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.

198 Poster

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

B. Anaya[1], M. Dominguez[1], A. Muñoz[1], S. Aguilar[1], F.J. Garcia-Cozar[1], M. Rodriguez-Iglesias[1]
[1]Puerto Real Univ Hosp, Research Unit, Cadiz, Spain

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.

199 Poster

Molecular subtyping of 159 stable microsatellite colon polyps

A. Neuville[1], N. Meyer[2], A. Schneider[3], M. Legrain[3], E. Guerin[3], D. Guenot[1], M. Kedinger[1], M.P. Gaub[1]
[1]Inserm U682, Physiopathology of Intestine, Strasbourg, France; [2] CHU Strasbourg, Sante Publique, Strasbourg, France; [3] CHU Strasbourg, Molecular Biology, Strasbourg, France

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.

200 Poster

Estrogen β receptor and colon cancer progression

L. Marascio[1], F. Castiglione[2], M. Baraghini[1], D. Rossi Degl'innocenti[2], F. Perna[1], M.N. Ringressi[1], G. Cavallina[1], M. Giannelli[1], P. Bechi[1], A. Taddei[1]
[1]Universita degli Studi di Firenze, Area Critica Medico-Chirurgica, Firenze, Italy; [2] Universita degli Studi di Firenze, Human Pathology and Oncology, Firenze, Italy

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.

201 Poster

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

M. Ioana[1], A. Saftoiu[1], D.I. Gheonea[1], F. Mixich[1], T. Ciurea[1]
[1]University of Medicine and Pharmacy Craiova, Research Center in Gastroenterology and Hepatology, Craiova, Romania

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

damage and execute cell cycle arrest through inhibiting the activity of cell cycle regulators.

PATIENTS AND METHODS: In order to detect the gene expression patterns we analyzed three sample types for each of our 30 patients: specimens from diverse sites of healthy gut, adenomatous polyps and malignant tissue. In order to asses the RNA quality we analysed the 18S and 28S ribosomal RNA bands integrity by electrophoresis on a denaturing agarose gel. For every sample 3.0 μg of total RNA were available at a concentration greater than 0.33 mg/ml. We used a Human DNA Damage Signaling Pathway Microarray that includes 113 genes associated with the ATR/ATM signaling network and transcriptional targets of DNA damage response. Genes related to cell cycle arrest, apoptosis, and the stabilization and repair of the cellular genome as a result of DNA damage signaling were represented as well. To complete our data analysis we used a specially designed web-based and a completely integrated Array Expression Analysis Suite.

RESULTS: We successfully performed focused microarray analysis showing that a dysfunction in DNA damage response contributes to genomic instability in colon samples. In 10 of our malignant samples we detected a significantly reduced expression of six DNA repair genes (ANKRD17, EXO1, MLH1, MLH3, MSH2, MSH3) than in normal colon specimens. Our obtained data were validated by quantitative RT-PCR.

CONCLUSION: Determination of gene expression profiles by using low density DNA microarrays is an ideal tool to improve our knowledge of CRC molecular pathways. However, defined gene signatures are highly variable among studies, none of the identified expressional patterns or molecular markers has been successfully validated as a diagnostic or prognostic tool applicable to routine clinical practice.

202 Poster

The first pilot study on characteristics and practice patterns of Kuwaiti breast cancer patients

F. Saleh[1], W. Reno[1], G. Ibrahim[2], A. Behbehani[2], H. Dashti[3], S. Asfar[4]
[1]Kuwait University Medical School, Anatomy, Kuwait, Kuwait; [2] Mubarak Al-Kabeer Hospital, Surgery, Kuwait, Kuwait; [3] Kuwait University Medical School, Anatomy and Surgery, Kuwait, Kuwait; [4] Mubarak Al-Kabeer Hospital and Kuwait University Medical School, Surgery, Kuwait, Kuwait

Background: Non-genetic breast cancer risk factors have never been evaluated in Kuwait. Accordingly, we aimed at examining these factors as well as the immune profile of the patients.

Materials and methods: Fifty-stage I-breast cancer patients and fifty age group-matched normal controls were assessed for the level of their peripheral blood lymphocyte subsets, and for risk factors associated with their demographic and reproductive characteristics, and with diet.

Results: The percentages of CD4+ T lymphocytes, CD4+:CD8+ ratio, and CD19+ B lymphocytes were significantly higher in the patients as compared to controls, while the percentages of CD8+ T lymphocytes and natural killer (CD56+) cells were significantly reduced. Risk factors associated with the disease included higher BMI, lack of regular exercise and physical activity in the past five years, early age at menarche, late age at first pregnancy, lack of previous information about breast cancer, hormonal therapy, and presence in Kuwait during the invasion/ liberation. Other parameters included significantly more frequent consumption of carbohydrate, sweets, animal fat, and vegetable oil (margarine), and less frequent consumption of fresh vegetables and olive oil.

Conclusions: This is the first study to highlight the environmental risk factors associated with breast cancer among the Kuwaiti women. We recommend introducing a nation-wide campaign to further investigate these factors, and addressing them accordingly.

