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The influence of the Ataxia-Telangiectasia mutated G5557A polymorphism on cervical cancer development

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Introduction: DNA double strand break (DSBs) is one of the most serious threats to the integrity of the eukaryotic genome. One key protein that responds rapidly to this threat is Ataxia-Telangiectasia Mutated (ATM) protein kinase.

Integration of Human Papillomavirus (HPV) DNA into the host genome is recognized as an essential step for the cell transformation and cervical cancer development. The aim of this study was to analyse the role of the ATM G5557A polymorphism in cervical cancer development.

Material and Methods: We developed a retrospective study considering 484 cervical specimens of women from the northern Region of Portugal, using a real-time polymerase chain reaction methodology (assay C__26487857_10). Statistical analysis was performed using SPSS software.

Results: No statistically significant differences were found, regarding the influence of the G5557A polymorphism with cytological classification, the presence or absence of HPV16 or other oncogenic high-risk HPV types ($p>0.050$). However, the ATM 5557A allele was found to influence the age at which the progression from LSIL to high-grade or invasive cervical cancer occurs (43.0 vs 59.0 years old; $p=0.001$).

Conclusion: Our study reveals, for the first time, that ATM 5557A allele may influence cellular transformation leading low-grade lesions to progress to high-grade or invasive cervical cancer.

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Association of Simian Virus 40 (SV40) with human breast carcinomas in Tunisian women

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Background: Breast carcinoma is the most common women's cancer worldwide, and the major cause of cancer mortality among women. Breast carcinoma is a multistep disease, and viral infection may play a role in one or more of the steps in its pathogenesis. Simian Virus 40 (SV40), which belongs to the polyomavirus family, is able to induce various specific tumor types in hamsters and other rodents, and to transform cells from different species. Over the last several years, different laboratories have reported the presence of SV40 in several types of human tumors. The present study was undertaken to investigate whether the SV40 is implicated in human breast carcinoma.

Methods: One hundred and nine invasive breast ductal carcinoma cases from Tunisia were tested for the presence of SV40 on paired tumor and normal frozen tissue specimens. Detection of SV40 genomic DNA was performed by polymerase chain reaction (PCR) assays targeting the Tag, the regulatory, and the VP1 regions. Immunohistochemistry was used to assess estrogen receptors, progesterone receptors, HER2, and P53 expression. The expression of large and small T-antigens of SV40 was investigated using Pab108 monoclonal antibody. We also examined the relationship between the presence of SV40 and clinicopathological data.

Results: Specific SV40 DNA sequences were detected by PCR in 24/109 (22%) of tumor and in only 2/109 (1.8%) of the matched non-tumoral tissues. Immunohistochemistry study has confirmed the presence of SV40 in tumor cells in all SV40 positive cases. Regarding clinicopathological data, we found that SV40-postive tumors were more frequently detected in patients aged over 50 years than in younger patients (34.7% vs. 12.7%; $p = 0.006$). With regard to immunohistochemical parameters, a significant correlation was found between SV40 presence and the accumulation of P53 protein (32.7% vs. 13.3%; $p = 0.015$). Furthermore, SV40 presence is inversely correlated with HER2 overexpresion (3.7% vs. 28%; $p = 0.008$).

Conclusions: In summary, our study demonstrates the presence of SV40 in a significant proportion of human breast carcinomas and provides data supporting a functional effect for this virus in these tumors. Further studies are required to elucidate the role of this virus in the pathogenesis of breast carcinoma. Additional investigations are necessary to evaluate the prevalence of SV40 in high risk breast carcinoma populations.

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Does cooked Western diet initiate colon carcinogenesis in rats?

