75 XRCC3 Thr241Met polymorphism influence on genotoxicity biomarkers frequency in workers occupationally exposed to formaldehyde

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Formaldehyde (FA) is ubiquitous in the environment and is a chemical agent that possesses high reactivity. Occupational exposure to FA has been shown to induce nasopharyngeal cancer and has been classified as carcinogenic to humans (group 1) on the basis of sufficient evidence in humans and sufficient evidence in experimental animals. The gene XRCC3 is involved in homologous recombination repair of cross-links and chromosomal double-strand breaks and at least one polymorphism has been reported in codon 241, a substitution of a methionine for a threonine. The goal of this study is to determine whether there is an *in vivo* association between genetic polymorphism of the gene XRCC3 and the frequency of genotoxicity biomarkers measured by cytokinesis blocked micronucleus assay (CBMN) in occupationally workers exposed to formaldehyde.

It was compared a sample of 56 workers exposed to FA in pathological anatomy laboratories with 85 controls, in order to investigate whether exposure to FA and of genetic polymorphism of XRCC3 Thr241Met is associated with the frequency of genotoxicity biomarkers such as: micronucleus (MN), nucleoplasmic bridges (NPB) and nuclear buds (NBUD) in human peripheral blood lymphocytes (PBL) and MN in buccal mucosa cells.

The mean of all genotoxicity biomarkers in study was higher compared with controls, a statistically significant difference (Mann–Whitney test, $p < 0.001$).

The exposed workers carrying the Thr/Met XRCC3 241 genotype were found to have higher mean in all biomarkers, MN in PBL (5.05 vs 2.92), NPB (3.91 vs 2.00), NBUD (1.50 vs 0.21) and MN in buccal mucosa cells (1.05 vs 0.95) and in controls it was the Met/Met genotype, MN in PBL (1.15 vs 0.70), NPB (0.25 vs 0.14), NBUD (0.20 vs 0.03) and MN in buccal mucosa cells (0.25 vs 0.11).

Multiple regression analysis indicated that the exposure to FA was an important variable affecting the genotoxic response, but the polymorphisms of XRCC3 at codon 241 were not found statistically significant, with the exception for NBUD.

Chromosomal instability has been associated to XRCC3 gene mutation and other genes involved in repair. Manifold studies suggest a direct role of XRCC3 Thr241Met polymorphism maybe associated, but not significant, to a reduce capacity of DNA repair. This study was verified that carriers of Thr241Met polymorphism have higher means of genotoxicity biomarkers.

76 Lifestyle factors influence in the frequency in buccal micronucleus

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Genomic damage is probably the most important fundamental cause of development and degenerative disease. It is also well established that genomic damage is produced by environmental exposure to genotoxins, medical procedures (e.g. radiation and chemicals), micronutrient deficiency (e.g. folate), lifestyle factors (e.g. alcohol, smoking, drugs and stress), and genetic factors such as inherited defects in DNA metabolism and/or repair. Tobacco smoke has been associated to a higher risk of development of cancer, especially in the oral cavity, larynx and lungs, as these are places of direct contact with many carcinogenic tobacco's compounds. Alcohol is definitely a recognized agent that influence cells in a genotoxic form, been citied as a strong agent with potential in the development of carcinogenic lesions. Epidemiological evidence points to a strong synergistic effect between cigarette smoking and alcohol consumption in the induction of cancers in the oral cavity. Approximately 90% of human cancers originate from epithelial cells. Therefore, it could be argued that oral epithelial cells represent a preferred target site for early genotoxic events induced by carcinogenic agents entering the body via inhalation and ingestion. The MN assay in buccal cells is used to study cancerous and precancerous lesions and to monitor the effects of a number of chemopreventive agents.

The study was carried out in Portugal in a sample 85 subjects without any occupational exposure and was asked about their smoking and drinking habits. The evaluation of genotoxic effects was conducted by applying MN test in exfoliated cells from buccal mucosa. The data were analyzed statistically using Kruskal-Wallis.

The analysis of the interaction between the alcohol consumption and smoking habits showed statistical signification ($p = 0.043$) with a stronger effect from tobacco smoke than alcohol consumption.

