of the neoplastic cells. In cervical intraepithelial neoplasia grade I (CIN-I) we did not find any alteration of HLA-I expression, although in CIN-III we found loss of these molecules in different levels, from 10 to 100% of the neoplastic cells. Approximately 34% of CIN-III show high or intermediate grade of expression loss of HLA-I. We also found up regulation of HLA-II expression in 45% of invasive tumors and in 34% of CIN-III. In some of these samples we observed concomitant expression of HLA-I and II. After analyzing the lymphocyte infiltrate, we found that 75% of CIN-III and 86% of invasive tumors exhibited from moderate to high number infiltrating lymphocytes and plasmocytes in adjacent stroma. In these samples we found T CD3+ and T CD8+ lymphocytes in similar quantities. Adenocarcinomas showed less lymphocyte infiltrate than epidermoid carcinomas. CONCLUSIONS: Human Papillomavirus infection and disease development

depends on intrinsic virus factors, as well as its control by the host immune system. Alterations in HLA-I and II expression were observed in HPV associated neoplasias, and can be an important mechanism of evasion of the immune system. Supported by: FAPESP

P-085

Potential Use of CD40L+ B-Cell Precursor Acute Lymphoblastic Leukemia Cell-Loaded DC to Trigger Antitumor Immunity Against Leukemia

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B-cell precursor Acute Lymphoblastic Leukemia (BCP-ALL) is the most common form of cancer in children. The current protocols lead to cure in more than 80% of patients. Nonetheless, some patients are still resistant to standard therapies. New strategies are needed to improve the overall survival and to decrease treatment related morbidity. Immunotherapy could represent a complementary approach in this subset of patients, even though these patients respond poorly to immunology-based approaches such as donor lymphocyte infusions. The finding that DC can present antigens derived from apoptotic cells prompted us to explore the feasibility of using DC for stimulating immune responses against residual BCP-ALL leukemic cells. We developed a protocol for induction of DC maturation while they phagocytose apoptotic CD40L+BCL-ALL cells. BCP-ALL cells acquired the CD40L antigen from the surface of the donor CD40L+ HBMS cell line during co-cultivation and were then induced to apoptosis by UV irradiation. After phagocytosis of CD40L+blasts, DC matured (> 70% CD83 expression). No CD83 up-regulation was observed if CD40L- cells were used. CD40L-stimulated DC showed functional APC characteristics such as the high expression of CD80/CD86 molecules, increased induction of proliferation on MLR and a Th1-inducing phenotype: IL-12p70 production (mean: 6, range 3-6.5 ng/ml, n=5) and high Th1 polarization of naive lymphocytes (e=30% IFN-g+ intra-staining, range 26-42%). T cells stimulated with CD40L+BCP-ALL-loaded DC showed a strong anti-leukemia cytotoxicity against unmanipulated BCP-ALL cells (mean values for 30:1 effector/target ratio: 78%, range 41-100%) while very low effect was seen if patient non-leukemic mononuclear cells or donor PBMC were used (<20% for both controls). Blocking MHC class I on target cells significantly abrogated leukemia lysis. The T cells obtained after the 21 days culture produced the Th1 cytokines IFN-g, TNF-a and IL-2, with absence of Th2 cytokines. CD4 and CD8 cells are equally expanded with a central effector and memory phenotype. These cells expressed also CD62L and CCR7, being able to migrate in response to CXCL12 and CCL19. In conclusion, after phagocytosis of CD40L+BCP-ALL cells, DC matured into potent APC able to generate cytotoxic

T lymphocytes reactive against leukemia, which are potentially able to reach leukemia infiltrated tissues, driving an anti-tumor response. This DC-based vaccine may be an attractive strategy for BCP-ALL immunotherapy. Supported by: Fundação M Tettamanti, CAPES, Fundação Ary Frauzino

P-086

Characterization of C21orf100 as a Novel Tumor Antigen

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Differentiation antigens are immunogenic proteins expressed in specific cell lineages or at specific stages of differentiation in a particular tissue. Generally, their expression in normal cells is preserved after neoplastic transformation and this feature has made such molecules useful markers in the immunopathological differential diagnosis of malignant neoplasias and also potential candidates for cancer immunotherapy. Through the alignment between expressed sequences and the human genome sequence, we have identified a novel gene on chromosome 21, named C21orf100. This transcript has 880 nucleotides, divided in two exons. It also has an open reading frame of 168pb and codes for a predicted protein of 55 amino acids. By RT-PCR analysis, we found that C21orf100 is exclusively expressed in normal prostate among 21 normal tissues. C21orf100 expression was also detected in 80% (20/25) of prostate tumors but was not detected in most of thyroid, stomach, uterus, melanoma and breast tumors. Taken together, these RESULTS suggest that C21orf100 may represent a novel prostate differentiation antigen. To investigate this possibility, we have expressed C21orf100 as a recombinant his-tagged protein and used the recombinant protein in immunoblotting assays to evaluate the presence of a humoral response against C21orf100 protein in plasma collected from prostate cancer patients. Antibodies against C21orf100 were detected in the plasma of 1/30 (3,3%) patients analyzed so far. Supported by: Ludwig Institute for Cancer Research / Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)

P-087

Type 1 Tumor Necrosis Factor Receptor Shedding Aminopeptidase Regulator Gene (ARTS-1) is Down Regulated in the Thymus of Tumor Bearing Mice

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INTRODUCTION: Clinical trials of cancer immunotherapy have resulted in disappointing results indicating that successful control of tumor growth may

not be an easy task. Tumor escape from immune system can be the main reason for this failure. Blocking factors secreted by tumor cells, among other mechanisms, can play an inhibitory role on innate immune reactivity facilitating tumor growth. OBJECTIVES: To gain new insights on the molecular genetic mechanisms, at gene expression level, that can be associated to failure in the immune system during the growth of a tumor. MATERIAL AND METHODS: We constructed a model-system in which Balb-3T3 fibroblasts transfected with a mutant Ha-ras oncogene (B61 cell strain) are subcutaneously injected in immunocompetent Balb-c mice. These cells allow the growth of a fibrosarcoma tumor at the site of injection that was accompanied by a curve weight vs time. During the tumor growth, the thymuses of mice were removed and processed for total RNA extraction that were then converted into ³²P-labeled cDNA complex probes (sham-injected controls and 3-15 days after injection of B61 cells). The thymus cDNA probes from tumor bearing animals and controls were then hybridized with nylon cDNA microarrays containing 1,536 thymus cDNA target sequences. The hybridization profiles were analyzed by bioinformatics programs (SAM and Cluster-TreeView) in order to pinpointing the differentially induced and repressed genes. RESULTS: Among the modulated genes, we have detected the repression of the Arts-1 gene (acc AK036559, clone ID640722) whose product is an aminopeptidase associated with antigen processing in the endoplasmic reticulum. The deregulation of this gene found by microarray hybridizations at day 15 of tumor growth was confirmed by northern-blotting during all phases of tumor growth. Conclusion: The repression of this gene in the thymus of the tumor-bearing mice may be associated to decrease the B61 tumor antigen processing and presentation contributing thus to tumor growth observed. Supported by: FAPESP, CNPq, CAPES and Pró-Reitoria of Pesquisa USP.

P-088

Monoclonal Antibody G12F2 Targets a Novel Surface Antigen Expressed by B16 Murine Melanoma

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OBJECTIVE: Studies on experimental melanoma have played a central role in the understanding of cancer immunobiology. The molecular characterization of melanoma antigens recognized by monoclonal antibodies has had important impact on this field. The B16F10 murine melanoma cell line is an experimental model largely used in melanoma immunology studies. However, few molecules have been characterized since these cells are usually lowly immunogenic. Herein we describe a monoclonal antibody which identifies a ~200 KDa molecule associated with murine melanoma which was produced after immunization of C57Bl/6 mouse. METHODS AND RESULTS: The IgM monoclonal

antibody G12F2 has demonstrated strong reactivity against B16F10 by immunoblotting and ELISA. This reactivity was not inhibited with metaperiodate addition to the antigen, suggesting recognition of the peptidic portion of the molecule and the Mab did not bind to purified fibronectin by immunoblotting assay. Moreover, cellular membrane of B16 cells were strongly labeled by confocal microscopy. By immunoblotting this Mab reacted with an epitope of Mr 230,000 on malignant murine cell extracts, but did not recognize the antigen in murine cells non-tumorigenic such as melan-a. This monoclonal antibody effectively inhibits tumor development *in vivo* and spheroid formation *in vitro*. Conclusion: G12F2 Mab will be further characterized with the purpose of better identification of the Mr 230,000 molecule, since it may represent a new marker of biological importance in the B16F10 experimental model. Supported by: FAPESP, CNPq, CAPES

P-089

Characterization of a Novel Cancer/Testis (CT) Antigen Gene Family Capable of Eliciting Humoral Response in Cancer Patients

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Cancer/Testis (CT) antigens are immunogenic proteins expressed in normal gametogenic tissues and in different types of tumors. CT antigens are promising candidates for cancer immunotherapy and the identification of novel CT antigens is a pre-requisite for the development of polyvalent cancer vaccines. Using alignments between ESTs and the Human Genome Sequence, we have identified a novel CT antigen localized on chromosome 21, named CTSP-1. This new transcript presents several alternative splicing and polyadenylation isoforms and has a very restricted expression pattern among normal tissues. CTSP-1 is exclusively expressed in normal testis and is aberrantly expressed in 47.6% (10/21) of tumor cell lines and in 44.4% (75/169) of tumors from different histological types. The highest percentages of positive expression were observed in melanomas (59.0%) followed by prostate (58.0%) and lung (57.0%) tumors. CTSP-1 is part of a highly conserved gene family and members of this family also have a restricted expression pattern and similar protein structure. Antibodies against members of this gene family were detected in 19.8% (28/141) of plasma samples from patients with a wide spectrum of tumors. The highest percentages of antibody response were observed in patients with breast (33.3%), prostate (33.3%) and thyroid (30.0%) tumors. To our knowledge, CTSP-1 is the most frequently expressed and

immunogenic CT antigen described so far and due to its very restricted expression pattern in normal tissues should be considered as a promising candidate for cancer immunotherapy. Supported by: FAPESP, CAPES, CNPq

P-090 Withdrawn by the authors

P-091

Anomalous Membrane Overexpression of the HLA-DRA Chain In Ovarian Cancer: Analysis of a Biomarker Candidate

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Tumor formation in immunocompetent hosts is believed to be dependent on the ability of tumor cells to evade the immune system, as suggested by the alterations of expression of the major histocompatibility complex (MHC) and related molecules. Our previous serial analysis of gene expression (SAGE) study revealed that HLA-DRA (HLA-DRA) is highly overexpressed in ovarian cancer (OVCA). This was unanticipated, as overexpression of MHC molecules would be expected to increase tumor immunogenicity, therefore compromising tumor growth. OBJECTIVES We aimed to study the proteomic expression profile of membrane HLA-DRA in OVCA and other cancers as a candidate of disease biomarker. METHODS *In silico* analysis of SAGE libraries was conducted to define HLA-DRA and HLA-DRB expression in several human cancers. Tissue array and immunohistochemistry was used to define protein expression in cancer samples. RESULTS We found HLA-DRA overexpressed in a majority of cancer tissues investigated. In contrast, mature HLA-DR was not synthesized in these tissues. Interestingly, HLA-DRA and HLA-DRB transcripts were both found expressed in many other cancer types, including OVCA, suggesting that the downregulation of HLA-DRB chain is a post-transcriptional or post-translational mechanism. We also observed high levels of the invariant chain (Ii/CD74) expression in both the cytoplasm and membrane of OVCA cells, possibly contributing to the lack of mature HLA-DR expression or to the a chain-mediated signaling. Conclusion Together, these data suggest that, while OVCA overexpresses HLA-DRA, perhaps as a result of inflammatory events in the tumor microenvironment, tumor cells may have compensatory mechanisms to reduce the production of functional MHC class II molecules, thus reducing immunogenicity and favoring tumor growth. We consider the possibility that HLA-DRA chain expression may exert a favorable effect on OVCA cell growth, thus possibly representing a novel biomarker for this deadly disease. Supported by: NIH, MD, EUA

P-092**Association Between the Expression of GLUT-1 and the Findings of ¹⁸F-FDG PET SCAN in Squamous Cell Carcinomas of the Head and Neck**

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INTRODUCTION: Pet scan (Ps) is valuable tool for metabolic imaging that has been used to staging, therapeutic planning and metastasis identification in squamous cell carcinoma of the head and neck. The physiological background of Pet scan is based on high glycolytic rates by the tumors cells. The most employed radiotracer uptake, 18-FDG, is a glucose analog. This uptake is mediated by a family of glucose transporters (GT) proteins known as GLUT. GLUT-1 is the main GT involved in tumor glycolysis. Thus the overexpression of these glucose carriers support the elevated metabolic consumption of high rates of glucose uptake by tumors cells. The aim of this study is correlate the GLUT-1 expression demonstrated by immunohistochemistry with Ps findings in patients with squamous cell carcinoma of the head and neck. **MATERIAL AND METHODS:** Retrospective analysis of GLUT-1 expression was performed by immunohistology in 34 cases with squamous cell carcinoma of the head and neck that were evaluated by Ps. The biopsy paraffin blocks were recovered from the files and submitted to immunohistochemistry with the polyclonal antibody MYM (anti-GLUT-1, DAKO, USA). These cases were considered positive when more than 50% of tumor cells showed a strong membrane immunostain. The results obtained were compared with the findings of Ps. **RESULTS:** The expression of GLUT-1 was observed in 28/34 cases. In 23 cases, the expression of GLUT-1 was positively correlated with the findings of Ps examination. Other five cases were GLUT-1 positive but the Pet scan did not show any activity of the disease. >From those, in 3 patients there was no tumor at the time of the Ps investigation. There were only 2 false-negative cases in Pet scan, one of them for technical reasons. **CONCLUSIONS:** There is a strong correlation between the expression of GLUT-1 and Pet scan findings. Cases Ps (+)/GLUT-1(-) might be implicate that other GT may be involved in the cellular glycolysis. One case was false negative in the Ps exam suggesting the possibility of molecular alterations of GLUT-1 in these tumors resulting in the no uptake of 18-F-FDG by tumor cells. Supported by: CAPES

P-093**G3BP2 and GPRC5A mRNA Expression in Oral Squamous Cell Carcinoma**

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PURPOSE: To identify whether or not Ras-GTPase activating protein SH3 domain-binding protein 2 (G3BP2) and G protein-coupled receptor, family C, group 5, member A (GPRC5A), among genes previously identified in a Differential Display RT-PCR done with primary tumors of patients (pts) with oral squamous cell carcinoma (OSCC) with/without compromised lymph nodes (pN+/pN0), were predictive of prognosis in this population. **METHODS:** We determined the expression of GPRC5A and G3BP2 mRNA expression in primary tumors and adjacent mucosa of 44 OSCC pts, by using Real time PCR, and correlated these data with clinical pathological parameters and survival. **RESULTS:** G3BP2 was found down regulated in primary tumors (mean 0.75±0.85 SD) as compared to adjacent mucosa (1.91±1.53, n = 26, p < 0.001 – paired Wilcoxon two-tailed test). We did not find any correlation between these markers and lymph nodal status or size of the primary tumor. However, G3BP2 negative pts (with expression below median relative expression) presented a trend towards shorter overall and disease free survival as compared to positive pts, as shown by Kaplan Meier survival curves (p=0.07 and p=0.06, respectively, log-rank test, univariate analysis). **CONCLUSION:** Our results and of the others showing that G3BP2 is a player in other signaling pathways involved with cancer, such as Ras, c-myc and NfκB pathways, indicate that G3BP2 gene may be further explored as a marker of prognosis in this population. Supported by: FAPESP 02/01738-9

P-094**Metallothionein Expression in Dermal Neoplasia**

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INTRODUCTION: Basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are the most frequent skin cancer in Brazilian people. Its pathogeny is linked to the genetic effects of actinic radiation exposure, specially to the ultraviolet rays ranging from 290 to 320 nm wavelength. Metallothionein (MT) are low molecular weight

proteins with high affinity for heavy metal, whose intracellular function is related to heavy metals and free radical detoxification. MT's actinic aggression protective action have been shown in others studies. In other way, its overexpression in different kind of tumors have been related to higher aggressiveness and worst prognostic. The proposal of this study was to evaluate the expression of MT in skin cancer associated to actinic radiation. MATERIAL AND METHODS: Eighteen BCC cases, five SCC and six normal skin fragments were investigated. Immunohistochemical assay were performed by streptavidin – biotin – peroxidase technique with anti-MT primary antibody (clone E9). RESULTS: In normal skin the immunostaining was observed in the the basal layer of the epithelium. In the epithelium adjacent to tumors, the suprabasal layer was also labelled. Six cases of BCC (33%) completely lack MT immunoreactivity, while all of SCC depicted strong MT immunostaining. Only one SCC case (20%) and eight BCC case (44%) shown low MT expression. However, 80% of SCC cases (4/5) had intense staining, while only 22% of BCC cases (4/18) presented the same staining pattern. The mean immunolabelling index was 0.69 ± 0.48 for BCC, and 1.55 ± 0.77 for SCC. CONCLUSION: Higher MT expression in SCC as compared to BCC suggests association with tumoral aggressiveness. Supported by: CNPq

