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In vitro functional analysis of missense mutations in hMLH1 and hMSH2 identified in Danish patients with colorectal cancer

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The present study, aim at the functional characterisation of rare missense mutations in hMLH1 and hMSH2, identified in Danish patients with colorectal cancer (CRC).

Germline mutations in hMLH1 and hMSH2 predispose to colorectal cancer. Approximately 30-40% of the patients with hereditary non polyposis colorectal cancer (HNPCC) carry mutations in these genes. Genotyping of hMLH1 and hMSH2 in Danish patients with HNPCC and in non-HNPCC families having a “strong” familiar accumulation of CRC have led to the identification of several missense mutations of unknown significance. Recently, we have performed a population based study to analyse the frequency of selected missense mutations in the Danish population. The results of that study showed that half of the analyzed missense mutations are very rare and are most likely only present in the families where they were identified originally. Some of the missense mutations were located in conserved regions of the hMLH1 and hMSH2 proteins indicating a relation to disease development. To further elucidate the pathogenicity of 10 (2 in hMLH1 and 8 in hMSH2) of these missense mutations, we carried out in vitro functional analyses. The missense mutations were constructed using site-directed mutagenesis and analyzed for their effect on protein expression/stability and repair efficiency. Eight missense mutations resulted in proteins with expression/stability and repair efficiency similar to the wild type. One missense mutation caused reduced protein expression/stability and one caused both reduced protein expression/stability and repair deficiency. The results of the functional analysis were correlated with clinical data on the families carrying the missense mutations. Three missense mutations were present in HNPCC families, 6 were found in non-HNPCC families and 1 was identified in a HNPCC family that also carried another disease causing mutation in hMSH2. The missense mutation resulting in both reduced protein expression/stability and repair deficiency was found in one of the HNPCC families. In conclusion, only 1/10 missense mutations displayed repair deficiency and could be classified as pathogenic. No final conclusion can be drawn on the one mutation causing reduced protein expression/stability. Given that hMLH1 and hMSH2 also play a role in other cellular processes than mismatch repair, some of the remaining missense mutations may still be predisposing mutations.

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BRCA1 and BRCA2 sequence variants in healthy women in Croatia analyzed by high-resolution melting and in silico analysis of variants of unknown clinical significance

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BRCA1 and BRCA2 are the major hereditary breast and/or ovarian cancer predisposing genes and their mutations increase the risk of developing cancer. Mutations in either of these tumor suppressor genes are associated with both sporadic and hereditary forms of breast cancer. At least ten percent of cases are attributable to familial inheritance. In Croatia, more than 2200 new cases of breast cancer are diagnosed each year, and about 900 women die of this malignancy.

We analyzed the distribution and occurrence of sequence variants in BRCA1 and BRCA2 genes on a healthy population of women in Croatia in an attempt to distinguish non-tumorigenic from tumorigenic changes in genomic sequences of BRCA1 and BRCA2 genes. The screening was performed by high resolution melting approach, which is based on differences in melting curves caused by variations in nucleotide sequence; detected variants were confirmed by direct sequencing.

In total, we analyzed 230 samples for BRCA1 gene and 140 samples for BRCA2 gene. We found 21 different sequence variants in BRCA1 (2 novel) and 36 variants in BRCA2 gene (7

At present, almost half of all BRCA1 and BRCA2 sequence variants found are unclassified variants (UVs) so their clinical significance is unknown or uncertain. That represents problem for risk assessment in genetic counselling. After revealing BRCA1 and BRCA2 sequence variants in healthy Croatian females, our aim was to find fast in silico method for assessing preliminary clinical significance of UVs newly found in patients.

We used different publicly available programs and web-based tools to identify UVs that may have deleterious effects with respect to different biomolecular functional categories (splicing regulation, transcriptional regulation, nonsynonymous amino acid SNP effect…) so their clinical significance in cancer etiology could be assumed.

We have found that several sequence variants with nonsynonymous amino acid change could have possible impact on the structure and function of a BRCA1 and BRCA2 proteins. Synonymous amino acid changes (silent mutations) could have impact on splicing regulation by disrupting exonic splice enhancers. Intronic sequence variants showed no potential impact on splicing because nucleotide changes at that positions likely make no changes in consensus splice sites.

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Major genetic risk factors in familial breast and colorectal cancers in North Tunisia

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Introduction: Cancers are multifactor diseases, due to additive effect of genetic and environmental risk factors, leading to somatic mutations in oncogenes and tumor suppressor genes. However, familial presentation of the disease led to the discovery of major genes mutated at germinal level. Oncogenetic consulting allows to identify families at risk of cancer as well as the search of mutations in the incriminated major genes, in proband and in healthy relatives. In this work we focused on the population of Northern Tunisia, considering familial breast and colorectal cancers, with the aim to set up oncogenetic consulting, since no previous studies have been performed on the subject.

