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TUMOR IMMUNOLOGY

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TUMOR EFFECTS UPON DENDRITIC CELL DIFFERENTIATION: INVOLVEMENT OF THE p38MAPK PATHWAY?

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Introduction: Dendritic cells (DC) are the most efficient antigen-presenting cells in the immune system. In cancer patients, however, these cells present functional and phenotypic disturbances that could be related to a stressful environment induced by the tumor. Heat Shock Proteins (HSP) and the p38MAPK pathway are involved in cell responses against stress and, interestingly, we noted that mature monocyte-derived dendritic cells (Mo-DC) from breast cancer patients present, indeed, higher levels of HSP27 mRNA. Furthermore, HSP27 is a phosphorylation target of the p38 pathway and high HSP27 levels have been associated with impaired Mo-DC differentiation. To investigate these phenomena we exposed monocytes from healthy donors to a tumor cell line supernatant and evaluated their differentiation into DCs and the activation of HSP27 and p38 in the differentiating cells. **Methods and Results:** Monocytes from healthy donors were negatively selected by magnetic beads and induced to differentiate into DCs (by treatment with GM-CSF and IL-4) either in the presence of 20% conditioned medium (CM) from the breast adenocarcinoma cell line SK-BR-3 (tDC) or not (cDC). The surface phenotype, the presence of total p38, total HSP27, phospho-p38 and phospho-HSP27 were evaluated at different time points of the culture by flow cytometry. At 24h tDC cells expressed less HLA-DR, CD11c and CD86 and showed higher levels of phospho-p38, but less phospho-HSP27. At 72h of culture tDC contained a higher frequency of PD-L1+ cells. **Conclusions:** CM from SK-BR-3 cells affected the differentiation of Mo-DCs, inducing a phenotype with lesser expression of molecules associated with the induction of immune responses (HLA-DR, CD11c and CD86) but higher expression of PD-L1, a molecule associated with the induction of immune suppression. This was concomitant to an early increase in p38 phosphorylation, but surprisingly lower presence of phospho-HSP27 in the same cells. Though preliminary, these data suggest that

the p38MAPK pathway may be involved, indeed, in the response negative effects of tumors upon the differentiation of Mo-DCs into effective inducers of anti-tumor immune responses.

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FASN INHIBITION WITH ORLISTAT REDUCE LEUKOCYTE INFILTRATE IN EXPERIMENTAL MELANOMAS

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Introduction: Melanoma is the most aggressive skin or mucous membranes malignant tumor and resistant to chemotherapy or radiotherapy. It has been should that melanoma presents increased fatty acid synthase (FASN) expression, which is associated with a poor prognosis for the patients. FASN inhibition with Orlistat reduces metastases in the experimental melanomas, inhibits cell proliferation and promotes apoptosis in B16-F10 cells. Our objective was evaluate whether Orlistat affects the immune response against experimental melanomas.

Methods and Results: The presence of macrophages, CD8, CD4 and regulatory T (Treg) cells from primary tumors of mice with experimental melanoma, treated or not with Orlistat, was quantified by flow cytometry. Plasmatic nitric oxide (NO) was quantified by Griess reaction and the number of metastatic lymph nodes were also evaluated in Orlistat-treated mice. Here, we observed that Orlistat treatment reduced macrophages, CD8, CD4 and Treg ($CD4^+CD25^+Foxp3^+$) cells in the intra-tumoral infiltrate. High levels of plasmatic NO and reduced number of metastases were observed in Orlistat-treated mice, when compared to controls.

Conclusion: FASN inhibition by Orlistat change immune response against melanoma.

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INTERACTIONS BETWEEN DENDRITIC CELLS AND T LYMPHOCYTES IN A NOVEL 3-D ENVIRONMENT

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Introduction: Dendritic cells (DCs) are the major antigen-presenting cells and, in situations of change in homeostasis, are able to initiate immune responses through the activation of T lymphocytes. Their immunomodulatory functions and generation *in vitro* are of great potential for immunotherapy of cancer. Among the many strategies under investigation, one, which our group has been using, is based on the fusion of DCs and tumor cells for therapeutic vaccination. To better understand the interactions between these fused DC-tumor cell hybrids and the different T cell populations, we are developing a three-dimensional (3D) culture system, whose first results are presented here. **Methods and Results:** Monocytes of healthy donors were purified from PBMCs by magnetic beads selection and induced to differentiate into DCs by treatment with GM-CSF and IL-4. Maturation of monocyte-derived DCs (mMo-DCs) was initiated by addition of TNF-alpha 48 hours or 2.5 hours before analysis. Allogeneic T lymphocytes were purified from PBMCs by magnetic beads selection and were co-cultured for 17 hours in the 3D Biotek scaffold, at 37 °C. These scaffolds are made from polystyrene and have a thickness of 600µm, fibers of 150µm and pores of 200µm. In this system, mMO-DCs activated for 2.5h did not interact with allogeneic T lymphocytes, while those activated for 48 hours did. The average time of interaction between mMo-DCs and T lymphocytes was 4 hours, and the average speed of the T lymphocytes in the scaffold containing 48h-activated mMO-DCs was of 1.38 µm/min. **Conclusions:** These data indicate that this 3D Biotek scaffold enables interactions between mMO-DCs and lymphocytes and may be useful for the characterization of these interactions, the cellular subtypes and patterns of response induced.

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EXPRESSION OF *SURVIVIN* IN HEAD AND NECK SQUAMOUS CELL CARCINOMA PATIENTS

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Introduction: Apoptosis is the fundamental process necessary for eliminating damaged or mutated cells. Alterations in the apoptotic pathway appear to be key events in cancer development and progression. Survivin, a novel inhibitor of apoptosis, it directly inhibits caspase-3 and caspase-7 activity. During tumorigenesis, survivin expression is inversely correlated with apoptosis and is positively correlated with proliferation and angiogenesis. Squamous cell carcinoma of the head and neck (HNSCC) is a relatively common human cancer characterized by high morbidity, high mortality, and few therapeutic options outside of surgery, standard cytotoxic chemotherapy, and radiation. The aim of this study was to investigate the expression of pro- and anti-apoptotic genes in oral cavity HNSCC patient samples.
Methods and Results: Cells isolated from HNSCC tumor were used for cDNA synthesis. Expression of pro- and anti-apoptotic genes was analyzed by Real Time RT-PCR. We observed the overexpression of surviving in tumor cells when compared to the adjacent tissue in all patients.
Conclusion: Survivin is overexpression in head and neck squamous cell carcinoma.

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B-1 CELLS EXPRESSING CCR5 LEADS TO AMELIORATION OF MURINE MELANOMA

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Introduction: Melanoma is a skin neoplasia arising from melanocytes that become malignant. After loss of keratinocytes control, transformed melanocytes leave the epidermis, establishing communication with stromal components of the dermis and other cells. It has been shown by our group that B16F10 murine melanoma cell line, after physical contact with B-1 cells have increased metastatic potential. However the mechanism by which B-1 cells migrate towards melanoma cells is unknown.

Objective: The aim of the work was to evaluate the influence of CCR5 expressed in B-1 cells on the course of B16F10 cells melanoma.

Results: The present study demonstrated that B16F10 cells release soluble factors that are chemoattractant for B-1 cells. It was demonstrated that 10% of peritoneal B-1 cells express the chemokine receptor CCR5 on their surface. These cells, when inoculated into animals $CCR5^{-/-}$, leads to in a 10-fold decrease in the number of metastatic nodules resulting from inoculation with B16F10 cells. Moreover, this approach promotes reduced rate of subcutaneous tumor growth, maintaining a stable volume of tumor while control animal presented exponential growth being that last day of measure in treated group show less tumor volume than first day of control group. Besides that, this treatment increased the survival of animals. When mice from treated group started to die, all mice from control groups already came to death.

Conclusion: Our data suggest that CCR5-expressing B-1 cells can change the development of murine melanoma, controlling it. So, this work contributed to a better understanding of B-1 cells chemotaxis and the influence of migration in evolution of melanoma.

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THERAPEUTIC EVALUATION OF MICE BEARING EHRLICH'S SUBCUTANEOUS CARCINOMA AND TREATED WITH EXTRACTS OBTAINED FROM JATOBÁ SEED OR SHELL (*HYMENAEA COURBARIL* L.)

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Introduction: Previous studies done with extracts obtained from Jatobá(*Hymenaeacourbaril* L.) show that their actions on the immune system are mediated, mainly, by modulation of macrophage effector functions. This way, the purpose of this study was to evaluate the therapeutic answer to the treatment with extracts obtained from jatoba in mice bearing Ehrlich's sc carcinoma. **Methods and Results:**The animals (male swiss mice, n=6), inoculated sc. with $1x10^6$ cells of Ehrlich's tumor. They were treated (intragastrically) daily with aqueous extracts (shell: 1,7mg/ml; seed: 2,2 mg/ml) or with ethanolic extracts 0,5% at the concentrations of 2 mg/ml, 5mg/ml or 10mg/ml (shell or seed) for 90 days. Weekly, the animals were evaluated for food consumption, body weight and estimate of tumor area. The evaluation of tumor development did not show statistical difference between the treated groups, however it is possible to infer that the treatment with aqueous extracts obtained from seed was able to reduce the tumor growth at the animals (tumor area: EHR:8,87±4,85; ERH/H_2O:7,74±4,49; EHR/seed/H_2O:5,92±1,58; tumor volume: EHR:14,42±8,52; ERH/H_2O:11,30±7,14; EHR/seed/H_2O:7,52±0,12). About the ethanolic extracts, we noticed that the treatment with jatoba, in spite of showing reduction in tumor development at the concentrations of 5mg/ml (area: Shell/OH:6,72±1,34; Seed/OH:5,01±3,95; volume: Shell/OH:11,16±4,28;

Seed/OH:7,87±8,00) e 10mg/ml (area: Shell/OH:4,17±2,37; Seed/OH:5,86±3,93; volume: Shell/OH:5,79±3,45; Seed/OH:7,75±5,58), did not show itself advantageous, since that the vehicle control(area: EHR/OH 0,5%: 3,78±2,49; tumor volume: EHR/OH 0,5%:3,81±3,19) showed lower values than the noticed in the treated groups. The clinical evaluation showed in general, that the groups treated with different extracts didn't change body weight (EHR:56,37±5,92; EHR/OH 0,5%:43,68±5,12; EHR/H_2O:50,96±6,07; Shell/H_2O:57,55±13,13; Seed/H_2O:45,28±3,83; Seed/OH 2mg/ml:46,93±3,37; Seed/OH 5mg/ml:48,50±9,93; Seed/OH 10mg/ml:49,76±6,21; Shell/OH 2mg/ml:49,20±3,13; Shell/OH 5mg/ml:51,47±7,58; Shell/OH 10mg/ml:48,58±9,22). In relation to the survival analysis, we noticed that the group treated with ethanolic extracts from the shell in 5mg/ml showed the lower survival rate (50%), while the HER control group showed a rate of 100%. **Conclusion:** The treatment withaqueous extracts from seed seems to show a promising effect at the reduction of Ehrlich's sc tumor development.

