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

regions as key members of the tanning process, targeting gene expression up-regulation of numerous pigmentation genes following UV-irradiation (2-3).

A combination of in vivo and in vitro experiments, including among others DNA-binding assays (ChIP, Band-shift) and gene expression experiments (Luc-assay, real-time PCR), using human keratinocytes (HaCaT), and a melanoma cell line (501mel) allowed us to identify a new USF-target. It is a member of the DNA-repair machinery that proved to be up-regulated following UV-radiation in a USF dependant manner.

Our data implicate for the first time the USF family in the DNA-repair process following UV-irradiation, giving new insights in understanding the complex function of USF in response to UV-stimulation.

1- CORRE S. and GALIBERT MD. Upstream stimulating factors: highly versatile stress-responsive transcription factors, PIGMENT CELL RES. 2005;18(5):337-48.

2- GALIBERT, MD., CARREIRA, S., GODING, C.R. The Usf-1 transcription factor is a novel target for the stress responsive p38 kinase and mediates UV-induced Tyrosinase expression, EMBO J. 2001, 20, 5022-5031.

3- CORRE S, PRIMOT A, SVIDERSKAYA E, BENNETT DC, VAULONT S, GODING CR, GALIBERT MD. UV-induced expression of key component of the tanning process, the POMC and MC1R genes, is dependent on the p-38 activated upstream stimulating factor-1 (USF-1). J. Biol. Chem., 2004, 279(49):51226-51233.

206 Poster

PF-4 causes down-regulation of PPAR gamma and increase formation of aggressive phenotype of MNU-treated breast cancer

H. Jaafar[1], F.M. Idris[1], T.A.D.T. Din[1], S.H. Shamsudin[1], S.N.M. Nafi[1]
[1]Universiti Sains Malaysia, Department of Pathology, Kubang Kerian, Malaysia

AIMS: To examine the effect of anti-angiogenic agent platelet factor-4 (PF-4) on the expressions of nitric oxide syntase (NOS), hypoxia-inducible factor-1alpha (HIF-1α) and peroxisome proliferator-activated receptor gamma (PPAR gamma) of methylnitrosourea (MNU)-treated rat mammary carcinoma. METHODS AND RESULTS: Breast carcinomas in Sprague-Dawley rats were induced by injecting intraperitoneally 70mg of MNU per body weight. The rats were divided into control group and PF-4 group where intratumoral injection of 10μg of PF-4 was given when the tumour size reached 1.2± 0.5cm. All the rats were sacrificed when the tumour in the control group reached 1.6 ± 0.5cm. Immunohistochemistry was performed to analyse the expression of NOS, HIF-1α and PPAR gamma in the tumour cells. Tumours injected with PF-4 showed a dramatic reduction in size compared to the control group. Histological study of the tumours in the control group showed cribriform (45%) and papillary (55%) type of breast carcinoma. In the PF-4 group, the phenotypes were cribriform (25%), papillary (63%) and diffuse infiltrating ductal carcinoma, no special type (12%). Necrosis is more prominent than in the control group. Positive expressions of NOS, HIF-1α and PPAR gamma in the control group were 100%, 100% and 91% respectively. In the PF-4 group, positive expressions of NOS, HIF-1α and PPAR gamma were 100%, 92% and 17% respectively. There was marked reduction of PPAR gamma expression in PF-4 treated group compared to the control group and this was statistically significant ($p < 0.001$). This trend was also observed in the intratumoural blood vessels. CONCLUSION: These results indicate the negative impact of PF-4 on the PPAR gamma and increase in the the number of aggressive type of breast carcinoma due to the anti-angiogenic activity of PF-4. It is possible that aggressive subclones developed from the suppression of blood vessels and PPAR gamma. Further study is needed to elucidate the mechanisms.

207 Poster

BRAF-induced papillary thyroid carcinoma – validation of microarray data

D. Rusinek[1], M. Wiench[1], D. Handkiewicz-Junak[1], M. Oczko-Wojciechowska[1], M. Kowalska[1], J. Zebracka-Gala[1], A. Pfeifer[1], B. Jarzab[1]
[1]Maria Sklodowska-Curie Memorial Institute, Department of Nuclear Medicine and Endocrine Oncol, Gliwice, Poland

Background: Papillary thyroid carcinoma, constituting 80% of all types of thyroid carcinomas, in most cases is effectively treated with the thyreidectomy combined with the radiotherapy. However there are PTC cases with poor prognosis which do not exhibit radioiodine sensitivity and dedifferentiate to anaplastic carcinomas. The recent findings suggest correlation between the aggressiveness of the PTC and the presence of the BRAF mutation V600E and the study of Giordano et al (2005) indicated the significant differences in gene expression profile of between PTCs harboring different initiating mutations.