203 Poster

Death receptors and p53 dependent impairment of UV-induced apoptosis in FADD knockouts cells

M. Radnic[1], I. Marijanovic[1], B. Nagy[1]
[1]Faculty of Natural Sciences and Mathematics, Department of Molecular Biology, Zagreb, Croatia

Ultraviolet (UV) irradiation is the cause of many adverse biological effects including development of cancer and aging. UV light targets both membrane receptors and nuclear DNA, thus evoking signals triggering apoptosis. In UV mediated apoptosis different molecular pathways are involved including DNA damage, activation of tumor suppressor gene p53, triggering of cell death receptors either directly or by autocrine release of death ligants, mitochondrial damage and cytochrome C release. Detailed knowledge about the interplay between these pathways will increase our understanding of photo-carcinogenesis.

To investigate comparatively the role of death receptors apoptotic signaling pathway and participation of the p53 mutation in the signaling cascade of UV induced apoptosis we used mouse embryonic cell lines from knockout mice deficient for death-domain-containing adaptor molecules FADD (Fas-associated protein with death domain). FADD is responsible for downstream signal transduction of death receptors belonging to the tumor necrosis factor (TNF) superfamily. Survival, apoptosis, and p53 mutations studies revealed that exposure of two cell lines, knockout and wild type, to UV-C radiation and TNF. As expected, FADD knockout cells were protected completely from death induced by TNF. The results indicate that apoptosis induced by UV-C light does not require FADD protein. The knockout cells were more sensitive than wild-type cells with respect to cell death. Allele-specific PCR detection of p53 in genomic DNA from UV-C irradiated knockout and wild type cells were analyzed by gel electrophoresis. The results show that UV-C induced apoptosis is independent of functional p53 for which the FADD knockout cells showed to be mutated. We challenge the hypothesis that UV carcinogenesis in wild type cells includes a loss of FADD function and generation of p53 mutations.

204 Poster

Bile reflux induced mutagenesis on esophageal epithelium in an animal model and the effect of low dose Aspirin

R. Anup[1], K. Anu[2], K. Sathish[1], K. Sathish[1], J. Kumar[1], B. Selvan[3]
[1]Christian Medical College Vellore, Welcome Research, Vellore, India; [2] Christian Medical College Vellore, Pathology, Vellore, India; [3] Christian Medical College Vellore, Department of Surgery, Vellore, India

Background: Barrett's esophagus and adenocarcinoma of the esophagus are related to long-standing duodeno-gastroesophageal reflux. The development of an animal model in which Barrett's esophagus and/or carcinoma is induced by duodeno-(gastro-)esophageal reflux could provide better understanding of the pathogenesis of the metaplasia-dysplasia-carcinoma sequence and would create the possibility of investigating new treatment strategies for this aggressive disease.

Aim: This study examines the incidence of bile reflux induced oesophageal metaplasia carcinoma sequence in an attempt to develop an animal model for Barett's esophagus & adenocarcinoma. We have also done the caspase 3 activity

Materials and Methods: Thirty Wistar rats weighing a minimum of 150 gms with an average age of 6 weeks were included in the study [Gp1 18 and Gp II 14]. Of these, 60% of the animals were subjected to side to side and 40 % were end to side oesophago-duodenostomy under intra peritoneal thiopentone sodium. Rats in group II received dissolvable aspirin at the dose of 15mg/Kg of the rats and from the third day till the day of sacrifice. Along with histopathology Caspase 3 activity was measured as an index of apoptosis.

Results. Mortality was higher in the end to side procedure. 18 rats without aspirin(Gp I)and 14(Gp II) with aspirin survived through one year. 8(45%)developed nodular lower esophagus[0.8x0.5cm on gross] and group2 none[$p<0.001$,with Fisher's exact]. GpII had 30 % small intestinal mocosa where Gp II did not have. basal cell hyperplasia, Epithelial hyperplasia, pappillamatosis were siginificantly in Gp II [$p<0.003$]. There was no difference in dysplasia rate Three rats did not show any changes as the side to side anastamosis was stenosed.. Carcinoma was present in one in Gp 1. The histopathologic evaluation was more suggestive of a reactive mucous producing lesion fitting the diagnosis of "esophagitis cystica profunda in Gp I and the incidence of carcinoma and dyspasia is not as high as that is been reported in the literature. However, no change in caspase 3 activity was evident under these conditions.

Conclusion: End to side oesophago-duodenostomy is the best animal bile reflux model and perioperative mortality is around 40%. Contrary to many studies reporting bile reflux induced carcinoma, Gp 1 developed "esophagitis cystica profunda." And one carcinoma. Low dose aspirin does have a role in reducing the incidence of bile induced changes in the oesophagus. This findings can be extrapolated in humans with barrettes and other reflux induced changes in esophagus

205 Poster

Implication of the upstream stimulating factor family in the DNA-repair process - identification of a new target in response to UV

Y. Baron[1], S. Corre[2], N. Mouchet[1], M.D. Galibert[1]
[1]CNRS UMR 6061 Institute of Genetics and Development of Rennes, Transcriptionnal Regulation and Oncogenesis Team, Rennes, France; [2] Marie Curie Research Institute, Signalling and Development Laboratory, Oxted, United Kingdom

The upstream stimulating factor –1 and -2 (USF1, USF2) are two distinct members of the evolutionary conserved basic-Helix-Loop-Helix Leucine Zipper transcription factor family (bHLH-LZ) that interact with high affinity to cognate E-Box regulatory elements (CANNTG) (1). USF genes are ubiquitously expressed, with their respective protein regulating a wide number of gene networks. We have previously implicated USF-1 transcription factor and specific E-Box elements located within promoter