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Background: Only a handful of studies have investigated the role of nutritional factors on colon carcinogenesis without prior chemical initiation. A synthetic Western diet (WD), representative of nutrient imbalance in Western populations, induces colonic tumours in mice, but not in rats (Newmark et al., 2001). Also, a heat-treated diet initiates aberrant crypt foci (ACF), putative precursors of colon cancer, in the colon of F344 rats (Dépeint et al, submitted). Hypothesis: We speculated that WD heated for 1h30 at170°C (CWD) can induce more colonic ACF than regular WD (NWD). We have also tested if ACF initiation was due to (i) Maillard compounds (MAL), (ii) lipid peroxides (PER), or (iii) vitamin deficiency (VIT), each achieved by heating or depletion of specific fractions of the WD diet. Material and Methods: F344 rats (n=20 per group) were fed one of the five WD-derived diets described above or a control AIN76 diet (AIN) for 130 days, without giving a colon carcinogen. Faecal water and urine samples were assayed for lipid peroxides and cytotoxicity. After 130d on experimental diets rats were sacrificed and their colon scored for inflammation and ACF formation. Lipid peroxides and mutagenic factors formed during the heating process were also measured directly in the diet. Results: Rats fed the CWD diet gained less weight than other rats, and showed obvious symptoms of rectal inflammation. The end of study is planned in April 2008, and full biomarkers and ACF data will be presented in Lyon, July 2008. Conclusion: Faecal thiobarbituric acid reactive substances (TBARS) and cytotoxicity have been correlated with ACF outcome (Pierre et al, 2003). Endogenous biomarker data collected strongly support the hypothesis of an increased risk associated with heat treatment of the diet. They also suggest a possible role of inflammation during the process, and that Maillard compounds may play a more important role in colon carcinogenesis than lipid peroxidation. No clear risk of vitamin deficiency has been found so far. However ACF data are needed for firm conclusion, and will help discriminate between the factors. Cooking is a common step in food processing. This study will shed some light on the risk associated with cooked foods and the investigation goes beyond the traditional culprits such as carcinogens produced during meat cooking, which are not involved here.

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E2F2 - a potential new molecular marker for colon carcinogenesis

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Colon cancers are classified in tumours of microsatellite instability phenotype (MIN) which results from mismatch repair gene alterations and in tumours of chromosomal instability phenotype (CIN) which present mostly aneuploidy. The genetic alterations found in CIN tumors are greatly heterogeneous suggesting that the signaling pathways required for colon carcinogenesis could be more complex than currently proposed. This could account for the development of tumors resistant to chemotherapy and for the difficulty to find efficient anticancer agents. Also, the heterogeneity of the cohorts analyzed could explain why despite numerous published studies, no molecular markers for tumour progression and metastatic invasion or for sensitivity to chemotherapy are available. To define a genomic profile of alterations linked to the tumour progression, a pan-genomic CGH array (aCGH) to detect genome deletions and amplifications at a high level of resolution has been performed on 62 CIN colon tumours. Among several alterations, a microdeletion in 1p36.12 region has been observed for 46/94 (49%) of the tumors of all clinical stages. This genomic region includes several genes and among them, E2F2. E2F family members are key regulators of the cell cycle, and the disruption of the pathway controlling E2F appears to be a necessary step in human oncogenesis as E2F family members can activate or repress transcription, stimulate or inhibit proliferation, and promote or suppress apoptosis depending on the context. Real-time PCR showed that deletion of the E2F2-targeting probes correlated with a decreased gene copy number and as a complement quantitative multiplex PCR of short fragment (QMPSF) showed that only one copy of the gene was deleted. Sequencing of the 8 exons did not evidenced gene mutation but a polymorphism in exon 4 was found. At the level of the gene gene expression, E2F2 mRNA was decreased in distal tumors but increased in proximal tumors. As E2F1, E2F2 and E2F3 have redundant functions in controlling cell cycle progression, messenger expression for E2F1 and E2F3 were also

evaluated but no compensation was observed. The functional impact of the deletion of E2F2 remains to be demonstrated in colon cancer by functional approaches like Gain- and Loss-of-function, in human colon cell lines and in vivo in human tumours xenografted into nude mice. Furthermore, as the clinical data are available, the potential prognostic value of this deletion, associated or not to other(s) alteration(s), will be evaluated.