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GENOTYPE AND PHENOTYPE OF BALB/c MOUSE STRAIN EXPRESSING H-2K^b-TsA58- SV40 IMMORTALIZING ONCOGENE.

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Introduction: The Immortomouse mouse strain expresses an immortalizing simian virus 40 (SV40) large T antigen tsA58 oncogene under the control of the interferon inducible murine H-2K^b promoter on chromosome 16. We want to establish a BALB/c strain of H-2K^b-tsA58 immortomice that could be utilized to investigate specific pathological and physiological patterns associated SV-40 oncogenicity and generation of conditionally immortal cells lines. **Methods and Results**: We have been crossing H2K^b-SV40-tsA58 CBA/CaxC57BL/10 hybrid immortomice with BALB/c mice to obtain a colony of transgenic mice with unique BALB/c background. We have used two pre-validated PCR genotype assays that can distinguish between wild-type, hemizygous, and homozygous animals. We characterized macroscopically and by immunohistochemistry in the F1-3 offspring of immortal hemizygous four females thymic hyperplasia which is a phenotypic pattern of SV40 tsA58 antigen overexpression. Moreover, we observed four small males with abnormality after birth. **Conclusion**: This transgenic mouse strain will help to isolate immortalizing cell lines growing under the permissive 33˚C temperature for the studies of the SV40 large T antigen transformation.

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ANTITUMOR ACTIVITY OF NEW THIOSEMICARBAZONE COMPOUNDS

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Introduction: Cancer is the major cause of death worldwide. In Brazil, 518,510 new cases are expected for 2012. Although, there is cure for some types of neoplasies, the treatment against cancer is very expensive, and the development of chemotherapy resistance has been shown as an obstacle to its efficacy. Thus, the discovery of new antitumor drugs is essential for the improvement of cancer treatment.
Objective: The aim of this study was to evaluate the anticancer activity of two new synthetic compounds of the Thiosemicarbazone class (CT2 and CT2 complex). Previous researches have described the pharmacological properties of the Thiosemicarbazone compounds, including their antiviral and anticancer activity.
Methods and Results: To investigate the capability of these compounds to induce cells death *in vitro*, the human erythroleukemia cell line K562 and Lucena I (Vincristine–resistant derivative K562) were used, both gently donated by PhD Vivian M. Rumjanek. Cells were incubated at different concentrations of CT2 and CT2-complex for 72h at 37°C with 5% of CO_2. An MTT assay enabled to observe CT2 induction of cell death in K562 (IC_{50} 20.5 ± 1.8 µM) and Lucena I (IC_{50} 12.8 ± 1.0 µM). Similar results were observed when treating Lucena I cells with CT2-complex (IC_{50} 9.1 ± 0.2 µM). These interesting outcomes led to the evaluation of CT2 and CT2-complex *in vivo* effects, using Ehrlich ascites as a model. However, the first step was to investigate the toxicity of CT2 and CT2-complex. Balb-c adult female mice were treated with 60 mg/kg of CT2 and CT2-complex. After 30 days, the animals were weighed, and glucose levels were analyzed, as well as weight of heart, kidneys, liver, spleen and thymus. Moreover, T lymphocyte subpopulations in lymph nodes were analyzed. Both compounds did not alter any parameter investigated, except for CT2-complex, which increased glucose levels. Therefore, we decided to investigate the anticancer activity of CT2. Two strategies were used to evaluate the effects of CT2 *in vivo*: 1 injection (intraperitoneal; i.p.) of CT2 24h after tumor inoculation; and 2 injections with half of dose of CT2 (i.p.) 24h and 7 days after tumor inoculation. 2.5 x 10^5 cells were inoculated i.p. in female Balb-c mice (6-8 weeks old). The mice were separated in 4 groups: control (vehicle;

PBS/DMSO), 15 mg/kg, 30 mg/kg and 60 mg/kg. Evidence of drug effect was described by %T/C = [Δ tumor volume of treated group]/[Δ tumor volume of control group] × 100%. 30 mg/kg of CT2 showed effective antitumor activity with T/C values of 264.2 % (1 injection) and 146.3 % (2 injections). Furthermore, after 70 days, the surviving mice did not show ascites. They were scarified, and the analysis of body, heart, kidney, liver, spleen and thymus weight matched healthy mice (without tumor).

Conclusion: Results suggested that CT2 is a promising compound with anticancer activity and reduced toxicity.

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MIGRATORY EFFECT OF GASTRIN-RELEASING PEPTIDE (GRP) ON A549 CELL LINE

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Introduction

Gastrin-releasing peptide (GRP) is considered to be a mitogenic agent capable of inducing proliferation and cell growth, since it acts as a growth factor of human tissues and tumors, and its up-regulated in cancer metastasis. GRP apparently alters the shape and increases cell adhesion through changes in the actin cytoskeleton. Nowadays, there are studies showing that the GRP and its receptor (GRPR) act primarily to regulate the morphology and tumor differentiation, rather than as mitogens. Because GRP is overexpressed in tumors and metastasis and we recently found that GRP can act as a chemotactic molecule for immune cells, we questioned if the effect of the tumor development is directed induced by GRP or by the immune cells recruited to GRP-producing tumors, where GRP can act as a chemotactic attractant for cancer cells. Thus, the objective of this study was to first confirm the expression of GRPR in the A549 cell line and investigate the migration effect of GRP on these cells.

Methods and results

A549 cells were grown in standard conditions. GRPR expression was quantified by real time PCR. Cells were exposed to concentrations of 10 nM, 50 nM and 100 nM of GRP in DMEM without FBS for 24 hours. It was also used 1μM of GRPR-antagonist RC-3095. Transwell migration assay was performed as described by Shi et al (2010) with some modifications. A549 cells showed high mRNA expression of GRPR receptor by real-time qPCR. There was no increase in the migration of cells exposed to concentrations of 10 nM and 50 nM GRP compared to controls. However, 100 nM GRP had a minor migration effect when compared to control ($p < 0.001$) and 10 nM GRP ($p < 0.01$).

Conclusion

In this study, we demonstrated that GRP did not increased migration of A549 lung cells through the transwell assay at concentrations of 10 nM, 50 nM and 100 nM. Interestingly, treatment with 100 nM GRP induced a minor migratory effect than 10 nM GRP. However, the transwell migration assay used in this study is not the most appropriate for tumor migration evaluation since the membranes were not covered by a gelatinous protein mixture (Matrigel) in order

to mimic extracellular matrix which would turn this *in vitro* assay closest to a situation *in vivo*. Therefore, we could not affirm that the GRP has migratory effect on these cells and further studies should be conducted using *in vivo* studies were immune cells could induce tumor development and metastasis.

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IMMUNOMODULATION BY HPV ASSOCIATED TUMORS

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Introduction: Human Papillomavirus (HPV) is the main etiologic factor for cervical cancer, responsible for virtually all cases of it. Tumors are complex structures, formed by several types of cells with different roles in proliferation, angiogenesis, immune system evasion and metastasis. Previous data from our laboratory showed that, in mice, HPV associated tumors recruit monocytes that differentiate into M2-like macrophages or TAM (tumor associated macrophages). TAM is important for the immune evasion mechanisms displayed by these tumors, secreting IL-10 and inducing regulatory T cell phenotype. Besides TAM, antigen presenting cells in the spleen of tumor bearing mice also promote regulatory T cell phenotype. Our observations show that tumor bearing mice have 2.5 fold more myeloid cells in the spleen than naïve mice. The objective of this project is to investigate mechanisms triggered by HPV associated tumors that may induce expansion of myeloid populations and the dynamics of these cells through lymphoid organs and tumors.
Methods and Results: Human cervical tumors derived cell lines HeLa (HPV18), SiHa (HPV16) and C33 (HPV-) were used for injections into RAG1-/- and Nude mice for bromodeoxyuridine (BrdU) incorporation and proliferating cells detection, and for protein lysates to identify possible factors involved in this process. Our results show an increase in CD11bGr1 proliferation in the bone marrow of SiHa or HeLa tumors in Nude and RAG1-/-, respectively. The Nude mice also shows an increase in proliferation of myeloid cell in the spleen and of B cells in the lymph nodes of mice bearing HPV positive tumor when compared with mice bearing HPV negative tumors. We performed cytokine proteome array assays to investigate differentially expressed cytokine in the different tumor cells lines. We observed an upregulation of CCL4, IL-5 and sICAM in HPV positive cells.
Conclusion: Tumors derived from HPV positive cell lines induced more myeloid cell proliferation, a possible evasion mechanism, and express cytokines which role we are still investigating.

