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.

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

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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.

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BRAF-induced papillary thyroid carcinoma – validation of microarray data

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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.

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Gene expression profile of follicular thyroid tumors

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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

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

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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

HLA_DR expression was evaluated. CD86 expression is considered the most relevant CD28 ligand. Furthermore CD86 is constitutively expressed in all activated DCs, showing a faster induction and reaching higher expression.

Materials: The L1 and L2 coding sequences were PCR-amplified from a plasmid containing the whole HPV-16 genome and cloned in a pENTR vector by means of the pENTRR /SD/D-TOPOR (InVitrogen). The expression cassette containing L1 or L2 coding sequences 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. Lentiviral supernatants were titrated by quantitating infection of Jurkat cells. Peripheral Blood Mononuclear Cells (PBMC) were purified by density-gradient centrifugation with Lymphocyte separation medium (Eurobio, Les Ulis, France). 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. CD11c+ cells were seeded in 24-well culture plates at 1x105 cells/ml and stimulated with 1 μg/ml LPS (Sigma). DCs were cultured with lentiviral supernatans and transduction efficiency was evaluated by FACS analysis (Dako Cytomation). More than 60% of DCs were efficiently transduced.

Results: A further, though slight increase of CD80 + CD86, mostly due to CD86 expression, is observed when GFP-L1 or GFP-L2 expressing cells are compared to cells expressing GFP alone which could be due to the costimulatory boosting effect of HPV capside proteins. Only minor differences in HLA-DR expression in cells transduced with either GFP-L1 or GFP-L2 being even more apparent in GFP-L2 expressing cells. Thus differences in HLA-DR expression could not explain the functional defects observed in HPV infected individuals.

Conclusions: Either GFP-L1 and/or GFP-L2 infected DCs express costimulatory surface molecules and HLA-DR, showing no significant differences with control DCs expressing GFP alone.

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Liver cancer caused by long-term infection with liver fluke, Opisthorchis viverrini in experimental hamster

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To elucidate cholangiocarcinogenesis caused by liver fluke infection, histopathology of the liver at different time point intervals up to 20 months post infection were studied in the experimental hamster infected with O.viverrini. Not only bile duct was tremendous affected during the infection but hepatocyte as well. Lymphocytic aggregation was found not uncommon in the liver section. Sclerosing cholangitis, liver fibrosis and cirrhosis were demonstrated. A small nodule of clear cell type liked hepatocellular carcinoma were found in hamster with 13 months post infection. Both benign dilated peribiliary cyst and high grade of dysplastic changes of peribiliary gland harmatoma were demonstrated. Two of infected hamsters could survived up to 20 months post infection, one was found to harbour a small solitary modules of well-differentiated intrahepatic cholangiocarcinoma. The other was found to harbour high grade dysplasia and carcinoma in situ of peribiliary gland harmatoma. Immuno-histochemical staining are needed to confirm.

Animal subjects used in this study has been obtained with permission from Animal house under regulation of Faculty of Medicine, KKU

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Cell cycle proteins in squamous cell carcinoma of oral cavity

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Background: Squamous cell carcinoma of the oral cavity (OSCC) is a common malignancy characterised by a high degree of local aggression and metastasis to cervical lymph nodes. Behaviour of this type of neoplasm is related to disturbance in several molecular cascades and cell cycle molecules play a central role. Cell cycle control is complex and involves numerous molecules. Phases of cell cycle progress aided by promoter molecules termed cyclins (A, B, E, C, D and H) and cyclin-dependent kinase (CDK). Inhibition of the cycle involves molecules called ckd-inhibitor (as p16, p21, p27, p57) and the classical proteins RB e p53. Other molecules also play essential role in critical processes of cell cycle and cell proliferation such as Ki-67 and Topoisomerase II. Alterations in these molecules are associated with poor prognosis in many human neoplasms and may be important molecular predictors of biological behaviour of OSCC. Methods: This study analysed cell-cycle related proteins - Cyclin D1, Cyclin B1, Cyclin A, p16, p21, p27, p53, Rb, Ki-67 and Topoisomerase using immunohistochemistry in tissue microarray of 136 cases of OSCC, and associated their expression with clinico-pathological features and survival rate, for predicting tumour prognosis. The results were evaluated quantitatively by the automated cellular imaging systems (ACIS III DAKO), which detects, counts, and classifies cells based on colour, shape, and size. Results: The results were compared to clinical-pathological features. Kaplan-Meier method and χ^2 tests were used for statistical analysis. Expression of Cyclin B1, Cyclin A, p16, p21, p27, RB, Ki-67 and Topoisomerase proteins had no significant association with clinical pathological parameters tested (age, sex, race, clinical stage, tobacco and alcohol consumption, histological grade, perineural invasion, vascular embolization, lymph nodes status and capsular rupture). Cyclin D1 overexpression was significantly correlated with advanced clinical stage (T3/T4) (p=0,003). Expression of p53 protein was correlated with poorly differentiated tumours (p=0,028). Significance between Cyclin D1 and p53 and other clinicopathological features was not statistically established. Tumours with p16 downregulation were related to patients with a smaller survival rate (analysis of a 10-year overall survival) (p=0,025). Conclusion: Our results suggest that overexpression of Cyclin D1 and p53 proteins and downregulation of p16 protein might be indicators of poor outcome in patients with OSCC. Supported by FAPESP.

POSTER SESSION

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PARP inhibition vs. PARP-1 silencing: different outcomes in terms of single-strand break repair

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Poly(ADP-ribose) polymerase-1 (PARP-1) and XRCC1 are crucial effectors in the short patch repair (SPR) branch of the base excision repair pathway (BER), an essential mechanism for the repair of DNA single-strand breaks (SSBs). We have determined the consequences of PARP-1 disruption on SSB repair (SSBR) during the S phase of the cell cycle using isogenic human HeLa cells exposed to a PARP inhibitor or stably silenced for PARP-1 (PARP-1KD) or XRCC1 (XRCC1KD) gene expression. We found that both PARP-1 inhibition or silencing prevented the recruitment of XRCC1 to DNA damage sites using laser microirradiation and live cell microscopy. Strikingly, alkaline elution analysis of DNA showed that PARP-1KD or XRCC1KD cells were able to rejoin radio-induced SSBs as rapidly as control cells. These data suggest that a PARP-1- and XRCC1-independent pathway operates to repair SSBs when SPR is deficient. The long patch repair (LPR) branch of BER appears to be the likely mechanism, as PCNA recruitment at sites of DNA damage was not affected by the absence of PARP-1. In contrast, inhibition of PARP-1 in HeLa cells exposed to γ-rays in S phase, dramatically slowed down SSBR as measured by alkaline elution. In addition, PARP-1 inhibition also triggered the accumulation of a large amount of PARP-1 and PCNA at sites of microirradiation which persisted for over 20 min. It is proposed that this accumulation results in steric hindrance and slows down the recruitment of other intermediates of the BER process. Thus we demonstrated that inhibiting or silencing the PARP-1 protein has different outcomes in terms of SSBR in the S phase of the cell cycle.

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Growth retardation and survival prolongation of experimental lung carcinoma by interstitial Ra-224 loaded wires releasing diffusing alpha-emitting atoms

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Background & Objectives: Alpha particles are substantially more effective in cell killing than photons and electrons. However, the short range of alpha