Cellular Oncology 32 (2010) 195-238
DOI 10.3233/CLO-2010-0518
IOS Press

Poster Sessions

Poster session 1: Cancer Predisposition

P01
EMBRYONAL RHABDOMYOSARCOMA IN A PATIENT WITH NOONAN SYNDROME AND A *SOS1* GERMLINE MUTATION

M. Jongmans[1], P. Hoogerbrugge[2], L. Hilkens[1], U. Flucke[3], I. van der Burgt[1], K. Noordam[4], M. Ruiterkam-Versteeg[1], H. Yntema[1], W. Nillesen[1], M. Ligtenberg[1], A. Geurts-van Kessel[1], R. Kuiper[1], N. Hoogerbrugge[1]
[1]*Radboud University Nijmegen Medical Centre, Human Genetics, Nijmegen, The Netherlands*
[2]*Radboud University Nijmegen Medical Centre, Paediatric Oncology, Nijmegen, The Netherlands*
[3]*Radboud University Nijmegen Medical Centre, Pathology, Nijmegen, The Netherlands*
[4]*Radboud University Nijmegen Medical Centre, Paediatric Endocrinology, Nijmegen, The Netherlands*

Noonan Syndrome (NS) is an autosomal dominant condition characterized by short stature, facial dysmorphisms and congenital heart defects, and is caused by mutations in either *PTPN11, KRAS, RAF1* or *SOS1*. Furthermore, NS is known for its predisposition to develop cancer, particularly hematological malignancies and specific solid tumors, mainly neuroblastoma and embryonal rhabdomyosacroma (ERMS). Until recently, however, cancer predisposition in NS patients with *SOS1* mutations was not reported.
Here we present a NS patient with a *de novo* germline *SOS1* mutation (p.Lys728Ile) and ERMS. This heterozygous germline mutation was homozygously present in the ERMS of this patient due to an acquired uniparental disomy (UPD) of chromosome 2. In addition, several other chromosomal aberrations were encountered, some of which are known to recurrently occur in ERMS.
Sequence analysis of the *SOS1* gene in 20 sporadic ERMS tumors failed to reveal any pathogenic mutations, implicating that *SOS1* is not a major player in the development of this tumor outside the context of NS.
In conclusion, we report a patient with a germline *SOS1* mutation who developed ERMS. The homozygous nature of this *SOS1* mutation in the tumor suggests that this mutation may underlie ERMS predisposition.

Poster session 2: Biomarker Discovery

P03
AUTOMATED SPUTUM CYTOMETRY FOR EARLY DETECTION OF INTRAEPITHELIAL NEOPLASIAS IN THE LUNG

G. Li[1], M. Guillaud[1], J. le Riche[1], A. McWilliams[1], A. Gazdar[2], S. Lam[1], C. MacAulay[1]
[1]*BC Cancer Research Centre, Integrative Oncology, Vancouver, BC, Canada*
[2]*UT Southwestern Medical Center, Dallas, Texas, USA*

There is currently no universally recognized lung cancer screening test, despite evidence of higher survival rates among lung cancers caught earlier. There is considerable interest in using biomarkers derived from automated image cytometry as a quick and reliable screen for lung cancer. We will focus here on an analysis of a novel sputum biomarker that uses automated image cytometric analysis of ploidy and nuclear morphology to detect subtle intraepithelial changes that precede lung tumours.
Methods: Data was collected from 1797 high-risk patients enrolled in ongoing chemoprevention trials, yielding 2270 sputum samples. Quantitative image cytometry using Feulgen-thionin staining and an automated imaging system was performed. Of the samples analyzed, 36 samples from normal patients were compared against 42 samples from carcinoma in situ and cancer patients to generate a Combined Score (CS).
Results: The CS was found to correlate with a number of known lung cancer risk factors, including histopathological grade, age, smoking status, and p53 and Ki67 immunohistochemical staining. At 50% specificity, CS could detect 75% of all high-risk patients, defined as those with histopathological grades

of moderate dysplasia or worse and highly abnormal nuclear morphology as determined by the Morphometric Index. CS also showed promising results in the ability to detect successful cancer treatment, although the sample size was too small to be conclusive.
Conclusions: This analysis demonstrates that sputum cytometry using the Combined Score is a powerful new tool for early detection of precancerous lung lesions at highest risk of developing into invasive cancer. This allows for selective enrichment of volunteers with dysplasia for chemoprevention trials, for example. The correlation of CS with other biomarkers suggests that CS may also find use as a surrogate biomarker, both for patient assessment and as an endpoint for chemoprevention clinical trials.

P04
ACTIVATED LEUKOCYTE CELL ADHESION MOLECULE SOLUBLE FORM (SALCAM): A POTENTIAL BIOMARKER IN EPITHELIAL OVARIAN CANCER

M. Fabbi[1], A.M. Orengo[1], D. Mezzanzanica[2], I. Bongarzone[2], A. Brizzolara[1], C. Andreani[1], L. Emionite[1], A. Puppo[1], M.G. Centurioni[1], M. Bruzzone[1], S. Canevari[2], S. Ferrini[1]
[1]*Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy*
[2]*Fondazione IRCCS Istituto Nazionale Tumori, Milano, Italy*

Background: ALCAM may act as cell surface sensor of growth saturation and regulate cell-cell interactions. In epithelial ovarian cancer (EOC) high membrane ALCAM expression significantly correlates with better prognosis. The metalloprotease ADAM17, which generates a soluble ALCAM form, regulates ALCAM membrane levels.
Methods: sALCAM was studied by immunoprecipitation and Western blot. Its concentration in serum and ascites was measured by ELISA. Luciferase+ human SKOV3 cells were grafted i.p. in NOD/SCID mice and human sALCAM serum levels monitored by ELISA.
Results: Two sALCAM forms (Mr 65 and 95 kDa) were shed by ADAM17 and detected in normal sera and in EOC sera and ascites. ALCAM-specific ELISA measured both forms. Serum sALCAM levels from 50 EOC were higher than those from 17 healthy age matched controls (44±21 vs 29±19 ng/ml; P=0.01). Correlation of sALCAM with biomarkers (Ca125 and IL-18) and with tumor characteristics is currently under evaluation. sALCAM in ascites were higher (P=0.007) than in sera simultaneously collected from the same patient (n.13). When sALCAM levels in 92 EOC ascites were correlated to clinical-pathologic variables, no significant association was found. Human sALCAM in sera and ascites of NOD/SCID mice bearing orthotopic xenografts increased with tumor burden (P=0.0001), with levels in ascites higher than in sera (P=0.0001).
Conclusions: Altogether these data indicate that increased sALCAM levels in EOC sera and ascites are generated by ALCAM shedding from the tumor and that sALCAM may represent an EOC biomarker. Data in a xenotransplant model indicate that sALCAM of tumor origin is found in the general circulation in proportion to tumor burden and not at early phases of tumor development. In addition, sALCAM levels in the sera of healthy controls are relatively high, suggesting that ALCAM shedding occurs in normal tissues. Recent data indicate that serum sALCAM is increased in breast cancer, thus emphasizing its possible role as non-specific biomarker for different epithelial cancers.

P05
TKI THERAPY RELATED PROTEOMIC PATTERNS IN SERUM FROM PATIENTS WITH METASTATIC RENAL CELL CARCINOMA

M. Walter[1], N. Kröger[1], T. Steiner[1], F. von Eggeling[2], H. Wunderlich[1], K. Junker[1]
[1]*University Hospitals Jena, Department of Urology, Jena, Germany*
[2]*University Hospitals Jena, Core Unit Chip Application, Institute of Human Genetics and Anthropology, Jena, Germany*

Question: Development of targeted therapies including tyrosine kinase inhibitors (TKIs) established new therapeutical options in patients with metastatic renal cell carcinoma. However, the primary clinical response, development of resistance as well as serious side effects is different in individual patients. Currently, no predictive biomarkers for these treatment options exist. Therefore, our aim was to define protein patterns in serum of patients treated by TKIs correlating with therapy benefit.
Methods: Thirty patients who received TKI therapy (Sorafenib or Sunitinib) were included in our study. Clinical response and follow-up data were known in all cases. Data were analyzed considering best response after 3 month and response or progression after 9 month for the respective therapy. To establish biomarkers pre-therapeutic samples were analyzed by Surface-enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (SELDI-TOF MS) in

combination with the ProteinChip®Arrays Q10, CM10 and IMAC30. Bioinformatic analysis was performed using the Fuzzy c-means method for clustering and the relevance index by Kiendl.
Results: For both TKI treatments it was possible to define specific protein patterns in serum which can be related to clinical response and development of drug-resistance. Using bioinformatics, candidate protein peaks were identified which are highly relevant for prediction of therapy response.
Conclusions: From our results it seems possible to predict therapy response analyzing serum samples obtained before targeted therapy. In this ongoing study, candidate proteins have to be identified to recognize their role in therapy response and development of resistance.

