the mean weight of NEIL1 males was significantly less than that of their wildtype counterparts ($p = 0.03$). In contrast, from 7 months onwards the NEIL1 knockout females weighed significantly more than the wildtypes ($p = 0.05$). Initial data from the myeloperoxidase assay suggests that whilst there is no difference in basal levels, there is significantly less neutrophil activity in the liver, heart and gut tissue of knockout animals when treated with 20 mg/kg lipopolysaccharide ($p = 0.05$).

Conclusions: The NEIL1 knockout mice show no obvious phenotypic change other than the weight differences described. No evidence of an obese phenotype was observed. The NEIL1 protein may, however, act in concert with other DNA repair proteins as a regulator of the immune system. In addition to the knockout mice, murine embryonic fibroblasts have been generated and together they should enable us to probe the biochemical and biological role of NEIL1 in genomic stability and the inflammatory process.

165 Array-based approach for early tumour detection in the biliary tract

K. Andresen[1], K.M. Boberg[2], M. Eknæs[1], H. Honne[1], E. Schrumpf[2], R.A. Lothe[1], G. Lind[1]. *[1]Department of Cancer Prevention, Institute for Cancer Research and Centre for Cancer Biomedicine Oslo University Hospital, Oslo, Norway, [2]Norwegian PSC Research Center, Medical Department Oslo University Hospital, Oslo, Norway*

Background and Aim: DNA methylation has been shown to play an important role in early tumourigenesis. So far, few DNA methylation target genes have been identified in hepatopancreatobiliary (HPB) cancers. Both cholangiocarcinomas and pancreatic carcinomas have a poor prognosis due to late clinical presentation. Cholangiocarcinomas in particular may be difficult to diagnose. In this study we aimed to identify novel methylated gene targets using HPB cancer cell lines.

Methods: Gene expression profiles of cholangiocarcinoma cell lines (n = 6) were analyzed before and after treatment with a combination of 5-aza-2′deoxycytidine and trichostatin A. CpG island containing genes upregulated after drug treatment in cell lines and simultaneously downregulated in cholangiocarcinomas compared with normal tissue, were selected for further analysis. Expression profiles of primary cholangiocarcinomas were acquired from published data sets [1,2]. The methylation status of these candidates was analyzed by methylation-specific polymerase chain reaction (MSP) in HPB cancer cell lines (n = 24).

Results: Fifty-seven candidate genes displayed increased expression in cholangiocarcinoma cancer cell lines and decreased expression in primary cholangiocarcinomas. Forty-one of these targets contained a CpG island in the promoter region and were subjected to DNA promoter methylation analyses. So far, twenty-one genes are analyzed in all cell lines. Four shared no methylation, others were methylated in one or few tumour types, and six genes including *SFRP1* and *ZSCAN18* were methylated across several HPB cancer cell lines.

Conclusions: By using a genome-wide approach we have identified several epigenetically regulated genes in HPB cancer cell lines. These target genes will be submitted for methylation studies in tumour and normal tissue as well as biliary brush cytology specimens in a hunt for early biomarkers.

Reference(s)

[1] Miller, G., et al., Genome wide analysis and clinical correlation of chromosomal and transcriptional mutations in cancers of the biliary tract. Journal of Experimental & Clinical Cancer Research 2009; 28: 62−74.
[2] Obama, K., et al., Genome-wide analysis of gene expression in human intrahepatic cholangiocarcinoma. Hepatology 2005; 41: 1339–1348.

166 Withdrawn

167 MicroRNA-34 family in triple-negative/basal-like breast cancer

J. Navratil[1], M. Svoboda[1], O. Slaby[1], P. Fabian[2], R. Vyzula[1]. *[1]Masaryk Memorial Cancer Institute, Department of Comprehensive Cancer Care, Brno, Czech Republic, [2]Masaryk Memorial Cancer Institute, Department of Diagnostic and Experimental Pathology, Brno, Czech Republic*

Background: Breast cancer is the most common malignancy of the woman after skin malignancies. “Basal-like” carcinoma is one of the most aggressive molecular subtype of breast cancer characterized by triple-negative (ER-, PR-, Her2-) and “basal-cell” phenotype and is associated with high grade, poor prognosis, and younger patient age. Adverse clinical outcome of these patients is also associated with frequent incidence of BRCA1 and p53 mutations. MicroRNAs have potential to post-transcriptionally regulate even one third of human genes, among them also significant number of important oncogenes, tumour suppressor genes and genes connected with invasion, dissemination and chemoresistance of tumours are involved. MicroRNA-34 family is under direct transcriptional control of p53 and seems to function as tumour suppressor. Mutations of p53 can induce decrease of microRNA-34 family and consequently apoptosis rate.