P-095

Metallothionein, MMP-2 and TIMP-2 Immunolocalization in Basal Cell Carcinoma

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INTRODUCTION: Basal cell carcinoma (BCC) is a slow growing skin cancer that rarely metastasize. Matrix metalloproteinases (MMPs) are proteins that cleave the extracellular matrix. Zinc-binding is essential for its function. MMPs are responsible for proteolytic degradation of the basement membrane which is required for cancer cells to invade the surrounding connective tissue and to metastasize. Metallothionein (MT) has been considered the major protein related to regulation of bioavailability of zinc. MT may potentially activate certain proteins by donating zinc. The aim of study was to assess quantitatively MT, MMP-2, and tissue inhibitor of metalloproteinase (TIMP-2) expression in BCC and to investigate correlation among MMP-2, TIMP-2 and MT expressions on this lesion. MATERIAL AND METHODS: Immunohistochemical expression of MMP-

2, TIMP-2 and MT antigens was assessed in 31 cases of BCC by a standard avidin-biotin-peroxidase technique. After morphological analysis of staining, quantitative indexes were constructed. Correlations among the immunolocalization indexes of these antigens were investigated by Spearman test. Comparisons of the mean indexes were performed to compare different BCC subtypes by Mann-Whitney test. RESULTS: Weak staining was observed for all antigens investigated in BCC. Mean immunolabelling indexes were $36.2\% \pm 32.7\%$, $74.5\% \pm 9.7\%$ and 77% for MMP-2, TIMP-2 and MT, respectively. It was not observed neither significant correlations among the expression of these antigens nor differences among the different BCC subtypes for each antigen. Conclusion: The RESULTS suggest that factors other than MT and TIMP-2 modulate the MMP-2 expression in BCC. Supported by: CNPq

P-096

G-Protein-Coupled Receptor, Family C, Group 5, Member A (GPCR5A) and Cyclin-Dependent Kinase 6 (CDK6) mRNA Expression in Tongue Squamous Cell Carcinoma

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PURPOSE: To identify whether or not the G-protein-coupled receptor, family C, group 5, member A (GPCR5A) and Cyclin-dependent kinase 6 (CDK6), among genes previously identified in a Differential Display RT-PCR done with primary tongue squamous cell carcinoma (SCC) with/without compromised lymph nodes (pN+/pN0), were predictive of prognosis in tongue SCC. METHODS: By using real time PCR, we determined the expression of these genes in 26 fragments of primary tumors (T) and adjacent mucosa (M) of different tongue SCC patients. These data were correlated with clinical pathological parameters. RESULTS: We did not find any differences between primary tumors and adjacent mucosa concerning the genes expression here studied: GPCR5A (T: 2.36 ± 2.00 vs 3.06 ± 5.28 ; M, $P = 0.65$, Wilcoxon test) or CDK6 (T: 3.54 ± 3.22 vs 1.69 ± 1.29 ; M, $P = 0.14$ Wilcoxon test). No correlations were found between lymph nodal status and expression of those markers. In relation to tumor size, GPCR5A mRNA expression was significantly lower in pT1/T2 (1.50 ± 1.43) as compared to pT3/T4 tumors (3.61 ± 2.11 , $P = 0.007$, Mann-Whitney test). For CDK6 mRNA no difference was found (2.76 ± 2.72 vs 4.80 ± 3.75 , $P = 0.18$, Mann-Whitney test). CONCLUSION: Even

if none of DDRT PCR indicated genes appears promising as markers of the presence of lymph node metastasis in tongue SCC, we have found increased expression of GPRC5A mRNA in pT3/T4 tumors, which suggests that this gene may be related to tumor progression in tongue squamous cell carcinoma. Further studies are necessary in this area to confirm these results. Supported by: FAPESP 02/01738-9

P-097

The Increased Expression of CCL19 and CCL21 May be Associated with Lymph Node Spread in Head and Neck Squamous Cell Carcinoma

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PURPOSE: The expression of chemokine/chemokine receptors by malignant cells and at the site of tumor spread suggests a role for these chemokines in malignant growth and metastasis. **METHODS:** In this work, we analyzed the mRNA expression of CXCL12, CCL19 and CCL21 chemokines, by real time quantitative polymerase chain reaction, in 18 fragments of primary tumor (T), in correspondent matched adjacent mucosa (M) and in 15 grossly compromised lymph nodes (LNC) of 18 patients with operable HNSCC. The results were correlated with clinical-pathological parameters. **RESULTS:** We did not detect any differences between primary tumors and matched adjacent mucosa concerning the chemokine ligands here studied. We have found higher expression of CXCL12 mRNA in primary tumors of patients with compromised lymph nodes as compared to correspondent from patients without compromised lymph nodes (8.1 ± 13.2 vs 0.2 ± 0.3 , $P=0.037$, Student T test). No relationship with lymph nodal status were found for CCL19 and CCL21. The compromised lymph nodes presented an increased CCL19 (LNC: 69.3 ± 120.2 vs 2.1 ± 4.3 : T, $P=0.045$) and CCL21 (LNC: 75.4 ± 110.6 vs 1.2 ± 2.2 : T, $P=0.021$) mRNA expression as compared to primary tumors. **CONCLUSION:** Based on these results, we suggest that CCL19 and CCL21 might be involved in tumor spread to lymph nodes in HNSCC. Supported by: CAPES

P-098

Analysis of Repetitive DNA in Skin Cancers

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INTRODUCTION: Microsatellites are repeated sequences spread all over genomes at defined loci. The number of internal repeats varies among individuals (heterozygosity) and may be used as polymorphic markers. Most cancers show microsatellite instability (MSI) and/or loss of heterozygosity (LOH). Our previous studies on skin cancers have demonstrated MSI and/or LOH on microsatellite D9S50 in 36 % basal cell carcinoma (BCC), 25 % squamous cell carcinoma (SCC) and 57 % malignant melanoma (MM). Microsatellite D9S52 was altered in 28.5 %; 42.8 %; and 71.4 % tumors, respectively. Microsatellites D6S252 and D9S15 concerning only BCCs (15 samples) presented only a single MSI for D9S15 (6%). **OBJECTIVE:** To compare DNA patterns of microsatellites from blood lymphocytes, biopsies from normal, pre-malignant and malignant skin lesions, in the aim of investigating genomic instability and possible molecular markers associated with tumor severity. **MATERIALS AND METHODS:** Normal and tumor samples from several BCC, SCC, MM, nevi and actinic keratoses (KA) were obtained at the Department of Dermatology from HC-FMUSP. Tumor tissues and normal skins were cryopreserved immediately after surgical removal; blood was stored in cryotube with heparin. DNA was extracted from all samples according to the current techniques. Four microsatellites (D9S180, D9S196, D6S251 and D6S252) were analyzed. PCR amplified products were visualized in 6% polyacrilamide gels. **RESULTS:** D6S251 microsatellite patterns revealed MSI and/or LOH in 50 % KA, 22 % BCC, 25 % SCC and 30 % MM. Other obtained data included: D6S252 (16% SCC tumors altered); D9S180 (70% BCC and 16% SCC tumors altered); D9S196 (33% BCC and 66% KA altered). There were no clear alterations in the remaining samples. **CONCLUSION:** Several alterations were found in the studied skin cancers genomes, reinforcing the assumption on the importance of repetitive DNA in chromatin organization and gene expression, often disrupted in cancer cells. Supported by: FAPESP

P-099

Expression of The Human Polycomb Group EZH2 Gene, a Transcriptional Repressor in Breast Carcinoma

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EZH2 (EZ) is a Polycomb Group protein homologous to Drosophila Enhancer of Zeste involved in gene silencing. Dysregulation of this gene silencing machinery can lead to loss of differentiation and development of breast cancer. OBJECTIVE: Our aim was to determine EZ expression in 23 primary breast carcinomas specimens and verify a possible correlation with lymph node involvement. METHODS: All 23 patients presented a clinical stage II invasive ductal carcinoma with positive immunohistochemistry expression of estrogen receptor (ER+). Patients were then classified according to presence (LN (+) (n = 14) or absence of lymph node (LN (-) (n = 9) involvement. No differences between groups were detected considering tumor histological grade, patient's age, and menopausal status. Gene expression was carried out by SYBR green quantitative Real Time PCR and analysis was performed in 23 samples of breast carcinoma and 7 specimens of perilesional breast tissue from women with benign lesions. RESULTS: EZ mRNA expression was significantly higher in tumors from patients presenting axillary lymph node involvement (0.67 ± 0.20) as compared to those without axillary lymph node involvement (0.20 ± 0.08) or normal breast tissue from patients with benign conditions (0.20 ± 0.15) ($p=0.001$)(Kruskal Wallis test). No differences were found in EZ levels between LN (-) tumors and normal controls. We have then analysed 6 patients with clinical stage III, LN(+), ductal invasive ER+ carcinoma and 83% of the samples showed EZ values higher than those observed in normal breast tissue (1.03 ± 1.02) ($p=0.005$) (Mann-Whitney test). CONCLUSION: EZ gene high expression in primary breast carcinomas seem to correlate with LN involvement. Supported by: FAPESP.

P-100

Analysis of Differentially Expressed Genes in Tongue Squamous Cell Carcinoma with/without Lymph Node Metastasis

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PURPOSE: Squamous cell carcinoma (SCC) of the tongue is a devastating disease. In these patients, the detection of compromised lymph nodes seems to be pivotal for the choice of more appropriate treatment. Because of this, our objective was to identify genes that could potentially act as prognostic factors or predictive for the presence of lymph node metastasis. METHODS: By means of Differential Display (DD)RT-PCR, we

identified differentially expressed genes comparing three RNA pools: one from 2 primary lymph node positive (pN+) tongue SCC, a second from 2 pN0 primary tongue SCC, and a third from adjacent mucosa of 3 pN0 patients. Northern blot/qPCR assays were used for validation in RNA from 30 tongue SCC. RESULTS: Of the 65 transcripts indicated by DDRT-PCR, here studied seven genes: SPRR2B, KTR6A, MTATP6, PLUNC, RPL7, SCARB2, RPS19 and WISP3. Of them, SCARB2 was overexpressed in primary tumors as compared to the adjacent mucosa ($P=0.012$, Wilcoxon test). No correlations were found between lymph nodal status and mRNA expression. In addition, MTATP6 and RPS19 mRNA expression were significantly lower in pT1/T2 as compared to pT3/T4 tumors (MTATP6: $P = 0.035$; RPS19: $P = 0.04$, Mann-Whitney test). We observed an inverse correlation between WISP3 mRNA values and histological grade (GradeI: 0.66 ± 0.57 ; GradeII: 0.21 ± 0.33 ; GradeIII: 0.015 ± 0.012 ; $P = 0.013$, Kruskal-Wallis test). Conclusion: Even if none of DDRT PCR indicated genes appears promising as a marker for the presence of lymph node metastasis in tongue SCC, we have found SCARB2 mRNA increased in tumors as compared to adjacent mucosa and should be studied further. Apart from that, the increased expression of MTATP6 and RPS19 mRNA in pT3/T4 tumors and the loss of expression of WISP3 in grade III tumors, suggest these genes might be related to tumor progression in tongue squamous cell carcinoma. Supported by: FAPESP 02/01738-9

P-101

Expression of SMAD2 and SMAD6 mRNA Seems to be Related to Prognosis in Oral Squamous Cell Carcinoma

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PURPOSE: Smads 1-8 are the main Transforming Growth Factor (TGF) β signaling transducers. Here we describe the mRNA expression of R-Smad 2 and 3, and I-Smad 6 and 7, by Ribonuclease Protection Assay (RPA), in 48 fragments of operable oral squamous cell carcinoma (OSCC) and 40 matched adjacent mucosa and these findings were correlated with the clinicopathological parameters and outcome. RESULTS: The Smad 2 ($p=0.013$), Smad 3 ($p=0.04$) and Smad 7 ($p<0.0001$) mRNA expression were higher in tumor as compared to adjacent mucosa. Non of the four Smads expression differed neither between patients categorized as pN0 (n=23) and pN+ (n=25) nor as pT1/T2 (n=17) and pT3/T4 (n=31). Next, patients (pts) were categorized as positive (above median) or negative (equal/below median) for each Smad, according to tumor median mRNA expression. Kaplan Meier survival curves revealed that

Smad2- pts (n=24, median overall survival - m.o.s. - not reached) presented a survival rate (s.r) of 66.7% against 29.2% of Smad2+ pts (n=24, m.o.s:14.60 months, Log Rank: 0.04). Otherwise, Smad6+ pts also presented a survival advantage (n=22, s.r: 68.2%, m.o.s: 52.13 months) over Smad6- pts (n=26, s.r: 30.8%, m.o.s: 14.6 months, Log Rank: 0.005). The Cox regression multivariate analysis revealed that Smad 2 and Smad 6 are independent prognostic marker (both p=0.007) and the combination of both markers, revealed that Smad2-/Smad6+ pts presented a favorable prognosis with a s.r. of 100% against 23% of Smad2+/Smad6- pts. No associations between the others Smad expression and survival were found. **CONCLUSION:** Taking these findings into account, we propose that the down regulation of Smad2 and the overexpressed Smad6 might be markers of good prognosis in oral squamous cell carcinoma. Supported by: FAPESP 02/0173 and CAPES

P-102

Glycosaminoglycans Analysis in Colorectal Cancer

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Glycosaminoglycans are a class of linear polysaccharides composed by repeating disaccharides units consisting of alternating hexosamine and uronic acid residues. Hyaluronic acid (HA), chondroitin sulfate, dermatan sulfate and heparan sulfate are involved with important biological activities such as cell adhesion, migration, remodeling of extracellular matrix, tumor development. **PURPOSE:** This study was designed to compare the glycosaminoglycans (GAG) concentrations in non-neoplastic, neoplastic and transition tissues from patients with colorectal cancer and correlates with clinical pathological parameters like presence of metastasis, stage and type of tumor. **METHODS:** The study included 40 patients subjected to resection of colorectal cancer, comparing with tissues obtained from non-neoplastic and transition areas on the same patient. A practical fluorescence-based assay method for determination of HA has been developed using HA-binding protein (HABP) and europium-labeled streptavidin. The quantification of chondroitin, dermatan and heparan sulfate were performed by agarose gel electrophoresis. **RESULTS:** The biochemical studies showed that colorectal tumors expressed higher amounts of glycosaminoglycans compared to transient and non neoplastic tissues and hyaluronic acid levels were significantly correlated with Dukes stage and were higher in patients with advanced stage of tumours. **CONCLUSIONS:** It was observed an increase on glycosaminoglycans expression and colorectal cancer compared to transition and non neoplastic tissues. Supported by: FAPESP, CAPES and CNPq.