Poster session 3: Identification of Molecular Predictive and Prognostic Factors

P08
DNA CONTENT FLOW CYTOMETRY, TOBACCO AND ANATOMICAL SUBSITE IN ORAL PRECANCER AND CANCER

M. Pentenero[1], W. Giaretti[2], R. Navone[3], A. Demurtas[4], L. Gassino[1], D. Malacarne[2], S. Gandolfo[1]
[1]*University of Turin, Dept Clinical and Biological Sciences, Orbassano (TO), Italy*
[2]*National Cancer Research Institute, Dept Advanced Diagnostic Technologies, Biophysics and Cytometry Section, Genoa, Italy*
[3]*University of Turin, Dept Biomedical Sciences and Human Oncology, Turin, Italy*
[4]*AOU S. Giovanni Battista, UOADU Anatomia Patologica 2, Turin, Italy*

Question: To date there are still no reliable biomarkers for oral potentially malignant lesions (OPMLs) to predict the risk of progression to squamous cell carcinoma (OSCCs). Aim of this study was to evaluate within a prospective clinical trial of patients with OPMLs, the relations among DNA content flow cytometry (DNA FCM), tobacco cigarette smoking and anatomical subsite.
Methods: DNA FCM in OPMLs and OSCC was evaluated using fresh samples obtained by microbiopsy.
Results: Among 60 OPMLs there were 6/42 OPMLs without dysplasia, but with DNA aneuploidy, versus 8/18 with both dysplasia and aneuploidy (p=0.02). When the tongue and the buccal mucosa, the two most common sites in this series of cases were compared, dysplastic OPMLs were mainly located on the tongue (p=0.01). Tobacco smokers, who preferentially developed OPMLs in the buccal mucosa at a younger age than non-smokers (p=0.002), had fewer dysplastic OPMLs than did non-smokers (p=0.01). Dysplasia was significantly linked to DNA aneuploidy (p=0.03) in smokers. When studying a larger series of patients with either OPMLs or OSCCs (n=123), the different location of lesions between smokers and non-smokers was confirmed, with more lesions in buccal mucosa among smokers (p=0.002) and in the tongue among non-smokers (p<0.0005). In particular, lesions in buccal mucosa were 6 times more likely to occur in smokers than in non-smokers (p=0.003).
Conclusions: The present data suggest that DNA aneuploidy is an early event in oral carcinogenesis and that the influence of tobacco varies according to subsite and patient age. Development of oral potentially malignant lesions or cancer among smokers was more likely to occur in the buccal mucosa and the floor of the mouth with respect to all other subsites, while it was predominant in the tongue among non-smokers. These associations, which were highly statistically significant, suggest that subsite mediate the effects of tobacco.

P09
PROTEIN PROFILING OF METASTATIC AND NON-METASTATIC RENAL CELL CARCINOMAS

C. Heinze[1], R. Pilchowski[1], C. Melle[2], N. Escher[2], D. Driesch[3], H. Wunderlich[1], K. Junker[1]
[1]*University Hospitals, Jena, Department of Urology, Jena, Germany*
[2]*University Hospitals, Jena, Core Unit Chip Application, Jena, Germany*
[3]*BioControl Jena GmbH, Jena, Germany*

Question: Presently the prognosis of patients with metastatic renal cell carcinoma (RCC) is in spite of a lot new kinds of therapies poor. Therefore it is necessary to identify prognostic parameters for an early detection of patients at high risk for metastasis.
The aim of our study is to identify specific protein patterns in tumor tissue of patients distinguish between metastatic and non metastatic tumors to define the metastatic potential of primary tumors.

Materials and methods: To establish specific protein pattern in tumor tissue we analyzed a pool of 45 patient samples including 25 metastatic and 20 non metastatic tumor samples. Protein lysates from tumor tissues were investigated by the Surface-Enhanced Laser desorption/Ionization Time-of-Flight Mass Spectrometry (SELDI-TOF-MS). The ProteinChip-Array Q10 (a strong anion exchanger) was used. The detected spectra were analyzed by the computer software XL-Miner 3.5 (Biocontrol Jena GmbH) with the Fuzzy c-means method for clustering followed by establishing rules and evaluation using the relevance index by Kiendl. This tool automatically generates rules with best prediction.
Results: The generated rule base for the Q10 surface at a significance level of α=0.90 showed a sensitivity of 95% and a specificity of 92%. This rule base permitted the classification of the groups metastatic / non matastatic tumors with a total reliability of 93%. This rule base contains 12 relevant protein peaks including highly significant peaks at 10348 Da, 10506 Da and 44565 Da.
Conclusion: The ProteinChip Technology including bioinformatic evaluation software XL-Miner 3.5 is suitable to predict the potential of a primary tumor to metastasize based on specific protein profiles. In the next step, the relevant proteins have to be identified by additional specific methods.

P10
RADIORESISTANCE IS ASSOCIATED WITH ALDH-1 ACTIVITY IN EWING´S SARCOMA *IN VITRO*

B. Greve[1], F. Sheikh-Mounessi[1], J. Weidner[2], T. Bölling[1], N. Willich[1], M. Götte[3]
[1]*University Hospital Münster, Radiotherapy, Münster, Germany*
[2]*Cytecs, Development, Görlitz, Germany*
[3]*University Hospital Münster, Gynaecology and Obstetrics , Münster, Germany*

Recently, authors described the existence of CD133 positive cancer stem cells in primary Ewing´s sarcoma tissue and were able to prove their high tumor-initiating and in vivo self-renewing potential. Here, we characterized the activity of aldehyde dehydrogenase 1 (ALDH-1), a stem cell feature with prognostic potential, in four different cell lines of Ewing´s sarcoma and investigated its influence on radiation response.
ALDH-1 positive cells were identified by using the ALDEFLUOR™ stem cell kit (STEMCELL Technologies, Grenoble, France) in four Ewing cell lines (ET1, VH64, RM82 and CADO). Cells were irradiated with 10Gy X-ray and apoptotic cell death was measured using the Annexin V assay.
ET1 and VH64 revealed the highest ALDH-1 positive cell content and showed low apoptotic cell death after irradiation (5.4% resp. 4.3%). RM82 showed higher proportion of ALDH-1 negative cells and apoptosis increases to 14.1%. In CADO ALDH-1 acitvity was not measureable and cells were determined as the most radiosensitive one with 50.2% apoptosis. In RM82 the proportion of ALDH-1 positive cells increases significantly after a single dose of 10Gy while the negative cell proportion declined.
In conclusion, an increased radioresistance of cancer stem cells is proposed but not proven so far. We were able to show that tumor cells with high ALDH-1 activity were more resistant against irradiation. Thus, the stem cell feature ALDH-1 activity determines a cell fraction with high radioresistance and it may be interesting whether ALDH-1 activity itself is responsible for this. Therefore, the current findings may open new options for the treatment of this aggressive childhood tumor.

P11
RETINOIC ACID SIGNALING ACTIVATES DIFFERENT TARGETS IN THE TUMORS OF LONG-TERM COMPARED TO SHORT-TERM SURVIVORS OF GLIOBLASTOMA

S. Barbus[1], B. Tews[2], M. Hahn[1], B. Radlwimmer[1], C. Hartmann[3], J. Felsberg[4], D. Krex[5], G. Schackert[5], R. Martinez[6], G. Reifenberger[4], P. Lichter[1]
[1]*German Cancer Research Center, Molecular Genetics, Heidelberg, Germany*
[2]*ETH/ University of Zürich, Brain Research Institute, Zürich, Switzerland*
[3]*German Cancer Research Center, Clinical Cooperation Unit Neuropathology, Heidelberg, Germany*
[4]*University of Düsseldorf, Department of Neuropathology, Düsseldorf, Germany*
[5]*University of Dresden, Department of Neurosurgery, Dresden, Germany*
[6]*University of Göttingen, Department of Neurosurgery, Göttingen, Germany*

Question: While the prognosis of glioblastoma patients is poor, a small fraction (3-5%) of patients, so-called “long-term survivors” (LTS), survive for 36 months or longer after diagnosis. The aim of the present work was to identify the pathomechanisms leading to long-term survival in glioblastoma.

Methods: Messenger-RNA expression profiling was performed in primary glioblastomas from 11 LTS and 12 short-term survivors (STS; overall survival < 6 months). Data validation was carried out by immunohistochemical staining of tissue sections. IDH1 and IDH2 genes were subjected to mutational analyses by direct sequencing.
Results: The data revealed an important role of the retinoic acid pathway in LTS tumors as indicated by significantly lower expression of RBP1, RARRES2 and FABP5. The ratio of FABP5 to CRABP2 is small in LTS and is associated with an activation of the anti-oncogenic retinoic acid receptors. This ratio is significantly higher in STS ($p<0.001$, t-test), resulting in PDK1 activation, which indicates that the primary target of retinoic acid signaling in these tumors is the pro-oncogenic nuclear receptor PPARD. High FABP5 protein expression in STS tumors is linked to highly proliferating tumor cells of astrocytic origin (assessed by anti-Ki67 and anti-GFAP double staining). Furthermore, the LTS tumors showed more frequently IDH1 mutations.
Conclusion: Retinoic acid signaling activates different targets in LTS and STS glioblastoma providing a novel option for future therapy development.

