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.

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

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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.

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

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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.

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

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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.

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

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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