P-103 Withdrawn by the authors

P-104

Analysis of Differentially Expressed Genes in Oral Squamous Cell Carcinoma Predictive of Metastatic Lymph Nodes

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PURPOSE: To identify genes, predictive of the presence of lymph node metastasis or of poor outcome in oral squamous cell carcinomas (OSCC). **METHODS AND RESULTS:** We first identified a panel of differentially expressed genes in OSCC with/without compromised lymph nodes (pN+/pN0) and pN0 adjacent mucosa by means of RT-PCR differential display (DDRT-PCR). Sixty six out of 99 recovered cDNAs matched genes/EST deposited at GenBank; 10 unmatched possibly represent novel genes. Pre-validation, done for 31 cDNAs using 2 RNA pools, one of 3 pN0 and another of 3 pN+ primary OSCC, by reverse-Northern hybridization, have shown that 15 genes were over expressed in tumors (with pN+/pN0 ratio above or equal 2, range: 2 to 9 fold). Six genes were down regulated in tumor (densitometric values below 60% of the values in pN0 mix). Of these genes, 12 were validated in 48 patients with OSCC, by using Northern blot. We confirmed the over expression of SCARB2 and casein kinase 1a1 (CSNK1A1) (p=0.03 for both by Mann-Whitney U-test) and the down regulation of small proline rich protein 2B (SPRR2B) (p=0.02) in the pN+ tumors as compared to pN0. A multivariate logistic regression model, in which SCARB2, CSNK1A1 and SPRR2B were included, revealed SCARB2 as an independent predictor of the presence of lymph node metastasis (p=0.04). Kaplan-Meier survival curves revealed that ATP synthase 6 (ATP6) and SPRR2B negative patients (with relative expression below median) present with shorter survival as compared to positive pts (p=0.04 and p=0.03, respectively, log-rank test, univariate analysis). **CONCLUSIONS:** Of the DDRT-PCR indicated genes and studied until now by Northern blot analysis, SCARB2 seems promising as a marker of lymph node status and ATP6 and SPRR2B appear to be potential biomarkers of outcome, and may represent potential targets for prevention and/or therapeutics in OSCC. Supported by: FAPESP 02/01738-9

P-105

Immunohistochemical Quantification of Heparanase in Colorectal Cancer

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Heparanase is an endo-beta-glucuronidase, that seems to be involved in extracellular remodelling during tumor development and metastasis promoting degradation of heparan sulfate proteoglycans (1,2,3,4). Our aim was to study the expression of heparanase in colorectal cancer and its correlation with tumor morphological variables. We analyzed heparanase expression in 20 patients subjected to resection of colorectal cancer at the "Hospital do Servidor Público Estadual" (São Paulo, Brazil) between 2002 and 2003, comparing with 13 tissues obtained from non-neoplastic areas from the same patients and one control tissue obtained from a normal person. Demographic and clinical data were obtained from patient charts for further analysis. Immunohistochemistry assay was performed using polyclonal antibody HPA-C17 (Santa Cruz, Ca, USA). We analyzed 10 microscopic fields performing 1×10^3 cells analyzed at a magnification of 400x using ImageLab 2000 software. The clinical and pathological variables considered were: sex, age, specific tumor localization, histologic grade, tumor size, presence or absence of metastasis and percentage ratio between positive cells for heparanase expression and total analyzed cells. Association between variables was assessed using Students t-Test for parametric variables. Data managements and analysis were done using the SPSS software, version 10 (SPSS Inc., IL, USA). This study involved 60% men and 40% women with an average age of 63.5 years. Considering the tumor size they presented a mean of 5.77cm. We observed a significant difference for heparanase expression between colorectal tumors (129.27 ± 47.5) and normal tissues (85.3 ± 40.65). Interestingly, we observed a decrease of heparanase expression in the metastatic cases ($p=0.03$). In addition, it was found that 10% of patients who presented locally advanced colorectal cancer showed a tendency of enhancement in heparanase expression compared to control tissues ($p=0.08$). We conclude that heparanase expression was significantly higher expressed in colorectal cancer compared to normal tissues ($p=0.004$) with a tendency to be also increased in tumors that presented local invasion. Curiously, patients with metastasis and the ones who died had shown lower heparanase expression. So, heparanase expression seems to be a good marker for colorectal cancer. References: [1] Vlodavsky I et al (1990) *Cancer Metastasis Rev* 9: 203-226. [2] Friedmann Y et al (2000) *American Journal of Pathology* 1167-1175; [3] Vlodavsky I et al (2002) *Cancer Biology* 12: 121-129; [4] Nobuhisa T et al (2005) *Clin Oncol*

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P-106

Characterization of Novel Ovarian-Specific Genes Identified by Serial Analysis of Gene Expression (SAGE)

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A better understanding of changes in gene expression during ovarian tumorigenesis and the identification of specific tumor markers may lead to novel strategies for diagnosis and therapy for this disease. OBJECTIVES: we aimed to characterize novel ovarian-specific tags (HOSTs) identified by SAGE methods: HOSTs identification was performed using NCBI SAGEmap database, while their ovarian cancer (OVCA) specificity was analyzed *in silico* among SAGE libraries. Corresponding ESTs were sequenced. The 5'-end of HOST2 was determined by 5'RACE. Northern blotting was conducted using RNA extracted from cell lines and primary tumor samples. Real-time RT-PCR was performed in a panel of microdissected OVCA. RESULTS: using our SAGE data, and public SAGE databases that contained a total of 137 SAGE libraries representing a wide variety of normal and neoplastic tissues, we identified 5 novel SAGE tags specifically expressed in OVCA. Database analysis, cloning, and sequencing of the corresponding ESTs revealed details about HOSTs: HOST1 is CA125 (MUC16); HOST2 is a novel gene containing multiple copies of retroviral-related sequences without an obvious open reading frame; HOST3 is claudin-16 (CLDN16); HOST4 is a proteoglycan link protein (LP); HOST5 is Na/PO4 type II (SLC34A2). Except for MUC16, these genes have not previously been shown to be expressed in ovarian or other cancers. Northern blot analysis confirmed that HOST are rarely expressed in normal tissues or nonovarian cancers, but are frequently expressed in OVCA-derived cell lines and primary tumors. Moreover, HOSTs are upregulated in all four major subtypes of OVCA compared to cultivated ovarian surface epithelial cells, as concluded by real-time RT-PCR using a panel of microdissected OVCA. HOST5/SLC34A2 expression was associated with increased differentiation in serous OVCA. CONCLUSION: while the roles of HOSTs in ovarian malignant transformation remain unclear, we propose that HOSTs may represent alternative targets for diagnosis, prognosis or therapy of this deadly disease. Supported by: NIH, MD, EUA

P-107

Genes from Fibroblasts of Involved and

Uninvolved Lymph Nodes Commonly Modulated by the Presence of Breast Cancer Cells

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Breast cancer (BC) establish metastasis in axillary lymph nodes (LN) where malignant epithelial cells may modulate stromal cells function to create a favorable microenvironment that will contribute to tumor development. Although fibroblasts from involved and uninvolved LN seem histologically similar, it is possible that their gene profile may be modulated by the presence of malignant cells. **OBJECTIVE:** Evaluate the influence of previous or present contact of BC cells on gene expression of LN fibroblasts from BC patients. **METHODS:** Primary culture of LN involved or uninvolved fibroblasts were established and co-cultures with MCF10A and MDA MB 231 cells were carried out for 72 h. Gene profile of fibroblasts, upon co-culture or not, was analyzed on c-DNA microarray glass slides containing 4,608 spotted sequences. Genes satisfying a significance level of $p < 0.01$ (Student t-test), False Discovery Rate (FDR) < 0.01 and at least a two fold variation between groups, were considered differentially expressed. **RESULTS:** Thirteen genes were commonly under-expressed and three over-expressed in fibroblasts from uninvolved LN co-cultured with BC cells MDA-MB-231, or involved LN co-cultured with mammary cells MCF10A or MDA-MB231. Some of these genes were related to regulation of transcription, apoptosis, metabolism and transport, and six of them code for proteins of unknown function. **Conclusion:** In fibroblasts from LN of BC patients, a few genes are commonly modulated upon the presence of malignant cells. Expression of these genes is probably influenced by soluble factors from malignant cells (as it was reproduced in co-culture with porous membrane) and has a lasting duration (as it is also observed in fibroblasts obtained from involved LN, which had a previous contact with tumor cells, co-cultured with non-malignant mammary cells). Supported by: FAPESP 02/09081-9 and CAPES.

P-108

Study of Mucins Expression in Esophageal Squamous Cell Carcinoma

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Esophageal squamous cell carcinoma (ESCC) is highly prevalent in Brazil and responsible for high mortality index. The mucins are proteins expressed by various epithelial cell types and are involved in signal transduction of various cellular processes. Podoplanin is a transmembrane glycoprotein of mucins family and is expressed on endothelial of lymphatic vessels. Alterations in these proteins expression have been associated with cancer development. There is no study on podoplanin expression and clinicopathological data in ESCC. This study investigated immunohistochemical expression of membrane-associated mucins (podoplanin and MUC1) and secreted mucins (MUC 2, 5AC and 6) in ESCC and correlated with clinicopathological data of 237 cases. Immunohistochemistry were carried out using monoclonal antibody for podoplanin, EMA (epithelial membrane antigen) and MUC 2, 5AC and 6. Cases represented by surgical resections were performed in three tissue microarray paraffin blocks spotted in duplicate. The cases were scored according frequency pattern, based on membrane (podoplanin and EMA) and cytoplasmic staining (MUC 2, 5AC and 6) of the tumor cells. All the cases studied showed no staining for MUC 2, 5AC and 6 immunoreexpression. It was observed 86 negative and 128 positive cases for podoplanin expression. Our results showed statistically significant correlation only between this protein expression and tumor location ($p=0.016$), being more frequently expressed in cases from the middle third of the esophagus. For EMA expression, 125 cases were positive and 83 showed no staining. Significant correlations were verified between histological grade and EMA expression ($p=0.034$), which was more expressed in well/moderate than in poor differentiated/undifferentiated cases. Although it were not significant, there was a tendency to correlate EMA expression and infiltration level ($p=0.075$), which positive cases for this protein were more frequent in deeper level. Univariate analysis showed no significant associations between the overall survival and expression of the podoplanin and EMA. These results suggest a role for EMA in tumoral progression in ESCC. Supported by: FAPESP 04/12360-2 and CEPID 98/14335-2.

P-109

DNA Ploidy, Nuclear Grade and Architectural Changes in Ductal Carcinoma In Situ and in Invasive Female Breast Cancer: a Contributory Study for the Model of Disease Progression

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INTRODUCTION: Carcinogenesis is a multiple stage phenomenon. Epidemiologic evidence indicates

that progression to invasive breast cancer begins in hyperplastic states of the epithelium passing by *in situ* carcinoma. The actual relationship that exists between ductal carcinoma *in situ* and invasive ductal carcinoma of the breast and the factors that lead to the tumor invasion process are questions that remain unanswered. **OBJECTIVE:** To study morphologically possible models of disease progression from intraductal carcinoma to invasive ductal carcinoma of the breast using nuclear and architectural parameters. **METHODS:** We studied retrospectively 46 tumor tissues of the female breast with coexisting ductal carcinoma *in situ* and ductal invasive carcinoma. DNA ploidy as well as nuclear grade was determined in both lesions. The architectural pattern of the *in situ* component and the degree of tubule formation of the invasive component were also determined. **RESULTS:** Our results showed that there are no statistically significant differences in both nuclear parameters studied (DNA ploidy, $\kappa=0,713, p<0,001$; nuclear grade, $\kappa=0,5018, p=0,0002$). However, when we compared the architectural features of ductal carcinoma *in situ* with the degree of tubule formation in the invasive component we found a statistically significant difference between the groups ($p=0,1033$). **CONCLUSION:** The nuclear features of a given invasive breast cancer are possibly determined in a pre-invasive level, while architectural changes probably develop in a late stage, after the acquisition of the invasive phenotype.

P-110

Coordinate Expression of Fatty Acid Synthase (FAS) And ErbB2 in Normal, Premalignant and Malignant Oral Keratinocytes

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Fatty acid synthase (FAS) is the cytosolic multifunctional enzyme responsible for the endogenous synthesis of saturated long-chain fatty acids from acetyl-CoA and malonyl-CoA. Its expression is up regulated in several human epithelial malignancies, including oral squamous cell carcinoma (OSCC) and soft tissue sarcomas. OSCC is the only tumor in which FAS expression has been shown to be more pronounced in well-differentiated than in undifferentiated lesions. Androgens or epithelial growth factor (EGF) up regulate FAS expression in prostate cancer cells and it has been recently shown a connection between the cell surface receptor ErbB2 and FAS expression in breast and ovarian cancer cells. The exact role of FAS in malignant cells is not known, however, it is clear that FAS specific inhibitors block cell cycle progression and induce apoptosis in several cancer cell lines. Herein we show by immunohistochemistry that FAS expression is higher

in oral hyperkeratosis, epithelial dysplasias and OSCC than in morphologically normal oral epithelium. According to previous results, FAS staining in well-differentiated OSCC was more intense than undifferentiated tumors. ErbB2 expression was observed at the cell surface of non-neoplastic and well-differentiated OSCC, whereas its cytoplasmic localization was mainly observed in undifferentiated tumors. The Ki-67 index was progressively higher from the normal oral epithelium to OSCC and positively associated with the ErbB2 cytoplasmic labeling. We also demonstrate, by employing a model for the differentiation of OSCC cell lines, that keratinization of SCC-9 cells changes ErbB2 expression from the cytoplasm toward the cell surface. Taken together, these findings indicate that FAS is involved in both neoplastic cell proliferation and oral epithelial cell differentiation and explain, at least in part, why well-differentiated OSCC express more FAS than their undifferentiated counterparts. Supported by: FAPESP (04/13903-0). SDS, ALCAR and MA were supported by FAPESP fellowships (04/06398-7; 04/06397-0 and 04/13904-6)

P-111

E-cadherin, β -catenin, MUC1, MUC2, MUC5AC and MUC6 Expressions: Association with Lymph Node Metastasis and Prognosis in Gastric Carcinomas

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OBJECTIVE: This study aimed to evaluate the expression pattern of some markers related to cellular adhesion (E-cadherin and β -catenin) and differentiation pathways of gastric carcinomas (MUC1, 2, 5AC and 6) and their relationship with lymph node involvement (pN) and patient overall survival. **METHODS:** Immunohistochemistry analysis was performed in a retrospective series of 446 gastric carcinomas using tissue array method (TMA). The cases were spotted twice in each TMAs and immunohistochemistry were carried out in two slides in different depth levels. The association between the variables and lymph node involvement was evaluated using the Pearson's chi square test or Fisher's exact test. Survival analyses were performed with the Kaplan-Meier method and the differences between the survival curves were tested using the log rank test. Multivariate survival analysis was performed using the Cox proportional hazards model ($P<0,05$ were considered statistically significant). **RESULTS:** The reduced/absent expression of E-cadherin was significantly associated with increased pN ($p=0,026$) and shorter survival than those with preserved membrane expression especially for patients with diffuse type tumors ($p=0,010$). Patients with nuclear/absent β -catenin

immunoreactivity showed association with pN ($p=0,088$) and worse survival index, but no statistical difference was found with overall survival. A high percentage of the evaluated tumors displayed MUC1 negative expression but no association was found with pN ($p=0,637$) or overall survival ($p=0,409$). The MUC2 and 5AC positivity was associated with better prognosis in specific histological groups -intestinal ($p=0,001$) and diffuse ($p=0,019$), respectively, and for MUC6 this association was independent to histological tumor type ($p=0,002$). The independent variables associated to lymph node metastasis E-cadherin (absent/reduced expression), MUC2 and MUC6 (absent expression). CONCLUSION: Our results indicate that E-cadherin, MUC2 and MUC6 expressions are possibly associated to the lymph node metastasis and suggest further investigations to access their prognostic significance in gastric cancer. Supported by: CNPq, FAPESP CEPID 98/14335-2

P-112

Comparative Study of Urinary Cytology in Urine and Bladder Washing Samples, and Between Simple Smear, Membrane Filter and Cyto centrifugation Techniques in Bladder Cancer

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OBJECTIVE: To verify if there is any influence on bladder transitional cell carcinoma detection when biologic specimens are obtained, directly, from urine samples or bladder washing, with variable volumes and analysed by three different procedures. **METHOD:** Ninety eight patients of University Hospital of São Paulo University with transitional cell carcinoma of bladder were evaluated for cytology in Division of Pathology, Adolfo Lutz Institute. Urine and bladder washing specimens were divided in 10 ml and 100 ml samples and they were processed to obtain slides to be studied by cyto centrifuge, membrane filter (Millipore) and simple smear techniques. They were staining in Papanicolaou and classified as positive, suspicious and negative. The surgical specimens were processed by standard histological techniques, and the tumors graded according to the Ash system. Data were analysed using Q Cochran's test and McNemar test, considering type one error of $\leq 5\%$. **RESULTS:** Urine samples obtained from patients with grades I, II, III and IV tumors were classified cytologically as positive, respectively, in 25%, 79,9%, 95% and 85,4% by membrane filter, in 29,2%, 73,5%, 81,8% and 68,3% by cyto centrifuge of 10 ml, in 18%, 68,2%, 79,7% and 75,6% by simple smear of 10 ml, in 44,4%, 84,6%, 88,8% and 75,6% by cyto centrifuge of 100 ml and in 32%, 75,1%, 85,5% and 78% by simple smear of 100 ml. Washing samples obtained from

patients with grade I, II and III tumors were classified cytologically as positive, respectively, in 94,7%, 96,8% and 100% by membrane filter, in 93,6%, 94,3% and 97,6% by cyto centrifuge of 10 ml, in 65,2%, 85,5% and 97,6% by simple smear of 10 ml, in 95,7%, 96,4% and 100% by cyto centrifuge of 100 ml and in 83,1%, 87% and 100% by simple smear of 100 ml. Comparative samples of urine and bladder washing: The mean positivity in the urine were 37,6%, 82,4% and 88,4%, and in the washing were 94,3%, 96,4% and 99,2%, in tumors grade I, II and III, respectively. **Conclusion:** The cytology on washing samples was superior to urine cytology and also cyto centrifuge or membrane filter over simple smear technique in washing or urine samples of grade I or II tumors. The increase of urine or bladder washing volume, improved the positivity of cytology by simple smear technique for grade I or II tumors or by cyto centrifugation technique for grade I tumor. In Grade III or IV tumors, the technique, the type or the volume of samples do not change results, except for simple smear of 10 ml of urine that was worse. Among all the techniques, samples or volume, the best results were obtained with cyto centrifuge of 100 ml and the worst was of simple smear of 10 ml. Supported by: Adolfo Lutz Institute