Methods: We identified 36 families of Breast cancer and 6 cases of Familial Adenomatous Polyposis (FAP). Starting from blood of probands, we analyzed by direct sequencing all the coding regions of the major genes: BRCA1 and BRCA2 for breast cancer families and APC and MHY genes for FAP.

Results: The results obtained allowed to reveal 6 deleterious mutations at heterozygous state, in 7 unrelated families. These frameshift mutations generate a truncated protein. Four mutations are observed in BRCA1 (c.211dupA, c.2551delG, c.5266dupC, c.4041delAG) among which one is new and two in BRCA2 (c.5382insC, c.1309del4). Phenotype/ genotype correlation showed that half of the cases with breast and ovarian presentation carry a deleterious mutation in BRCA1, while half of families with male cases present a deleterious mutation in BRCA2.

For the 6 cases of FAP, we have identified 3 deleterious mutations in APC gene at heterozygous status (3183_3187 delACAAA, c.1636_1639 delAGTG, c.2514 G>T) and one at homozygous state in MYH gene (c.1145 G>A). Half of these four mutations are newly described.

Conclusion: These results show the absence of recurrent mutations in cancer Tunisian families. For 29 breast families studied and 2 FAP, we do not show any obvious deleterious mutations in the analyzed genes. However, we identified UV mutations and SNP in these genes and it cannot be excluded that some SNP haplotypes are responsible for the disease. Moreover, other major or minor factors could be identified.

Additional functional and genetic analysis is necessary to conclude upon these questions.

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Apoptosis and senescence are triggered by Lamellarin-D in cancer cells

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The marine alkaloid lamellarin-D (Lam-D) shows interesting anticancer properties against cell lines. We previously discovered that Lam-D is a potent poison of human topoisomerase I (Cancer Res. 2003 Nov 1; 63(21):7392-9) and also demonstrated that cancer cell mitochondria are direct pro-apoptotic targets for Lam-D (Cancer Res. 2006 Mar 15; 66(6):3177-87). Here, the cellular consequences and mechanisms of Lam-D were investigated in vitro. Using several cancer cell lines including camptothecin –resistant topoisomerase I mutated leukemia cells, we demonstrated that the inhibition of topoisomerase I by Lam-D mediated DNA damage as measured by gamma H2AX staining, which in turn induced a classical anti-proliferative effect associated with G2/M cell cycle arrest. Cellular levels of the p21waf1/cip1 protein and p21waf1/cip1 mRNA were increased through a p53-independent pathway. In this context, the inhibition of topoisomerase I by Lam-D treatment induced a senescent-like phenotype mediated by reactive oxygen species (ROS). In contrast, at higher concentrations (at the micromolar range), Lam-D promoted a rapid cell death irrespective of its nuclear effect on topoisomerase-I. Using cancer cells lacking mitochondrial DNA we also showed that mitochondrial ROS were required for Lam-D-induced apoptosis. In conclusion, our study indicates that Lamellarin–D may act as a potent pro-oxidant agent responsible for both senescence and apoptosis in cancer cells.

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Polymorphisms in glutathione S-transferases increase colorectal cancer risk

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Background: Colorectal cancer (CRC) is one of the most frequent malignancies. CRC incidence in Czech republic ranks third worldwide. The majority of CRC are sporadic cases accounting for over 85% of all cases. High mortality is probably caused by diagnosis of CRC in advanced stages when prognosis is bad. Low-penetrance genes, e.g. genes coding xenobiotic-metabolizing enzymes (XME) belong to relevant factors modifying an individual susceptibility to CRC. This hospital-based case-control study followed functional polymorphisms in XME as potential CRC susceptibility factors.

Materials and methods: Polymorphisms in glutathione S-transferases GSTM1 (deletion), GSTT1 (deletion), GSTP1 (Ile105Val), NADP(H)-quinone oxidoreductase NQO1 (Pro187Ser), cytochrome P450 CYP1B1 (Asn453Ser and Leu432Val) and epoxide hydrolase EPHX1 (Tyr113His and His139Arg) were assessed by PCR RFLP in 784 CRC patients and 493 unrelated colonoscopy cancer-negative controls of Czech Caucasian origin.

