Results: N-octanoyl-glucosylceramide (GC) was identified as a potent enhancer of drug uptake in vitro. Enhanced cellular uptake strongly correlated with amphiphilicity. Whereas the sphingolipid analogue itself was not toxic, incorporation of 10 mol% GC in doxorubicin-containing liposomes significantly enhanced their cytotoxicity in A431 cells resulting in an increase in EC50 values up to 10 fold, as compared to standard liposomal doxorubicin. In vivo studies confirmed the in vitro observations. Enhanced efficacy of GC-enriched doxorubicin liposomes over standard doxorubicin liposomes towards A431 human tumor xenografts in nude mice was demonstrated. With respect to tumor growth and toxicity the optimal concentration of GC-enriched and standard doxorubicin liposomes was set at 6 mg doxorubicin/kg bodyweight. The tumor growth delay for reaching 200% initial volume was 6 and 11.5 days (2-fold delay) for mice treated with standard liposomal doxorubicin and GC-enriched liposomes, respectively, as compared to untreated animals.
Conclusions: Short chain sphingolipids can be used as enhancers for delivery of amphiphilic compounds. GC-enriched doxorubicin liposomes displayed superior in vitro and in vivo anti-tumor activity, as compared to standard doxorubicin liposomes. Liposomal formulations enriched with short chain sphingolipids represent an advanced and versatile technology and provide opportunities for improving drug delivery of anti-cancer agents.

340 POSTER

In vitro and in vivo activation of the tumor suppressor Lats1 by the transcriptional regulator CDP/Cux

R. Siam[1], R. Harada[2], C. Cadieux[2], L. Leduy[3], A. Nepveu[4]. *[1]American University in Cairo, biology, Cairo, Egypt; [2]McGill University, Molecular Oncology Group McGill University Health Center Biochemistry, Montreal, Canada; [3]McGill University, Molecular Oncology Group McGill University Health Center, Montreal, Canada; [4]McGill University, Molecular Oncology Group McGill University Health Center Biochemistry Oncology and Medicine, Montreal, Canada*

The transcription regulator CDP/Cux binds to distinct promoter regions and up-regulates or down-regulates transcription of several genes involved in cell cycle and tumorogenesis. We tested distinct promoter regions of several genes involved in tumorogenesis and found the CDP/Cux regulates transcription of the tumor suppressor gene Lats1. We utilized scanning ChIP and identified the exact region bound by one of the short isoforms of CDP/Cux (p110) in the Lats1 gene region. We therefore tested regulation of Lats1 transcription in vitro by both short isoforms of CDP/Cux (p110 and p75) and demonstrated that the short isoforms up-regulates transciption of this tumor suppressor. Several tumor cell lines over-expressing distinct short isoforms of CDP/Cux were tested for Lats1 expression and likewise revealed an increase in Lats1 transcription. Additionally, transgenic mice over-expressing the short isoforms of CDP/Cux and developing mammary gland tumor, uterine tumors and myeloproliferative disease like myeloid leukemia show an enhance in transcription of Lats1. We postulate that CDP/Cux regulation of the tumor suppressor gene Lats1 in cell lines and tumor cells may express an altered Lats1 protein that has an altered role in tumorogenesis.

341 POSTER

−308G>A TNF-alpha polymorphism is a genetic susceptibility marker for nasopharyngeal carcinoma development

H. Sousa[1], E. Breda[2], R. Catarino[1], D. Pinto[1], P. Canedo[3], J.C. Machado[3], R. Medeiros[1]. *[1]Instituto Português de Oncologia do Porto FG EPE, Grupo Oncologia Molecular – CI Lab 4º Piso, Oporto, Portugal; [2]Instituto Português de Oncologia do Porto FG EPE, Otorrinolaryngology Department, Oporto, Portugal; [3]IPATIMUP, Cancro Gástrico, Oporto, Portugal*