P-113

DNA Methylation in Multiple Myeloma

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INTRODUCTION: Multiple Myeloma (MM) is characterized by neoplastic proliferation of monoclonal plasma cells. These neoplastic cells arise from post-germinal center B-cells, which migrate to bone marrow (BM), adheres to the marrow stroma, and triggers subsequent bone resorption. Despite improvements in treatment, the disease remains incurable; new therapeutic targets are therefore urgently needed. Molecular studies have identified alterations in cytokines, adhesion molecules, oncogenes, and tumor suppressor genes (TSG) in MM. The epigenetic control of gene transcription in cancer has been the theme of many recent studies and therapeutic approaches. Carcinogenesis is frequently associated with aberrant methylation and consequent down-regulation of TSG. Although silencing of TSG by aberrant methylation contributes to the pathogenesis of various human malignancies, little is known about the methylation of TSG in MM. **OBJECTIVES:** To determine the methylation status of a panel of 7 genes (*CDH1*, *CDKN2A*, *DAPK1*, *GSTP1*, *MGMT*, *p14*, *RARB*, *MINT25* and *MINT31*) in 50 samples of MM. **METHODS:** DNA was isolated using the TRIzol reagent (Invitrogen), as recommended by the manufacturer, from BM aspirates

from MM patients and from individuals without cancer (givers of BM for allogeneic transplant). The methylation patterns of the selected genes and CpG islands were determined by methylation-specific polymerase chain reaction analysis (MSP). RESULTS: Up to now, 17 MM patient samples and 3 normal controls have been analyzed. The frequency of aberrant methylation among the patient samples was 71% for CDH1, 47% for RARb, 12% for CDKN2A, 12% for DAPK1, 12% for MGMT, and 0% for genes GSTP1, p14, MINT25 and MINT31. Conclusion: Our partial results suggest that CDH1 and RARb are frequently methylated in MM and, for these reason, these genes could have a participation in the physiopathogenesis of the disease. However, the evaluation of a larger number of patients is necessary to confirm these RESULTS. Supported by: FAPESP

P-114

SATR-1 Hypomethylation Is a Common and Early Event in Breast Cancer

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Genome stability and normal gene expression are maintained by a fixed and predetermined DNA methylation pattern, which becomes abnormal in malignant cells. Hypomethylation of satellite DNA sequences is frequently found in tumors and has been associated with an increased frequency of DNA rearrangements and chromosome instability. In this work, we used Methylation Sensitive Arbitrarily Primed-PCR (MSAP-PCR) to identify differentially methylated DNA fragments between normal and tumor breast samples. We identified a novel differentially methylated fragment located on chromosome 5 and with high similarity to a SATR-1 satellite sequence. This fragment was found to be hypomethylated in 63% of breast tumor cell lines and in 86% of breast tumors relative to normal breast tissue. Interestingly, we found that normal tissue adjacent to breast tumors displayed a variable percentage of methylation decrease and that the decrease observed for most of these adjacent samples was higher than the one observed for normal breast tissue derived from reduction mammoplasty. The methylation decrease was, however, significantly higher in tumor samples when compared to adjacent tissue (Qui square=154, 1d.f. $p < < 10^{-4}$), suggesting that SATR-1 hypomethylation frequently occurs in the early stages of tumor development. Our results highlight the importance of global DNA hypomethylation as a contributing factor in breast tumorigenesis. Supported

by: FAPESP

P-115

Identification of Genes Silenced by Methylation in Breast Tumor Cell Lines

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Breast tumors represent the main cause of death in women in Brazil. When rapidly and precisely diagnosed, chances of cure may reach 95%. Genetic and epigenetic abnormalities associated with cancer can be explored for the development of more specific and sensitive diagnostic tools. The aim of this project is to identify genes silenced by methylation in breast tumor cell lines, in order to increase the number of tumor markers and improve diagnosis and prognosis in breast cancer. For this purpose, three breast tumor cell lines (MCF-7, MDA-435 and MDA-231) were treated with the demethylating agent 5-aza-2'-deoxycytidine (5AZA), which reactivates genes silenced by methylation. Total RNA extracted from cell cultures before and after 5AZA treatment was used to construct subtractive cDNA libraries using the RaSH (Rapid Subtraction Hybridization) methodology. In silico analysis of the sequences originated from the subtracted libraries allowed the identification of 68, 96 and 339 genes reactivated by 5AZA treatment in the MCF-7, MDA-435 and MDA-231 cell lines respectively. As a criteria to select candidates for further validation, the promoter region of the reactivated genes was analyzed for the presence of CpG islands. Approximately 80% of the reactivated genes contain a CpG island within their promoter region, confirming the role of DNA methylation in the regulation of their expression pattern. The reactivation of some of these genes is currently being evaluated by semi-quantitative RT-PCR and so far we were able to validate 4 out 10 candidate genes for the MCF-7 cell line and 5 out 10 candidates for the MDA-435 cell line. Validation of candidates selected from the MDA-231 subtracted library is in progress and future plans include the validation of some candidates by quantitative Real Time-PCR, reverse-northern blot analysis of the MDA-231 clones and analysis of the methylation pattern within the promoter region of the validated genes. Supported by: Ludwig Institute for Cancer Research, FAPESP, CAPES

P-116

Gelatinolytic Activity of Matrix Metalloproteinases in Basal Cell Carcinoma of Skin and Its Inhibition by Plant Extracts

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INTRODUCTION: Basal cell carcinoma (BCC) is a malignant disease with slow growing and rarely metastatic behavior. The reasons for such facts remain widely unknown. In many malignancies, acquisition of invasive potential is accompanied by increased of matrix metalloproteinases (MMPs) activity both in the tumor and in the adjacent stroma. MMPs activity is suppressed or reduced by tissue inhibitors (TIMPs) and are related to lost of invasive behavior *in vitro*. The aim of this study was to verify the MMP-2 and -9 activity in BCC and to investigate the potential inhibition of MMPs by natural products. **MATERIAL AND METHODS:** Initially, aqueous extracts of *Aloe vera* and commercial black-tea was obtained. Twenty samples of skin BCC was submitted to protein extraction. The collagenolytic activity of MMP-2 and -9 produced by the tumors was investigated by zymographic assays. After incubation with the aqueous extracts, this activity was submitted to new zymograms. The inhibition of proteolytic activity was observed by digital comparison between both zymograms. **RESULTS:** MMP-2 and -9 was only detected in 13 and 4 BCC samples, respectively. Both aqueous extracts were able to inhibit MMP-2 and MMP-9 activity, but this action was much more noticeable for black-tea. **CONCLUSION:** The non metastatic behaviour of BCC can be due in part to low MMPs activity. Black-tea seems to be a more potent inhibitor of MMP activity when compared to *Aloe vera*. Supported by: CNPq/CAPES

P-117

Homocysteine and Global DNA Methylation in Breast Cancer

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BACKGROUND: Alterations in DNA methylation profile, an important epigenetic mechanism regulating gene expression, are associated with initiation and progression of a variety of neoplasias, including breast cancer. Methyl groups are derived from S-adenosylmethionine (SAM) in methionine cycle and recent studies in humans have correlated alterations in this cycle components with higher risk of developing cancer. Homocysteine, one of these components, may reflect SAM consumption and, consequently, a methylation imbalance. **OBJECTIVE:** The purpose of this study is to correlate global DNA methylation with plasma homocysteine levels and prognostic factors from breast cancer patients. **MATERIALS AND METHODS:**

Global DNA methylation levels were determined by a methyl acceptance assay in tumor biopsies samples from 30 breast cancer patients. Homocysteine levels were determined by HPLC and relevant clinical data was obtained from patients charts. Pearson's correlation test, Kruskal-Wallis t tests and unimodal ANOVA were performed for statistical analysis. **RESULTS AND CONCLUSION:** We observed an inverse correlation between tumor DNA methylation and plasma homocysteine and age. Tumor global DNA hypomethylation is correlated with prognostic factors associated with a poor outcome, such as larger tumor diameter, which was confirmed in our study. This pilot study suggests that homocysteine levels may be used as a surrogate marker for tumor methylation status. A broader analysis of these and others parameters is ongoing in our institution to confirm this findings. Supported by: CNPq

P-118

Inducing Epigenetically Silenced Genes in Breast Tumor Cell Lines Using 5AZA-DC

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Breast cancer is among the major causes of death from cancer in woman. It is also the most common malignancy and ranks as the second cause of death in Brazilian woman. Although, progress has been made towards a better understanding of breast tumorigenesis a few molecular markers to improve diagnosis, prognosis and treatment have been put into clinical practice. Aberrant methylation patterns have been associated with several pathological states including neoplasias. Two general types of DNA methylation changes can concurrently occur in tumors: large-scale hypomethylation of the genome and local hypermethylation within CpG-rich regulatory regions of cancer-related genes. DNA methylation has been used as a diagnostic tool to detect cancer cells in several body fluids, to define different clinical phenotypes as well as to identify patients who will be sensitive to chemotherapy. The identification of aberrantly methylated genes within the tumor genome may thus be a valuable tool to transfer research from the bench to the bedside. In this study, 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 with this demethylating agent, cDNA microarray analysis (4,800 genes) was used to identify epigenetically inactivated genes induced upon

treatment. We identified 37 genes that were induced by 5Aza-dC treatment in at least two of the three breast tumor cell lines. Of the 37 induced genes, 31 harbor dense CpG islands in their promoter region and are likely to be regulated by DNA methylation. Twenty six of the 31 induced genes harboring CpG islands were selected for Real Time PCR validation (qRT-PCR). Using this quantitative technique, we were able to confirm the induction of 17 out of the 26 genes selected for validation. The methylation status of the promoter region of the genes validated by qRT-PCR is currently being analyzed by bisulfite treatment followed by sequencing in the breast tumor cell lines. Once confirmed the role of DNA methylation in the inactivation of these genes, the presence of DNA methylation will be analyzed in a large collection of breast tumor samples in order to evaluate its potential as a molecular marker in breast cancer. Supported by: FAPESP/Instituto Ludwig de Pesquisas sobre o Câncer/Fundação Antônio Prudente

P-119

Aberrant Methylation of Gene Promoter in Childhood Myelodysplastic Syndrome

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The myelodysplastic syndrome (MDS) encloses a heterogeneous group of clonal hematopoietic stem cell disorders and it is characterized by ineffective hematopoiesis and by a high incidence of progression to AML (Acute Myeloid Leukemia). Despite the rarity in childhood, the disease prognosis is poor and the majority of the patients die in consequence of the leukemic transformation or complications involved in the evolution of the MDS (infections and hemorrhage). The abnormal methylation in the promoter region of some genes is related to gene silence. The abnormal methylation could be involved with initiation and progression of cancer when it occurs in tumor suppressor genes. For this reason it has been evaluated as potential markers for the detection of the cancer in early stages (before the clinical manifestation), for the prognostic determination, for the follow-up of the disease progression and for the determination of the response to therapy. We analyzed the aberrant methylation profile of the promoter region of 14 genes (*CDKN2B*, *CDKN2A*, *CALCA*, *CDH1*, *HIC1*, *GSTP1*, *p73*, *p14^{ARF}*, *MGMT*, *APC*, *RAR²*, *CDH13*, *DAPK* e *TIMP-3*) with tumor suppression features in 12 bone marrow aspirate samples and 13 bone marrow smear samples of pediatric patients diagnosed with MDS. DNA of these samples was extracted and submitted to MSP. The most frequently methylated genes were *CALCA* (76%), *GSTP1* (52%) and

CDKN2B (50%). For the other genes the methylation frequency was below 25%. Our RESULTS suggest that the methylation pattern in childhood MDS could be similar to the methylation pattern in adults MDS and that the methylation of the genes presented here can be used as a tool to improve the diagnostic and treatment of the MDS. Supported by: CNPq, FAPESP, CEPID

P-120

ARHGAP21 Gene Expression Is Modulated by Chromatin Remodeling Agents in Myeloid Cell Lines

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INTRODUCTION: RhoGTPases mediate many aspects of cell biology including proliferation, apoptosis/survival, adhesion and events related to actin cytoskeleton dynamics. In the last years, an increasing amount of evidence indicates that several members of the Rho family are important players in tumor biology and several other human pathologies. Rho-GTPase activating proteins (Rho-GAPs) are negative regulators of RhoGTPase signaling pathways. We identified a novel human gene, termed ARHGAP21, which codes for a 1957-aminoacid RhoGAP containing in addition to a RhoGAP domain, a PH and PDZ domain. We demonstrated that this gene is upregulated during myeloid and erythroid differentiation, suggesting a potential role for this RhoGAP in regulating cell differentiation through a gradual inactivation of GTPase cascades. The preliminary data suggest that this protein could be involved in the progression of malignant process of hematopoietic cells. The discovery that hypermethylation and acetylation contributes to the malignant process indicated that chromatin remodeling agents could be used as therapeutic strategy in cancer. MATERIALS AND METHODS: Myeloid cell lines (HL60 and KG1) were grown to subconfluence and treated with two different agents: DAC (Decitabine) and TSA (Trichostatin A) during 72h. Real Time PCR assays were used to analyze the differential expression of ARHGAP21. RESULTS: We have shown that ARHGAP21 transcript levels are upregulated in myeloid cell lines after treatment with chromatin remodeling agents, in a dose-dependent way. CONCLUSION: Analysis of the anti-tumor therapies used nowadays in the clinical trials for the hematological diseases and the data published for RhoGAPs suggest that ARHGAP21 could be involved in the regulation of different cellular process that are important for the development and progression of myelodysplasia and leukemias, aiming this gene as important candidates for anti-tumor therapy. Supported by: FAPESP and CNPq

P-121

Expression of Nuclear Factor Kappa-B (NFκ-B) Pathway Genes In Multiple

Myeloma Patients

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NFκ-B is a transcription factor that controls the expression of a variety of genes, including regulation of apoptosis, cell proliferation and expression of adhesion molecules. NFκ-B is regulated by an inhibitory protein (IκB), which is degraded by Proteasome 26S. In multiple myeloma (MM) patients, NFκ-B activity confers longer survival and drug resistance to tumor cells. Osteoprotegerin (OPG), RANK and RANKL also act in this pathway and are involved in myeloma bone disease pathogenesis. Therefore, a better understanding of molecular mechanisms involved in NFκ-B pathway can contribute to identify new therapeutic targets. PURPOSE: To evaluate the expression of NFκ-B pathway genes in MM patients by real-time quantitative PCR. PATIENTS AND METHODS: Expression of NFκB, IκB, RANK, RANKL, OPG and IL6 were analyzed in 3 normal and 42 bone marrow samples from newly diagnosed MM patients using *Taqman* system (Applied Biosystems). Genes were considered hyper or hypo-expressed in tumor samples when their expression levels were more or less than two times when compared to normal samples. RESULTS: 90% had stage III and 24% of cases were classified as International Prognostic Index (IPI) 1, 33% IPI 2 and 36% IPI 3. Gene expression analyses demonstrate that RANK, RANKL and IL6 were overexpressed in 29%, 31% and 50% of MM samples, respectively. On the other hand, IκB e OPG presented hypo-expression in 26% and 38% of MM cases. Only 16% of MM samples presented NFκB overexpression but we did not find hypo-expression of this gene in any tumor sample. CONCLUSION: 1) most of our MM patients were diagnosed with advanced stage disease and high IPI scores, i.e., unfavorable clinical prognosis; 2) RANK and RANKL overexpression and OPG hypo-expression in at least 1/3 of cases confirm the involvement of this pathway in advanced stage bone disease; 3) overexpression of IL6 in 50% of cases confirms its role in tumor growth and survival in MM; 4) our findings suggest that drugs acting in NFκ-B pathway, such as bortezomib, must have an important role in MM control and should be tested in association with other agents in MM front-line therapy.