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THE ROLE OF GALECTIN-3 IN DIFFERENTIATION AND NEOPLASTIC TRANSFORMATION OF B LYMPHOCYTES IN PRISTANE-INDUCTION MODEL

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Introduction: Recent studies of our group show that galectin-3-/- (gal-3-/-) animals present larger amount of plasma cells in the spleen, bone marrow and mesenteric lymph nodes. Furthermore, in B cell neoplasms, high concentrations of gal-3 provide greater tumor resistance and progression. An important model for the induction of plasmacytomas is the injection of mineral oils in the peritoneal cavity of mice, such as pristane. This oil causes a chronic granulomatous inflammation rich in macrophages, plasma cells and peritoneal B cells. The use of gal-3-/- animals in the study of plasmacytoma can be considered a singular model as it permits detailed evaluation of malignant transformation in B cells by chronic inflammation. Thus, the objectives of this study were to evaluate quantitative and qualitatively peritoneal cavity and lymphoid organs such as bone marrow and spleen, and analyze the formation of lipogranulomas due to injection of pristane.
Methods and Results: To this end, we used wild (WT) and gal-3-/- animals, both with Balb/c background. The animals were injected intraperitoneally with pristane or maintained under physiological conditions and sacrificed 2 months pos-injection. The spleen and lipogranulomas were surgically removed, fixed in 10% paraformaldehyde, embedded in paraffin and stained with hematoxylin and eosin or prepared for immunohistochemistry. Gal-3-/- mice presented large amounts of lipid droplets in the spleen and less lymphoid structures in lipogranulomas compared to WT animals. Furthermore, CD138+ plasma cells, were differentially distributed in gal-3-/- animals comparing to WT group, forming cell clusters in the splenic parenchyma. Fenotypical and cell cycle analysis were performed by flow cytometry. The distribution of T and B cell populations in peritoneal cavity were modified in the absence of gal-3 and PI+ dead cells were increased in bone marrow. Significant differences in cell cycle were not observed in peritoneal cavity and lymphoid organs. Bone marrow cells were maintained in culture and both WT and gal-3-/- stroma supported hematopoietic differentiation through a system of co-culture, however, the

stroma derived from gal-3-/- animals resulted in a greater differentiation of eosinophils compared to WT stroma.
Conclusions: The results obtained allow us to conclude that the kinetics of inflammatory cells occurs differently in the absence of gal-3, modulating lymphoid and myeloid populations.

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SERUM AMYLOID A PROTEIN AFFECTS AND CAN BE AFFECTED BY TUMOR MICROENVIRONMENT

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Introduction: The tumor microenvironment has been implicated in the regulation of tumor growth. Cytokines, growth factors, matrix metalloproteinases (MMPs) and reactive oxygen species (ROS) are secreted by cells of the stroma as well as by cancer cells, driving the impact in the tumor. Furthermore, the importance of hypoxia in driving tumor growth has receiving increased attention. Serum amyloid A (SAA) is a protein expressed and produced by tumors and whose impact on tumor cells has been studied by our group. We have shown that SAA is able to affect proliferation, migration and tumor invasion. In this study we examined how SAA can modulate molecules present in the tumor microenvironment, such as, MMPs and ROS and how SAA can be modulated by molecules present in the tumor microenvironment, as pro-inflammatory cytokines and also by hypoxia conditions.
Methods and Results: We perform measurement of MMP-2 and -9 activity and superoxide anion in glioblastomas (GBMs) cells - A172 e T98G; and qRT-PCR and ELISA for SAA expression and production by the addition of IL-6, TNF-a and IL-1b and hypoxia condition. SAA inhibited activity of MMP-2 ($p<0.05$) and -9 ($p<0.001$) in A172 cells while increased these activities in T98G cells ($p<0.001$). SAA induced the production of reactive oxygen species in both GBMs ($p<0.001$). IL-6, TNF-a and IL-1b increased the transcription of the different genes of SAA ($p<0.001$), but increase in protein level was observed only for A172 when treated with IL-1b ($p<0.001$). Hypoxia for 6 hours did not affect the production of SAA in both GBMs.
Conclusion: Our findings show that for some tumor types SAA affects and can be affected by tumor microenvironment.

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LOW CONCENTRATIONS OF PACLITAXEL INDUCE THE EXPRESSION OF HEAT SHOCK PROTEINS AND ENHANCE THE SENSITIZATION OF DENDRITIC CELLS

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Introduction: Our previous studies showed that the treatment of HCT-116 human colorectal cancer cells with non-toxic concentrations of paclitaxel (PAC) increased the gene transcription of heat shock proteins (HSP40 and HSP70) and improved tumor cell immunogenicity and sensitivity to cytotoxic T cells. In this study, we aimed to evaluate whether: a) changes on the gene transcription of HSP translate into increased protein expression and b) the treatment of tumor cells increases the efficiency of their lysates on the up-regulation of the phenotype and functions of dendritic cells (DCs). **Methods and Results:** HCT-116 cells were treated with decreasing concentrations of PAC. Minimum effective concentration (MEC) was determined as the lowest concentration capable of stopping tumor cell growth (3,5 nM). Non-toxic concentration (NTC) neither prevented cell proliferation nor stimulated cell growth (0,5 nM) (n=4). Analysis of HSP in cell lysates by ELISA showed that the treatment of tumor cells with both concentrations of PAC induced the expression of HSP40 (MEC: 0,444±0,074; NTC: 0,412±0,058; CONTROL: 0,225±0,035). Productions of HSP70 were induced only by MEC (MEC: 0,722±0,284; CONTROL: 0,222±0,047) whereas HSP90 was induced by NTC (NTC: 0,526±0,125; CONTROL: 0,273±0,025). Pulsing monocyte-derived DC with these lysates showed that the treatment of tumor cells with low concentrations of PAC enhanced the antigen-presenting function of DC in the mixed lymphocyte reaction (lymphocyte proliferation: CEM: 2,672±1,016; CONTROL: 0,384±0,014, $p<0.001$). The statistical analysis was performed by analysis of variance (ANOVA) followed by Tukey test for multiple comparisons. Differences were considered significant when error probabilities were lower than 5% ($p<0.05$). This project is part of a larger project, already approved by the Ethics Committee of our Institution (Proc. 3537-2010). **Conclusion:** Our data indicate that pre-treatment of colorectal cancer cells with MEC, but not NTC of PAC,

improves the efficiency of the lysate to sensitize DC, enhancing their antigen-presenting function.

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EFFECTS OF SERUM AMYLOID A ON PROLIFERATION AND INVASIVENESS OF MELANOMA CELLS

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Introduction: Serum amyloid A (SAA) is a protein involved in inflammatory processes that has called attention due its high concentrations in cancer. In melanoma, the most aggressive skin cancer, there is a positive correlation between serum concentration of SAA and tumor grade. Recently, we verified that SAA increase proliferation of glioma lines and affect invasiveness processes. To clarify why tumor cells produce SAA as well as its roles in tumor progression we choose two melanoma cell lines to verify the effects of SAA on proliferation, migration and invasion processes and the receptors for SAA in these cells.
Methods and Results: We stimulated SK-MEL 19 and SK-MEL 147 with SAA (5 µg/mL) during 48h and evaluated proliferation using [^{3}H]-thymidine method. Migration and invasion processes were made using scratch test and transwell assay, respectively. Expression of toll-like receptors (TLR) -2 and -4 were made by real time PCR and the results were expressed as mean ± S.E.M. We observed an inhibition in proliferation of SK-MEL 19 (C = 7100 ± 20.50cpm; SAA = 3800 ± 5.48cpm; $p<0.05$; n=3) with no effects in migration (migrated area: C = 23 ± 1.45%; SAA = 25.4 ± 2.98%; n=5) and invasion (invasion index: C = 1.0; SAA = 1.2 ± 0.037; n=5). For SK-MEL 147, we did not observed effects of SAA on proliferation (C = 1450 ± 4.58cpm; SAA = 1280 ± 10.70cpm; n=3) but it caused an inhibition in migration (migrated area: C = 82 ± 2.10%; SAA = 43 ± 3.45%; $p<0.001$; n=5) and invasion (invasion index: C = 1.0; SAA = 0.65 ± 0.022; $p<0.05$; n=5) processes. Furthermore, both lines express TLR-2 and TLR-4, however, SK-MEL 147 have the highest expression of both receptors (SK-MEL 19: TLR-2 = 1.0; TLR-4 = 3.9 ± 0.2; SK-MEL 147: TLR-2 = 4.1 ± 0.04; TLR-4 = 14.2 ± 1.3; n=3).
Conclusion: In conclusion, we verified that SAA was able to inhibit tumor progression in both cell lines studied, but in different ways. These cells present a very different genetic background and the different effects of SAA may be related with the different mutations and the differences in toll-like receptors profile.

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EFFECTS OF ORLISTAT ON THE IMMUNE RESPONSE AGAINST EXPERIMENTAL MELANOMAS

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Introduction: Orlistat has anti-neoplastic properties by irreversibly inhibiting fatty acid synthase (FASN). Since there is no information in literature about the importance of FASN activity for the immune response against cancer cells, our work aimed to study the effects of Orlistat on the percentage and activation of $CD3^+$ $CD8^+$ T lymphocytes ($CD8^+$ TL), $CD3^-$ $CD49b^+$ natural killer (NK) and $CD11c^+$ dendritic cells (DC) present in the primary tumors (PT) and their metastases to mediastinal lymph nodes (mLN) in a B16-F10 melanoma mouse model.

Methods and Results: Flow cytometric immunophenotyping was used to analyze the effects of Orlistat on the percentage/activation of $CD8^+$ TLs, NK cells, and DCs present in PT and mLN metastases. Orlistat-treated C57BL/6 mice (n=20) had a lower percentage of $CD8^+$ TLs in PT (1.74 ± 0.72 vs. 2.73 ± 0.71 in control group) [$p=0.001$] while the expression of granzyme b (30.04 ± 10.14 vs. 16.86 ± 8.07 in control group) [$p<0.0001$] and perforin (48.96 ± 14.19 vs. 31.76 ± 13.48 in control group) [$p<0.001$] were significantly higher in these cells. In addition, the treatment with Orlistat was able to reduce the expression of MHC I (58.83 ± 16.42 vs. 84.57 ± 5.54 in control group) [$p<0.0001$] on DC cells, which were more numerous compared to control group (4.03 ± 1.04 vs. 2.97 ± 1.13) [$p=0.016$]. No significant differences were observed regarding Ly49A expression in PT between control and treated animals ($p=0.937$). Among mLN pools (n=3 – Orlistat and n=3 – control, total of 20 animals for each group) we did not find significant differences between the percentage of immune cells and expression of their activation markers, except for granzyme b (1.06 ± 0.31 vs. 0.83 ± 0.17 in control group) [$p<0.05$] and perforin (2.19 ± 0.99 vs. 0.54 ± 0.19 in control group) [$p<0.05$] that showed higher levels on $CD8^+$ TLs from treated mice. Moreover, although not statistically significant, inhibitory NK cell receptor Ly49A expression was reduced in Orlistat-treated mLN pools (n=4, total of 25 animals) (15.63 ± 4.36 vs. 25.44 ± 6.42)

[p=0.063].