Introduction: Nasopharyngeal carcinoma (NPC) is a viral associated neoplasia, extremely rare in western populations, on which genetic polymorphisms related to immune response have been associated to its development. The Tumor Necrosis Factor Alpha (TNFα) is a pro-inflammatory cytokine that has been associated to several cancers, especially to viral associated neoplasia. We have designed a study to analyse the role of a common Single Nucleotide Polymorphism (SNP) on the promoter region of the TNFα (−308G>A) on the development of NPC.
Material and Methods: We developed a cross-sectional study considering a total of 547 individuals from the Northern Region of Portugal, including 101 patients with the undifferentiated type of NPC (UNPC) and 446 healthy individuals without evidence of neoplastic disease. The genetic analysis was performed by Real-Time PCR with a TaqMan® SNP Genotyping Assay from Applied Biosystems (Assay C___7514879_10).
Results: This study revealed an increased frequency of the −308A TNFα allele in patients with UNPC rather than in healthy individuals, which represents almost a five-fold risk increase for −308A homozygous (p = 0.002; OR = 4.67; 95% CI 1.21–5.90). Moreover, logistic regression analysis revealed that having −308A homozygosis (p = 0.010; OR = 4.24; 95% CI 1.41–12.73), being male gender (p = 0.002; OR = 2.11; 95% CI 1.31–3.40) and having age >49 at diagnosis (p = 0.001; OR = 2.12; 95% CI 1.36–3.32) can represent predictive factors for the development of NPC.
Conclusions: These results confirm that in a Portuguese population −308A TNFα homozygosis can represent a risk factor for NPC development, and also corroborate data from previous studies where male gender and age >49 at diagnosis are known as specific markers for NPC, contributing for the knowledge of NPC aetiology.

342 POSTER

P53 codon 72 PRO/PRO genotype is a genetic susceptibility maker for gastric adenocarcinoma development

M. Dinis-Ribeiro[1], H. Sousa[2], S. Oliveira[2], A.L. Pinto-Correia[2], C. Faustino[3], P. Perreira[3], M. Fragoso[3], R. Medeiros[2]. *[1]Instituto Português de Oncologia do Porto FG EPE, Gastroenterology Service, Oporto, Portugal; [2]Instituto Português de Oncologia do Porto FG EPE, Grupo Oncologia Molecular – CI Lab 4º Piso, Oporto, Portugal; [3]Instituto Português de Oncologia do Porto FG EPE, Medical Oncology Department, Oporto, Portugal*

Introduction: Gastric adenocarcinoma (GC), a major public health problem worldwide, has been associated with several gene deregulations. TP53 gene encodes for the p53 protein, one of the key genes on cancer development. Moreover, p53 has been suggested as altered in over 50% of all human cancer cases. Genetic polymorphisms have been analyzed by several studies as genetic markers for cancer development. A common polymorphism on p53 codon 72, which causes a replacement of an Arginine by a Proline aminoacid, has been suggested as a susceptibility factor for the development of several cancers. We have attempted to evaluate the role of the p53 codon 72 polymorphism in the development of GC in a population from the Northern region of Portugal.
Materials and Methods: A cross-sectional study was performed in 427 individuals, considering both patients with histological confirmed GC (n = 126) and healthy blood donors without evidence of neoplastic disease (n = 281). DNA was extracted from peripheral blood leucocytes and genetic analysis was performed by Real-Time PCR with a TaqMan® SNP Genotyping Assay from Applied Biosystems (Assay C___2403545_10).
Results: Our results revealed and increased frequency of the Pro allele in patients with GC than in healthy donors (46.0% vs 36.8%). We also found an almost three-fold increase risk of GC development among Pro/Pro genotype (p = 0.015; OR = 2.58; 95% CI 1.18–5.66), which is also confirmed by logistic regression adjusted for age (p = 0.015; OR = 2.72; 95% CI 1.21–6.07). Moreover, Pro/Pro homozygous seem to have s shorter median time-to-onset of GC (68.0 months vs 75.0 months; p = 0.030).
Conclusions: TP53 is an import gene in cell regulation and has a major role on cancer development. Previous studies have revealed that the p53 codon 72 polymorphism seems to influence the risk for cancer development since the two polymorphic variants of p53 might different regulations. Our study reveals that p53 codon 72 Pro allele represents a susceptibility marker for GC development and might contribute to the understanding of GC etiology.

343 POSTER

ERK activity in B-1 cells is important for increasing the metastatic potential of murine melanoma cells

E.C. Pérez, J. Machado Jr., M. Mariano, J.D. Lopes. *Escola Paulista de Medicina, Microbiology Immunology and Parasitology, Sao Paulo, Brazil*

Introduction and Objectives: B-1 cells are the most prevalent linage of B cells in the peritoneal and pleural cavities of mice. Previous studies in our group demonstrated that co-cultivation of B-1 cells with B16F10 murine melanoma cells increases the metastatic potential of the latter. However, the mechanisms underlying how B1 cells influence the intracellular signaling pathways involved in this effect have not yet been addressed. Among the signaling pathways, activation of the extra-cellular signal-regulated kinase (ERK) and protein kinase C (PKC) have been correlated with metastatic spread in several cancer types, including melanoma. Therefore, the aim of this work was to investigate whether B1 cells increases the metastatic potential of B16F10 melanoma cells by modulating the activation of ERK and/or PKC in these cells.
Methods and Results: Protein expression and phosphorylation status of PKC and ERK were evaluated in lysates of melanoma cells co-cultured or not with B-1 cells in the presence or absence of pharmacological inhibitors of either PKC (Gö6976) or ERK (PD98059) by western blotting. The biological effects of these inhibitors were studied by experimental metastases assays in vivo. We showed that (1) ERK is constitutively