P-122

Signature Expression as Predictors for Response to Concomitant Chemoradiotherapy in Head and Neck Squamous Cell Carcinomas, Using cDNA Microarray

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Combination of chemotherapy and radiotherapy became the gold standard for treatment of locally advanced SCC of the larynx, with disease-free survival similar to that obtained with total laryngectomy and adjuvant radiotherapy. However, non-responders suffer from toxicity, delay in salvage surgery and risk of complications, with a significant increase in the cost of the treatment. Thirty-five patients were enrolled in a phase II study in which patients were selected for weekly paclitaxel and cisplatin with concurrent radiotherapy, to a total dose of 7040cGy in 180cGy daily fractions. Prior to treatment, a biopsy sample was obtained and used as source of mRNA for gene expression profiling. Concurrent treatment led to a complete response with preservation of the larynx (CR) in 60% of patients. Using statistical and mathematical tools, we defined expression signatures from individual samples that could be predictors of therapeutic response after a followed-up from 3-5 years. We identified 3 trios and 21 quartets of genes that could precisely predict responsiveness and bootstrap analysis using random sets of genes and samples indicated that such classifiers are not due to chance. We also applied relevance networks and identified sets of genes with altered behavior in the two sample classes that can be used as information for generating data-based hypothesis for the understanding of molecular events underlining resistance to treatment. Our data demonstrate that molecular classifiers can be applied in order to select patients for chemoradiotherapy or surgical treatment of locally advanced SCC of larynx or hypopharynx. Supported by: FAPESP

P-123

Expression Profile of Malignant and Non-Malignant Lesions of Esophagus and Stomach: Identification of Active Expression Modules Related to Inflammation and Lipid Metabolism

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A common feature in the pathogenesis of gastric and esophageal adenocarcinoma is chronic inflammation,

manifested as gastritis associated to *Helicobacter pylori* infection and esophagitis. However, not all individuals with these symptoms develop cancer, suggesting that other factors are critical in determining whether an individual is at risk for neoplastic progression. One promising approach in experiments of cDNA microarrays exploits the idea that alterations in gene expression might manifest at the level of biological pathways or co-regulated genes sets rather than investigations centered on individual genes. Using cDNA microarrays, we determined the expression profile of 71 samples representing normal stomach mucosa, intestinal metaplasia of the stomach, gastric adenocarcinomas, normal esophagus mucosa, Barrett's disease, and adenocarcinomas of the esophagus. Based on the expression profile of 4600 genes, grouped according to the Kyoto Encyclopedia of Genes and Genomes, we determined functional modules that are differentially active in the various sample groups. The glycerolipid metabolism pathway was very active in intestinal metaplasia tissues and not active in adenocarcinomas. The cytokine-cytokine receptor interaction process was active in intestinal metaplasia and adenocarcinomas tissues and not active in normal mucosa of stomach and esophagus, including esophagitis mucosa. The genes involved in these two processes were selected to perform cluster analysis by *K-means* algorithm. The best result was obtained with $K=4$ and, for both processes, we observed a clear separation between squamous tissue samples (normal esophagus mucosa and esophagitis mucosa); tumor samples; and all metaplastic and columnar samples (normal body and antrum mucosa and normal cardiac mucosa). In a different approach, we used Pearson's Linear Correlations in order to find pair of genes with altered correlation between distinct clinical conditions. The nonparametric test *Mann-Whitney* was used to determine the statistical significance of the observed differences. Altered expression of IFNAR2, IL4R, CXCL12 and CCL18 was sufficient to discriminate between squamous and columnar tissues. cKIT was a marker for normal gastric mucosa. The expression of IFNAR2 and IL1R2 were augmented in intestinal metaplasia tissues (stomach and esophagus), whereas the expression of CCL25 was augmented only in intestinal metaplasia of stomach. Finally, the expression of TGFB2, CXCL9, IL6, INHBA and INHBB were significantly altered in adenocarcinomas tissues. The role of lipid metabolism and inflammation might be relevant to better understand the process of chronic inflammatory response associated to intestinal metaplasia of the stomach and esophagus and its subsequent progression to adenocarcinoma. Supported by: FAPESP

P-124

Gene Expression Modulation in Human Melanoma Cells Treated with TNF and/or Melphalan

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Cutaneous melanoma is a malignant tumor derived from melanocytes, neural-crest originated cells situated in the basal layer of the epidermis, specialized in the synthesis of melanin pigment, responsible for skin, eyes and hair color. Early-stage tumors are in most cases curable by surgical resection, but the treatment of advanced tumors is very difficult due melanoma's radio and chemio resistance. In irresectable melanoma of the extremities, amputation is required. Isolated Limb Perfusion (ILP) with melphalan is an alternative procedure to avoid amputation of the limb. This therapy showed an great improvement when melphalan is associated with Tumor Necrosis Factor (TNF). The systemic administration of this cytokine results in serious toxic effects on cardiovascular and respiratory systems, but when confined to the limb (as seen on ILP), in association with melphalan, TNF significantly improved tumor's response rates, resulting in rehabilitation of many patients that otherwise would be submitted to amputation of the limb. Some hypothesis have been made to establish the exact role of TNF and its combination with melphalan in the treatment of malignant melanoma through the ILP procedure. Previous studies about melanoma regression induced by TNF aimed to explain the anti-cancer activity of this cytokine based on the TNF-induced alterations on the tumor endothelial cells. In the presented study we used the cDNA microarray technology to analyze the gene expression profile of melanoma cell lines in response to TNF, melphalan or the combination of the two drugs. After the identification of a panel of induced and repressed genes in response to each treatment, special attention was given to those already described on the apoptosis pathway. In all cell lines tested, NFKBIA induction was detected after treatment with TNF, suggesting that the tumor regression process is also related to TNF activity on melanoma cells. Supported by: FAPESP

P-125

Suppression Subtractive Hybridization and Microarray Analyses Reveal Novel Genes Potentially Involved in Melanoma Progression

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INTRODUCTION: Melanoma is one of the most aggressive malignancies whose molecular determinants are not precisely understood. Melanoma progression occurs through three major stages: radial growth phase

(RGP), characterized by horizontal growth typically confined to epidermis, vertical growth phase (VGP) when tumor cells are capable of growing vertically into the dermal layer, and metastasis. **OBJECTIVE:** Identify novel genes involved in melanoma progression through differential expression analysis. **METHODS:** A microarray of ORESTES cDNA mapping chromosomal regions associated to melanoma development was used to compare the expression profiles of RGP, VGP and metastatic cell lines. Using suppressive subtractive hybridization we construct two subtractive libraries representing the genes differentially expressed between an RGP cell line and a pool of metastatic cell line, and 800 clones from both libraries were sequenced and annotated. **RESULTS:** The microarray analysis indicate a small, but statistically significant group of genes presenting differential expression, among them two genes with unknown function (C6ORF51 and C9ORF82) with potential tumor suppressor role, once their expression was maintained only in a less aggressive RGP cell line. The analysis of subtractive libraries indicated that among the genes represented we have several for which a role in tumor progression has already been characterized, as well as genes presenting some functional characterization, but no association to cancer and others whose function is completely unknown. Through Northern blot, RT-PCR and flow cytometry we confirmed the expected expression pattern of all genes selected for validation revealing novel genes with remarkable differential expression during melanoma progression. **CONCLUSIONS:** Through the combination of two approaches of differential gene expression analysis we identify novel genes with potential involvement in melanoma progression. Supported by: FAPESP, CAPES, CNPq

P-126

Detection of the Noncoding Transcript in T Cells (NTT) in Human Melanoma Cells

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INTRODUCTION: The NTT (noncoding transcript in T cells) gene was found serendipitously during a search for genes that are differentially expressed among subsets of human T cells. The product of this novel gene is a 17-kb transcript which is selectively expressed in activated CD4⁺ T cells. The NTT transcript is polyadenylated, but is not spliced and has no open reading frame larger than 270bp. Thus, NTT transcript is a noncoding RNA detected only in activated CD4⁺ cells and its function is still unknown. We speculate that the NTT transcript could also be found in other types of activated cells such as tumor cells. **OBJECTIVE:** In this study, we investigate the presence of NTT transcript in human melanoma cells at different stages (radial, vertical and metastatic) of tumor development. **MATERIAL AND METHODS:** The RNA was extracted from cell lines of human melanoma corresponding to different stages of melanoma

development. Total cellular RNA was used for cDNA synthesis and the primers for amplification were designed based on the sequence of NTT (GenBank Access No. U54776). The PCR product (426 bp) was subsequently cloned into the pGEM-T vector and sequencing. **RESULTS** and **CONCLUSION:** We detected the PCR product of 426bp in all stages of melanoma development and the NTT transcript was confirmed by sequencing. It is accepted that the NTT gene is only expressed in activated CD4⁺ T cells. To our knowledge, this is the first time that the noncoding NTT transcript is detected in tumor cells. It is known that around 98% of the transcriptional output in mammalian cells is noncoding RNA. The detection of the noncoding NTT transcript in human melanoma is relevant since recent studies have indicated that noncoding RNAs regulate important cellular processes such as cell growth, differentiation, apoptosis and gene expression. Additional work is required to elucidate the function of the noncoding NTT transcript in human melanoma cells. Supported by: CAPES and FAPESP

P-127

RNA Interference Mediated PKR Gene Silencing in Melanoma B16-F10 Cells

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INTRODUCTION: With the complete sequencing of the human genome, research priorities have shift from the identification of genes to the elucidation of their function. RNA interference (RNAi) has become a powerful tool to investigate the function of mammalian genes by degrading a specific mRNA target. The mediators of sequence-specific mRNA degradation are double-stranded small interfering RNAs (siRNAs). The RNA-dependent protein kinase (PKR) is a serine-threonine kinase activated by intermolecular autophosphorylation upon binding RNA. Accumulating evidence during recent years has implicated PKR in cell growth, differentiation and apoptosis. It has also been suggested that PKR acts as a tumor suppressor, which is still controversial. **OBJECTIVE:** In this study, we examine the ability of RNAi to inhibit the expression of PKR gene of melanoma B16-F10 cells in culture of 24 hours. **MATERIAL AND METHODS:** We have used a short hairpin RNA (shRNA) expressing plasmid (psiSTRIKE U6 hairpin cloning systems, Promega) controlled by Pol III U6 promoter. The B16-F10 cells were transfected with the PKR-specific siRNA and lipofectamine 2000, and the PKR gene silencing was monitored by RT-PCR and Western blot analysis after 24 hours of culture. **RESULTS AND CONCLUSION:** Our RESULTS showed that the levels of both PKR mRNA and PKR protein were significantly reduced in transfected B16-F10 melanoma cells, indicating that specific siRNA was effective in PKR gene silencing in these tumor cells. However, we did not observe differences in terms of number of B16-F10 cells before and after transfection at least in culture of 24 hours. Further work in vivo is required to evaluate

whether the PKR gene acts as tumor suppressor by using mice injected with transfected B16-F10 cells in this model of experimental metastasis. Supported by: CAPES and FAPESP

P-128

Differential Expression of Genes in Multiple Myeloma (MM) and Identification of Candidates to Targeted Therapy

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Considering the heterogeneity of genetic aspects related with MM pathogenesis, recent studies have been focused on prognostic factors that could define the best therapeutic approach for each case or to contribute for the development of new therapies. In this way, gene expression profiling analysis can be very helpful to define such prognostic factors. Serial analysis of gene expression (SAGE) allows not only the identification of genes expressed in a given tissue, but also their expression levels. Until now, there was no data of gene expression in normal or neoplastic plasmacytes in public SAGE databases. PURPOSE: The aim of this study is to create two SAGE libraries using normal and neoplastic plasmacytes and to identify genes differentially expressed in MM. METHODS: Normal plasmacytes were obtained from palatine tonsils of children who underwent tonsillectomy. MM library was obtained from bone marrow plasmacytes of two IgGκ newly diagnosed MM patients. Purified normal and neoplastic plasmacytes were obtained after magnetic sorting of CD-138-positive cells (MACS - Magnetic Cell Sorting of Human Cells, Miltenyi Biotec). Both SAGE libraries were obtained using the kit I-SAGE (Invitrogen), according to manufacture's instructions. RESULTS: We generated 4,000 clones from each library and, after automatic sequencing, we got 29,918 tags from normal and 10,340 tags from tumoral libraries (corresponding to 8,241 and 2,359 unique tags, respectively). After comparison of unique tags expression levels, we selected 15 overexpressed (at least 10 times) genes in the MM library (ZFHX1B, XBP1, PIM2, LGALS1, RANBP2, HSPC132, PRDM2, DDX5, ARMCX3, LSM5, JUND, MAPKAPK2, SP140, KLF2 and NTN1). CONCLUSION: Once these genes are related with transcription, signaling, cell proliferation and/or apoptosis, they can have important role in the tumorigenesis process and may be possible therapeutic targets for MM control. Supported by: FAPESP # 04/13213-3

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Functional Analysis of PPM1D and FLJ: Two Genes Identified as Differentially

Expressed in Prostate Tumors

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Prostate cancer is the leading cause of cancer-related deaths in the Western male population. Several important pathways of prostatic tumor progression are well understood, but, unfortunately, the malignant transformation process remains largely unknown. In order to elucidate new pathways involved in prostate tumorigenesis, we constructed subtracted cDNA libraries using the RDA (Representational Difference Analysis) technology, which allowed the identification of a new gene (FLJ) as being overexpressed in tumor samples (5/11 matching pairs of prostate tumor and adjacent normal tissue from the same patient). To understand the biological importance of this gene in prostate cancer, we analyzed its expression in the LNCaP prostate cell line upon treatment for different periods of time with a synthetic androgen (R1881). Preliminary results show that FLJ is downregulated upon a 4h treatment with androgen. These results are surprising since androgens are mitogenic agents for prostate cell lines and, also, because genes which are downregulated by androgens usually are downregulated in prostate tumor as well. One possible explanation for these results is the presence of new isoforms of FLJ with different responses to cell transformation and androgen treatment. This hypothesis was partially confirmed by identification of at least two new isoforms of FLJ. The second selected gene for functional analysis was PPM1D, which had previously been identified, by cDNA microarray, as being overexpressed in prostate tumors of higher malignancy grades (Reis et al, 2004). To understand the physiological role of PPM1D in prostate cells, we analyzed PPM1D expression upon androgen treatment of LNCaP cells. We observed that androgen treatment for 4 to 24h induces PPM1D expression and this response seems to be independent of protein synthesis. We are currently cloning the complete PPM1D cDNA for functional analysis in prostate cancer cell lines. Supported by: CNPq, FAPESP, FINEP and PRP-USP

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Characterization of a Novel Conserved Human Gene Expressed in Melanoma Cell Lines

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INTRODUCTION: Previous differential expression studies in our laboratory using ORESTES clones led to

the identification of a gene located in the human chromosome 1p36 and annotated as KIAA0090, that shows higher expression levels in some metastatic melanoma cell lines when compared with cell lines from earlier tumor progression stages. The gene encodes a highly conserved 993 amino acid polypeptide present in all eukaryotes from yeast to humans for which no function was determined. OBJECTIVES: Determine the subcellular localization of the KIAA0090 protein and the phenotypes generated by overexpression of multiple recombinant forms of this protein fused with EGFP, in melanoma cell lines. METHODS: The full-length coding region from KIAA0090 cDNA was cloned in mammalian and bacterial expression vectors. Subcellular localization of the protein in melanoma cells was analyzed by expression of KIAA0090 fused to EGFP and associated to immunocytochemistry using markers for different organelles. The bacterially expressed protein was used to generate polyclonal antibodies against KIAA0090. RESULTS: Expression of KIAA0090 fused to EGFP in the N or C terminal (KIAA-EGFP and EGFP-KIAA) showed that the protein is exclusively cytoplasmic. KIAA-EGFP co-localizes with endoplasmic reticulum, without affecting the normal distribution of the organelle, whereas EGFP-KIAA rapidly aggregates in a perinuclear region promoting a dramatical mitochondrial co-aggregation, and causing pronounced cell death. Both fusion proteins, although in different levels, affected cell viability in a way that seems to be dependent on caspase activation. Transfected apoptotic cells were shown to be TUNEL positive. Besides that we generated a polyclonal antibody against the KIAA0090 that is now been used to analyze the expression pattern of the endogenous protein. CONCLUSIONS: We show here the initial characterization of KIA0090, a novel predicted protein, in melanoma cells. The KIA0090 is highly conserved in all eukaryotes, and expression of recombinant forms of this protein affects cell viability, indicating that KIAA0090 is probably involved in important cellular processes with potential importance in cancer. Supported by: FAPESP, CAPES, CNPq, FAEPA.