Epidemiological evidence points to a strong synergistic effect between cigarette smoking and alcohol consumption in the induction of cancers in the oral cavity.

77 Germline allele-specific expression of TGFBR1 as a susceptibility factor for sporadic colorectal cancer

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Germline Allele-Specific Expression (ASE) of the transforming growth factor-B type I receptor gene (*TGFBR1*) has been proposed to be a major risk factor for colorectal cancer (CRC). Germline ASE resulted in specific down-expression of one of the *TGFBR1* alleles, occurring in about 20% of informative familial and sporadic CRC cases. Nevertheless, the implication of ASE appeared to be present mainly in familial CRC cases. Recently, it has been reported that the ASE is a very rare event.

The aim of the present study was to assess the implication of ASE in *TGFBR1* with CRC, in the Spanish population.

We tested the insertion/deletion polymorphism *TGFBR1*9A/6A*. This polymorphism has been reported to be in linkage disequilibrium with the ASE phenomenon in *TGFBR1*. A total number of 1116 individuals were genotyped for this polymorphism: 409 controls; a cohort of 311 individuals with suspicion of Lynch syndrome (Bethesda guidelines), and 396 sporadic CRC individuals. We found 180 heterozygous individuals (16%): 67 for the control group, 52 for the hereditary and 61 for the sporadic CRC cohorts. These informative cases were then analyzed for ASE in *TGFBR1*. ASE experiments were performed by PCR-capillary electrophoresis using cDNA samples from peripheral blood (controls and hereditary cases), or normal colorectal tissue (sporadic CRC). We tested each sample in triplicates, and included a five point-standard curve constructed with dilutions of cDNAs from **9A* and **6A* homozygous individuals. Besides, three heterozygous cases were used as calibrators to correct the potential inter-experimental variations. Relative quantification of allele expression was extrapolated from the standard curve. Thus, highly quantitative results were obtained. Cases were considered positive for the presence of ASE if demonstrating an allelic expression ratio <0.67 or >1.5.

We detected three control individuals displaying modest ASE (4.5%). None from the hereditary CRC group showed ASE; and found evidences of ASE in 9 of the sporadic CRC cases (14.7%). The calculated OR was 3.7 (95% CI: 1.0–13.2). Seven of these cases had a relative over-expression of the **6A* allele and two cases over-expressed **9A* allele. No association was found between ASE and age, sex, tumour location or stage.

Our results suggest that ASE may be a risk factor for sporadic CRC in the Spanish population. Further studies should be considered to confirm these findings and unveil the mechanisms involved in the germline ASE in *TGFBR1*.

78 Analysis of raf kinase inhibitor protein (RKIP) expression as a prognostic marker for glioblastomas

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Background: Glioblastomas (GBM) are the most common and malignant form of primary adult brain tumours, with a very poor prognosis and with no effective treatment options available. The raf kinase inhibitory protein (RKIP) negatively regulates the Raf/MEK/ERK pathway by interfering with the activity of Raf-1. Besides regulating intracellular signaling cascades, RKIP has been shown to be also involved in cell cycle regulation. Down-regulation of RKIP has been associated with tumour progression and metastasis, being categorized as a metastasis suppressor gene. More recently RKIP has also shown to be an important prognostic factor in some neoplasms, such as gastric, prostate and colon cancer. The expression levels and prognostic role of RKIP protein in GBMs is completely unknown.

Methods: RKIP expression levels in 138 GMB samples and 18 normal brain samples were studied by immunohistochemistry. Prognostic impact of RKIP was assessed by log-Rank test using Kaplan–Meier curves.

Results: We found that RKIP was highly expressed in all the 18 normal brain tissues studied (12 of them are normal tumour-adjacent tissues). Concerning GBM tumours, we observed RKIP positive expression in 89.1% (123/138) of cases and in the remaining 10.9% (15/138) cases, RKIP expression was scored as negative. A trend for a poor prognosis was observed for patients with RKIP negativity, however statistically significance was not reached ($p = 0.096$). No associations were found with other clinic-pathological data such as age,

gender and treatment (79 of the patients were treated with standard stupp protocol).
Conclusions: This is the first study assessing RKIP expression levels in GBMs. We conclude that, in contrast to other solid tumours, the percentage of RKIP negative GBM cases is low and that the absence of RKIP expression seems not to be associated with poor survival in GBMs patients.