Conclusion: Our results indicate that inhibition of FASN with Orlistat changes the percentage and activation state of intratumoral and lymph node immune cells by increasing the percentage of activated $CD8^+$ TLs in PT and mLN pools. It can be hypothesized that this drug contributes to enhance the immune response against experimental melanomas.

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HLA CLASS I AND II GENOTYPING AND *IN SILICO* ANALYSIS TO SELECT PRECURSORS DENDRITIC CELLS TO STUDY THE IMMUNOBIOLOGY OF KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS (KSHV)

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Introduction: The Kaposi-associated herpesvirus (KSHV) is associated with the development of Kaposi's sarcoma. Even though the virus is considered lymphotropic, it is able to infect others cell type such as macrophages, monocytes and dendritic cells. The viral protein K1 of KSHV, which is encoded by viral ORF-K1, interfers in the cellular signaling inducing proliferation and supporting cellular transformation. It shows high variability between different genotypes of KSHV. So far, it is not clear whether different isoforms of K1 have specific immunobiological features. The KSHV latency is maintained under strict control by the immune system supported by an adequate antigen presentation involving Human Leucocyte Antigen (HLA) class I and II. Polymorphisms of HLA class I and II genes confer an enormous variability in molecules that recognize a large amount of antigens, but also can increase the susceptibility to autoimmune diseases. The determination of HLA molecules which confer better response to K1 epitopes can help the study of immunobiology of the KSHV. This study aims to genotyping HLA class I and II from healthy volunteers to identify HLA haplotypes that can provide better response to K1 epitopes from different KSHV genotypes. **Methods and Results:** First of all, twenty volunteers were selected to genotyping HLA class I and II (AB/DR/DQ SSP UniTray kit, Invitrogen, CA, USA). Then, *in silico* analyses were performed using BIMAS and SYFPEITH databases. Is important to note that these databases couldn't analyze ours HLA class II molecules, so our study was restricted to HLA class I molecules. In our results we observed prevalence of certain HLA class I haplotypes as HLA-A1, HLA-A2, HLA-A24, HLA-A26, HLA-B8, HLA-B18 and HLA-B44. Furthermore, after *in silico* analysis, we observed high scores for epitopes from the B genotype of KSHV, indicating high affinity of these peptides to the HLA class I molecules. Additionally, we found some HLA molecules that potentially can be good presenters of K1 peptides - HLA-A1, HLA-A3, HLA-A11, HLA-B16, HLA-B38 and HLA-B51. **Conclusion:** Therefore, we can conclude that at least 9 of 20 volunteers analyzed are potentially good presenters of K1 peptides, since both HLA-A and HLA-B molecules shows high affinity to these epitopes, allowing select precursors dendritic cells to posterior immunobiology studies of KSHV.

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ROLE OF B LYMPHOCYTES MODULING ANTI-TUMOR RESPONSES

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Introduction: Human Papillomavirus (HPV) infection is the main cause for cervical cancer and a percentage of other anogenital and oropharingeal tumors. Using the HPV16 associated tumor model, TC-1, we have previously demonstrated that there is a significant increase in myeloid populations in the spleen of tumor bearing mice and that tumor associated macrophages, representing up to 10% of the total tumor
cell populations, secrete IL-10 and can induce regulatory phenotype on T cells. We have also shown an increase in B lymphocytes in the lymph nodes of tumor bearing mice in comparison to naïve mice. B cells and macrophages, together with dendritic cells are professional antigen presenting cells (APC) that have an essential role in modulating T cell responses. The cross-talk between APCs and T cells is mediated by cytokines, receptors, adhesion molecules, among them CD40/CD40L. Since we have characterized the role of macrophages in tumor growth and immune responses in our HPV16 associated tumor model, the objective of this work is to determine if B cells also have a role in the modulation of anti-tumor immune responses.
Methods and Results: We observed that as macrophages, B cells in mice bearing TC-1 tumors express CD40. *In vitro*, we have observed that stimulation of spleen isolated B cells with anti-CD40 induces increase in CREB and Erk phosphorylation, but not STAT3 phosphorylation, and also increase of CD86, CD80, MHC-II expression, revealing an augmented antigen presentation capacity. *In vivo*, using C57Black/6 / RAG1$^{-/-}$ chimeras, we observed that T cells from C57 Black/6 HPV immunized or tumor bearing donors display more efficient anti-tumor responses when transplanted into TC-1 RAG1$^{-/-}$ injected mice together with anti-CD40 stimulated B cells, than with unstimulated B cells derived from tumor bearing donors.
Conclusion: These results suggest that activated B lymphocytes may be used to break T cell tolerance toward tumor antigens. As the B lymphocytes can be derived from the donor and are easily manipulated, we believe this may be an useful tool in anti-tumor immunotherapy.

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SUSCEPTIBILITY TO TUMORIGENESIS IS ASSOCIATED WITH HIGH ANTIBODY PRODUCTION

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Introduction: Skin is an organ under intense physical and chemical stress resulting in disorders, including cancer. Applications of the procarcinogen 7, 12-dimethylbenzanthracene (DMBA), a polycyclic aromatic hydrocarbon, provide a system for study skin tumorigenesis. Lines of mice genetically selected according to High (H) and Low (L) antibody production can be used to investigate how humoral immunity can affect tumor susceptibility. The aim of this work was to study the influence of genetic factors relevant to antibody production on tumor development.
Methods and Results: Skin tumor was induced at the shaved back of mice by epicutaneous application of DMBA (50µg in 0,1mL acetone) for 5 consecutive days, and controls were treated with acetone at the same time. Skin samples had RNA isolated and cytokine gene expression was analyzed by Real Time PCR. After DMBA application, both strains showed an intense superficial cutaneous inflammation around 15 days, but between 30 – 60 days, L mice healed skin lesions (74%, n=31) while H mice developed papillomas (76%, n= 33). Analyzing skin and lung, L mice were more resistant, showing few lesions and tumors. Skin tumor multiplicity increased and the incidence around 90 days post treatment was significantly higher ($p< 0,0001$) in H mice (76%, n= 30) than in L mice (14%, n=29). Lung tumors were observed in all H male animals (100%, n=8). Gene expression of *il-1β, il-6* and *tnf-α* was carried out in skin tissue with 120 and 240 days after DMBA application, and no difference was observed between the strains.
Conclusion: Our results suggest that genes related to high antibody production provide susceptibility for chemical tumorigenesis in this model.

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CHARACTERIZATION OF THE INFLAMMATORY RESPONSES IN HUMAN PAPILLOMAVIRUS ASSOCIATED CERVICAL LESIONS

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Introduction

Cervical cancer is the second cause of women mortality in the world. Human Papillomavirus (HPV) is responsible for virtually all cervical cancer cases and the greatest burden of HPV induced cancers occurs in developing countries. Although these viruses display several immune evasion mechanisms, most infected women are naturally capable of eliminating the infection, even when precursor lesions have developed. A fraction of the infected women, however, display persistent HPV infections that may progress to high grade lesions or invasive cancer. Tumors are complex environments consisting of tumor cells, endothelial cells, inflammatory infiltrate, and acellular elements like extracellular matrix and molecules secreted by different cell types. In lesions produced by HPV, many studies show that the inflammatory infiltrate tends to increase proportionally to the lesion grade, although its role is not known. The inflammatory infiltrate may play a pro or anti-tumoral role. This role may change as lesions progress. The objective of this project is to characterize the phenotype and activity of the inflammatory infiltrate in HPV associated cervical low grade, high grade and invasive tumors. We also intend to characterize the potential of antigen presenting cells in the peripheral blood of the same patients.

Methods and Results

Cervical biopsies of HPV-associated lesions were collected in parallel with a blood sample. Biopsies were mechanically disrupted and enzymatically digested. Single cell suspensions were incubated with antibodies against markers for several leukocyte populations and analyzed by flow cytometry. PBMC were collected from the blood samples and frozen in liquid nitrogen to study the phenotype of antigen-presenting cells and monocytes.

Until now, we have analyzed 10 lesions: 2 low grade, 8 high grade lesions. The low grade lesions displayed low numbers of inflammatory cells compared to high grade lesions. Among the observed inflammatory populations, $CD16^{+}CD8^{-}CD19^{-}$ and $CD16^{-}CD8^{+}$ populations were detected in all high grade lesions.

Other populations were not consistently represented, although $CD16^{+}CD8^{+}$, potentially CTLs, appeared in 75% of the high grade lesions.

Conclusion

Our results reveal the presence of both lymphoid and myeloid populations within the infiltrate of HPV-associated cervical lesions.