Poster session 4: Telomere Biology

P14
TELOMERE SHORTENING AND CHROMOSOMAL INSTABILITY IN MYELODYSPLASTIC SYNDROMES

K. Lange[1], L. Holm[1], K. Vang Nielsen[2], A. Hahn[3], W. Hofmann[1], H. Kreipe[4], B. Schlegelberger[1], G. Göhring[1]
[1]*Hannover Medical School, Institute of Cell and Molecular Pathology, Hannover, Germany*
[2]*Dako Denmark A/S, Glostrup, Denmark*
[3]*Institute of Biometrics, Hannover Medical School, Germany, Hannover, Germany*
[4]*Institute of Pathology, Hannover Medical School, Germany, Hannover, Germany*

Telomere shortening and chromosomal instability are believed to play an important role in the development of myeloid neoplasia. So far, published data are only available on the average telomere length in myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML), but not on the telomere length of individual chromosomes. We used a new technique, telomere/centromere-fluorescence in-situ hybridization (T/C-FISH), which combines fluorescence R-banding and FISH using a probe against the telomere repeats to measure the telomere length of each chromosome arm in patients with MDS (Perner at al, 2003).
T/C-FISH was validated by comparing the telomere length of 5 healthy controls with published data: the measured T/C values, the standard deviation and the telomere length profile, especially the telomere length expected according to the patient's age, agreed with Mayer et al. 2006.
In line with previous results, our measurement of 78 patients with MDS and different cytogenetic or morphological subgroups showed significantly shorter telomeres than those of healthy controls with a median of 7.0 kb (range 3.7-11.4 kb) compared to 9.5 kb (range 8.0-12.0 kb), respectively ($p<0,05$). Telomere lengths did not differ significantly between distinct morphological subtypes of MDS. However, there was a significant difference in telomere length between patients with an isolated monosomy 7 (7.6 kb) and patients with a normal karyotype (6.5 kb) ($p<0.05$).
Although patients with MDS showed short telomeres compared to the healthy controls, there was no correlation between telomere length and structural aberrations. For example, patients with an 5q-syndrome did not show strinkingly shorter telomeres at chromosome 5 than at other chromosomes. However, all patients shared relatively short telomeres at the short arm of the acrocentric chromosomes and showed a higher variability in telomere lengths between different metaphases than healthy controls.
In some MDS subtypes, like MDS with isolated monosomy 7, telomeres may be stabilized and even increase in length due to the activation of telomerase or alternative mechanisms (ALT). Telomerase measurement using TRAPeze RT Telomerase Detection Kit (Millipore/Chemicon, USA) showed a 3-fold increase in telomerase activity comparing patients with normal karyotype and patients with an isolated monosomy 7. However, the significance of this analysis is restricted due to the limited patient material. Moreover, an elongation by alternative telomere lengthening (ALT) cannot be excluded.

Poster session 5: Cancer Genomics

P16
COMBINATION OF TWO NON INVASIVE URINE TESTS (TRAP AND FISH) GREATLY INCREASES ACCURACY IN DIAGNOSING BLADDER CANCER IN SYMPTOMATIC PATIENTS

S. Bravaccini[1], V. Casadio[1], D. Amadori[1], W. Zoli[1], R. Gunelli[2], O. Nanni[1], R. Silvestrini[1], D. Calistri[1]
[1]*Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (I.R.S.T.), Oncology Department, Meldola, Forli, Italy*
[2]*Morgagni-Pierantoni Hospital, Urology Department, Forlì, Italy*

Introduction: Previous case-control studies have shown the role of urine telomerase activity by the telomerase repeat amplification protocol (TRAP) assay for the detection of early bladder cancer. However, according to international guidelines the TRAP assay should be validated in a large and consecutive series of symptomatic patients before being translated in clinical practice. The aim of the present study was to verify whether a multiple approach based on first-level TRAP assay analysis and successive evaluation with UroVysion test, limited to patients with TRAP-positive urine samples, could unmask false-positive results and improve diagnostic accuracy in terms of specificity. *Patients and methods:* Three hundred individuals with urinary tract symptomatology (50 females, 250 males), but without prior history of bladder malignancy were consecutively enrolled between January 2007 and June 2008. All urine samples were processed for cytological examination and for telomerase activity determination using a quantitative TRAP assay. FISH analysis was carried out only in TRAP-positive samples. *Results:* At a cut-off of 50 arbitrary enzymatic units (AEUs), shown in previous case control-studies as the most accurate diagnostic discriminant, the positive predictive value (PPV) and negative predictive value (NPV) using TRAP assay were 48% and 86%, respectively, with an overall accuracy of 71%. Following the two-step TRAP-FISH approach, PPV increased to 75% and overall accuracy to 83%. *Conclusion:* Our results indicate that TRAP assay by itself could make an important contribution to early bladder cancer diagnosis in patients with urinary tract symptomatology, and that its combination with FISH strongly improves specificity by unmasking false-positive TRAP results.
Sara Bravaccini and Valentina Casadio contributed equally to this work.

P17
WHOLE GENOME GENE EXPRESSION ANALYSIS OF CANCER CELLS GROWING IN CONVENTIONAL 2-DIMENSIONAL VERSUS 3-DIMENSIONAL CELL CULTURE

O. Zschenker[1], T. Streichert[2], N. Cordes[1]
[1]*TU Dresden, OncoRay, Dresden, Germany*
[2]*University Hospital Hamburg-Eppendorf, Department of Clinical Chemistry/Central Laboratories, Hamburg, Germany*

Growth of cells in three-dimensional (3D) cell culture models reflects physiological growth conditions enabling the examination of cell behavior, signal transduction and responsiveness to external stimuli in a way similar to in vivo. Although gene expression in tumor biopsies of patients has been associated with tumor progression and response to radio- and chemotherapy, it is still an open question which exact molecular mechanisms contribute to the therapy resistance of tumors. This study in 2D and 3D cell culture systems was performed to determine differences in gene expression profiles of cells, which may be mechanistically linked to the radiation response of cancer cells. In lung carcinoma and squamous cell carcinoma cell lines, genome-wide cDNA microarray analysis was executed using the Affymetrix HG U 133 Plus 2.0 gene chip. Significant analysis of microarray (SAM) and t-test analysis revealed significant changes in gene expression profiles of 3D as compared to 2D cell cultures. These changes were mainly associated with signaling pathways involved in the regulation of cell size, cell-cell signaling and integrin-mediated cell adhesion. Gene expression was validated exemplarily for the genes BCL2A1, CTGF, ErbB3, Fibronectin (FN1), TGFbeta1 and SIRT2 using real time PCR, Western Blot analysis and immunofluorescence staining technique. In conclusion, our data show cell growth-dependent modifications which are related to cellular morphology and interactions with the microenvironment. The findings strongly support our hypothesis that cell morphology and cell-cell contact are two of the major determinants of cellular responsiveness to external stress. Future studies in optimized 3D cell culture models will be needed to address the mechanisms of tumor cell biology, cancer

therapy responsiveness and evaluation of novel cancer targets.

P18
CHARACTERIZATION OF THE GENETIC CHANGES IN THE DIFFERENT HISTOLOGICAL COMPONENTS OF GLIOBLASTOMAS WITH OLIGODENDROGLIAL FEATURES (GBMO)

B. Klink[1], S. Patt[2], E. Schrock[1]
[1]*Institut für Klinische Genetik, TU Dresden, Dresden, Germany*
[2]*Institut für Pathologie, Friedrich-Schiller-Universität Jena, Jena, Germany*

Glioblastomas (WHO grad IV) are the most common and most malignant brain tumors of adults. A small subgroup of glioblastomas contains areas with histological features of oligodendroglial differentiation. Oligodendroglial tumors, in contrast to astrocytic tumors, show a better prognosis and increased therapeutic responsiveness and are is associated with the combined loss of 1p and 19q. The genetic hallmarks of glioblastomas are the gain of chromosome 7 and the loss of chromosome 10. Our objective was to genetically characterize the oligodendroglial and the astrocytic parts of GBMOs and correlate morphologic and genetic features with clinical data.
We analyzed the oligodendroglial and the classic glioblastoma parts of 13 GBMOs separately by interphase fluoreszence *in situ* hybridization (FISH) on paraffin sections using our own probe set (regions 1p, 1q, 7q, 10q, 17p, 19q, cen18, 21q) and by comparative genomic hybridization (CGH) of microdissected paraffin embedded tumor tissue. Additionally, we studied ten classical GBMs by interphase FISH using the same markers.
We identified four distinct genetic subtypes in 13 GBMOs: an astrocytic subtype (9/13) characterized by +7/-10; an oligodendroglial subtype with -1p/-19q (1/13); an intermediate subtype showing +7/-1p (1/13), and an other subtype having none of the former aberrations typical for gliomas (2/13). In contrast, all ten classical GBMs correspond genetically to the astrocytic subtype. The diagnostic determination of these genetic signatures may allow for a better prognostication of the patients.
The different histological tumor parts of GBMO revealed common genetic changes in all tumors and showed additional aberrations specific for each part. These findings demonstrate the monoclonal origin of the tumor followed by the development of the astrocytic and oligodendroglial components.

P19
COPY NUMBER ABERRATIONS IN PAPILLARY THYROID CARCINOMA (PTC) DETECTED BY ARRAY-CGH

J. Hess[1], K. Unger[2], H. Braselmann[1], G. Thomas[2], H. Zitzelsberger[1]
[1]*Helmholtz Zentrum München, Department of Radiation Cytogenetics, Neuherberg, Germany*
[2]*Imperial College London, Department of Histopathology, London, UK*

An increasing incidence in childhood thyroid cancer is a main consequence of the Chernobyl accident in the contaminated regions. Previous studies revealed a distinct genetic heterogeneity of RET/PTC-rearrangements in a subgroup of PTC. Since these findings suggest multiple clones involved in tumour development, we studied a PTC-cohort to analyse whether genes other than RET are involved in thyroid carcinogenesis. In particular, we aimed to identify candidate genes either interacting with RET/PTC or pointing to alternative routes of tumour development. Another aim was to identify genetic alterations that correlate with the patients' exposure status to radioiodine fallout.
52 PTC from the Chernobyl Tissue Bank were analysed. The cohort was matched for age at diagnosis and residence, and consisted of patients both exposed and not exposed to radioiodine fallout. 33 patients were born before the Chernobyl reactor accident and have been exposed to radioiodine fallout. The other 19 patients were born after January 1st 1987 and have not been exposed to radioiodine fallout (sporadic PTC). In order to detect copy number aberrations (CNA) we performed array-CGH using 1-Mb BAC-Arrays. Cases with similar aberration patterns were identified by hierarchical cluster analysis (HCA). FISH analysis on FFPE tissue sections was applied to validate frequent CNA.
HCA revealed three distinct tumour subgroups that correlated with the presence of RET/PTC rearrangements ($p=0.0096$), tumour size ($p=0.00087$) and lymph node status ($p=0.04$). Statistical analysis identified chromosomal aberrations that were significantly associated with exposure to radioiodine fallout, female sex, histological subtype follicular, tumour size T3 and tumour size T1. Commonly altered as well as tumour phenotype specific altered regions, have been searched for candidate genes. Overall, we

identified 201 tumour related candidate genes that are involved in various molecular pathways such as apoptosis- and PI3K-pathway. Amplifications of the candidate genes CCND1, WNT1, CDK5 and CTTN were exemplarily confirmed by FISH.
Distinct aberration patterns in RET/PTC-positive and -negative cases indicate different mechanisms of tumour development. The identification of new candidate genes in thyroid tumourigenesis and their involvement in different pathways, as well as the identification of a new potential marker for radiation-induced PTC, may serve as a starting point for further expression and functional studies on thyroid cancer.