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Hypermethylation Profile Analysis of Gene Promoters in Head and Neck Squamous Cell Carcinoma

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Head and neck cancer is one of the most frequent tumor types and the most common variant is the

squamous cell carcinoma of the upper aerodigestive tract (HNSCC). The therapeutic strategies for HNSCC are based on the TNM stage and tumor site. However, tumors showing the same clinical stage and site treated by the same protocols present different recurrence and survival rates. A better knowledge about the biological behavior of HNSCC would guide to a more precise delineation of tumor progression and biological potential of invasion and metastasis. In this context, the identification of molecular markers plays an important role in the determination of the prognosis and follow-up of cancer patients adjusting the treatment to the aggressiveness behavior of the tumor. OBJECTIVES: Determine the hypermethylation profile of promoter regions of tumor suppressor genes in HNSCC samples from American and Brazilian patients in order to evaluate the utility of hypermethylation of these genes as molecular markers for HNSCC. METHODS: Ten genes (*PI6INKa*, *MGMT*, *DAPK*, *MINT31*, *CCNA1*, *CCND2*, *TIMP-3*, *ESR*, *AIM-1* and *DCC*) were selected for the hypermethylation analysis in 104 samples of HNSCC from patients treated at the Johns Hopkins University. The hypermethylation profile analysis was performed by quantitative methylation specific PCR. RESULTS: Regarding the Johns Hopkins University patients, hypermethylation for at least one gene was detected in 101 (97.1%) cases and the most frequent methylated genes were *DCC* (78.8%), *DAPK* (74.0%), *ESR* (70.2%) and *TIMP-3* (70.2%). It was also possible to correlate the hypermethylation profile of some genes to the anatomic site, alcohol and tobacco consumption. All the genes were considered good markers for HNSCC. PERSPECTIVES: Determine the hypermethylation profile of the same 10 genes in 104 tumor samples of HNSCC collected from patients treated at the Hospital do Câncer A.C. Camargo to evaluate the utility of the promoter hypermethylation of these genes as molecular markers for Brazilian patients. Supported by: FAPESP e SPORE (USA)

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Gene Expression Profile in Cutaneous Melanoma

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INTRODUCTION: Cutaneous melanoma, the most aggressive common skin tumor, originates from the malignant transformation of melanocytes that is located in the basal layer of the epidermis. Cutaneous melanoma develops in three different stages, from radial to vertical growth phases and metastatic disease. In order to contribute to the understanding of melanoma

progression and to the basis of variability in clinical behavior, which is only partially explained by clinical and histological data, we are determining the gene expression profile of primary and metastatic melanomas using cDNA microarray technology. This effort should lead to the identification of rational targets for drug development and biomarkers for disease monitoring, which should impact positively on detection and treatment of melanoma. **OBJECTIVE:** With the aid of mathematical and statistical tools we shall determine the association between a given expression profile and clinical outcome. **METHOD:** Patients are being recruited at the department of Skin Oncology of Hospital do Câncer AC Camargo. All patients sign a pre-informed consent and the study was approved by the internal ethics committee. Thus far, 61 tumours samples (21 primary and 40 metastatic melanomas) were analyzed. Total RNA was extracted using RNeasy Midi-Kit® and amplified by a T7-based protocol. We performed indirect cDNA-labeling as described by Cox et al. (2004). We used glass arrays containing 4,800 cDNA sequences that correspond to ORESTES cDNA fragments derived from FAPESP/LICR Human Cancer Genome Project. Data were extracted with ScanArray Express software using histogram method. For data analysis we used R (<http://cran.r-project.org>) and tools from the Bioconductor project (<http://www.bioconductor.org>). **RESULT:** After non-linear normalization, log (base 2) transformation, and hierarchical clustering we determined the p-values for genes differentially expressed when primary and metastatic samples were compared. Thus far, we only have a list of some genes with biological relevance. We are looking forward to associating them with clinical outcome. Supported by: FAPESP; CEPID; Ludwig Institute for Cancer Research

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Gene Expression Profile of Non-Malignant Melanocytic Lesions

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Cutaneous melanoma arises from the malignant transformation of melanocytes. Although early melanoma is curable during the early stage, the prognosis of advanced melanoma is poor due to high metastatic potential and therapeutic resistance. A sequence of steps of tumor progression has been proposed: common nevi, dysplastic nevi, RGP and VGP primary melanomas, and metastatic melanomas. Nevi are composed by melanocytes with focal proliferations that are ordered in nests and temporally restricted in

their growth. They are both precursors and markers for melanoma; thus, by studying nevi we may be able to identify genes involved in the early steps of melanocytic differentiation. To improve our knowledge of the molecular profile of nevi, we have been doing a systematic analysis of genes expressed by 04 benign compound nevi (acquired) and 10 benign intradermal nevi (acquired and congenital) using microarray technology. Using a set of differentially expressed genes (pvalue ≤ 0.005 , fold change ≥ 1.5) between compound and intradermal nevi we obtained hierarchical cluster that completely separates samples by histological type, whereas all but one cases of congenital and acquired intradermal nevi comparison have clustered according to the histogenesis. Some of the differentially expressed genes identified have already been reported in cells and tumors, suggesting a possible role in the malignance potential of these lesions. The preliminary data raises the possibility to clarify the type of nevus which can potentially progress to melanoma. In addition, data from acquired and congenital nevi suggest that features of melanoma may differ according to their histogenesis. However, these findings will be confirmed using a larger number of cases. Supported by: FAPESP and CNPq

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Gene Expression Comparison Between Ductal Carcinoma *In Situ* and Invasive of Breast Showed Potential Genes Involved in Invasiveness Process

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Breast cancer progression is likely a multistep process involving genes whose regulation is altered. Cellular invasion is the first step for metastasis contributing to high death-rate among women with breast cancer. Therefore, the identification of genes capable to predict the cellular invasion and also the understanding of tumor progression has still been a challenge. **OBJECTIVE:** To study breast carcinoma ductal *in situ* (DCIS) and invasive (IDC) we intend to identify potential key genes involved in invasion process. **MATERIAL AND METHODS:** 16 ductal carcinoma samples and 4 non-neoplastic breast samples were laser capture microdissected to obtain homogeneity of cells and also the separation of both histological types of the same sample. The RNA was extract and amplified in 2 rounds using T7 based methodology. cDNA microarray technology was performed using a customized cDNA platform containing 4,608 human genes. Comparison between 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 p -value < 0.05. RESULTS: High similarity between DCIS and IDC from the same sample was found, obtaining 33 genes differentially expressed with maximum differential expression of 2.5 fold. For the identification of relevant biological processes and molecular functions we applied Gene Ontology analysis. This analysis showed cell-cell signaling, apoptosis and chemotaxis as biological process while molecular function showed heparin binding and extracellular matrix structural constituent. Among the differentially expressed genes 10 of them showed a trend in down or up-regulation when compared with normal sample class, where some of them have been previously reported as involved in invasiveness. CONCLUSION: Even though a high similarity of gene expression pattern between both lesions was found, we identified 33 invasion related-genes that may be tested as potential invasiveness markers in breast cancer. Supported by: CAPES and CEPID/FAPESP

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Analysis of Breast Cancer-Associated Exons Through Microarray Technology

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Current analyses have shown the occurrence of alternative splicing (AS) in at least 70% of human genes contributing to wide diversity of transcripts and increasing the proteomic complexity. It is known that some variants generated by AS or aberrant splicing is preferentially expressed in human tumors. The importance of alternative mRNA splicing in the generation of genetic diversity resides on the possibility to correlate specific variants with clinical information in order to identify tumor markers. OBJECTIVE: To identify molecular markers in breast cancer using a microarray platform containing exons from splicing variants. METHODOLOGY: 270 tumor-associated exons were selected from several tumor tissues by a bioinformatics pipeline, where 75 are overexpressed in breast tumors compared to its normal counterparts. Exons were immobilized on a nylon membrane and hybridized with 27 tumoral and 5 non-neoplastic breast tissues. Samples were manually microdissected and total RNA amplified in 2 rounds using T7 based methodology. Five mg of amplified RNA was labeled with 60 mCi of $\pm^{32}$ P-dCTP and hybridized in duplicate. Generated signal was captured by Phosphorimager and analyzed by Array Vision program. Normalization data was performed using intensity means of 24 replicates of constitutive

genes (*gapdh* and β -*actin*). T Student test was applied in order to determine differentially expressed exons (FDR < 0.01). RESULTS: From 75 breast cancer-associated exons, 33 (44%) were overexpressed (fold ranging from 1.23 to 3.14). Functional annotation of 3 of the higher expressed exons were analyzed and may be involved in tumorigenesis: MK-STYX, related to MAP kinase pathway; nuclear prelamin A recognition factor, involved in the maintenance of nucleus structural rigidity; and membrane-bound transcription factor protease, involved in survival programs of the cells mediated by retinoic acid. CONCLUSION: These analyses (bioinformatics and experimental) identified 33 exons overexpressed in breast tumor. These exons will be tested as potential molecular markers. Supported by: CAPES, CEPID-FAPESP

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Differential Gene Expression Profile of Fibroblasts Originated from Breast Cancer as Compared to Those Derived from Normal Adjacent Tissue from Benign Disease

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The homeostasis of normal breast depends on interactions between epithelial cells and their associated stroma. Previous studies indicated that in breast carcinoma, tumor associated stromal cells with different functions appear to be emerged. These stromal elements include fibroblasts which modulate tumor behavior providing various growth factors and extracellular matrix components. OBJECTIVE: Our aim was to evaluate the differential gene expression between fibroblasts derived from malignant breast tumors (CAF), ECII and normal adjacent tissue from benign breast lesions (NAF) using Serial Analysis of Gene Expression (SAGE). METHODS: After establishment of primary cultures, vimentin and smooth muscle actin imunocytochemistry were evaluated in order to verify the purity of culture. The RNA was then extracted and SAGE was performed. RESULTS: We obtained 20424 unique tags in CAF library and 31849 unique tags in NAF, between these tags 14205 and 20305 have annotation in CAF and NAF respectively. Upregulated genes in CAF included those related to adhesion and cytoskeleton; proteases, oncogenes, protooncogenes and genes involved in signaling transduction. Interestingly the most upregulated gene in these fibroblasts coded for early growth response 1 protein (fold of 64,5) which was demonstrated to be involved in carcinogenesis and cancer progression, especially metastasis. The most downregulated gene was TIMP3 followed by genes

coding for extracellular matrix proteins and proteins related to membrane trafficking. **CONCLUSION:** In spite of these cells have been cultured up for 2 months, fibroblasts derived from malignant breast tumors retained an intrinsic modification of gene profile. Supported by: FAPESP 01/13515-1 and 04/04607-8, CNPq 477538/2003-7 and Fundação Faculdade de Medicina.

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Global DNA Methylation Analysis in Breast Tumors Using Promoter Microarray

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Aberrant DNA methylation of CpG sites in promoter regions is among the earliest and most frequent 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 treatment, abnormally methylated sequences occurring in the tumor genome need to be identified. Recently the microarray-based strategy was adapted to allow genome-wide assessment of methylation status of promoter regions. Human RefSeq promoter sequences were retrieved from GenBank and promoter fragments amplified by PCR were spotted onto glass slides resulting in a promoter microarray containing triplicate spots of 12,800 promoter sequences. Genomic DNA from a reference source (HB4a normal breast cell line) and a test source (MDA-MB-231 tumor breast cell line) were digested with the methylation sensitive enzyme HpaII. Oligonucleotide linkers were ligated to the digested fragments. Repetitive sequences were then removed by hybridization with biotin labeled Cot-1 DNA, followed by affinity capture with streptavidin magnetic particles. PCR using linker-specific primer was then used to separately amplify the test and reference DNA fragments. Test and reference DNA products were labeled with Cy3 or Cy5, respectively, and hybridized to the promoter microarray. 117 genes appeared to be differentially methylated between the HB4a normal cell line and the MDA-MB-231 tumor cell line. Among the differentially methylated genes, we were able to identify several genes that have already been reported to be hypermethylated in breast tumors (eg. RB1, RASSF5, EGFR, CDKN2C, WNT2B, BAX, CDC20 and GADD45G). Thus, the use of a method that directly amplifies the CpG regions of interest combined with a promoter microarray analysis seems to be a very useful strategy to genome-wide analysis of methylation patterns. We are now applying this technique to identify differentially methylated fragments between node positive and node negative breast tumors, which could be used in the future as molecular marker for disease

management. Supported by: FAPESP

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Identification of Expressed Sequence Tags Representing Putative Human Micro-RNA Transcripts

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INTRODUCTION: Alterations of miRNAs expression have been associated to human cancers. Also, it has been shown that miRNA profiles vary dramatically across tumor types and reflect the differentiation state of the tumor. **OBJECTIVE:** In an effort to identify micro-RNAs expressed in normal and/or tumor human tissues, we performed an *in silico* analysis searching for ESTs representing putative micro-RNAs transcripts. **METHODS:** We compared a set of 1,3 million ORESTES and ~5 million NCBI ESTs with a set of 510 ESTs of monkey microRNAs precursors. **RESULTS:** We identified 32 ORESTES clusters and 38 NCBI-EST clusters with significant match to monkey microRNA ESTs. Using the BLAT tool, to localize these 70 ESTs clusters in the human genome, we verified that all of them are derived from regions of predicted microRNA genes, 31 of which were experimentally validated by northern blot. 42 of the EST clusters are located in introns of coding genes, 20 are intergenic and a smaller group mapped in exons (3) or intron/exon boundaries (3) of coding genes. Among the ORESTES clusters representing the putative human miRNAs precursors, we found 18 containing sequences derived from normal and tumor tissues while 14 are derived exclusively of either normal or tumor tissues. Sequences corresponding to mir-181c, mir-15b, let-7a-1, let-7f-1, mir-135-1 and mir-205 were found only in tumor samples. mir-127, mir-221, mir-125-b-1, mir-26a, mir-141, mir-23, mir-214 and mir-143 were represented only in libraries of normal tissues. **CONCLUSIONS:** The analysis identified 14 miRNAs candidates to be differentially expressed in normal and cancer tissues. The set of miRNAs found only in normal tissues are especially promising, once the amount of sequences derived of normal tissue libraries is about 1/3 of the total. We are now selecting some of these miRNA genes for further characterization and expression analysis in different types of tumors. Supported by: FAPESP, CNPq, CAPES, FUNDHERP

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Soft Tissue Sarcomas with Atypical Response to Chemotherapy and Rare Metastasis Pattern

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OBJECTIVES: To present two cases of leiomyosarcoma with uncommon metastasis spread, and atypical response to chemotherapy. **CASE REPORTS:** The former case is a 44-year-old female with a diagnosis of a 9 cm high grade leiomyosarcoma of retroperitoneum. Six months later, metastasis to the lungs were diagnosed. Six cycles of doxorubicin and Ifosfamide and more six cycles of dacarbazine were administered, however, she presented with atelectasia of her right lung. Vincristine 1,5 mg/m² in one day and dactinomycin 4,5 mg/m² in three days were initiated. Clinical benefit was observed and the patient receive 6 more cycles before dying, 3 years after diagnosis. The second case is a 48-year-old female with a 19 cm high grade leiomyosarcoma in her leg. She undergone surgery and six cycles of Doxorubicin and Ifosfamide. She presented with pancitopenia and a bone marrow biopsy showed leiomyosarcoma. Immunohistochemistry (IHC) was positive to SMA and vimentin. Computed tomography showed lung metastasis and two lymph node conglomerates. The patient died 20 months after surgery. **Discussion:** Unusual response observed with third line chemotherapy questions if adjuvant treatment would benefit STS of retroperitoneum. Epithelial IHC markers did not explain this metastasis pattern. Perhaps, gene expression analysis will be able to predict clinical outcome in leiomyosarcomas. **Key Works:** soft tissue sarcoma, leiomyosarcoma, chemotherapy, metastasis.