The purpose of the study was the analysis of differences in gene expression profile of BRAF-positive and RET/PTC-positive PTCs and validation of microarray data using the real time QPCR.

Methods: A meta-analysis of joint sets of 39 our papillary thyroid carcinomas and 51 PTC cases analyzed by Giordano et al. was performed. Two-class comparison (PTCs with RET/PTC rearrangements vs PTCs with BRAF mutation) was carried out and genes with univariate significance level lower than p=0.001 were selected. The verification of the selected genes was carried out on an independent group of 58 PTCs (among them 27 are BRAF-positive) by quantitative real-time PCR.

Results: 3383 probesets were differentially expressed between PTCs with RET/PTC rearrangements and BRAF mutation. Nine genes were selected to be validated: BRAF, IGF1, MAP2K1, MAPK14, MAPK1, PGF, PHLDA1, TM7SF4.

TM7SF4, with high significance in microarray data was strongly over-expressed in PTCs with V600E BRAF mutation ($p<0.001$). BRAF gene was up-regulated in BRAF(+) PTCs, but the dispersion of the results was higher ($p=0.0346$). Remaining three genes were down-regulated in BRAF-positive tumors: IGF1 ($p=0.000018$), PGF ($p=0.000257$), PHLDA1 ($p=0.0051$). For MAP2K1, MAPK14, MAPK1 genes we noted no significant difference ($p>0.06$).

Conclusions: There are distinct differences between BRAF-positive PTCs and BRAF-negative cases in gene expression profile. The function of selected genes is still to be investigated. The diminished expression of PHLDA1 may contribute to IGF-1 induced apoptosis while TM7SF4 may take part in antigen presentation by dendritic cells, thus, influence the immune response to PTC.

Supported by Ministry of Science and Higher Education (no:. N401 101 32/2138) and the 6th FP Genrisk-T (no 036495)

208 Poster

Gene expression profile of follicular thyroid tumors

M. Oczko-Wojciechowska[1], A. Kukulska[1], D. Lange[2], E. Chmielik[2], M. Kowalska[1], E. Gubala[1], A. Pfeifer[1], B. Jarzab[1]
[1]Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Department of Nuclear Medicine and Endocrine Oncology, Gliwice, Poland; [2] Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Department of Tumor Pathology, Gliwice, Poland

Background: Morphological differences between thyroid benign lesions — follicular adenomas (FA) and malignant follicular carcinomas (FTC) are based only on the cellular invasion features. Genomic approaches have been undertaken to determine the genes relevant for differences in biology of these tumors, which may be also of utmost diagnostic importance. The aim of the study was to compare gene expression profiles of FTC and FA.

Material and Methods: We applied high density oligonucleotide microarrays (HG-U133A, Affymetrix). We included 22 follicular tumors from our own collection (10 malignant and 12 benign) and compared them both to the gene expression profile of other benign and malignant thyroid tumors, analyzed by us (in total approx. 100 specimens) as well as to published microarray study by Weber et al. (JCEM 2005).

We use bioinformatic techniques based both on unsupervised (SVD – Singular Value Decomposition) and supervised approach.

Results: When the genetic distance between the different types of thyroid tumors is evaluated by the number of genes with significantly changed expression, the difference between follicular adenomas and carcinoma is much smaller by each of the tests applied (290 significant genes, combined our and Weber's dataset) than the distance to other benign/malignant thyroid tumors. Some of the genes differentiating FA and FTC, obtained in our analysis were previously described, among them FOXO1A (forkhead box O1A, rhabdomyosarcoma) and LARP1 (La ribonucleoprotein domain family, member 1). Our attention was focused on sigificant changes within the genes related to MAP kinase regulation by dual specificity phosphatases, especially dipeptidylpeptidase 8, down-regulated at FDR 0.5% in FTC. Class prediction analysis allowed to properly classify 13 of 14 follicular tumors by 150 gene set (cross-validation approach).

Conclusion: Gene expression profiling reveals important differences between transcriptome of benign and malignant follicular thyroid tumors

The study was supported from Polish Ministry of Science and the 6th FP Genriskt-T

209 Poster

Capside proteins (L1 and L2) of human papillomavirus type 16 not increase the expression of costimulatory molecules and HLA-DR on dendritic cells

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: In order to evaluate the effect of Human Papillomavirus type 16 (HPV) capside proteins (L1 and L2) on the expression of costimulatory molecules, CD11c+ DR+ dendritic cells (DCs) were infected with lentiviral vectors expressing GFP-L1 or GFP-L2, and then CD80 + CD86 and