P20
ESTABLISHMENT AND GENETIC CHARACTERIZATION OF TWO CELL LINES DERIVED FROM SINONASAL SQUAMOUS CELL CARCINOMAS.

C. García-Inclán[1], M. Alonso-Guervós[1], F. López[1,2], C. Suarez[1,2], J. Llorente[1,2], M. Hermsen[1]
[1]I.U.O.P.A., Hospital Universitario Central de Asturias. Otolaryngology.Universidad de Oviedo, Oviedo, Spain
[2]Servicio de Otorrinolaringología, Hospital Universitario Central de Asturias. Universidad de Oviedo, Oviedo, Spain

Background: Sinonasal malignancies that originate in the respiratory epithelium are unusual pathologies, representing about 3% of all head and neck cancers. The most frequent tumor types in this localization are squamous cell carcinoma (SCCNC) and intestinal-type adenocarcinoma, both of which etiologically related to professional exposure to textile, leather, wood or aluminium, but not to tobacco smoking. However, little is known about the genetic pathways underlying SCCNC development nor are cell lines available as a model system for studying these tumors. Here we describe two SCCNC cell lines established in our lab and compare the genetic characteristics with those of their original primary tumors**.**
Material and Methods: Cell line NC1 was derived from a T3N1M0 tumor of the ethmoid sinus and NC2 from a T4N1M1 tumor of the maxillary sinus. Freshly obtained biopsy samples from two primary tumors were cut into small fragments, transferred to a dry culture flask and covered with culture medium. Initial outgrowth was observed after a few days and co-cultivated fibroblasts were removed by differential trypsinizations. Growth and invasive properties were analyzed with the Matrigel Invasion Assay. Genetic analyses of the cell lines (passage 50 or later) and their original primary tumors included microarray CGH, MLPA and FISH.
Results: Both cell lines demonstrated many copy number losses, gains and also high level amplifications, which were virtually identifical in the original primary tumors. Surpringly, the overall pattern of gains and losses, i.e. loss 3p/gain 3q, gain 7p, gain 11q13 and 20q, was similar to smoking-related head and neck squamous cell carcinomas. Two amplifications, ERBB1 (EGFR) NC1 and ERBB2 (Her2/neu) in NC2, detected by microarrayCGH and MLPA were confirmed by FISH. Matrigel Invasion Assay showed that NC1 is less invasive than NC2, in spite of its higher growth rate.
Conclusion: Two immortal cell lines were established from squamous cell carcinoma of the nasal cavities and may serve as model system for the study of this type of carcinoma.

P21
ESTABLISHMENT AND GENETIC CHARACTERIZATION OF AN IMMORTAL CELL LINE DERIVED FROM AN INTESTINAL-TYPE SINONASAL ADENOCARCINOMA

J. García Martínez[1], J. Pérez-Escuredo[1], B. Vivanco[2], F. Fresno[2], C. Suárez[1], J. Llorente[1], M. Hermsen[1]
[1]IUOPA, Hospital Universitario Central de Asturias, Otolaryngology, Oviedo, Spain
[2]IUOPA, Hospital Universitario Central de Asturias, Pathology, Oviedo, Spain

Intestinal-type sinonasal adenocarcinoma (ITAC) are rare tumors, etiologically related to professional exposure to wood dust. The overall prognosis is poor, mainly due to the difficulty to resect the tumor completely in this anatomically complex region. Therefore, there is great need for alternative treatments. However, the lack of a good tumor model system for ITAC has hampered the development and testing of new therapeutic agents. Here we report the establishment and characterization of the first human ITAC cell line named ITAC-3.
The cell line was initiated from small explants of a T5N0M0 tumor and grew as an adhering monolayer in HuMeC culture medium. Growth and invasiveness parameters as well as genetic characteristics were analyzed.
The doubling time was 23 hours and the cell line was capable of invasion in matrigel. The cell line did not express cytokeratin 7 or 20. Chromosomal analysis showed a modal number of 65 chromosomes. High resolution microarray CGH analysis showed a

multitude of copy number alterations, including homozygous deletions, but no high level amplifications. Immunohistochemical analysis showed overexpression of p53 and EGFR, but normal expression of B-catenin and p16.
This is the first report of the establishment of an ITAC cell line derived from a representative of ITAC in general, genomic profile, growth and invasive potential and it will be a very useful tool that will allow better understanding of the carcinogenesis of this tumor type.

P22
A ROLE FOR CHRONIC INFLAMMATION IN INTESTINAL-TYPE SINONASAL ADENOCARCINOMA

J. Pérez-Escuredo[1], B. Vivanco[2], J. García Martínez[1], F. Fresno[2], C. Suárez[1], J. Llorente[1], M. Hermsen[1]
[1]IUOPA, Hospital Universitario Central de Asturias, Otolaryngology, Oviedo, Spain
[2]IUOPA, Hospital Universitario Central de Asturias, Pathology, Oviedo, Spain

Intestinal-type sinonasal adenocarcinomas (ITAC) are epithelial tumors of the nasal cavities and paranasal sinuses related to professional exposure to wood dust. Despite its clear etiology, it is unknown by what molecular mechanism ITACs develop. The present model suggests that inhaled wood dust particles larger than 5µm become trapped in the mucosa of the middle turbinate and ethmoid. Since these particles do not have mutagenic properties, it may be hypothesized that prolonged exposure to and irration by wood dust particles stimulate cellular turnover by inflammatory pathways. Chronic inflammation is recognized to be an important mechanism in tumor initiation and progression in various cancer types, such as colorectal and stomach adenocarcinoma. Our goal was to find out if chronic inflammation plays a role in the development and progression of these tumors.
We studied samples of ITAC from 66 patients, 54 of wich related to professional exposure to wood dust. For immunohistochemical protein expression analysis we constructed a tissue array containing triplicates of 66 ITACs. RT-PCR was employed for relative quantification of mRNA sequences, extracted from snap-frozen tumor material of 32 patients. We analysed the expression of several genes described to be important in inflammatory pathways.
P53 protein overexpression was detected in 63% of the cases, COX-2 in 47%. COX-2 overexpression was associated with EGFR expression (p=0.004). P65 and p50, components of NF-kB transcriptor factor, were found in 44% and 35% cases respectively. The gene expression study showed elevated expression in TNF-α, IL-8, COX-2, IL-6, MMP1, MMP9, MMP12 and MMP13. COX-2 gene expression was associated (p=0.043) with solid and mucinous type tumors, carrying a bad prognosis.
This study present preliminary evidence of a role for chronic inflammation in ITAC. It remains to be clarified whether this role is causal or rather the consequence of tumor development.

P23
DNA NEAR-DIPLOID ANEUPLOIDIZATION AND ENDOREDUPLICATION BY HIGH RESOLUTION DNA CONTENT FLOW CYTOMETRY SEPARATE PROGRESSION STEPS IN ORAL PRECANCER AND CANCER.

A. Donadini[1], M. Maffei[1], A. Cavallero[2], M. Pentenero[3], D. Malacarne[1], E. Di Nallo[1], M. Truini[4], R. Navone[5], P. Mereu[6], M. Scala[6], A. Santelli[2], S. Gandolfo[3], W. Giaretti[1]
[1]National Cancer Research Institute, Dept. Diagnostic Oncology, Genoa, Italy
[2]San Martino Hospital, Department of Otolaryngology, Genoa, Italy
[3]Oral Medicine and Oral Oncology Section, University of Turin, Department of Clinical and Biological Sciences, Turin, Italy
[4]National Cancer Research Institute, Department of Diagnostic Oncology, Pathology Section, Genoa, Italy
[5]University of Turin, Department of Biomedical Sciences and Human Oncology, Pathology Section, Turin, Italy
[6]National Cancer Research Institute, Department of Surgical Oncology, Oral Surgery Section, Genoa, Italy

Oral potentially malignant lesions (OPMLs) with dysplasia and aneuploidy are thought to have a high risk of progression into oral squamous cell carcinomas (OSCCs). Non-dysplastic oral clinically normal appearing mucosa in OPML distant fields" (ODFs) and non-dysplastic OPMLs, however, may also represent an early pre-cancerous state. ODFs, OPMLs without and with dysplasia and OSCCs were investigated by high resolution DNA content flow cytometry (hr-DNA-FCM). ODFs and OPMLs without dysplasia were DNA aneuploid respectively in 7/82 (8.5%) and 25/109 (23%) cases. "True normal oral mucosa" samples and human lymphocytes from healthy donors used as sex specific DNA diploid controls were DNA diploid in all cases. Dysplastic OPMLs and OSCCs were DNA aneuploid in 12/26 (46%) and 12/13 (92%) cases. The DNA aneuploid sublines were characterized

by the DNA Index (DI). DI aneuploid values (DI≠1) in ODFs and in non-dysplastic and dysplastic OPMLs were near-diploid (DI<1.4) respectively in all, 2/3 and 1/3 of the cases. DNA aneuploid OSCCs, instead, were characterized prevalently by multiple sublines (67%) and commonly (57%) by high-aneuploidy (DI≥1.4). DNA near-diploid aneuploid sublines in ODFs and OPMLs appear to reflect mechanisms of loss of symmetry in cell division. High DNA aneuploid and multiple sublines in OPMLs with dysplasia and OSCCs suggest, instead, mechanisms of tetraploidization of earlier near-diploid aneuploid cells and chromosomal loss. Hr-DNA-FCM seems to enable separation of subsequent progression steps of the oral carcinogenesis.