Material and Methods: In our study we examined expression levels of microRNA-34 family in 41 specimens of “basal-like” carcinoma by use of Real-Time PCR. Invasive breast carcinomas were immunohistochemically analysed for oestrogen receptors (ER), progesterone receptors (PR), cytokeratin 5/6 (CK5/6), epidermal growth factor receptors (EGFR), Ki67, p53 and vimentin. Tumours were considered to have basal-like phenotype if they were ER negative and HER2 negative, but positive for CK5/6 and/or EGFR and/or vimentin.

Results: Expression levels of miR-34b and miR-34c were markedly lower than those of miR-34a. We identified significantly higher levels of miR-34a in primary tumours disseminated to regional lymph nodes ($p = 0.0209$). Further, we observed increase of miR-34b levels in patients with significantly shorther overvall survival ($p = 0.05$). We did not proved association of microRNA-34 and grade, BRCA1 or clinical stage of breast cancer.

Conclusion: Our results suggest potential significance of miR-34a in invasiveness and dissemination of “basal-like” carcinoma and usage of miR-34b in diagnostic and predictive oncology of this aggressive molecular subtype of breast cancer.

Supported by grants: Internal Grant Agency of Czech Ministry of Health (IGA MZ CR) NS10357.

168 Grp78 activity is associated with Androgen Receptor status and upregulated in Hormone-Refractory prostate cancer

S. Tan[1], I. Ahmad[1], L. Singh[1], C. Nixon[1], J. Edwards[2], H. Leung[1]. *[1]Cancer Research UK Beatson Labs, Urology, Glasgow, United Kingdom, [2]University of Glasgow, Division of Cancer Studies, Glasgow, United Kingdom*

Background: Prostate Carcinoma (PC) is the most commonly diagnosed cancer of men in the developed world and relapse of PC following androgen deprivation remains a major clinical problem. Better prognostic biomarkers may facilitate treatment stratification and improve patient outcome. The aim of our study is to investigate if Grp78 expression in prostate cancer is associated with clinic-pathologic parameters including survival and the development of castrate resistance.

Materials and Methods: Immunohistochemical analysis was performed on formalin-fixed, paraffin embedded tissue microarrays containing PC specimens. 259 primary PC samples along with 36 matched pairs of hormone naïve and castrate resistant prostate cancer (CRPC) samples were studied for Grp78 expression.

Results: Using the weighted Histoscore method graded by independent observers, upregulated Grp78 expression was found to be associated with prostate carcinogenesis. Immunohistochemical expression of Grp78 in malignant tissue (n = 164) was significantly higher than benign tissue (n = 23) ($p = 0.000$). CRPC specimens also contained a significantly greater Grp78 staining than their matched hormone naïve specimens ($p = 0.028$). A higher Grp78 stain was significantly associated with 39 samples expressing androgen receptor positivity in the nucleus ($p = 0.010$). A Kaplan-Meier Survival analysis for androgen receptor positive tumours revealed a greater median survival time of 8.011 years in samples with low Grp78 stain as compared to 4.506 years in samples with high Grp78 stain ($p = 0.049$). Grp78 did not have any correlation with the degree of metastasis in patients ($p = 0.724$). It also did not have any influence on the biochemical relapse rate ($p = 0.501$) and time to death ($p = 0.653$) in the transition of hormone-naïve to castrate-resistance.

Conclusion: Grp78 expression is significantly associated with androgen receptor status and is upregulated in CRPC. It may play a key role in prostate carcinogenesis and further investigations are warranted to validate its use as a prognostic marker.

169 Reverse phase protein arrays: a powerful tool for cancer proteomics

L. De Koning[1], B. Marty-Prouvost[1], T. Dubois[1]. *[1]Institut Curie, Department of Translational Research, Paris, France*

Tumourigenesis implies major changes in cell signaling pathways involved in cell adhesion, proliferation and apoptosis. Activation of these pathways largely depends on post-translational modifications, such as phosphorylation, and thus cannot be analyzed at the mRNA level in transcriptome profiling. Therefore, high-throughput analysis at the protein level seems an absolute requirement to get a better insight in tumour biology. Yet, the technology required for such proteomics approaches has become available only very recently.

At the Translational Research Department of the Institut Curie (France), we developed a platform specialized in Reverse Phase Protein Arrays (RPPA). This highly quantitative technique consists of depositing in an automated manner very small amounts (1 ng) of cell- or tissue lysates onto microscope slides covered with nitrocellulose. Proteins of interest are subsequently detected using specific antibodies, directed against either modified (e.g. phosphorylated) or total protein pools. In this manner, up to 3000 samples can be analyzed simultaneously for the presence and the activation status of selected targets.