Results: Heterozygous genotypes in CYP1B1 (OR=0.68, CI=0.52-0.90, P=0.007) and GSTP1 (OR=1.30, CI=1.00-1.67, P=0.049) and deletion polymorphism in GSTM1 (OR=1.31, CI=1.04-1.66, P=0.024) were associated with CRC risk. The risk was further enhanced in carriers of combination of variant alleles in GSTM1 and GSTP1 (OR=1.80, CI=1.24-2.60, P=0.002) and in smokers carrying the deletion in GSTM1 (OR=2.08, CI=1.42-3.04, P<0.001). Polymorphisms in EPHX1, NQO1 and GSTT1 were not associated with CRC risk.

Conclusions: The results of this study further support the concept that interaction between metabolism and environment contributes to colorectal carcinogenesis. Glutathione S-transferases should be further followed as candidate risk-associated genes in carefully controlled studies with special attention to exposure status of participants.

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Gene expression signatures in BALB3T3 fibroblasts exposed to cobalt micro/nano-particles and cobalt ions

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Nanoscale materials are increasingly employed in several industrial applications as well as in biology and medicine. Despite their wide predicted utilization, little research has been carried out on the potential toxicity of metal-based micro/nano-materials. To explore possible biological effects relevant to toxicity that could depend on the physical characteristics of compounds, we investigated the global gene expression patterns of BALB3T3 A31-1-1 cells exposed or not to 1 microM concentrations of cobalt microparticles (Co-micro), cobalt nanoparticles (Co-nano), and cobalt ions (Co++). All exposures were carried out at semi-confluence for 72 hrs, as determined based on toxicity assays performed for toxic metals. Global gene expression analyses were carried-out using 70 mer murine 13.443 oligonucleotide microarrays (Operon version 1.1). Data were normalized using the MIDAS program and elaborated using the SAM program. Molecular interaction networks were constructed applying gene ontology, canonical pathway and functional network analyses with the Ingenuity Pathways Analysis (IPA) tools software (http.//www.ingenuity.com).This showed that the involved molecular pathways differed according to exposure: the interferon, apoptosis, JAK/STAT and death receptor signalling pathways were involved following Co-nano exposure; nucleotide sugar metabolism, G1/S cell cycle check point, hypoxia signalling and ubiquitination following Co-micro exposure; no statistically significant alterations in molecular pathways were detected after exposure to Co++. Real-Time PCR analysis confirmed that exposure to Co-micro, Co-nano, and Co++ down-modulated Rab18, a member of the RAS oncogene family, and that Co-micro exposure down-regulated CAV2, a key constituent of caveolae, integral membrane components. Rab18 and CAV2 represent candidate biomarkers of possible effects on the regulation of intracellular membrane trafficking after Co-micro, -nano, and -ions exposure. In addition, our results show that exposure to Co-micro, Co-nano and Co++ differentially activates cellular pathways that control defense and repair mechanisms.

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Lung cancer in never smokers in Florida: 1981-2003

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Lung cancer is the leading cause of cancer death among men and women. (1) Recently there has been increased awareness of lung cancer in never smokers, especially women (2-5). It has been estimated that 10% of lung cancers occur among never smokers (2). Studies have been inconsistent however in finding gender differences in lung cancer risk (2, 6, 7).

The study investigated whether lung cancer is increasing among never smokers and if any increase is primarily among female never smokers. The study also considered the related issues of gender differences in age at diagnosis, stage, grade and histology.

This study included histologically confirmed primary carcinoma of the lung in the Florida Cancer Data System, 1981- 2003. Data in the de-identified file included gender, race, smoking status, age at diagnosis and tumor characteristics. Odds ratio were used to estimate relative risks, logistic regression was used to calculate adjusted odds ratios and ninety-five percent confidence intervals for the odds ratios.

In Florida, during the study period, 10618 histologically confirmed primary carcinomas of the lung were reported among men and 13134 among women. The number of lung cancers among never smokers increased from 4841 for 1981-85 to 6061 for 1996-2000. Despite this increase, the proportion of lung cancers that occurred in never smokers decreased over time for each major cell type, among men and women. Differences by gender remained however and a never smoker who developed lung cancer was more likely to be female than male. The odds ratio for being a never smoker if female was 1.93 (1.87-1.98) that of a male. Odds ratios were greatest for adenocarcinoma OR=2.28 (2.19-2.37) and least for small cell carcinoma OR=1.14 (1.06-1.23). Further analyses are underway.

As lung cancer continues to increase among women, the proportion of cases among never smokers decreases while the absolute number of lung cancers increases, resulting in the perception of increased risk of lung cancer among women who never smoked.

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