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Two Substances Isolated from *Crotalus durissus terrificus* (Cdt) Snake Venom Induce Antinociception and Inhibit Tumor Growth in a Rat Model of Cancer Pain

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AIM OF INVESTIGATION: Cdt venom induces analgesia mediated by opioid receptors. Recently, a synthetic analgesic product, CNF 021.03 (CNF), was obtained based on the analgesic factor isolated from the venom. The antinociceptive action of CNF in a model of cancer pain induced by the growing of Walker 256 carcinoma cells injected into Wistar rat hind paws was presently investigated. Also, the antitumoral effect of

crotoxin (Ctx), the main venom toxin, was evaluated. **METHODS:** Walker 256 carcinoma cells (1x10⁶) were injected by intraplantar route. Hyperalgesia and allodynia were assessed using a paw pressure test and von Frey filaments, respectively. For observation of spontaneous pain, the duration of lifting and licking of paws was measured. Tumor cell growth was analyzed by determination of paw volume increase and histopathological analysis. **RESULTS:** The injection of Walker 256 cells caused hyperalgesia and spontaneous pain on day 5 after cell inoculation. Low-threshold mechanical allodynia was significant 2 days after tumor cell injection, increasing in magnitude on day 5. The volume of paws started to increase on day 2-post inoculation, increasing up to 40% on day 5. On day 5 after injection, there is a marked proliferation of tumor cells, with the presence of anaplastic and pleomorphic cells, evident nucleoli and atypical mitotic figures. CNF (6 µg/kg, p.o) administered at day 5, produced analgesia that lasts for two days. Nor-BNI (50 mg/paw) blocked the CNF effect, whereas ICI 174,864 (10 mg/paw) partially reversed this effect. CTOP (20 mg/paw) did not modify this effect. Long-term treatment (5 days) with Ctx (18µg/kg, s.c.) inhibited tumor growth (30%), neovascularization and hyperalgesia. Naloxone did not interfere with Ctx-induced analgesia. **CONCLUSIONS:** These results indicate that i.pl. injection of Walker 256 cells causes cancer pain. CNF induces long-lasting antinociception mediated by k and d-opioid receptors. Ctx alleviate cancer pain by suppression of tumor growth. Supported by: CAPES, CAT/CEPID/FAPESP, COINFAR

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Prion Protein Expression and Its Role in Protection Against Doxorubicin Toxicity

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The chemotherapeutic agent doxorubicin has a very important side effect in the heart, causing a severe dose-dependent cardiotoxicity by oxidative stress, which is a limiting factor for its usage in humans. The cellular prion protein (PrP^c), is a polymorphic protein presenting a 4 or 5 octarepeat domain in humans, with the former being present in 7 to 10% of the population. PrP^c is involved in protection against oxidative stress due to a copper binding site present at the octarepeat domain. Since PrP^c is highly expressed in heart, we decided to evaluate its role in protection against the damage induced by doxorubicin treatment. We initially evaluated the toxicity carried by acute intra-peritoneal injection of doxorubicin in PrP^c null mice (Prnp^{0/0}) and transgenic animals expressing ten times more PrP^c (Tg-20) than wild-type animals (Prnp^{+/+}). We observed that at high doses (50 mg/kg), doxorubicin is extremely toxic and all of the animals died within 48 hours, with no differences between the groups (at least 15 animals per group). Nevertheless, when 30 mg/kg was injected, the transgenic mice (Tg-20) presented survival higher than

the two other groups (*Prnp*^{0/0} and *Prnp*^{+/-}) ($p < 0.05$, minimum of 15 animals per group) which, in this case, presented similar elevated mortality to the treatment. Remarkable, 15 or 20 mg/kg doxorubicin treatment were more toxic to the *Prnp*^{0/0} mice than to the wild-type ones ($p < 0.05$, at least 10 animals per group) while Tg-20 animals never reached death. When 10mg/kg was injected, only the *Prnp*^{0/0} mice died throughout the 42 days of the treatment ($n=13$ in the *Prnp*^{0/0}, $n=15$ in the *Prnp*^{+/-} and $n=10$ Tg-20). As for the assessment of cellular doxorubicin toxicity, we are also performing immunohistochemistry analysis to evaluate any signal of cardiotoxicity in the myofibrils. These results indicate that toxicity produced by doxorubicin treatment is dependent on the PrP^c expression levels in mice. Therefore, sensitivity to doxorubicin toxicity in humans may be associated to partial lost-of-function due to polymorphisms present at the PrP^c protein. This issue deserves further intensive studies. Supported by: FAPESP

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High Pathologic Response Rate of Neoadjuvant Chemoradiation with Gemcitabine in Locally Advanced Vulvar Carcinoma

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OBJECTIVE: To evaluate the response rate of chemoradiotherapy with gemcitabine in treatment-naïve patients with locally advanced vulvar carcinoma. **Patients and METHODS:** Patients were accrued from May 2002 until December 2004. The scheduled dose of radiotherapy was 50.4 Gy and the dose of gemcitabine was 300 mg/m²/week. At the end of treatment, surgical resection was performed and response rate was evaluated. **RESULTS:** Five patients were enrolled. The median age was 72 years, ranging from 59 to 79. Four patients were stage III and one was stage II (FIGO). All patients received and chemoradiotherapy. Median number of gemcitabine cycles was 5 (2 to 10). Clinical complete response was observed in four patients, and pathologically confirmed in 3. Grade III skin toxicity developed in 2 patients. One patient received only two cycles of chemotherapy because of a stroke. **Conclusion:** Neoadjuvant chemoradiation in vulvar carcinoma with weekly gemcitabine shows high rates of clinical and pathological complete response with acceptable toxicity. These positive findings suggest that further evaluation of this therapeutic approach is needed to confirm this benefit. **Keywords:** cancer, vulva, chemotherapy, radiotherapy.

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Microarray of ORESTES cDNA Allows

the Identification of Novel Genes with Differential Expression in Melanoma Tumor Progression

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INTRODUCTION: Tumor progression is a complex process involving changes in a diverse range of cellular pathways. In previous studies, we have demonstrated an elevated proliferation rate and resistance to anoikis associated with a higher degree of tumorigenesis and metastasis in the WM1617 cell line (derived from a lymph node metastasis) in comparison with the cell line WM278 (derived from a primary tumor) of the same patient (Meenhard Herlyn's laboratory). **OBJECTIVES:** Identification of novel genes with differential expression in melanoma tumor progression, using as models these two paired human melanoma cell lines. **METHODS:** We selected 1207 clones (351 corresponding to predicted and 856 to provisional RefSeqs, NCBI database) from 90,000 ORESTES (Open Reading Frame ESTs), generated by the RC1 and RC3 laboratories during the Human Cancer Genome Project, to synthesize a cDNA microarray on a nylon membrane. Hybridization assays were performed for WM278, WM1617 and a reference pool of four cell lines, using as probes radioactively labeled cDNA obtained from total RNA. **RESULTS:** Digital quantification and statistical analysis of the microarrays indicated 5 up-regulated and 75 down-regulated genes in the metastatic cell line. Expression analysis of a group of these genes by Northern blots of this pair of cell lines plus a cell line of the radial growth phase (WM1552) showed that the genes LRPAP1 and CD63 are overexpressed in WM1617 in comparison with the other two cell lines. Among the genes with underexpression confirmed in the metastatic cell line, CYR61, BUB3, HMG2 and MGC5576 demonstrated the greatest differences in expression. **CONCLUSIONS:** In agreement with published evidence, loss of CYR61, BUB3, HMG2 expression is compatible with the phenotype of these melanoma cell lines, suggesting that further efforts will be worthwhile in order to characterize the importance of these genes as well as of the uncharacterized genes identified in this study as tumor suppressors in melanoma. Supported by: Human Cancer Genome Project, FAPESP, CAPES, CNPq and FAEPA.

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Pharmacokinetics and Antineoplastic Effect of a 7-Ketocholesterol-Containing Emulsion (LDE/7KC)

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understood, and effective treatment options are limited. To investigate the role of central AMPK/ACC signaling in the pathogenesis of cancer anorexia, we assessed the effects on food intake of the AMPK agonist AICAR administered into the third cerebral ventricle of Wistar rats that were anorexic from Walker-256 cancer. In anorexic tumor-bearing rats, daily treatment with AICAR (2mM, intracerebroventricularly) prevents reduction in food intake from 38 $\pm$ 2% to 81 $\pm$ 5% of preanorectic baseline, we also observed that the median overall survival in the control group was 7 days, as compared with 12 days in the AICAR group. Because AICAR partially reverses cancer and IL 1b induced anorexia, increased central nervous system AMPK/ACC signaling is implicated in the pathogenesis of this disorder. This suggests that new targets for the treatment of cancer anorexia may be found in the AMPK/ACC signaling pathways. Supported by: FAPESP, CAPES

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Activation of Hypothalamic AMPK Decreases Fever Induced by IL-1b and Lipopolysaccharide

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The increase in cytokines production as IL-1b occurs in response to peripheral tumor development, causing anorexia, weight loss, and fever. Fever is one of the most common symptoms in advanced cancer. However the molecular mechanism of fever induced by cytokines has not been examined. 5-Aminoimidazole-4-carboxamide riboside (AICAR), a pharmacological activator of AMP-activated protein kinase (AMPK) reduces the cytokines production mediated by LPS. To evaluate the AMPK/ACC pathway in the fever induced by cytokines, male Wistar rats were injected into third cerebral ventricle with or without AICAR and IL-1b or LPS. Rectal temperature and Western Blot were used to evaluate the fever and AMPK/ACC pathway, respectively. Our RESULTS demonstrated that activation of hypothalamic AMPK/ACC pathway reduced fever induced by IL-1b or LPS. This suggests that new targets for the treatment of tumoral fever may be found in the AMPK/ACC signaling pathways. Supported by: FAPESP/CNPq.

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Are PrP^C Polymorphisms Associated to Cardiotoxicity in Post Pediatric Cancer Patients Treated with Doxorubicin?

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The antracyclines are chemotherapeutic agents widely applied in pediatric oncology treatment due to their unquestionable efficacy. However, their potential cardiotoxic effect increases mortality and decreases quality of life in cancer cured patients. Together with the cumulative dose, this cardiotoxicity is also associated to individual drug susceptibility. PrP^C is a GPI-anchored membrane protein highly expressed in brain and myocardium and has a repetitive octapeptide region that is involved with cellular protection against oxidative stress. The octarepeat domain is a polymorphic site where 7 to 10% of the population has 4 octarepeats and the rest 5 octarepeats. Similarly, a polymorphism is also present at codon 129, that seems to regulate protein folding and function. Since the antracyclines cardiotoxicity is dependent on the concentration of free radicals in the myocardial cells, it is possible that these polymorphisms at PrP^C can predispose to higher susceptibility to cardiac damage. Records of patients that were submitted to doxorubicin (an antracyclic type) therapy at least three years ago were analyzed regarding demographic data and cumulative doses of this drug. These patients were submitted to ecodopplercardiographic study for evaluation of the cardiac function and PrP^C gene polymorphisms analysis by DHPLC. We analyzed 72 patients being 59% white and 51% male. The age at diagnosis of cancer ranged from 0.3 to 194.0 months. The cumulative total dose of doxorubicin varied between 61 and 1630 mg/m². Ejection fraction was altered in 23.6% of patients. The octarepeat domain polymorphism (4 repeats) was present in 7.2% and that at codon 129 was distributed as 23.1% M129M, 17.4% V129V and 59.4% M129V. Our data demonstrated that alterations in the cardiac function are dependent on the doxorubicin cumulative dose, as expected. Nonetheless, this alteration was not correlated with the presence of the PrP^C polymorphisms. Our perspective is to increase the study population to at least 300 patients since experimental data in mice demonstrates that PrP^C expression is directly correlated to the doxorubicin toxicity. Supported by: FAPESP; CNPq

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Citotoxic Effect of Synthetic Chalcones on Lymphoblastic Leukemia Cells L-1210

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INTRODUCTION: Chalcones are essential intermediate compounds in the flavonoids biosynthesis, and they are easily found in arboreal or smaller plants. Many works have demonstrated the anti-inflammatory, analgesic and antitumoral activities of the chalcones. Our objective was to study the antitumoral activity of

eleven chalcones chemically derived from 3,4-(metilendioxibenzaldeide) in L1210 cells. MATERIAL AND METHODS: Murine L-1210 lymphoblastic leukemia cells were incubated with the chalcones at 1, 10 and 100 μ M for 24 hours. The cellular viability was evaluated by the MTT method, and apoptosis was analysed by DNA fragmentation in agarose gel 1,5% eletrophoresis stained with ethidium bromide. The liperoxidation was evaluated through TBARS method. RESULTS: We observed that 100 μ M of the the compounds 3-(1,3-benzodioxol-5-yl)- 1-phenylprop-2-en-1-one (A), 3-(1,3-benzodioxol-5-yl)-1-(2,5-dimethoxyphenyl)prop-2-en-1-one (B), 3-(1,3-benzodioxol-5-yl)-1-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one (C) and 3-(1,3-benzodioxol-5-yl)-1-(3-nitrophenyl)prop-2-en-1-one (D) reduced the number of viable cells in $83 \pm 3,8\%$, $82 \pm 3,2\%$, $80 \pm 3,5\%$ and $82 \pm 3,1\%$, respectively, when compared with the control (100% of viable cells) and caused DNA fragmentation. Only the compound 3-(1,3-benzodioxol-5-yl)-1-(2,5-dimethoxyphenyl)prop-2-en-1-one (B) produced lipid peroxidation. CONCLUSION: Our results showed that the synthetic chalcones named A, B, C and D caused apoptosis in L-1210 Lymphoblastic leukemia cells and suggest that the chalcone B induced cell death through the peroxidation of the membrane.

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Natural Compounds Sensitizes Melanoma Cells to Radiotherapy and Abolishes Intracellular Anion Superoxide Levels

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BACKGROUND. Oxidative stress caused by ionizing radiation produces a variety of highly reactive free radicals that damage cells, initiate signal transduction pathways and alter gene expression. Interestingly, melanoma cells are extremely resistant to radiotherapy, but have been shown to produce high levels of superoxide anion (O_2^-), which has a protective role against death in these cells. OBJECTIVE. We are interested in studying propolis and garlic properties regarding their anti-tumoral effects, in special those related to melanoma radiation sensitivity and reactive oxygen species ROS production. METHODS AND RESULTS. Murine melanoma cells (Tm5) present high levels of intracellular O_2^- when compared to non-tumorigenic melanocytes (melan-a), as demonstrated by flow cytometry using dihydroethidium. Propolis treatment completely abolishes O_2^- production in melanoma cells, but does not affect hydrogen peroxide (H_2O_2) production by either melanocytes or melanoma cells. Either propolis or garlic in aqueous solution inhibited melanoma cells proliferation *in vitro*. *In vivo*, combined propolis and garlic treatment was significantly more efficient in inhibiting tumor growth than either compound alone. Preliminary results show that the addition of garlic/propolis mixture in culture medium sensitizes melanoma cells to radiotherapy effects, as demonstrated

by DNA fragmentation and proliferation assays at specific time points after ionizing radiation treatment. CONCLUSIONS. Combined treatment of melanoma with garlic and propolis is a promising complementary treatment against this deadly disease. Supported by: Private Funding.