P24
CHROMOSOMAL CHANGES CHARACTERISE HEAD AND NECK CANCER WITH POOR PROGNOSIS

V. Bauer[1], H. Braselmann[1], M. Henke[2], K. Unger[3], H. Zitzelsberger[1]
[1]*Helmholtz Zentrum München, Department of Radiation Cytogenetics, Neuherberg, Germany*
[2]*University of Freiburg, Clinic for Radiation Therapy, Freiburg, Germany*
[3]*Imperial College London Hammersmith Hospital, Department of Histopathology , London, UK*

It is well established that genetic alterations may be associated with prognosis in tumour patients. We investigated chromosomal changes that predict the clinical outcome of head and neck squamous cell carcinoma (HNSCC) and correlate with characteristic clinico-pathological and radiotherapeutic parameters.
We performed comparative genomic hybridization (CGH) on tissue samples from 117 HNSCC patients scheduled for radiotherapy. Genomic aberrations occurring in more than five patients were tested for correlation with locoregional-progression-(LPR)-free survival. Significant alterations were further analysed by array CGH and Fluorescence in situ Hybridization (FISH).
In multivariate survival analysis gains on chromosomes 1q and 16q predicted reduced LPR-free survival after radiotherapy independently from known prognostic factors. Cluster analysis separated the patients into two clusters that are characterised by significant differences in copy number in 13 chromosomal regions. Array-CGH revealed 16q24.3 to be the region of interest on chromosome 16. This was verified by FISH analysis which also identified an increased copy number of the gene Fanca, a member of the FA/BRCA pathway. Quantitative RT-PCR confirmed increased Fanca expression on RNA-level. To determine the effect of Fanca on the radiosensitivity of cells, two cell lines were stably transfected with a Fanca-overexpressing vector and tested for differences in cell survival (colony forming assay) and chromosomal instability (three colour FISH) after irradiation with different doses of γ-radiation in comparison to non-transfected cells.
The findings of our study demonstrate that chromosomal gains on 1q and 16q represent prognostic markers in HNSCC and that these alterations may explain to some extent the unfavourable prognosis of a subgroup of patients after radiotherapy. Preliminary results of cell survival experiments indicate reduced radiation sensitivity in Fanca-overexpressing cells.

P25
EXPRESSION PROFILE OF MMR GENES IN CARCINOMA PROSTATE IN INDIAN SUBCONTINENT

A. Soni[1], A. Bansal[1], L.C. Singh[1], A.K. Mishra[1], N.K. Mohanty[2], S. Saxena[1]
[1]*Institute of Pathology, Tumour Biology, New Delhi, India*
[2]*Safdarjung hospital, Urology, New Delhi, India*

Question: Mismatch repair (MMR) system plays a role in promoting genetic stability by repairing DNA replication errors, inhibiting recombination between non-identical DNA sequences and participating in responses to DNA damage. Cells deficient in MMR exhibit a mutator phenotype and microsatellite repeat instability. In addition, these cells are hyper-recombinogenic because MMR normally imposes a barrier to recombination between divergent sequences. MMR genes are potential markers for detection and prognosis since their expression is linked to various malignancies in tumor predisposition, progression and response to chemotherapeutic agents. Previous studies have identified MMR gene mutations, MMR deficiency and differential MMR gene expression in Carcinoma Prostate (CaP). In this study, we hypothesize that MMR gene expression is down-regulated during pathogenesis of CaP in Indian population. To test this hypothesis, MMR gene expression was analyzed in tissue obtained from patients of CaP and Benign Prostatic Hyperplasia (BPH). We further correlated MMR gene expression with clinicopathological parameters to evaluate their role as potential prognostic marker in CaP.

Methods: Applying quantitative real time PCR analysis, expression of six important MMR genes *viz. hMLH1*, *hMSH2*, *hPMS1*, *hPMS2*, *hMSH3* and *hMSH6,* was evaluated. The study included 25 cases of CaP and 30 cases of BPH. Multiple endogenous control strategy was utilized to enhance the accuracy of expression analysis.
Results: Our results demonstrated that *GAPDH* and *TBP* genes could be used as endogenous control genes in CaP while *18S RNA* gene showed altered expression and hence could not serve as appropriate endogenous control gene in CaP. A significant down regulation of expression of *hMLH1*, *hMSH2* and *hPMS2* genes in CaP was observed as compared to BPH. Further, a trend of down regulation of *hPMS1*, *hMSH3* and *hMSH6* genes was noted but was not found to be statistically significant.
Conclusion: Our study suggests that altered MMR gene expression has important biological and clinical significance in CaP. The data indicates that *hMLH1*, *hMSH2* and *hPMS2* genes has a key role in causation of CaP in Indian population. Detailed exploration of these MMR genes could reveal the intriguing pathogenesis of CaP and may facilitate development of novel treatment strategies.

Poster session 6: Genomics and Proteomics of Colorectal Cancer

P31
DNA COPY NUMBER ALTERATIONS IN FLAT COLORECTAL ADENOMAS

Q. Voorham[1], B. Carvalho[1], A. Spiertz[2], N. van Grieken[1], H. Grabsch[3], B. Rembacken[4], M. Kliment[5], M. van de Wiel[6], A. Bruïne[2], C. Mulder[7], M. van Engeland[2], G. Meijer[1]
[1]*VU University Medical Center, Dept of Pathology, Amsterdam, The Netherlands*
[2]*GROW-School for Oncology and Developmental Biology, Maastricht University Medical Center, dept of Pathology, Maastricht,The Netherlands*
[3]*Leeds Institute of Molecular Medicine, University of Leeds, Pathology and Tumour Biology, Leeds, UK*
[4]*Leeds General Infirmary, Centre for Digestive Diseases, Leeds, UK*
[5]*Hospital Vitkovice, dept of Gastroenterology, Ostrava, Czech Republic*
[6]*VU University Medical Center, Dept of Epidemiology and Biostatistics, Amsterdam, The Netherlands*
[7]*VU University Medical Center, Amsterdam, Dept of Gastroenterology, Amsterdam, The Netherlands*

Introduction: Flat colorectal adenomas are associated with an aggressive clinical behaviour. In literature it is described that these lesions have a different molecular pathogenesis than regular polypoid-shaped lesions. Although a number of studies have investigated molecular alterations (such as mutations and methylation) still little is known about the tumourigenisis of these lesions. The aim of the present study was to identify, based on high resolution genome wide DNA copy number profiling, chromosomal regions that differ between flat and polypoid adenomas and could be linked to the two different phenotypes.
Material and Methods: Formalin-fixed paraffin-embedded (FFPE) material of 27 polypoid and 55 flat adenomas (classified according to the Paris classification) was isolated and analyzed by genome wide array comparative genomic hybridization (Agilent, 180K array). DNA copy number profiles were evaluated and correlated to the different phenotypes.
Results: Preliminary analysis of the data showed that overall all adenomas showed little chromosomal aberrations, 30 of the 82 adenomas (36.5%) showed less then 1% aberrant probes. Of the lesions that showed aberrations the most frequent ones were losses on chromosomes 1p and 18 and gains on chromosomes 7, 8, 9, 12, 13, 20 and X in both types of adenomas. For these regions no significant difference were found. However, the proportion of tumours with less then 1% aberrant probes was higher in flat adenomas when compared to polypoid adenomas, 23 out of 55 (42%) and 7 out 27 (26%), respectively (P = 0.01). Chromosomal regions found to be significantly different between polypoid and flat lesions were 3p12.3, 8p11.23, 14q32.33, 16p12.3, 17q12 and 21q11.2. (FDR<0.1).
Conclusion: Overall flat and polypoid adenomas are showing the same aberrations. Flat adenomas had significantly more profiles with little chromosomal aberrations. The regions 3p12.3, 8p11.23, 14q32.33, 16p12.3, 17q12 and 21q11.2 were significantly different between the two groups (FDR<0.1). Further investigations are warranted to uncover which genes located on these regions are involved in the different biological mechanisms leading to the different phenotypes of these lesions.