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EVALUATION OF NEUTROPHIL FUNCTION IN A PEDIATRIC CANCER PATIENT WITH SYSTEMIC FUNGAL INFECTION

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Introduction: Neutrophils are key cells in host defense against pathogens and cancer patients undergoing chemotherapy (CT) often develop neutropenia. This condition is implicated as responsible for their increased susceptibility to infections, but patients with the same neutrophil counts may have or not complicated infections. Thus, it is safe to assume that neutrophil counts are not enough to predict susceptibility to infection. So, the current study aims to analyze neutrophil function in cancer patients, before, during and after CT, in an attempt to study neutrophil function disturbances in pediatric cancer patients subjected to CT. Here, we present preliminary data describing neutrophil function in a patient that had undergone chemotherapy for acute myeloid leukemia and presented a systemic fungal infection unresponsive to antifungals. **Methods:** Neutrophils oxidative burst was determined by flow cytometry using dihydrorhodamine 123 (DHR), which, when oxidized by H_2O_2, gives rise to the fluorescent compound, rhodamine 123; the oxidative burst was measured without stimulus or after cell treatment with lipopolysaccharide, phorbol-12-myristate-13-acetate, *E.coli*, *S.aureus* or *C.albicans*. Phagocytic activity of *E.coli*, *S.aureus* or *C.albicans* previously stained with propidium iodide (PI) by neutrophils was also determined by flow cytometry. All assays were done in parallel with cells from a healthy young donor. **Results:** The total amount of H_2O_2 produced by neutrophils was similar between patient and control, for all stimuli but *Candida*. When the fungus was used to induce the oxidative burst, patient's neutrophils presented a near 50% reduction in H_2O_2 production. When the production was analyzed on a per cell basis, patient's neutrophils produced less H_2O_2 after all stimuli tested. Overall phagocytosis of the three pathogens was very similar between control and patient, but, when analyzed again on a per cell basis, it distinguished clearly patient and control cells: patient neutrophils phagocytosed less bacteria but twice as much fungi as neutrophils from the control. **Conclusion:** This preliminary data suggest that neutrophils in

cancer patients may have unique functional aberrations that might explain their clinical complications and evolution.

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SYNTHETIC PHOSPHOETHANOLAMINE HAS AN ANTITUMORAL EFFECT ON MURINE MELANOMA

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Introduction: The low responsiveness of melanoma to traditional treatments together with its increasing incidence makes the development of new therapeutic strategies against this type of cancer extremely important.

Methods and Results: In this study, we used a murine melanoma model to evaluate the effects of synthetic phosphoethanolamine (PEA) on the development of this tumor. C57BL/6 mice bearing B16F10 melanoma tumors were treated orally with PEA (2.5; 5 and 10 mg/ml) during 20 consecutive days (N = 10/group). *In vitro*, PEA had an inhibitory effect on B16F10 cells, inducing 36.1% ± 7.1 of apoptosis after 48h of stimulation (control: 4.8% ± 0.32; $P<0.001$). *In vivo*, the treatment of animals induced macroscopic differences in tumors and resulted in a tumor volume reduction of at least 70% (control: 9.72 mm^3 ± 1.22; animals treated with 2.5; 5 and 10 mg/ml: 1.92 ± 0.63; 1.98 ± 0.72 and 1.91mm^3 ± 0.75 respectively; $P<0.001$). An increase of 44 and 64% was observed in the numbers of erythrocytes in animals treated with 2.5 (control: 30 ± 1.9; treated: 43.5 ± 2.9; $P<0.003$) and 5 mg/ml (control: 30 ± 1.9; treated: 49.4 ± 5.4; $P<0.01$) respectively. The number of platelets was also increased, 23 and 48%, in animals treated with 5 (control: 3.5 ± 0.3; treated: 4.3 ± 0.2; $P<0.04$) and 10 mg/ml of PEA (control: 3.5 ± 0.3; treated: 5.2 ± 0.4; $P<0.008$). All concentrations of PEA induced an increase of more than 30% in the number of leukocytes when compared to the untreated group ($P<0,01$). In addition, the treatment led to IL-6 and TGF-β levels 200% higher than control ($P<0.03$), while IFN-γ production was reduced by approximately 50% ($P<0,03$). No differences were observed in the levels of IL-1β, TNF-α and IL-12p70 upon treatment.

Conclusion: Our preliminary results indicate an inhibitory role of PEA in the development of melanoma and other studies are being conducted to better understand this effect.

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Flavonoids as modulators of glia/glioma interaction: role of inflammatory cytokines and MEC/MMP expression

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Por favor extenda o resumo com últimos resultados, pois pode ser bem mais amplo, até 2000 palavras.

Introduction: Studies have been described glioma/microglia interaction involving regulation of MEC expression and immunologic responsiveness as responsible for their aggression and tumor invasion. Our previous study have been suggested that rutin, a flavonoids extracted from seeds of the Brazilian plant *Dimorphandra mollis,* act as inhibitor of growth of human glioblastoma cell lines and modulator of imumodulatory agents as TNFα and NO in glial cells. Hence we have been evaluated the effect of flavonoids rutin, and its derived aglycone quercetin, on growth and migration of isolated human (GL-15) and rat glioblastoma cells (C6), and in interaction microglial/macrophages.

Methods and Results:Phase contrast microcopy in a monolayer wound assay of synchronized glioma cells treated with flavonoids (50 µM) showed that the closure of the wounded area was significantly slower indicating inhibition on glioblastoma cells migration. Flavonoids induced reduction of MMP-2 activity an expression, and an increase on production and secretion of fibronectin, proteins related to glioma migration and adhesion. Moreover, OX-42 positive cells in rat microglia cultures were elevated after flavonoids exposure, indicating activation. It reflected in changes in cellularity and morphology of C6 glioma cultures interacting indirectly with these phagocytes, through conditioned medium derived from microglial cultures treated with flavonoids, or directly in a model of co-cultures of C6/microglia cells. ELISA performed with the medium of microglia cultures showed that levels of TNF-α were increased in cultures treated with 100 µM rutin or 50-100 µM quercetina

Conclusions: This findings suggest that flavonoids rutina and quercetina can induce changes on regulatory profile of glial response during glioma interaction.

Key words: Flavonoids, Glioma, MEC, Microglia, MMPs.

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ANTITUMORAL AND IMMUNOMODULATORY EFFECT OF ALKALOID DIHYDROCHELERYTHRINE ON GLIOBLASTOMA CELLS LINAGES

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Introduction: Gliomas the most common and aggressive tumor of central nervous system. Although microglia represents the first line of defense against brain tumors, their immune function as APCs is inhibited in the tumors microenvironment because gliomas secrete some cytokines such as TGFb, IL-10 which modulate the host immune response, resulting in an immune suppression. This study evaluated the anti-proliferative and immunomodulatory effects of alkaloid dihydrochelerythrine (DHC) extracted from *Zanthoxylum stelligerum* on of human (U-251) and rat (C6) glioma cells, and in C6 cells interacting with microglial/macrophages

Methods and Results: We observed by MTT test that DHC (10 to 200 µM) induced a time and dose dependent reduction on the cell viability. Flow cytometry analysis after Annexin-V staining showed that DHC also induced apoptosis in glioma cells. CBA performed to cytokines IL-12p70, INFg, IL-2, IL-10, IL-8, IL-6, IL-4, IL-5, IL-11b, TNFa and TNFb with the medium of cultures showed that, treatment with 100µM DHC induced after 48 h a change on profile of cytokines secretion with a significant increase on secretion of IL-4, IL-10 and INFg. Moreover, we observed in rat C6/microglia co-cultures, after staining with isolectin B4-FITC (specific for microglia) and nuclear chromatin co-staining with Hoeschst-3325, a total suppression of glioma cells after 48 h treatment with 30mM DHC. ELISA performed with the medium of C6/microglia co-cultures showed that levels of IL-10 were increased after 48 h treatment with 30-100mM DHC.

Conclusion: DHC induce death on glioma cells mainly when co-cultured with microglial cells, suggesting an antitumor potential. DHC also presented an immunomodulatory potential since it induced secretion of a regulatory cytokine IL-10 in glioma/microglia co-cultures. Studies are being conducted to determine its molecular targets and mechanisms of action, to find future applications on glioma therapy.

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PHOSPHOETANOLAMINE MODULATES THE INFLAMMATORY MICROENVIRONMENT OF COLON CANCER

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Introduction. Colon cancer is the second leading cause of cancer-related death in the Western World. It has a natural history of transition from normal crypts through adenoma to overt adenocarcinoma, providing a great opportunity for prevention and intervention strategies. The controversial role of phospholipids in tumor development has been recently demonstrated. Experimental evidence indicates that these molecules participate in leukocyte proliferation, cytokine synthesis, as well as cell activation, commonly leading to an increased antitumoral response. In this study, we evaluated the effects of synthetic phosphoethanolamine (PEA) on the early stages of colonic oncogenesis. **Methods and Results.** C57BL/6 mice were given 4 intrarectal deposits of 0.1 ml solution of MNNG (5mg/ml) twice a week for 2 weeks. Twelve weeks after carcinogen administration, animals were treated orally (0.1ml/mouse) with PEA (0.05 mg/ml solution) twice a week for 4 weeks. At sacrifice, the colons were opened longitudinally, fixed and processed for histological and immunohistochemical methods. Treated animals (n=10) exhibited increased rates of tumor cell apoptosis (from 4.149 ± 0.3942 to 2.545 ± 0.3821, $p<0.05$), reduced numbers of CD4+ (from 3.433 ± 0.1242 to 2.545 ± 0.0321, $p<0.05$) and CD8+ cells (from 4.148 ± 0.2226 to 2.535 ± 0.1616, $p<0.01$) and increased numbers of macrophages (from 1,280 ± 0,1083 to 2,533 ± 0,3501, $p<0.001$). Furthermore, we observed a decreased production of the proinflammatory cytokines IL1b (from 70,55 ± 7,703 to 30,51 ± 3,179, $p<0,001$), TNF-α (from 304,2 ± 23,15 to 211,3 ± 41,35, $p<0,05$), IFN-γ (from 11,74 ± 1,940 to 3,571 ± 0,9352, $p<0.05$), IL12 (from 737,4 ± 45,02 to 533,2 ± 76,95, $p<0.05$), and an increase of the anti-inflammatory cytokines IL10 (from 33,46 ± 5,162 to 217,6 ± 59,62, $p<0,05$) and TGF-β (from 7860 ± 3247 to 642,8 ± 168,9, $p<0,05$). **Conclusion.** These preliminary results indicate that PEA affects the tumor-associated inflammatory response. Further study is currently being undertaken to better understand the immunomodulatory effect of phosphoethanolamine.