79 Association of JAK-STAT pathway related genes with lymphoma risk

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Background: Non-Hodgkin Lymphoma (NHL) belong to the seventh most common cancer in Europe and constitute the tenth most commonly diagnosed cancer worldwide. Apart from risk factors such as certain infectious agents and immunodeficiency syndromes, genetic variants related to immunity have been associated with lymphomagenesis. Previous studies suggested an important role of the JAK-STAT signalling pathway in tumour development. Therefore, we explored genetic variants in the JAK-STAT pathway associated with lymphoma risk.
Material and Methods: In total, 1481 lymphoma cases and 1491 age, sex and study centre matched controls of the EpiLymph study, a multi-centre case-control study on the aetiology of lymphomas among adults in Europe, were genotyped for 1536 single nucleotide polymorphisms (SNPs) using GoldenGate BeadArray™ Technology (Illumina, San Diego, CA).
Association between selected SNPs and haplotypes of the JAK-STAT pathway and risk of Hodgkin lymphoma (HL), NHL and most frequent NHL subtypes were estimated by calculating Odds Ratios (OR), the corresponding 95% confidence intervals (CI) and p-values using unconditional logistic regression using SAS (version 9.2).
Results: Among 220 relevant JAK-STAT pathway SNPs, polymorphisms in several genes (*STAT3, STAT6, IFNG, BMF, STAT5A*) were significantly associated with lymphoma risk. Reduced risk for NHL overall and diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL) were seen in association with seven *STAT3* SNPs in high linkage disequilibrium and respective haplotypes. Variant rs4103200 conferred an about 20% reduced NHL risk (OR_{CG} 0.79, 95% CI 0.66–0.94, OR_{GG} of 0.78, 95% CI 0.66–0.91, ptrend = 0.002). Reduced risk in association with this variant was also evident for DLBCL and FL. A putatively functional variant in STAT6 previously associated with IgE levels (rs324011) was inversely associated with HL risk ($OR_{TC/CC}$: 0.61, 95% CI 0.45–0.82, p = 0.001).
Conclusion: Our results implicate a relevant role of the JAK-STAT signalling in the development of lymphoma. Furthermore, our data support previously found associations between genetic variants of STAT genes and immune phenotypes.

80 NAT2 gene polymorphisms and risk susceptibility to childhood acute leukemia

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Background: Maternal exposures to a variety of carcinogens, such as those found in cigarette smoke, diet, drugs and environment during pregnancy play a role in the etiology of childhood leukemia. These compounds are acetylated by NAT2 resulting in activation or detoxification of a variety of heterocyclic amine drugs and carcinogens. Individuals may be classified as rapid or slow acetylators according to the rates at which drugs are acetylated by NAT2. Epidemiological studies suggest that the *NAT2* acetylation polymorphisms may modify the risk of developing childhood acute leukemia. To identify the distribution of *NAT2* polymorphisms in Brazilian children and the effects of the polymorphisms on the development of childhood acute leukemia, we performed a case-control study.
Material and Methods: DNA samples from a total of 194 childhood acute leukemia cases and 285 age-matched controls were analyzed. The genotypes of polymorphisms were assessed by PCR-RFLP and the phenotypes of subjects were defined as fast- or slow-acetylators based on their genotypes. Unconditional logistic regression methods were used.
Results: Point mutations at positions 191 and 341 were more frequent in children with acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) than in control group (7.3% and 9.7% vs. 3.7%, respectively of 191 position; and 46.5% and 48.6% vs. 34.3%, respectively of 341 position). We found an association of *NAT2* slow-acetylation alleles and increased risk of ALL and AML (odds ratio [OR] = 2.29; 95% confidence interval [CI], 1.69–3.11; and OR = 2.80; 95% CI, 1.55–5.07; respectively), due to a high frequency of *NAT2*5A* allele within the leukemia group. On the other hand, because of the underrepresentation of *NAT2*4* and **12A* alleles in leukemia group, *NAT2* rapid-acetylation alleles were associated with a protection role of ALL and AML (OR = 0.44; 95% CI, 0.32–0.59; and OR = 0.36; 95% CI, 0.20–0.65; respectively).
Conclusions: In conclusion, ours findings suggest that *NAT2* slow-acetylation phenotype modifies the risk of ALL and AML development in Brazilian children.