P-159

Melanoma Model in Mice: Efficiency and Mechanisms of Photodynamic Therapy with Methylene Blue (MB) and a Non-Coherent Light Source

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PURPOSE: We have recently obtained important results in treating six melanoma lesions of a 93 years patient by using local injection of MB and non-coherent light irradiation (RL50[®]). This performance has been related with the photochemical and photobiological properties of MB. In order to perform a detailed study on the effect of MB concentration, light dose, and characterize the effect of this therapy in the progression of melanoma tumors we developed an animal model in our laboratory. We choose to use B-16 melanoma cells injected intra-dermal in nude mice. METHODS: Ten mice were inoculated with melanoma B-16 cells. Five were treated with three PDT sessions, at eight, fourteen and twenty one days after the inoculation, and the other five mice were used as control group. 0.3mL of a 2% MB with 2% lidocaine solution was injected intra-tumoral and after 30s RL50[®] was applied in the final dose of 18J/cm². Pictures were taken every session and the lesion sizes (LS) were estimated by measuring and multiplying the two highest diameters of the lesions. Five days after the last irradiation the mice were sacrificed in a CO₂ chamber. Tumors were excised and fixed in formal. Histological examinations of the tissues were done by hematoxylin and eosin (H-E) staining and the proliferating cell nuclear antigen (PCNA) was tested by usual immunohistochemistry protocol. RESULTS: The group treated with PDT had a mean tumor growth (LS at the end of the experiment divided by the LS before the first PDT) of 15.48 times, and the control group had a mean growth of 28.49 times. Therefore, control group presented tumor growth 84% higher than the group treated with PDT. H-E stained cells show extensive necrosis areas in the group treated with PDT, which were absent in the control group. PCNA positive cells were predominant in the control group and decrease inversely with the tumor evolution, i.e., the tumors that presented the best response for PDT were those that had the smallest expression of PCNA. CONCLUSIONS: The combination of MB and RL50^a

provides an efficient PDT protocol in the animal model. Effect of MB concentration and light dose will now be evaluated. Therefore, there is a great potential for this combination to treat melanomas "in vivo". Supported by: FAPESP, CAPES and CNPq

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Photodynamic Therapy with Methylene Blue and Red Light Source RL50® In Superficial Tumors

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PURPOSE: Photodynamic therapy (PDT) represents the use of a photosensitizing agent excited by a specific light source to promote tumor damage and death. We devised an inexpensive polychromatic light source named RL50® that produces light able to excite the photosensitizing agent Methylene Blue (MB). The purpose of this work is to evaluate performance of PDT with MB and RL50® in superficial tumors. **METHODS:** After ethical approval and obtained informed consent from ten patients with seventeen superficial tumors that could not be treated systemically, we employed PDT with MB and RL50®. PDT was applied in multiple sessions every week or every other week. 30 seconds after intra-tumoral injection of MB 2% solution, light irradiation from RL50® was applied in doses between 18 J/cm² to 36 J/cm². Pictures were taken and tumor sizes were measured every session. **RESULTS:** 82% response rate was observed (64,70% complete response and 17,64% partial response) in 6 metastatic melanoma lesions, 6 basal cell carcinoma lesions, 1 squamous cell carcinoma lesion, 3 breast carcinoma lesions and 1 Kaposi's sarcoma. **CONCLUSION:** We conclude that PDT using the RL50® and MB, is an effective alternative for treatment of superficial tumor lesions. It has several advantages: It works on virtually all types of cancers, lacking the specificity of chemo-therapeutics and radiation; The procedure can be repeated several times, because there is no cumulative toxic effect. It is an out-patient procedure; Because of its lower risk profile, it can be used even in the elderly or in people who are too sick for surgery. Considering the low costs of MB and RL50®, this therapy can be used for medically underserved populations. Supported by: FAPESP, CAPES and CNPq

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Reversal of Cancer Anorexia by Blockade of Central PI(3)Kinase In Rats

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Anorexia is a debilitating manifestation of many

malignancies. The etiology of cancer anorexia is poorly understood, and effective treatment options are limited. To investigate the role of central PI(3)kinase signaling in the pathogenesis of cancer anorexia, we assessed the effects on food intake of the PI(3)kinase antagonists LY294002 and Wortmannin administered into the third cerebral ventricle of Wistar rats that were anorexic from Walker-256 cancer. In anorexic tumor-bearing rats, daily treatment with LY294002 (50mM, intracerebroventricularly) and Wortmannin (100nM, intracerebroventricularly) prevents reduction in food intake from 42 +/- 3% to 83 +/- 6% and 65 +/- 4% of preanorectic baseline, respectively, we also observed that the median overall survival in the control group was 7 days, as compared with 11 days in the LY294002 group and 12 days in the Wortmannin group. In accordance with these data pretreatment with LY294002 partially reversed the anorexia induced by acute infusion of IL1b intracerebroventricularly from 33 +/- 4% to 77 +/- 4% of control animals baseline. Because LY294002 and Wortmannin partially reverses cancer and IL 1b induced anorexia, increased central nervous system PI(3)Kinase signaling is implicated in the pathogenesis of this disorder. This suggests that new targets for the treatment of cancer anorexia may be found in the PI(3)kinase signaling pathways. Supported by: FAPESP, CNPq, CAPES

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Effect of TNF and Melphalan on Tumor Growth and Survival in a Model of Murine Melanoma

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INTRODUCTION AND OBJECTIVES: Isolated limb perfusion (ILP) with melphalan (MEL) is an alternative approach for treatment of unresectable, locally advanced cutaneous melanomas of extremities, avoiding limb amputation. The efficacy of ILP is improved substantially by simultaneous addition of TNF, raising complete response rates up to 80%. The molecular mechanisms underlining this synergistic effect are poorly understood. The aim of our study is to determine the gene expression profile in melanoma after the treatment with TNF, MEL, TNF + MEL and correlate the differential pattern of gene expression with *in vivo* response. **METHODS:** C57BL6 mice were injected s.c., on both flanks, with 5.0x10⁵ of B16F1 or B16F10 melanoma cells. When tumors reached 1.0cm³, TNF (0.05mg/Kg), MEL (12mg/Kg), TNF+MEL or saline (SAL) were administered i.v. (n= 9-12 mice/group). Tumor from the right flank was surgically removed 3h after treatment for RNA extraction and histological analysis. The size of remaining tumor was

measured every 2 days. RESULTS: MEL or TNF+MEL administration led to impairment in tumor growth observed at day six ($p < 0.001$). A significant increase in the progression-free survival (PFS) was also observed for mice treated with TNF+MEL in comparison with SAL ($p < 0.001$) or TNF ($p = 0.001$) groups, although no differences were found between MEL and TNF+MEL administration. Treatment with TNF was associated with more necrosis. A reduction on the number of mitosis was observed in all treatment groups when compared to SAL. Treatment with melphalan was associated with less CD34 positive blood vessels per tumor area. The addition of TNF to MEL doubled the necrotic area and the necrotic area/total tumor area ratio, although these parameters did not reach statistical significance. Conclusion: In our model, systemic administration of MEL in mice bearing subcutaneous melanoma delayed tumor growth and improved PFS. Simultaneously administration of TNF and MEL did not alter these parameters. TNF led to increased tumor necrosis and inhibition of cell proliferation. The correlation of these data with gene expression profile modulated by each treatment will allow us to identify candidate genes related to the mechanism of action of MEL and TNF in the treatment of melanoma. Supported by: FAPESP

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Evaluation of the Photodynamic Efficiency of Photosensitizers for Photodynamic Therapy

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Photodynamic Therapy (PDT) is a developing cancer treatment involving the administration of a photosensitizing compound and its subsequent activation by light to destroy tumor cells. Hematoporphyrins derivatives as Photofrin, Photogem and Photosan, are the most clinically used and investigated group of photosensitizers. Photodithazine is a chlorin-e6-derivative, a second-generation drug. The present work provides a comparative study between these photosensitizers, using albumin (BSA) and uric acid (UA) as chemical dosimeters. BSA (2 μ M) and photosensitizer (1 μ g mL⁻¹) solutions in phosphate buffer (pH=7.4), were illuminated with LED ($\lambda=630$ nm, I=25 mWcm⁻²). The decrease in the 340nm fluorescence was used to calculate the photooxidation rate constant (k_N ,

min⁻¹). In UA test, a mixture of UA (30 μ mol) and photosensitizer (15 μ g mL⁻¹) was illuminated with LED ($\lambda=630$ nm, I=13mW cm⁻²). The decrease in the 293nm absorbance of UA was measured and the photodynamic activity coefficient (PA, m².J⁻¹) was determined. The results of both methodologies suggest the photodynamic efficient ranking: Photodithazine > Photogem > Photofrin > Photosan, based on PA values (64 $\pm$ 14); (37 $\pm$ 9); (9 $\pm$ 2) and (4 $\pm$ 1) while k_N values 14,8; 9,8; 3,2 and 2,9 respectively. Supported by: CAPES, FAPESP

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Interleukin-10 Increases the Metastatic Potential of B16F10 Murine Melanoma Cells Induced by Contact With B-1 Lymphocytes

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B-1 cells are the major subtype of B cells in the peritoneal and pleural cavities of adult mice. These cells have a strong self-renewal capacity and are one of the main sources of interleukin-10 (IL-10). Moreover, IL-10 is involved in B-1 cell survival, suggesting a positive autocrine regulatory loop. Previous studies from our group demonstrated that co-cultivation of B-1 cells with B16F10 murine melanoma cells increased the metastatic potential of the later and that physical contact between these two cells was necessary for this effect. Since B-1 cells constitutively secrete IL-10, we asked whether this interleukin also plays a role in the increased metastatic effect of B16F10 melanoma cells after contact with B-1 lymphocytes. To test this, B16F10 melanoma cells were co-cultured for 48 h with B-1 cells either from wild-type or IL-10 knock-out C57BL-6 mice. After this period, B16F10 melanoma cells were isolated from B-1 lymphocytes and injected in the tail vein of both wild-type and IL-10 knock-out C57BL-6 mice for evaluation of pulmonary nodules as indicative of metastases. We showed that a significant increase in the number of pulmonary nodules was observed only when B16F10 melanoma cells were co-cultured with B-1 cells from wild-type but not from IL-10 knock-out mice. These RESULTS suggest that IL-10 favors the effect of B-1 cells in the increase of the metastatic potential of murine melanoma cells induced by previous contact with B-1 lymphocytes. Supported by: FAPESP

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DIMORF® Comprimidos - DIMORF® Solução Oral - DIMORF® Solução Injetável - DIMORF® LC - DIMORF® SP (sem conservante) - sulfato de morfina - INDICAÇÕES: *Dimorf® Comprimidos e Dimorf® Solução Oral:* alívio da dor aguda e crônica grave. *Dimorf® Solução Injetável e Dimorf® SP:* alívio da dor que não responde a nenhum outro analgésico narcótico sistêmico. Aplicado por via peridural ou intratecal promove o alívio da dor por períodos mais longos. Indicado também no alívio da dor do parto quando administrado via intratecal. Na suplementação da anestesia geral, regional ou local. *Dimorf® LC Cápsulas com microgrânulos de liberação cronogramada:* alívio da dor crônica por via oral. **CONTRA-INDICAÇÕES:** *Dimorf® Comprimidos, Solução Oral e Dimorf® LC:* hipersensibilidade à morfina. Insuficiência ou depressão respiratória, depressão grave do sistema nervoso central, crise de asma brônquica, insuficiência cardíaca secundária, doença pulmonar obstrutiva crônica, arritmias cardíacas, aumento da pressão intracraniana ou traumatismo crânio-encefálico, tumor cerebral, alcoolismo agudo, delírium tremens, desordens convulsivas, após cirurgia do trato biliar, cirurgia no abdômen, anastomose cirúrgica, administração conjunta com inibidores da MAO ou após um período de 14 dias com este tratamento. *Dimorf® Solução Injetável e Dimorf® SP:* pacientes alérgicos à morfina ou outros opiáceos, asma brônquica ou obstrução das vias aéreas superiores. Problemas de coagulação causados por terapia anticoagulante ou doenças hematológicas. Dependência de analgésicos opiáceos. A administração por via peridural ou intratecal está contra-indicada na presença de infecção no local da injeção, terapia anticoagulante, diátese hemorrágica, administração parenteral de corticosteróides num período inferior a duas semanas ou outra droga ou condição médica que contra-indique as técnicas peridural ou intratecal. **CUIDADOS E ADVERTÊNCIAS:** Os pacientes devem estar sob monitoramento de depressão respiratória. Administração intravenosa rápida pode resultar em rigidez da parede peitoral. Em administração peridural ou intratecal, o médico deve estar familiarizado com as condições do paciente. Pelo fato de que administração peridural é associada a menor potencial em relação aos efeitos adversos imediatos ou tardios que a administração intratecal, a primeira deve ser escolhida sempre que possível. Manter monitoramento por pelo menos 24 horas após administração peridural ou intratecal inicial. Deve-se ter disponível equipamento de ressuscitação para os pacientes que tenham recebido sobredose, e pessoal experiente no uso e limitações de antagonista narcótico específico como a naloxona. Extrema cautela em pacientes que apresentam: Pressão intracranial elevada ou enxaqueca, Doença Pulmonar Crônica, doença hepática ou renal, cirurgia ou doença do trato biliar, desordens no sistema urinário, pacientes hospitalizados (com reduzido volume de sangue circulante, cardiopatias ou drogas simpatomiméticas). Gravidez - Categoria C. Trabalho de Parto: Administração via peridural, intratecal ou intravenosa, pode causar depressão respiratória do recém-nascido (naloxona e equipamento de ressuscitação devem estar disponíveis para reversão do quadro). Amamentação: O sulfato de morfina é excretado no leite materno. Uso Pediátrico: Seu uso não é recomendado. Uso em Idosos: Os efeitos farmacodinâmicos do sulfato de morfina neuraxial em idosos são mais variados que na população jovem. **INTERAÇÕES MEDICAMENTOSAS:** depressores do SNC como álcool, sedativos, anti-histamínicos ou drogas psicotrópicas (IMAO, fenotiazinas, butirofenonas e antidepressivos tricíclicos) potencializam os efeitos depressores da morfina. **REAÇÕES ADVERSAS:** *Dimorf® Comprimidos, Dimorf® Solução Oral e Dimorf® LC:* depressão respiratória; depressão circulatória; parada respiratória; choque e parada cardíaca. As reações adversas mais observadas incluem tontura, vertigem, sedação, náusea, vômito e transpiração. SNC: euforia, desconforto, fraqueza, dor de cabeça, insônia, agitação, desorientação e distúrbios visuais. Gastrointestinais: boca seca, anorexia, constipação e espasmo no trato biliar. Cardiovasculares: rubor na face, bradicardia, palpação, desmaio e síncope. Genitourinárias: hesitação ou retenção urinária, efeito antidiurético, redução da libido e impotência. Alérgicas: prurido, urticária, outras erupções cutâneas, edema. Podem ocorrer também: prurido - a administração de dose peridural ou intratecal de *Dimorf® Injetável* é acompanhada de prurido generalizado e está relacionado com a dose; retenção urinária - a retenção urinária é um efeito colateral frequente; constipação - a constipação é frequentemente encontrada durante a infusão contínua de sulfato de morfina. *DIMORF® SP:* o mais sério efeito colateral é a depressão respiratória. A administração, na forma de bolus, pela via intratecal ou peridural, pode apresentar uma rápida depressão respiratória. A excitação do SNC resultando em convulsões aparece após administração de altas doses intravenosas de morfina. **POSOLOGIA:** *Dimorf® Comprimidos:* adultos: 15 a 30 mg cada 4 horas. *Dimorf® Solução Oral:* cada 1 ml contém 10 mg de sulfato de morfina que corresponde a 26 gotas. Dose oral para adultos: de 10 a 30 mg a cada 4 horas. Dose oral pediátrica: a dose deve ser individualizada pelo médico de acordo com a gravidade da dor, levando-se em consideração a idade e o peso do paciente. A dose média recomendada, na administração por via oral é de 0,3 a 0,6 mg/kg. *Dimorf® Solução Injetável:* dosagem para administração peridural em adultos: a dose inicial de 5 mg na região lombar pode proporcionar alívio da dor por até 24 horas e, se o adequado alívio da dor não for conseguido dentro de 1 hora, administrar cuidadosamente doses incrementais de 1 a 2 mg. Para infusão contínua, uma dose inicial de 2 a 4 mg/24 horas é recomendada. Doses complementares de 1 a 2 mg podem ser administradas se o alívio da dor não foi conseguido inicialmente. Administração intratecal: a dose é normalmente 1/10 da utilizada na administração peridural. Dosagem para via intratecal em adultos: injeção de 0,2 a 1 mg proporciona satisfatório alívio da dor por até 24 horas. *Dimorf® LC:* uma cápsula de 30 mg, 60 mg ou 100 mg a cada 12 horas (após titulação da dose) ou segundo orientação médica. *Dimorf® SP:* administração intramuscular: a dose inicial deverá ser de 5 a 20 mg/70 kg de peso. Administração intravenosa: a dose inicial deverá ser de 2 a 10 mg/70 kg de peso. Administração peridural: dosagem para via peridural em adultos: a posologia é a mesma que para *Dimorf® Solução Injetável*. Administração intratecal: a dosagem intratecal é normalmente 1/10 da dosagem peridural. Dosagem para via intratecal em adultos: a posologia é a mesma que para *Dimorf® Solução Injetável*. A Naloxona (Narcan®) é o antídoto para as reações adversas graves de *Dimorf®*. **CRISTÁLIA** - Produtos Químicos Farmacêuticos Ltda - Farm. Resp.: Dr. Joaquim A. dos Reis - CRF-SP nº 5061- Rod. Itapira-Lindóia, km 14 - Itapira - SP - CNPJ nº 44.734.671/0001-51 - SAC 0800-7011918 - Nº do Lote, data de Fabricação e Prazo de Validade: vide rótulo/cartucho - **Classificação:** Apresentações injetáveis em caixas com 50 amp: Venda sob Prescrição Médica - Restrito a hospitais - Com notificação de receita "A" / Apresentações injetáveis em caixas com 5 amp: Venda sob Prescrição Médica - Venda sob retenção de receita "A" / Apresentações orais: Venda sob Prescrição Médica - Venda sob retenção de receita "A" - Reg. MS nº 1.0298.0097 - APERSISTIREM OS SINTOMAS O MÉDICO DEVERÁ SER CONSULTADO.