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CD4+ t cells stimulated in the presence of dendritic cells transduced with human papillomavirus GFP-L1, showed a decreased progression towards s and m phase of the cell cycle

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Background: To demonstrate the effect of Human Papillomavirus type 16 (HPV) capside proteins (L1 and L2) on to arrest the cell cycle on T cells obtained from normal human blood.

Materials: HPV16 L1 and L2 coding sequences were cloned in a pENTR vector by means of the of the pENTRR /SD/D-TOPOR (InVitrogen). The expression cassette containing L1 or L2 were subcloned into the pHRLVGateway (GatewayR Invitrogen). GFP-L1 or GFP-L2 expressing plasmids were co-transfected with plasmids coding for HIV gag/pol. HekFT cells were used as packaging cell lines. CD11c+ cells were purified from PBMC with CD1c (BDCA-1)+ bound magnetic beads using the Dendritic Cells Isolation Kit (Miltenyi Biotech GmbH) according to manufacturer's instructions. Dendritic cells (DCs) were cultured with lentiviral supernatans and transduction efficiency was evaluated by FACS analysis. PBMCs were stimulated with 1µg/ml PHA (SigmaR) and maintained for 5 days in DMEM complete medium containing 10U/ml IL2. Subsequently CD4+ or CD8+ T cells were purified by immunomagnetic positive selection using anti-human CD4 or anti-human CD8 IMag DM particles respectively (BD Biosciences). Cell cycle analysis was performed in a CyanADP-MLE flow cytometer (DakoCytomation).

Results: T cells stimulated in the presence of GFP-L1 transduced DCs, showed a decreased progression towards S and M phases of the cell cycle, with a higher percentage of cells arrested in G0/G1 phase. In contrast T cells stimulated in the presence of GFP-L2 transduced DCs did not show significant. Purified CD4+ T cells failed to proliferate upon stimulation with PHA when the accessory DC expressed GFP-L1, while they progress through the cell cycle when stimulated in the presence of GFP expressing GFP-L2. A defect in progression to S and M phases could be due either to a cell cycle block or to cell death. There are no significant differences in cell death when T cells stimulated in the presence of GFP expressing DCs were compared with those stimulated in the presence of GFP-L1 expressing DCs.

Conclusions: T cells, stimulated with PHA in the presence of GFP-L1, but not GFP-L2 expressing DCs showed a reduced progression to S and M phases of the cell cycle. CD4+ purified cells stimulated with PHA in the presence of GFP-L1, but not GFP-L2 expressing DCs showed a reduced progression to S and M phases of the cell cycle. T cells stimulated with PHA in the presence of GFP-L1 expressing DCs do not show a significant increase in cell death.

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Molecular subtyping of 159 stable microsatellite colon polyps

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Introduction: The two main pathways of carcinogenesis currently allowed in colon cancer comprise genomic aberrations whose accumulation is correlated with the transition adenoma-carcinoma. However no predictive factor useful in clinic has been validated. This absence of consensus comes mainly from the heterogeneity of the patients included in the studies, due either to the tumor localization (colon, rectum) or to their genomic status (allelic instability -MIN, chromosomal instability -CIN).

Our team showed that the allelotyping on a cohort of 104 colon adenocarcinoma comprising all the clinical stages and of a cohort of 38 colon adenocarcinoma with synchronous liver metastasis, all of CIN phenotype, reveals existence of three CIN cancer subtypes without correlation of their frequency with the evolutionary stage.

Objective: Our project aims at determining molecular subtypes of a cohort of colon polyps using allelotyping and MGMT gene methylation status, in correlation with histology and grade.

Material: 159 colon polyps resected between 1996 and 2006 in Strasbourg University Hospital were frozen and stored in the tissue bank.

Methods: allelotyping of 33 microsatellites targeting 18 chromosomes and methylation status of the MGMT gene by MSPCR.

