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,