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MUTATION EFFECT OF HSPB1-PROTEIN CLEAVAGE SITE ON *IN VIVO* TUMOR GROWTH

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Introduction: HspBP1 is a cochaperone with nucleotide exchange activity that binds and regulates Hsp70. Hsp70 is elevated in numerous types of cancer cells and has anti-apoptotic activity. We have previously shown that HspBP1 levels are also elevated in a variety of cancer types. Increased expression of HspBP1 did not alter tumor cell growth in culture. However, injection of these cells into either normal or immunodeficient mice resulted in decreased tumor growth compared to controls. HspBP1 and mutants of the HspBP1-protein cleavage site (C22S, C199S, C22S and C199S) were over expressed in B16F10 cancer cell lines. Therefore, the aim of this study evaluates the HspBP1 mutants effects on the tumor growth and compare to intact HspBP1 protein.

Objectives: Evaluate the role of expression of HspBP1 mutants (C22S, C199S, C22S and C199S) in murine melanoma tumor growth in vivo.

Methodology: B16F10 melanoma cells line were transfected with the plasmid pcDNA4/TO encoding the sequence of murine HspBP1 and HspBP1 mutants (C22S, C199S, C22S and C199S), using 4D-Nucleofactor (LONZA), or only with the vector as a control. The expression of the protein was confirmed using western blot analysis. After 24hs, C57Bl/6 mice were implanted subcutaneously in the thigh with B16F10 HspBP1 transfectant cells at a concentration of $2x10^5$, after anesthesia with 83 mg/Kg of ketamine and 17 mg/kg of xylazine. Tumor growth will be evaluate using a caliper.

Results: The murine HspBP1 mutants were transfected successfully in BF16F10 melanoma cells line and the cells were implanted in the animals. This experiment is in process.

Conclusions: Previous results, obtained in our laboratory, demonstrate that HspBP1 can inhibit tumor cell growth in an animal but not in cultured cells by a mechanism that does not involve binding to Hsp70. We are now doing experiments with HspBP1 mutants in order to elucidate the real mechanisms involved in the tumor regression mediated by HspBP1.

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IMMUNOMODULATORY ACTIVITY OF LQB-118, AND ITS ANTINEOPLASTIC EFFECT IN EHRLICH SOLID TUMOR MODEL

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Introduction: Immune system plays a crucial role in protecting the host against cancer, and solid tumors are known to promote alterations in its microenvironment, using negative regulatory mechanisms. Novel chemotherapic agents with minimal effect on immune system are of great interest. Among natural products with pharmacological effects, Pterocarpans and Naphthoquinones stand out, known for their antineoplasic activity. Based upon these molecules, our group proposed a new synthetic substance, the hybrid pterocarpanquinone LQB-118. Since LQB-118 was proven to be effective *in vitro*, this study aims to describe the immunomodulatory and antineoplastic effect of LQB-118 *in vivo*. **Methods and Results**: Two-month female swiss mice received a single intraperitoneal (I.P.) injection of LQB-118 in a acute dose (3.8 mg/kg). After 24 h animals were sacrificed and bone marrow, spleens and lymph nodes were excised. Absolute cell count and viablity was obtained by Trypan Blue exclusion. Also, splenocytes were stimulated by Concanavalin A (ConA) and viablility was measured by MTT assay (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). $5x10^5$ splenocytes/well were incubated at 37ºC, 5% CO_2 for 24 h and 72 h in different concentrations of LQB-118, with or without ConA addition. To evaluate the antineoplastic effect of LQB-118, two-month female swiss mice were injected subcutaneously (S.C.) with 10^7 Ehrlich Ascitic Tumor (EAT) cells, and then treated with I.P. injections of 0.35 mg/kg/day of LQB-118 for two weeks. It was observed that an acute dose of LQB-118 as pre-treatment did not show toxicity for immune system since I.P. administration seems not to change absolute number of cells, and has no effect in both spleen cells viability and proliferation capacity under ConA stimulation.

In vivo LQB-118 administration proved to be effective, since volume and tumor mass weight were reduced after two weeks. **Conclusion**: Data showed that the synthetic molecule LQB-118 presents clinical potential, because even at acute doses it was not able to produce negative effect in immune system cells. Furthermore, it showed a potential antineoplastic effect against a solid tumor *in vivo*.

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ROLE OF B1 CELLS IN TUMOR PROGRESSION OF MOUSE ADENOCARCINOMA CELLS (4T1)

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Introduction

Previous data from our group shows that B1 cells interact physically with B16 murine melanoma cells, *in vitro*, inducing an increase in their metastatic potential. However, the B1 cell role in the development of different tumors has not been investigated yet. Thus, the aim of this project was to investigate the role of B1 cells in the growing and development of 4T1 mouse mammary adenocarcinoma.

Methods and Results

To evaluate the effect caused by the interaction with B1 cells *in vitro*, 4T1 cells, maintained in RPMI-1640 medium supplemented with 10% fetal calf, were co-cultivated with B1 cells from BALB/c wild type mice for 72 hours. After this period 4T1 cells were harvested and injected in tail of vein of BALB/c wild-type animals. Fourteen days passed, mice were sacrificed and lungs surgically removed to determine the number of metastatic colonies. The counting of metastatic colonies demonstrated that 4T1 cultivated with B1 cells have more metastatic colonies than the culture of 4T1 cells only.

Conclusion

These experiments suggest that B1 cells elevated the metastatic potential of mouse mammary adenocarcinoma cells in *in vitro* co-culture system.

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JACALIN-ACTIVATED MACROPHAGES PRESENT AN ANTI-TUMOR PHENOTYPE

CLÁUDIA DANELLA POLLI[1]; THAIS HERRERO GERALDINO[1]; LUCIANA CHAIN VERONEZ[1]; LUCIANA PEREIRA RUAS[2]; MARIA CRISTINA ROQUE-BARREIRA[2]; GABRIELA SILVA BISSON[1,3].

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Introduction: Tumor-associated macrophages (TAMs) have an ambiguous and complex role in the carcinogenic process, since these cells can be polarized into different phenotypes by the tumor microenvironment. The interaction between tumor cells and macrophages involves several players such as cytokines, chemokines, growth and angiogenic factors. A better understanding of the regulation and function of TAMs is crucial to interfere with their differentiation in order to drive TAM polarization into cells with an anti-tumor phenotype. The aim of this study was to analyze the modulation of macrophage tumoricidal activities by the lectin jacalin.

Methods and results: We showed that *in vitro*, jacalin (2.5 to 40µg/ml) induced the production of both pro- and anti-inflammatory mediators by human macrophages. All concentrations of the lectin induced the secretion of low levels of the anti-inflammatory cytokine IL-10. On the other hand, jacalin (10 to 40 µg/ml) induced high amounts of the pro-inflammatory cytokines TNF, IL-6 and IL-12. Macrophages stimulated with jacalin (20µg/ml) also produced low levels of nitric oxide. Moreover, the lectin activated the NF-κB signaling pathway in macrophages. As assessed by MTT assays, when supernatants from macrophages stimulated with higher, but not with lower concentrations of jacalin were added to cultures of human colon (HT-29) or breast adenocarcinoma cells (MCF-7), up to 25% and 70% reduction of cell viability was observed,

respectively. Flow cytometric analysis using annexin V showed that the reduction in cell viability induced by jacalin was due to apoptosis of tumor cells.

Conclusion: In conclusion, these results indicate that jacalin, through its ability to exert a pro-inflammatory activity, can direct macrophages to an anti-tumor phenotype.

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THERAPEUTIC TARGETS IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

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Introduction: Apoptosis or programmed cell death plays an essential role in the development and homeostasis of multicellular organisms. Because many anticancer drugs kill tumor cells by inducing apoptosis, mutations or deregulation of pro- and anti-apoptotic proteins can contribute to the acquisition of chemoresistance. Head and neck cancer is a relatively well characterized human tumor, more than 90% of this cancer type has squamous origin and common sites include hypopharynx, larynx, oral cavity, nasopharynx, oropharynx, paranasal sinus, nasal cavity, parathyroid and salivary glands. The cause of head and neck squamous cell carcinoma (HNSCC) is multifactorial and, despite recent advances in treatment, the long-term survival rate has remained at 50% with high rates of associated mortality. Late presentation of lesions, lack of suitable markers for early detection and failure of available chemotherapy response in advanced lesions contribute to a poor outcome of HNSCC. In addition, little is known about the molecular mechanisms underlying this type of cancer. The aim of this study was to investigate the anticancer efficacy of drugs in HNSCC cells treatment.
Methods and Results: HNSCC cells (HN30, HN31, 6, 6.1, FaDu, SCC-25 and HaCat) was maintained in DMEM containing 10% fetal bovine serum in a humidified incubator with 5% CO2 and were treated with actinomicin-D, ara-C, etoposide and cycloheximide (CHX), for 18, 24 and 48 hours and the apoptotic cells were detected by DNA fragmentation analysis. We observed that tumor cells treated with apoptogenic stimulus *in vitro (*actinomicina-D and CHX) showed decreased in the resistance of death.
Conclusion: HNSCC cells are sensitive when treated with *a*ctinomicina-D and CHX.
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ANTITUMOR EFFECT OF LAMINARIN DERIVED FROM *LAMINARIA DIGITATA* IN SOLID EHRLICH TUMOR

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Introduction: Laminarin is a β-glucan derived from brown seaweed *Laminaria Digitata* and *Laminaria Cloustroni*. This β-glucan is capable of active immune system by binding a pattern recognition receptor, Dectin-1, present in cells of the innate immune system. Activation of this receptor is related with production of IL-6, NF-kB, IL-2, IL-10 and reactive oxygen species (ROS). Ehrlich tumor is a breast adenocarcinoma, and in this work was used in its solid form. Tumor cells are capable of interact with your microenvironment causing a oxidative stress in associated fibroblast, by ROS production and release. The oxidative stress causes genomic instability in adjacent cells that induce a more aggressive phenotype. Also, by this stress fibroblast releases lactate, which act as energy source for tumor cells. **Methods and Results:** C57BL/6 mice were used (five per group). Ehrlich cells were inoculated in the rind right paw. Treatment was done by intraperitoneal inoculation or gavage of Laminarin at alternated days up to five treatments with a dose of 200mg/kg of Laminarin. Tumor development was measured by a digital caliper until twenty days after cells inoculation. Laminarin cytotoxicity was performed by MTT test. ROS production by Ehrlich cells was evaluated using DCFH-DA probe when incubated with Laminarin associated or not with PP2 by flow cytometer. The paws were analyzed histologically. Test t *Student* was performed in the statistical analysis. Treatment with Laminarin via gavage shows a reduction of tumor growth when compared with control group. Incubation of Laminarin in serially concentrations (5mg/ml to 32,5µg/ml) with Ehrlich cells did not show any cytotoxicity effect. Surprisingly, the Ehrlich cells produces high amount of ROS and Laminarin diminished this production. The use of SRC family inhibitor (PP2) restored the ROS production. Histological analyses shows that the treatment with Laminarin reduced the tumor field compared with control group. **Conclusion:** Laminarin seems to reduce the growth of tumor, and this effect

can be related with reduction in ROS production. Besides, this inhibitory effect seems to depend of SRC family kinases.