P32
DNA INTEGRITY ANALYSIS FOR COLORECTAL CANCER EARLY DIAGNOSIS

D. Calistri[1], C. Rengucci[1], A. Casadei Gardini[1], E. Scarpi[1], G.L. Frassineti[1], W. Zoli[1], F. Falcini[2], R. Silvestrini[1], D. Amadori[1]
[1]*I.R.S.T., Meldola, Forlì, Italy*
[2]*Morgagni-Pierantoni Hospital, Cancer Prevention Unit, Forlì, Italy*

Question: Of all the diagnostic tools currently available in colorectal cancer (CRC) screening programs, colonoscopy has shown great potential in reducing disease morbidity and mortality. In contrast, although the widely used fecal occult blood test (FOBT) is non-invasive, it is characterized by a high number of false-positive results, with psychological consequences for the patient as well as higher costs due to the need for second-level tests. We evaluated the potential of the fecal DNA assay as an alternative or in addition to the widely used fecal occult blood test (FOBT) for the early diagnosis of colorectal cancer (CRC).
Methods: Five hundred and sixty subjects (50- 69 years of age) with a positive FOBT were enrolled from the FOBT Regional Screening Program run by the Cancer Prevention Unit of Morgagni-Pierantoni Hospital in Forli, Italy. Twenty-six were diagnosed with adenocarcinoma, 264 with high-grade adenoma and 54 with low-grade adenoma, while 216 subjects did not have any premalignant or malignant lesions. Fecal DNA integrity was analyzed blindly by the fluorescence long DNA (FL-DNA) test.
Results: FOBT and FL-DNA were largely independent variables (r_s = 0.036, P = 0.42), with values ranging from 101 to 5826 ng/mL and from 0 to 515 ng, respectively. Median values of both variables were significantly higher in cancer patients than in patients with non neoplastic lesions or in healthy individuals. FOBT values were significantly higher in patients with pedunculated rather than sessile adenomas (P = 0.003), and in those with larger (≥2 cm) rather than smaller adenomas. In contrast, FL-DNA values were significantly higher in patients with sessile than pedunculated adenomas, in those with mixed rather than single localization adenomas, and in patients with rectal cancer compared to other intestinal sites.
Conclusions: Of note, FL-DNA proved capable of identifying subgroups at different risk of cancer in FOBT-positive individuals. Our results indicate that fecal DNA furnishes additional, accurate diagnostic information in FOBT- positive individuals.

P33
GAIN OF CHROMOSOME 13 IS A CAUSE OF CDK8 OVEREXPRESSION ASSOCIATED WITH COLORECTAL ADENOMA TO CARCINOMA PROGRESSION

B. Carvalho[1], C. Postma[1], S. Mongera[1], P. van Diemen[1], B. Ylstra[1], G.A. Meijer[1]
[1]*VU University medical center, Pathology, Amsterdam, The Netherlands*

Introduction: Colorectal adenomas are common precursors of colorectal cancer (CRC). About only 5% of adenomas progress to cancer and this progression is mostly associated with overall onset/increase of chromosomal instability. Gain of 13q is often implicated in this progression of adenoma to carcinoma (1). Until recently however no genes were identified to be the drivers of this amplicon. Recently, Firestein and collaborators (2) showed that cyclin 8 (*CDK8*), at 13q12.13, functions as an oncogene in CRC.
The aim of this study was to evaluate *CDK8* gene dosage effects in colorectal adenoma to carcinoma progression.
Material and methods: Sixty seven colorectal tumours (34 adenomas and 28 carcinomas) were analysed by array CGH (5k BAC platform, including contig coverage of 13q) and by expression microarray (30k Compugen library). Integration of DNA copy number dosage and gene expression was performed using the Ace-it tool (3).
Results: In the tumours analysed we observed 13q copy number gain in 9% and 46% of adenomas and carcinomas, respectively. Integrating copy number and mRNA expression with the differential upregulation of genes between carcinomas and adenomas, provided us with a list of 43 genes. Within this list, *CDK8* ranked 7th in significancy (p=0.003).
Conclusions: Copy number gain of 13q has a gene dosage effect on *CDK8* mRNA expression, indicating a role of this gene in colorectal adenoma to carcinoma progression.

(1) Hermsen M, Postma C, Baak J, Weiss M, Rapallo A, Sciutto A, Roemen G, Arends JW, Williams R, Giaretti W, De Goeij A, Meijer G. Colorectal adenoma to carcinoma progression follows multiple pathways of chromosomal instability. Gastroenterology 2002 Oct;123(4):1109-19.
(2) Firestein R, Bass AJ, Kim SY, Dunn IF, Silver SJ, Guney I, Freed E, Ligon AH, Vena N, Ogino S, Chheda MG, Tamayo P, Finn S, Shrestha Y, Boehm JS, Jain S, Bojarski E, Mermel C, Barretina J, Chan JA, Baselga

J, Tabernero J, Root DE, Fuchs CS, Loda M, Shivdasani RA, Meyerson M, Hahn WC. CDK8 is a colorectal cancer oncogene that regulates beta-catenin activity. Nature 2008 Sep 25;455(7212):547-51.
(3) van Wieringen WN, Belien JA, Vosse SJ, Achame EM, Ylstra B. ACE-it: a tool for genome-wide integration of gene dosage and RNA expression data. Bioinformatics. 2006 Aug 1;22(15):1919-20.

P34
IDENTIFICATION OF 8Q AND 20Q GAINS IN THE DNA DIPLOID COMPONENTS OF ORAL POTENTIALLY MALIGNANT LESIONS.

M. Maffei[1], P. Scaruffi[2], A. Donadini[1], E. Di Nallo[1], D. Malacarne[1], G.P. Tonini[2], M. Pentenero[3], S. Gandolfo[3], P. Castagnola[4], W. Giaretti[1]
[1]National Cancer Research Institute, Department of Diagnostic Oncology, Genova, Italy
[2]ational Cancer Research Institute, Department of Translational Paediatric Oncology, Genoa, Italy
[3]University of Turin, Department of Clinical and Biological Sciences, Turin, Italy
[4]National Cancer Research Institute, Department of Translational Oncology Research, Genoa, Italy

Background and aims: The identification of the earliest events and related biomarkers during oral cancer genesis and progression is under intense study. To identify genomic abnormalities and candidate biomarkers, we investigated diploid fields of distal normal appearing mucosa (ODFs) among patients with oral potentially malignant lesions (OPMLs), non-dysplastic and dysplastic OPMLs and oral squamous cell carcinomas (OSCCs) by high-resolution array-comparative genomic hybridization (a-CGH).
Methods: We used flow cytometry (FCM) to evaluate the DNA Index (DI) and to sort DI specific aneuploid sublines. In particular, we compared the DNA diploid and aneuploid components among 13 ODFs, 10 non-dysplastic and 15 dysplastic OPMLs and 17 OSCCs. Furthermore, a-CGH profiles were obtained from DNA extracted from 19 aneuploid FCM sorted and 36 diploid sublines using 105K oligonucleotide microarrays (Agilent Technologies). In addition, "true normal oral mucosa" from healthy donors were considered as negative control for aberrations.
Results: The aCGH analysis revealed DNA copy number changes in 89% (17/19) DNA aneuploid sublines and in 52% (12/23) DNA diploid components. Remarkably, the diploid ODFs showed DNA copy number changes in 61% (8/13) cases. The average number of aberrations in the DNA aneuploid sublines increased gradually from OPMLs without and with dysplasia up to OSCCs. Loss of chromosome regions were more frequent (p=0.01) in the aneuploid sublines than in the diploid ones. Most of the structural aberrations were observed among the diploid components from OPMLs without and with dysplasia and OSCCs, whereas the DNA aneuploid components showed both structural and numerical aberrations. ODF sublines were characterized by gains of 7p22.2-pter, 8q24.3-qter, 11p15.4-pter, 20q13.33-qter, and loss of 9p21.3. Remarkably, 8q and 20q gains were present at high frequency in the diploid sublines of the ODFs and in non-dysplastic OPMLs.
Conclusions: The present findings suggest that 8q and 20q gains are early events of the oral carcinogenesis. Related biomarkers may provide useful clinical applications to predict the preneoplastic-neoplastic transition.

P35
CHROMOSOMAL COPY NUMBER ABERRATIONS INVOLVED IN THE DIPLOID TO ANEUPLOID TRANSITION IN COLORECTAL CANCER.

M. Maffei[1], L. Terpstra[2], S. Mongera[2], B. Carvalho[2], A. Donadini[1], P. Castagnola[3], G.A. Meijer[2], W. Giaretti[1]
[1]National Cancer Research Institute, Genoa, Department of Diagnostic Oncology, Genova, Italy
[2]VU University medical center, Department of Pathology Tumor Profiling Unit, Amsterdam, The Netherlands
[3]National Cancer Research Institute, Department of Oncology and Translational Research, Genoa, Italy

Background and aims: Chromosome 20q amplification was reported to elicit multiple putative oncogenes contributing to chromosomal instability (CIN) and colorectal cancer (CRC) progression [1,2]. The aim of this study was to further investigate the possible role of 20q gain and, in addition, of 8p loss, 8q, and 13q gain in the diploid-aneuploid transition in sporadic CRC.
Methods: Sixteen aneuploid sporadic CRCs were selected, among which six were Dukes B, ten were Dukes C and all with microsatellite stable phenotype. Multiple superficial and intratumor samples obtained from the 16 CRCs have been used to provide nuclei suspensions for flow cytometric (FCM) analysis of the DNA Index (DI) and for FCM-sorting of 16 epithelial enriched DI diploid and the corresponding 16 aneuploid sublines. All samples were analysed by MLPA using a probe mix targeting chromosome arms 8p, 8q, 13q and 20q. Gains and losses were assigned by

averaging the values for all the probes used for the same chromosome arm.
Results: The MLPA detected aberrations were 8p losses (7) and gains (4) and respectively 14, 15 and 18 gains for 8q, 13q and 20q. The most frequently detected aberration was gain of 20q (20q+) among the DNA aneuploid sublines (14/16, 87.5%) versus 4/16 (25%) for the corresponding DNA diploid sublines. The corresponding figures for 13q+ and 8q+ aneuploid and diploid sublines were respectively 75% and 19% and 69% and 19%. For 8p- we had respectively 31% and 12.5%.
Conclusion: These preliminary data suggest that 20q+ as well as 13q+ and 8q+ are to be considered relatively late events in the CRCs analyzed in the present series since their DNA diploid components contained statistically significant lower incidences of these aberrations compared to the corresponding aneuploid sublines. The hypothesis, in particular, that 20q amplification in tumor diploid cells may specifically elicit multiple putative oncogenes contributing the diploid-aneuploid transition [2] seems not to be reinforced by our present data. The aneuploid component results in both studies are not contradicting. Nevertheless, we believe that more probes on 20q chromosome arm are needed to investigate more thoroughly the involvement of this chromosomal region in the diploid to aneuploid transition in colorectal cancer. Work is in progress to analyze the same subclones also by high-resolution CGH analysis.