activated in melanoma cells and is up-regulated after contact with B-1 cells; (2) treatment with either Gö6976 or PD98059 during co-cultivation of B-1 with melanoma cells inhibited the increased metastatic potential induced by B-1 cells; (3) previous treatment of melanoma cells with Gö6976 but not with PD98059 before their co-cultivation with B-1 cells inhibited this effect, and (4) inhibition of ERK activity in B1 cells prevents their capacity to increase the metastatic potential of melanoma cells.
Conclusion: ERK activity in B-1 cells is important for increasing the metastatic potential of murine melanoma cells.
Financial support: FAPESP.

344 POSTER

Effects of cancer cachexia on heart function

J. Springer[1], S. Palus[1], A. Hilmann[1], M. Rauchhaus[1], V.C. Flach[1], K. Hartmann[1], T. Braun[1], K. Schmidt[2], J.M. Argiles[2], S.D. Anker[1]. *[1]Charité Campus Virchow, Divsion of Applied Cachexia Research, Berlin, Germany; [2]Universitat de Barcelona, Department de Bioquimica i Biologia Molecular, Barcelona, Spain*

Background: Cachexia is a very common co-morbidity in cancer patients which drastically reduces quality of life and survival. These patients also develop shortness of breath of unknown reason. Here we assessed the cardiac function in terminally ill rats with cancer.
Methods: Rats (weight approx. 195 g) were inoculated intra-peritoneally with 10^8 AH-130 hepatoma cells (n = 15) or saline (sham, n = 8). Heart function (high resolution echocardiography) was assessed before inoculation and on day 11 of the 14-day protocol. Weight and body composition (NMR-scan) were assessed on day 0 and day 14 after sacrifice (without tumour).
Results: Animals with cancer displayed severe cachexia (164±5 g vs sham: 258±5 g, $p < 0.0001$) and lost fat (−9.1±0.9 g vs sham 9.2±1.1 g, $p < 0.0001$) and lean tissue (−19.6±3.3 g vs sham 42.2±2.6 g, $p < 0.0001$). Heart weight was reduced in cancer animals (515.5±15.8 mg vs 751.9±9.0 mg; $p < 0.0001$). Heart function was significantly impaired in tumour rats compared to sham and compared to pre-inoculation (see table).

	Tumor		Sham	
	Day 0	Day 11	Day 0	Day 11
LV ejection fraction (%)	79±3##	64±2***	78±3	81±2
Fractional shortening (%)	50±3##	36±2*	48±3	51±3
LVD dia (mm)	6.47±0.11	6.28±0.25	6.24±0.25	6.38±0.13
LVD sys (mm)	3.27±0.19##	4.06±0.23*	3.23±0.11	3.12±0.21
PWT dia (μm)	1588±61	1452±78*	1566±59	1732±74
PWT sys (μm)	2598±126###	2032±110***	2508±159	2885±152
LV Vol dia	273±11	251±13	256±28	280±15
LV Vol sys	65±8##	110±6*	83±10	89±5
LVSV (μl)	208±11	141±11*	172±21	190±14
LVmass (mg)	451±16	413±12***	437±25	529±12

LV: left ventricular, D: diameter, dis: diastole, sys: systole, PWT: posterior wall thickness, Vol: volume, SV: stroke volume. *: $p < 0.05$, ***$p < 0.001$ tumour vs sham (t-test). #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ tumour day 0 vs tumour day 11 (paired t-test).

Conclusion: Cardiac function is severely impaired in terminally ill rats with cancer cachexia. The nature of these impairments is comparable to processes in chronic heart failure and hence ACE-inhibition and beta blocker approaches in cancer cachexia may be useful.

345 POSTER

A novel statistical method for analyzing standard tumor growth curves

C. Murayama[1], M. Isozaki[1], A. Shintani[2], A. Kamijo[3], Y. Maeda[3], Y. Ohizumi[4], H. Kobayashi[1], S. Sadahiro[3]. *[1]Tokai University, Clinical Pharmacology, Isehara, Japan; [2]Vanderbilt University, Biostatistics, Nashville, USA; [3]Tokai University, Surgery, Isehara, Japan; [4]Tokai University, Radiation Oncology, Isehara, Japan*