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EXPRESSION OF CLAUDIN-10 ON B-1 CELLS TRIGGERS THE HIGHER METASTATIC POTENTIAL OF B16 MURINE MELANOMA CELLS

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B-1 cells comprise a small fraction of the B cell family found preferentially in peritoneal and pleural cavities. Considering that B-1 cells are one of the main sources of interleukin-10 (IL-10), we previously demonstrated that cocultivation of B16 melanoma cells with B-1 cells from C57BL/6 mice (wt), but not with C57BL/6 IL-10 knockout B-1 cells (IL-10KO), increases the metastatic potential of melanoma cells. However, the molecule expressed on B-1 wt cells able to affect the metastatic potential of B16 cells has not yet been fully addressed. Therefore, the aim of this work was to identify the molecule expressed by B-1 wt, but absent in B-1 IL-10KO cells, which triggers increased metastatic potential of melanoma cells.

Materials and Methods: To investigate differential gene expression between B-1 cells from wt and IL-10KO mice, expression profiles were generated using the *Affymetrix GeneChip Mouse Genome 430 2.0 Array.* Results were validated by western blot analysis and interference RNA assays were realized to evaluate biological function of differentially expressed molecules.

Results: Three independent experiments of microarrays analyses demonstrated differential mRNA expression of seven (7) genes between wt and IL-10KO B-1 cells. Among these genes, claudin-10, involved with cell communication and cell adhesion, was upregulated in B-1 wt cells. Data was

confirmed by western blot analysis and interference RNA assays were performed to evaluate the biological function of claudin-10 in this model. Interestingly, silencing of claudin-10 expression on B-1 wt cells prevent their capacity to increase the metastatic potential of melanoma cells.

Conclusion: These results suggest that claudin-10 is one of the molecules present on B-1 cells responsible for the interaction that triggers the phenotypic changes of melanoma cells during coculture.

Financial support: Fapesp

Insulin-like growth factor-II mRNA-binding protein 3 as a potential prognostic biomarker for squamous cell carcinomas of the head and neck

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Introduction: Insulin-like growth factor II mRNA-binding protein 3 (IGF2BP3 aka IMP3), a member of the insulin-like growth factor-II (IGF-II) mRNA-binding protein (IMP) family, is overexpressed in embryonic tissues and several epithelial malignancies. In adition, peptides derived from IGF2BP3 inducing human leukocyte antigen-A2-restricted cytotoxic T lymphocytes reactive to cancer cells have been identified. Thus, it is suggested that IGF2BP3 represents a promising cancer biomarker and immunotherapeutic agent. However, analysis of IGF2BP3 expression in head and neck squamous cell carcinomas (HNSCC) is little known and the clinical, pathological, molecular and prognostic features of IGF2BP3-positive HNSCC remain unclear. The aim of this study was to asses the IGF2BP3 expression in 186 HNSCC, including 153 oral and oropharyngeal primary tumors surgically resected and 33 lymph node metastasis.
Methods and Results: The expression was demonstrated by both mRNA and protein measurements, using combination of real time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and immunohistochemistry on a tissue microarray and in individual lesions. The expression levels of IGF2BP3 mRNA and protein were greatly increased. qRT-PCR analysis showed that IGF2BP3 was up-regulated in 50% of investigated HNSCC. Immunohistochemical analysis demonstrated a cytoplasmic presence of IGF2BP3 in 129 (84.3%) primary HNSCC. The positive staining was “weak” in 39 primary tumors (25.5%) as well “moderate” in 46 (30.1%) and “strong” in 44 (28.8%). In lymph node metastasis of HNSCC, IGF2BP3 protein expression was positive in 23 (69.7%) cases. In relation to clinicopathological analysis, IGF2BP3 protein expression was associated with clinical stage III-IV (p=0.036) and positive lymph node metastasis (p=0.004).

Conclusion: Our results indicate that IGF2BP3 is a potential prognostic biomarker in HNSCC.

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ROLE OF DERMCIDIN IN TUMOROGENICITY OF G361 HUMAN MELANOMA CELL LINE. BEATRIZ A. SANGIULIANO[1], MARCELA PEREZ[1], ALINE C. CUSTODIO[1], JOSÉ E. BELIZÁRIO[1]. Department of Pharmacology, Institute of Biomedical Sciences, University of São Paulo. Av Lineu Prestes 1524 - CEP 05508-900 - São Paulo, SP.

Introduction: Previous studies have demonstrated that Dermcidin (DCD) is a potential candidate oncogene for breast cancers and melanomas. DCD is produced mainly by dark cells of eccrine sweat glands and benign melanocytic naevi and proteolytic processed and transported via sweat to the epidermal surface to act as peptide antibiotic and/or growth factor. **Methods and Results**: We have been examining the role of DCD in growth and survival by silencing DCD expression via shRNA in human melanoma G361 cell line. Down-regulation of DCD significantly reduced cellular resistance to H_2O_2 in vitro cell culture. In vivo model, Balb/c Nude mice inoculated with $1x10^6$ G-361 cells underwent a progressive increase (30 days period) in the tumor size (volume) and body weight loss, whereas animals inoculated with G-361-IBC I melanoma cells expressing DCD shRNA there was significant reduction in tumor growth and final weight. Moreover, administration of rabbit polyclonal antibody against DCD in mice bearing G361 xenografts for four weeks delayed tumor growth. To examine tumor heterogeneity we isolated by FACSAria-III cell sorter specific melanoma and cancer-stem cells based on commonly used markers (S-100, HMB-45, melan-A, ABCB5, CD133, etc), non-adherent sphere-forming and adherent cells and examined tumorigenic capacity in Nude mice. In parallel, we are determining by Affymetrix microarray ST 1.0 chip, RT-PCR and target-Seq exome sequencing the genes that are up- and down-regulated in activated signaling pathways, (example: EGFR/HER2, C-MYC, PI3K/AKT/mTOR, VEGF, BRAF/RAS) as well as genetic modifications, SNP and mutations in genes involved in G361 melanoma cell tomorogenicity. **Conclusion**: These results are suggesting that DCD functions as tumor growth and cell survival factor.

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DIFFERENCES OF NOX MODULATION ON MDR PHENOTYPE IN HTLV-1 INFECTED AND NON-INFECTED T CELL LINEAGES

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Introduction: Human T-cell lymphotropic virus type 1 (HTLV-1) is an etiologic agent of adult T-cell leukemia/lymphoma and HTLV-1-associated myelopathy. It has been estimated that HTLV-1 infects 20 million people worldwide. The acute ATLL symptoms develop rapidly, and quite quickly ATLL becomes life-threatening if not treated. ATLL cells exhibit a multidrug resistance phenotype, but the mechanisms involved with this characteristic remain unknown. Among the various mechanisms identified with the MDR phenotype, the best characterized involves the expression ABC transporters, such as multidrug resistance related protein 1 (ABCC1). ATLL cells overexpress ABCC1. ABCC1 is related with transport of glutathione, collaborating with the cellular redox status. The NADPH oxidase (NOX) is an enzymatic multi component that produces reactive oxygen species (ROS), which are associated with cancer development. There are no studies correlating NOX expression and/or activity and ABCC1. In this study, we evaluated the effects of NOX activity inhibition in ABCC1 expression and activity, as well as, the effects of ABCC1 activity inhibition in NOX expression. To this study we used a HTLV-1 infected cell line, MT-2, and a non-infected cell line, Jurkat.

Materials and Results: Cells were cultivated with or without NOX inhibitor: DPI (0,1-1 μM); ABCC1 inhibitors: Indomethacin (1-300 μM) or MK571 (25 μM); chemotherapy drug: Daunorubicin (5 ng/ml); or a combination of these substance. Using flow cytometry, we observed that the incubation of MT-2 or Jurkat cells with DPI reduced the expression of ABCC1 in about 50% DPI (MIF values: MT-2: CT = 11,3; DPI 0,1 μM = 6,3; DPI 1 μM = 5,9; Jurkat: CT = 66,0; DPI 0,1μM = 27,7; DPI 1 μM = 37,5). The reduction of ABCC1 expression may modulate the of MDR phenotype. Thus, we evaluated the cell viability by anexina-V and PI assay. The incubation the Jurkat cells with DPI and

Daunorubicin combination induced an increase 20% higher than Daunorubicin alone in cell death. However, this effect did not observe in MT-2 cells, suggesting that HTLV-1 infection induces others important MDR mechanisms. On the other hand, NOX expression was higher in cells (Jurkat or MT-2) cultivated with Indomethacin or MK571.

Conclusion: Our results suggested an important correlation between ABCC1 and NOX in leukemic cells. The results indicated the NOX as a new chemotherapeutic target in HTLV-1 non-infected cells.

