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