We previously reported that combining preoperative chemo-radiotherapy with oral UFT and intraoperative electron-radiation therapy (IORT) significantly reduced local recurrence and improved prognosis for clinical T3−4Nx rectal cancer (Sadahiro et al., EJSO 2004, Hepato-gastroenterology 2007). A new oral fluoropyrimidine (S-1) has recently shown promise in treating metastatic colorectal cancer with a response rate of 35%. We now present preclinical results concerning the combined therapeutic effect of S-1 and radiotherapy evaluated by standard growth curve analysis methods in a murine model using human colon xenografts. We studied four groups (Control, S-1 only, Radiotherapy only, and S-1 plus Radiotherapy) over a period of 43 days to assess treatment effect on tumor growth time (TGT). Table shows synergistic and radio-modifying measurements of treatment effect when comparing TGT for 3-fold and 5-fold increases in tumor volume. In both cases, therapeutic benefit was observed by combination of S-1 and radiotherapy treatments. Our results showed a synergistic effect for 3-fold growth TGT, but only an additive effect at 5-fold growth TGT assessment. This discrepancy illustrates a fundamental problem with growth curve analysis methods in that results vary depending upon the chosen tumor magnification level. Tumor control assay methods (e.g. TCD_{50}) are more reliable, but the increased time required for data collection and associated costs are often prohibitive. Given these limitations, we are developing novel statistical methods for assessment of treatment effect using all measurements collected for standard growth curve analysis procedures. In this result, our novel statistical approach constructed from the bootstrap method and R for analysis of standard tumor growth curve data points supported an additive effect at 5-fold growth TGT assessment.
Conclusion: In preclinical studies, novel statistical methods using all data points are robust and eliminate problems associated with choosing just one tumor magnification level.

	Synergy ratio		Radio modifying factor		Chemo modifying factor	
	mean	95%CI	mean	95%CI	mean	95%CI
3x#	1.33	1.08−1.58	2.13	1.28−2.98	1.47	1.12−1.82
5x##	1.06	0.73−1.39	1.19	0.18−2.19	1.09	0.6−1.58

#TGT = time required for the initial tumor volumes to grow to 3-times original size. ##TGT = time required for the initial tumor volumes to grow to 5-times original size.

346 POSTER

The role of N-myc downstream regulated gene 1 (NDRG1) in human prostate cancer cells

F. Fares, T. Chosha. *Carmel Medical Center-Technion Faculty of Medicine, Molecular Genetics, Haifa, Israel*

Background: NDRG1 is a member of the N-myc down-regulated gene family, which belongs to the α/β hydrolase superfamily. This gene was previously identified as an up-regulator of cellular differentiation, and was found to be down regulated during colon, breast and prostate tumor progression. NDRG1 is regulated by several factors including androgen, p53 and N-myc. The biological function of NDRG1 and the physiological relevance of its role in the cellular context remain elusive. To clarify the functional role of NDRG1 in prostate cancer cells, we over-expressed the NDRG1 in three prostate cancer cell lines with different differentiation levels.
Methods: Normal trophoblast cells were used in order to clone the NDRG1 cDNA. The coding sequence of NDRG1 was amplified by PCR and cloned into the eukaryotic expression vector, PCDNA 3.1. The plasmid was transfected into human prostate cancer cell lines; LNCaP (well differentiated), DU145 (moderately differentiated) and PC3 (poorly differentiated). Expression of NDRG1 was detected in the transcription and translation levels. The differentiation factors p21 and cytokeratin 8/18 were detected by western blotting.
Results: All the three cell lines expressed NDRG1. High levels were detected in poorly and moderately differentiated whereas low levels were found in the well differentiated cell line.
The cDNAs from all cell lines were used as templates for amplification of the NDRG1 by PCR. Sequencing results of NDRG1 showed that there are no mutations in the coding sequence of the gene.
To investigate the role of NDRG1 in the prostate cancer cell lines, the cells were transfected with molk vector and with NDRG1 expression plasmid. The expression of NDRG1 in the cloned cells was tested by RT-PCR and further confirmed by Western blot.
The NDRG1 transfected cells were examined for the rate of proliferation in comparison to molk transfections. The results revealed that there were no significant differences from the control cells. However, it was found that over-expression of NDRG1 up-regulated p21 and c8/18, therefore causing or progressing differentiation in cancer cell lines.
Conclusions: In this study we showed that human prostate cancer cells expressed basal levels of NDRG1 and that there are no mutations in the coding sequence of the gene. The NDRG1 CDNA was cloned into eukaryotic expression vector. Over expression of NDRG1 by transfection studies into human prostate cancer cells induced the expression of the differentiation markers; p21 and c8/18. However overexpression of NDRG1 did not influence the proliferation rate of the cells. Further studies are needed in order to clarify the role of NDRG1 in prostate cancer cells.