References
1. Carvalho B et al. Gut *2009;58;79-89) 2. De Angelis P et al. Int. J. Cancer: 120, 2734-2738 (2007).*

P36
GENOME WIDE DNA ALTERATIONS OF STAGE II COLON CANCER IN PATIENTS WITH AND WITHOUT RELAPSE ASSESSED BY ARRAY CGH

O. Krijgsman[1], E. Belt[2,3], D. van Essen[1], B. Carvalho[1], G. Meijer[1], B. Ylstra[1]
[1]*VU University medical center, Pathology, Amsterdam, The Netherlands*
[2]*VU University medical center, Surgery, Amsterdam, The Netherlands*
[3]*Kennemer Gasthuis Hospital, Surgery, Haarlem, The Netherlands*

Patients diagnosed with curable stage II colon cancer undergo surgery but are generally not treated with adjuvant chemotherapy. However, 20-30% of these patients develop a relapse. Identification of patients with a high risk of relapse after surgery could aid a decision to treat these stage II colon cancer patients with chemotherapy.
The aim of this study was to analyze copy number changes observed in stage II colon tumors from patients with and without relapse in order to find a (set of) biomarker(s) to predict recurrence.
A case-control pilot study was conducted using DNA from 35 microsatellite stable (MSS) stage II colon carcinomas with or without relapse (18 and 17, respectively). DNA copy number analysis was performed on high-resolution CGH oligonucleotide arrays containing 180,000 probes with DNA isolated from matched normal mucosa as a reference. Matched reference reduced noise in the data and allows us to differentiate between somatic aberrations and germ-line variations. Data pre-processing was performed using "DNAcopy" to segment the data and "CGH call" to estimate if a chromosomal region is gained, lossed or amplified. Both packages are available in R through Bioconductor.
Overall, copy number aberrations pattern observed were in agreement with literature, namely gains of chromosome 13 and 20 in 70%, gain of chromosome 7 in 50% and deletion of chromosome 18 in 90% of the carcinomas. No differences were observed in large chromosomal aberrations between the two groups. However, because these samples were analyzed on a high resolution CGH platform, further analysis into the focal (< 3Mb) chromosomal aberrations might yield a better insight into the genomic differences between patients with and without relapse and identify potential driver genes which explain the differences in the risk of relapse.
In conclusion, further analysis of the samples in this pilot study, including differences in focal genomic aberrations, will be conducted to uncover the genomic alteration underlying the differences in patients with and without relaps.

Poster session 7: Genomics and Proteomics of Breast Cancer

P41
CENTROSOME MORPHOLOGY AND TUMOR PROGRESSION IN BREAST CANCER

K. Friedrich[1], D. Otto[1], M. Toma[1], M. Wolfdietrich[1], G. Baretton[1]
[1]*University Hospital TU Dresden, Institute of Pathology, Dresden, Germany*

Question: The purpose of the study was to analyse centrosome morphology in breast cancers with different different degrees of chromosomal instability, reflected by DNA ploidy and with different stages of tumor progression, reflected by clinicopathological markers.
Methods: The centrosomes of 45 invasive breast cancers were stained immunhistochemically with an antibody against gamma-tubulin (GTU-88; SIGMA) and quantitatively analysed for number, size and shape with Spectracube SD-200H (Applied Spectral Imaging). The DNA ploidy was estimated with an OPTIMAS® based image cytometry workstation. The statistical analysis was done by t-Test according to Student ($p<0,05$).
Results: Centrosomes were larger in non-diploid tumors than in peridiploid tumors. Centrosomes of breast cancers larger than 2 cm differed in number and shape from those of smaller breast cancers. Lymph node positive breast cancers showed larger centrosomes than lymph node negative tumors. Centrosomes in grade 1 tumors differed in their shape from centrosomes in tumors with a histopathological grade 2. Further differences in size and shape of centrosomes were detected in breast cancers with different expression status of estrogen receptor and p53.
Conclusions: The number and morphology of centrosomes in breast cancer is not only associated with DNA-ploidy, but also with different expression of clinicopathological markers. Thus, the analysis of centrosomes may contribute to improvement of prognostication in breast cancer. Furthermore, some chemotherapeutic drugs work by the influence on the mitotic spindle. Knowledge about the morphology of centrosomes as an import part of the mitotic spindle may be helpful for selection of chemotherapeutic drugs. However, this requires the analysis of a considerably higher number of cases under standardised conditions.

P42
MOLECULAR HETEROGENEITY IN G3 N0 BREAST CANCER - BETTER TREATMENT TAILORING FOR PATIENTS OF DIFFERENT AGES?

K. Unger[1], S. Oliveros[2], H. Zitzelsberger[3], S. Riley[1], W. Williams[1], R. Leonard[2], G. Thomas[2]
[1]*Singleton Hospital, Department of Histopathology, Swansea, UK*
[2]*Imperial College London, Surgery and Cancer, London, UK*
[3]*Helmholtz-Zentrum Muenchen, Department of Radiation Cytogenetics, Neuherberg, Germany*

*Background:*It is already known that there is an age-related difference in the relative proportion of Grade 1 and 3 (G3) breast cancer (BC), with G3 BC being more common in younger women. The aim of this study was to examine these differences more extensively, concentrating solely on G3, node negative (N0) BC in two distinct populations selected on the basis of age - one aged under 43 and the other aged over 70 at diagnosis. In this study we used BAC array CGH to study genomic copy number alterations (CNA) of the tumours to investigate whether G3N0 BC shows significantly different patterns with respect to age.
Materials and Methods: Ethics approval for the study was obtained from the South West Wales Research Ethics Committee and sections from routine diagnostic formalin fixed paraffin embedded (FFPE) blocks were obtained from 39 patients with G3N0 BC (18 patients < 43 years and 21 > 70 years, all IDC). DNA was extracted using the QiAmp FFPE tissue system and integrity of DNA assessed by multiplex PCR. We used 1Mb BAC array CGH to identify genomic copy number alterations. Spatial normalisation, circular binary segmentation and the CGHcall algorithm was used to generate CGH profiles. Unsupervised hierarchical clustering, supervised and correlation were carried out within the R statistical platform.
Results: Three distinct groups were identified on the basis of their CNA. One group of 12 patients and one of 13 were identified which significantly correlated ($p=0.015$, Fisher's Exact test) with young (8/12) and old age (11/13) at diagnosis. The main CNAs that distinguished the two groups on age involved small regions on chromosomes 1, 9, 10, 14 and 20. The remaining patients formed a group which showed no

correlation with age. There was no significant difference with respect to ER or Her2 status among these groups.
Conclusions: Our results show considerable heterogeneity in CNA in G3N0 breast cancer, some of which associated with younger and older groups of patients. Other studies have suggested that breast cancer in elderly women is more indolent than in younger patients, although few have dissected this as a function of histological grade. Further studies breaking down these differences may result in better targeting of therapy in pathologically similar BC, and may lead to differing treatment options based on age-associated changes in biology.

P43
TWIST1 ONCOGENE: COMPARATIVE STUDIES, SEQUENCE VARIATIONS AND MRNA EXPRESSION IN CAT MAMMARY GLAND CARCINOMAS

C. Baptista[1,2], E. Bastos[2], S. Santos[2], I. Gut[3], H. Guedes-Pinto[2], F. Gärtner[4,5], R. Chaves[2]
[1]*ICBAS-Porto University, Veterinary Clinics, Porto, Portugal*
[2]*University of Trás-os-Montes and Alto Douro, Institute for Biotechnology and Bioengineering, Centre of Genetics and Biotechnology (CGB-UTAD/IBB), Vila Real, Portugal*
[3]*CEA/DSV/IG-Centre National de Génotypage, Evry Cedex, France*
[4]*ICBAS-Porto University, Department of Pathology and Molecular Immunology, Porto, Portugal*
[5]*Institute of Pathology and Immunology - University of Porto (IPATIMUP), Porto, Portugal*

Feline mammary carcinoma (FMC) is highly aggressive, mainly hormone receptor-negative cancer, proposed as a model for poor prognosis human breast cancer (HBC). Germline mutations in *TWIST1* may predispose to breast cancer and when increased in breast cancer cells it has been shown to promote metastatic ability in *in vivo* animal models. The aims of this study were to partially isolate the *TWIST1* gene in *Felis catus,* perform comparative studies, to screen spontaneous FMC for sequence variations and evaluate its mRNA expression.
Primer combinations were selected based on the alignments of homologous DNA sequences. After PCR amplification using cat gDNA, 3 bands were obtained (around 300, 800 and 1000 bp), purified and sequenced. Several bioinformatic tools were used to perform comparative studies. 30 spontaneous FMC were screened for polymorphisms. To evaluate the *TWIST1* expression in 7 FMC, RNA extraction/purification and cDNA synthesis were performed. Primers were designed and hybridization probes selected according to major homology for each transcript.
There is a higher similarity between the isolated *TWIST1* gene in *Felis catus* and *Homo sapiens* (86%) than between *Homo sapiens* and *Rattus norvegicus* or *Mus musculus* (75%). The partial amino acid sequence showed no change in these four species. This inferred coding sequence presented high similarity (~96%) between *Homo sapiens* and *Felis catus*. No sequence variations were identified in all tumours analyzed regarding the predicted coding region. *TWIST1* was downregulated in all carcinomas.
We believe that this investigation is the first one to study the *TWIST1* gene in cat regarding all the aspects here reported. The comparative studies evidenced a higher similarity between cat and man than between man and other widely used animal models such as rat or mouse. These results suggest that cat is as an attractive model, at least for *TWIST1* studies, and that may be used instead of the classical animal models. *TWIST1* downregulation in all carcinomas is, however, an unexpected result. Nevertheless numbers are small suggesting future directions for further investigations. In conclusion, although we present here some challenging results, we also give the first insights regarding the *TWIST1* gene in cat that may contribute to establish a feline spontaneous model to study HBC.