Results: Histological examination identifies 14 hyperplastic polyps, 110 tubular adenoma with 17 high grades and 35 tubulo-villous adenoma with 15 high grades. The distribution on right and left side of colon is homogenous (48,5% vs 51,5%). 68% of polyps have allelic imbalances (AI) corresponding to CIN status. All of the microsatellite loci have AI. The frequency of AI on each microsatelitte is ranging from 3,1 % to 25%. 37% of polyps are methylated on the MGMT gene. 39% are only CIN, 14% are only methylated, 26% are CIN and methylated and 21% are not CIN, nor methylated. Tubulo-villous adenoma and high grade adenoma are correlated with AI (p=0,005, p=0,0001), specifically with microsatellites targeting chromosomes 1, 6 and 9. The clustering analysis of the AI identifies three subgroups of polyps: those with very few AI (<10%), those with few AI (10-25%) and those with many AI (>25%).

Conclusion: This study shows evidences for different modes of tumor initiation of the preneoplasic colon lesions of MSS phenotype. There is a large proportion of polyps with no AI, nor methylation of MGMT gene. The microsatellite allelotyping showed an important heterogeneity of genomic alterations in colon polyps and the clustering data show three subtypes of polyps with AI, like it was seen in colon carcinoma. These alterations are correlated with the histological subgroup of tubulo-villous adenoma and the high grade of adenoma. Microsatellite loci on chromosomes 1, 6 and 9 should represent target to identify predictive factor of evolution.

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Estrogen β receptor and colon cancer progression

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Estrogens are implicated in the development and progression of Colon Cancer (CC) and their effects are mediated by 2 Estrogen Receptor (ER), α and β. ERβ, described in 5 splice variant, is the dominant receptor type in normal colonic tissue and its down-regulation is related with the progression of disease. Only isoforms 1,2 and 5 have been demonstrated in normal colorectal mucosa. The aim of this study is to analyze the expression of the estrogens β, β1, β2, β5 receptors in colon adenocarcinomas (AC) and compare them with normal mucosa to evaluate a possible correlation between their expressions and Dukes staging.

Colonic mucosa fragments from 40 patients were obtained from operative specimens of patients undergoing colon resection for cancer, then conserved in RNAlaterTM and kept overnight at 4°C and stored at -80°C until analysed. All RNA samples were reverse transcribed to cDNA using iScript Select cDNA Synthesis Kit. TaqMan real-time quantitative PCR was performed on an ABI PRISM 7000 Sequence Detector System, using gene-specific primers.

We analyzed 80 samples from 40 consecutive patients. 20 samples were tumors of sigma and 20 of rectum. 5 had Dukes A stage disease, 17 Dukes B, 13 Dukes C and 5 Dukes D. ER-β1 expression is been shown in 80% of AC (32 cases) and in 87% of normal mucosae (35 cases). ER-β2 gene is expressed in all samples except 1 CC and 2 normal mucosae. The mean of ER-β1 and ER-β2 expression in tumour tissue was 89% lower respect to normal colonic mucosa. The expression of ER-β5 gene is present in all specimens. 18 tumor cases showed an expression higher than the normal mucosa, 22 cases lesser; the mean of ER-β5 gene expression was 8% lower in cancer tissues respect to normal mucosa. The expression means of all estrogen β receptor was higher in the cases with Dukes A and B stages regarding to the cases with C and D stages.

Normal tissues showed a receptors expression greater than pathological tissues and the ERβs were most expressed in the lower disease stages. We hypothesize a possible protecting role of these receptors in colon mucosa, regarding cellular turn-over in tumor development derived from p53 control loss on the cellular cycle. The ER-β5 isoform was expressed in normal tissue in only 50% of the cases and it could suggest that estrogens are not exclusively protecting but also a possible risk factor in colon carcinogenesis.

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Expressional patterns for DNA damage signaling pathway genes in human colon cancer

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BACKGROUND: DNA damage checkpoint is one of the surveillance systems to maintain genomic integrity. Checkpoint systems sense the DNA