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THE ROLE OF SIGIRR IN THE TUMOR AND IMMUNE SYSTEM CROSSTALK

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Introduction: The oncogene ERBB2 is overexpressed in 30% of breast tumors and is associated with rapid disease progression and poor prognosis. Transcriptional changes associated with ERBB2 pathway were investigated using an immortalized mammary epithelial cell (HB4a) and its ERBB2 overexpressing variant (HB4a-C5.2). Global analysis of gene expression using MPSS identified SIGIRR as a gene upregulated in the HB4a-C5.2 variant. SIGIRR is a negative modulator of pro-inflammatory signals triggered by IL-1R and TLRs. Giving the dual role of inflammation in tumor microenvironment, we hypothesize that SIGIRR may fine-tune the inflammatory response and attenuate the anti-tumoral adaptive response. **Methods and Results:** SIGIRR upregulation in HB4a-C5.2 cells was confirmed by qRT-PCR and Western blot and was significantly reduced upon treatment with the tyrosine kinase inhibitor Lapatinib, confirming that SIGIRR is a novel ERBB2 target gene. Ablation of SIGIRR expression in the HB4a-C5.2 cell variant using shRNA resulted in a 2-fold increase of NF-kB DNA binding capacity upon IL-1β stimulation, detected by EMSA, showing that SIGIRR is negatively modulating IL1R-dependent NF-kB activation. To address the role of SIGIRR upregulation in the tumoral milieu we also investigated the differential expression of inflammatory cytokines IL-1β, IL-6, IL-8 and TNF-α after IL-1β stimulus. The expression of all the above mentioned cytokines were 2 to 3-fold upregulated in SIGIRR knock-down clones and higher levels of IL-6 and IL-8 were also detected in conditioned medium (CM) from these clones. Higher expression of chemotactic cytokines (CCL2, CSF2, CXCL10 and CSF3) was also observed in SIGIRR knockdown clones. Consistent with these observations, using *in vitro* chemotaxis assays, we observed that monocyte and neutrophil recruitment were more prominent when cells were incubated with CM from SIGIRR knockdown clones when compared to CM from HB4a-C5.2. **Conclusion:** Our results suggest that SIGIRR upregulation in ERBB2 overexpressed tumors might fine-tune inflammation by

modulating cytokine levels and the recruitment of inflammatory cells to the tumoral microenvironment.

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IL-17 PROMOTES TUMOR PROGRESSION IN BREAST CANCER

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Introduction and objective: Breast cancer is the leading cause of malignancy in women worldwide. IL-17 is a pro-inflammatory cytokine present in several types of tumors, but their role in the tumor immunity is not known. **Methods and results:** In tumor biopsies collected from patients with invasive ductal breast carcinoma, we found higher expression of IL-17 and Th17-related inflammatory molecules (IL-6, IL-1β, IL-23, TGF-β and CCL20) compared with control tissues by qPCR and immunohistochemistry. IL-17 was produced by CD4 (±13,3%) and CD8 (±8,14%) T lymphocytes. The expression of RORC and CCR6 mRNA was positively correlated with the expression of IL-17 mRNA. By microarry, we identified 74 up and 168 down-regulated genes, involved with proliferation and survival of tumor cells in breast tumor samples with higher level of IL-17 than those low levels. Moreover, we also found that murine breast carcinoma cell line expresses IL-17R, and the IL-17/IL-17R signaling pathway induces TGF-β expression in tumoral cells. To evaluate the role of IL-17 in invasive breast cancer, we used a model experimental of metastatic mamary tumor and we found that IL-17 was produced for CD4 and CD8 T cells during the development of invasive tumor. At 35 days after tumor induction, in both primary tumor and secondary site, such as liver and lung, we found increase of numbers neutrophils and a high expression of TGF-β, IL-10, arginase-1 and MMP-9 genes related with protumor neutrophil. Anti-IL-17 antibody treatment reduces

tumor load and number of IL-10-producing neutrophils infiltrating the lung and consequently improves the survival of animals. **Conclusion:** Our data indicate that IL-17 promotes tumor progression via the induction of TGF-β in tumor cells or via the recruitment of neutrophils, opening new perspectives for the development of therapies using anti-IL-17 antibody in patients with invasive breast cancer.

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STUDY OF CANDIDA ALBICANS DEVELOPMENT IN THE TUMOR SITE USING A MURINE MODEL OF EHRLICH SOLID TUMOR.

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INTRODUCION: The host response against infection by *Candida albicans* is a complex interaction between innate and adaptive immunity, effective in healthy individuals, but not in immunocompromised patients. As the tumor condition affects the host immune response, it may favor the spread of fungus. In previous studies we have studied these events and their complications in Ehrlich solid tumor bearing mice (TBM). However the mechanisms involved in these processes are not fully understood. In order to evaluate the interaction between microorganisms and host's immune cells in the tumor microenvironment, we investigated the presence of fungus in the tumor site by classical staining for fungi and fluorescent assay using PKH-26, a vital fluorescent dye that provides strong fluorescent signals without affecting cell viability. To evaluate the spread of PKH-26+ *C. albicans* in TBM and to compare this method with standard histological and microbiological analysis.

METHODS AND RESULTS: Male C57BL/6 mice were inoculated subcutaneously with 1×10^7 viable Ehrlich tumor cells. After seven days, the animals were intraperitoneally inoculated with *C. albicans* (5×10^6 viable yeasts) that were previously stained with PKH-26. After 3, 6 and 12 hours after inoculation, the mice were euthanized and samples of tumor, spleen, brain, liver, lung and kidney were collected and submitted to microbiological evaluation in Sabouraud agar. The tumor samples were spliced in two parts which one was submitted to formalin fixation, routine paraffin embedding procedures and Periodic Acid-Schiff (PAS) staining; and the other one to imprint technique for fresh immunofluorescence analyses. Our results demonstrated that the fungal load in tumor site was lower compared to internal organs and we

observed PKH-26+ yeasts, but not hyphae (pathogenic form), in tumor samples in all moments. Furthermore, the PAS staining analyses also showed typical yeast and no hyphae in the tumor site, but only 12 hours after the inoculation.

CONCLUSION: Our results showed that the C. albicans migrated to the tumor site; still demonstrate that the hyphal-form transition was impaired, suggesting that tumor milieu affects the fungal development. Studies in our lab are ongoing to extend this finding. Furthermore, our results confirm that fungal stained with a vital fluorescent dye is an interesting option for fungal trafficking and when it is associated with imprint technique provides a fast and more sensitive fungal detection.

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Uptake multi-wall carbon nanotubes by dendritic cells and macrophages increase of expression of MHC I, MHC II, co-stimulatory molecules and inflammatory cytokines.

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Introduction: There is an urgent need for new therapeutic approaches for treating tumors. Nanotechnology is a new science that has been growing in recent years and comes as a new promise in the treatment of various types of diseases, including cancers. Carbon nanotubes (CNT) have attracted global interest and are regarded as a novel material with broad applications in many areas. Several studies have demonstrated the ability of single-walled carbon nanotubes (SWCNT) and multi-walled (MWCNT) to penetrate into the membrane of various cellular types. However little is known about the behavior of these cells after internalise these nanoparticles.

Objective: The goal of this study the effect the uptake of MWCNT in dendritic cells and macrophages.

Material and Methods: Dendritic cells are differentiated through cultivation of bone marrow of mice stimulated with GM-CSF. Intraperitoneal macrophages

were extracted after the thioglycollate 4% stimulus. MWCNT autoclaved and dispersed in PBS + Pluronic 127 (sterile solution). The cells were incubated at different times with 10ug of MWCNT.

Results: Through the data submitted was possible to observe that the uptake of MWCNT not functionalized activate antigen-presenting cells. We observe through flow cytometry a significant increase in the expression of major histocompatibility complex molecules I and II, and also of co-stimulatory molecules such as CD 80 and CD 86. This result was accompanied by an increase in the expression of genes for inflammatory cytokines such as TNFα, IL1β and IL 12, with decrease of TGFβ and IL10.

Discussion: This study demonstrates that the uptake of MWCNT activate antigen presenting cells such as dendritic cells and macrophages. The uptake of MWCNT by dendritic cells and macrophages stimulates the maturation of dendritic cells and activate macrophages intraperitoneal. This was demonstrated through the expression of MHC molecules and co-stimulatory cytokines. These data raise questions about the use of these nanoparticles for possible therapies against cancer.

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The administration of multi-wall carbon nanotubes (MWCNT) stimulate the accumulation of lymphocytes, dendritic cells and NK cells infiltrated in the tumor and reducing tumor growth.

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Introduction: Immunotherapy using single-walled carbon nanotubes (sigle-walled-SWCNT) or multiple (multi-walled-MWCNT) as drug carrier has aroused the interest of many researchers mainly in the field of Oncology. The Administration "in vivo" of MWCNT, even without being functionalized, stimulates the appearance of a normal immune response in mice. Studies show that the uptake of these nanoparticles by immune system cells can change their signaling, so, activate or suppress the immune response.

Objective: Study the effect of systemic administration of MWCNT on immune response of mice with Lewis lung carcinoma.

Material and Methods: The MWCNT were synthesized in laboratory of Nanoengenharia and Diamante (NanoEng)-UNICAMP. Mice were inoculated in the back with 1.0 x 10^5 cells in 0.1 mL HBSS sterile by animal (subcutaneous

injection). After the tumor growth animals received through the bloodstream 100 MWCNT + PBS/Pluronic 127. Tumor growth was evaluated on alternate days. The immune response was measured 7 days after inoculation.

Results: The results show that the *in vivo* administration of MWCNT in animals with Lewis carcinoma increase significantly lymphocytes (32% CD8 and 29% CD4), dendritic cells (28,2%) and NK cells (51%) in the tumor microenvironment. This increase resulted in reducing tumor mass of these animals and decreased expression of genes for VEGF and osteopontin.

Discussion: The data are consistent because it shows that the production of inflammatory cytokines possibly stimulated tumor antigen presentation to lymphocytes, activating an immune response against the tumor. The increase of these cells was observed mainly in the lymph nodes and within the tumor mass. The increase of NK cells was observed only within the tumor. This corroborated to a reduced production of factors that could participate in tumor progression increasing angiogenesis.

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