81 Catumaxomab: a causal therapy for malignant ascites

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Background: Malignant ascites is a typical complication of several epithelial tumours due to the spread of malignant cells into the peritoneal cavity and is associated with a poor prognosis. Catumaxomab (Removab®, anti EpCAM x anti-CD3) is the first approved causal therapy for malignant ascites. The safety and efficacy data reported here are from the international pivotal trial (NCT00836654).
Material and Methods: The trial was a two-arm, randomized (2:1) open-label, phase II/III study. Patients (pts) received either paracentesis plus catumaxomab or paracentesis alone (control). Following randomisation, pts were stratified into groups with ovarian and non-ovarian cancer. Catumaxomab treatment consisted of 4 i.p. infusions of 10, 20, 50, and 150 μg on day 0, 3, 7, and 10. The primary endpoint was puncture-free survival, defined as time to first therapeutic puncture or death, whichever occurred first. Main secondary endpoints were time to next therapeutic puncture, and overall survival (OS).
Results: Overall, 258 pts (129 ovarian and 129 non-ovarian cancer pts) were randomized. Statistical significant improvement was demonstrated for the catumaxomab group for both median puncture-free survival ($p < 0.0001$, 46 days versus 11 days for control), as well as time to first need of therapeutic puncture with 77 versus 11 days in the control group, ($p < 0.0001$). The study was neither designed nor powered for overall survival, however the pooled analysis showed a positive trend for catumaxomab, and statistical significant OS was shown in the gastric cancer subgroup. The benefit of catumaxomab was confirmed independent of the primary tumour or prognostic factors like number of previous chemotherapies or presence of distant metastases. Catumaxomab was well tolerated with more than 80% of the pts receiving all four infusions. The observed safety profile was expected due to the mode of action and consisted of cytokine release related symptoms like pyrexia, nausea or vomiting. The side effects were generally mild to moderate and fully reversible.
Conclusions: Catumaxomab treatment resulted in a clear clinical benefit in patients with symptomatic malignant ascites. Based on these results catumaxomab (Removab®) was granted approval in the European Union April 2009 for the treatment of malignant ascites in patients with EpCAM-positive cancer.

82 PET and MRI determination of the effects of Sunitinib on hypoxia and vasculature on a rat brain tumour model

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Background: The use of anti-angiogenic treatments has proven highly efficient for solid tumour including brain tumours [1,2]. However, it has also been shown that these treatments led, paradoxically and at least transiently, to a normalization of the vasculature instead of its disappearance [3]. Although the normalization process should result in more functional vasculature associated with a decrease in tumour hypoxia, until now, no direct proof has been brought in vivo for a correlation between hypoxia and vasculature following an anti-angiogenic treatment. Consequently, the aim of the present study was to analyse, using MRI and PET imaging, the effects of an anti-angiogenic treatment (Sunitinib) on tumour growth, vasculature and hypoxia.
Methods: A rat brain tumour model has been used after inoculation of C6 glioma cells in Wistar rats (5.10^4 cells/3μl). Rat received Sunitinib orally from Day 17 to Day 24 daily (20 mg/Kg) and underwent MRI and PET imaging on Day 17 and 24. MRI was performed on a 7 teslas magnet (Bruker) using (i) T2w RARE imaging; (ii) T2 maps; (iii) T2* maps; (iv) ADC maps and (v) T1w imaging. T2 and T2* maps were performed prior and after an intravenous injection of Sinerem (200 μmol/kg; Guerbet SA) to compute CBV and VSI maps [4]. Hypoxia detection was performed using a microPET imaging (Inveon, Siemens) 120–150min after injection of ^{18}F-FMISO (600 μCi/rat).