P44
HYPOXIA INDUCES DOWNREGULATED EXPRESSION OF SERINE/THREONINE KINASE-15 (STK15) IN BREAST CANCER CELL LINES

L.R. Corsini[1], D. Fanale[1], V. Amodeo[1], M. Terrasi[1], L. La Paglia[1], L. Insalaco[1], G. Bronte[1], S. Rizzo[1], G. Cicero[1], V. Bazan[1], A. Russo[1]
[1]*University of Palermo, Oncology, Palermo, Italy*

Background: STK15 (Aurora A/BTAK) is an oncogenic serine/treonine kinase playing a key role in centrosome separation and in mitotic bipolar spindle formation during mitosis. This role appears consistent with the high incidence of its misregulation in cancer; it is highly expressed in many types of human tumors including breast cancer, and its overexpression induces aneuploidy and centrosome amplification. Several studies suggested that the hypoxia is a determinant factor involved in *STK15* up-regulation in hepatoblastoma cell lines. Since the hypoxia is a

typical tumoral condition which influences the expression of various proteins involved in proliferation and cell cycle progression, the aim of our study was to obtain new insights into STK15 regulation *in vitro,* evaluating the possible HIF-1 role in its transcriptional control of expression.
Methods: A microarray analysis using Affymetrix platform, was performed in MCF7, MDA-MB-231 and SKBr3 breast cancer cell lines cultured in normoxic and hypoxic conditions in order to compare the differential gene expression profile in response to hypoxia. This analysis allowed us to obtain a statistically significant ($p < 0.05$) differential expression genes, which made it possible to select a set of genes involved in cell cycle progression, angiogenesis and tumor pathogenesis.
Results: By focusing our attention on genes involved in cell cycle progression, we found a downregulated expression of *STK15* gene in MCF-7, MDA-MB-231 and SKBr3 breast cancer cell lines. We confirmed this downregulation of the *STK15* gene, showing a reduction of mRNA levels and of related protein, by means of Real-Time PCR and Western Blotting. We investigated the HIF-1 role in the transcriptional control of *STK15* expression by means of the ChIP assay.
Conclusions: Our data suggest that hypoxia induces a reduction of *STK15* expression and in discordance with previous reports, we hypothesize that this specific downregulation of the *STK15* gene might be able to suppress its proliferation and lead to the apoptosis of breast cancer cell lines.

P45
HYPOXIA AND HUMAN GENOME STABILITY:DOWNREGULATION OF BRCA2 IN BREAST CANCER CELL LINES

D. Fanale[1], L.R. Corsini[1], M. Terrasi[1], L. La Paglia[1], V. Amodeo[1], L. Insalaco[1], G. Bronte[1], S. Rizzo[1], G. Cicero[1], V. Bazan[1], A. Russo[1]
[1]*University of Palermo, Oncology, Palermo, Italy*

Background: Hypoxia is a key tumor microenviromental factor. Many studies have demonstrated that hypoxia inhibit the DNA repair process and promotes genetic instability in human cancers. Very little is known regarding the functional consequences of hypoxia in the expression of proteins involved in DNA double-strand break repair in human breast cancer. Therefore aim of our studies is to evaluate the effects of hypoxia on genomic stability in breast cancer cell lines, to obtain new insights on role that the tumor microenviromental have on DNA repair and on genetic instability in breast cancers.
Methods: A microarray analysis, using Affymetrix platform, was performed in MCF7, MDA-MB-231 and SKBr3 breast cancer cell lines, cultivated in normoxic and hypoxic conditions, to identify genes showing a differential gene expression profile in the examined conditions. Among all the genes, we selected those involved in DNA repair mechanisms, to obtain new knowledge about the mechanisms that regulate genomic instability in response to hypoxia.
Results: We found a downregulated expression of *BRCA2* and other genes involved in DNA repair process both MCF-7, MDA-MB-231 and SKBr3 breast cancer cell lines. Focusing on *BRCA2* our results were confirmed evaluating the reduction of mRNA levels and the related protein by Real-Time PCR and Western Blotting.
Conclusions: Our data suggest that the hypoxia, decreasing the DNA repair capacity by downregulated expression of *BRCA2* and other genes involved in DNA repair, could be responsible for the continuous changes that affect the DNA during the process of tumorigenesis favoring the progression to stage more advanced of breast cancer.

P46
QUANTITATIVE REAL-TIME PCR OF BASAL MARKER EXPRESSION IN FORMALIN-FIXED PARAFFIN EMBEDDED BREAST CANCER SECTIONS

D. Haag[1], M. Hahn[1], E. Veltrup[2], K. Pfleger[1], H.-P. Sinn[3], R. Penzel[3], P. Lichter[1], A. Schneeweiss[4], R. Wirtz[2]
[1]*German Cancer Research Center, Molecular Genetics, Heidelberg, Germany*
[2]*Siemens Healthcare Diagnostics Products GmbH, Molecular Research, Cologne, Germany*
[3]*University of Heidelberg, Institute of Pathology, Heidelberg, Germany*
[4]*National Center for Tumor Diseases University of Heidelberg, Department of Gynecology and Obstetrics, Heidelberg, Germany*

Background: Gene expression measurement of biomarkers on RNA level is valuable for prognosis and prediction of treatment response in primary breast cancer. In addition, distinct transcript patterns have been shown to be characteristic for intrinsic subtypes. However, the infrastructure required for collecting and processing fresh tumor samples yielding in high quality RNA remains challenging for clinical routine. RNA

extraction from formalin-fixed paraffin-embedded (FFPE) tissue offers a promising alternative. Therefore, the accuracy and reliability of data generated from such specimen has to be verified.
Methods: A fully automated, bead-based RNA-isolation procedure was applied to 101 FFPE breast cancer tumor samples and expression of five basal marker genes (*ESR1, PGR, ERBB2, KRT5* and *MMP7*) was measured using quantitative real-time PCR (RT-PCR). For comparison, basal-marker gene expression was accordingly examined in RNA extracted from corresponding freshly frozen (FF) biopsies. Finally, a correlation analysis with previously generated microarray and immunohistochemistry (IHC) data of the same samples was conducted.
Results: We observed a significant concordance of gene expression measured on transcript level for FFPE and FF derived RNA and across platforms ($r>0.5$, $p<0.05$) for all five genes. Three of these showed even a stronger correlation ranging from $r=0.71$ to $r=0.95$.
Furthermore, IHC-score was significantly associated with transcript abundance in all cases ($r>0.5$, $p<0.05$).
Conclusion: Gene expression analysis on transcript level using RT-PCR is feasible on total RNA extracted from FFPE tumor sections, providing accurate and reproducible results as compared to freshly frozen material and an independent platform. At least five tested common markers can be utilized to assess transcript levels in breast cancer using routinely acquired tumor specimen. These assays might therefore provide reliable parameters for breast cancer classification applicable in clinical practice.

P47
ATM GERMLINE MUTATIONS IN WOMEN WITH FAMILIAL BREAST CANCERAND A RELATIVE WITH HAEMATOLOGICAL MALIGNANCY

L. La Paglia[1,2], L. Anthony[2], G. Weber[2,3,4], J. Champ[2,3,4], E. Cavaciuti[5,6], J.L. Viovy[3,4], A. Russo[1,7], D. Stoppa-Lyonnet[2,8,9]
[1]*University of Palermo, Oncology, Palermo, Italy*
[2]*Curie Institute , Oncology, Paris, France*
[3]*Curie Institute, Research Center, Paris, France*
[4]*CNRS Unit, UMR 168, Paris, France*
[5]*Curie Institute, Biostatistical Science , Paris, France*
[6]*INSERM , U900, Paris, France*
[7]*Temple University, Sbarro Health Research Organization, Philadelphia, USA*
[8]*INSERM , Unit U830, Paris, France*
[9]*Université Paris Descartes, Paris, France*

Background: Germline biallelic mutations of the Ataxia Telangiectasia Mutated (*ATM*) gene are the cause of ataxia telangiectasia (A-T), a complex neurological disease associated with an immune deficiency and a high risk of leukaemias and lymphomas. The relative risk of breast cancer estimated for obligate *ATM* heterozygote mutation carriers is about 3.
The frequency of *ATM* mutation carriers in women with a Breast Cancer (BC) family history and affected with this cancer has been estimated to be 2.70%.
Methods: The study focused on the search of a possible link between haematological malignancies and BC,. We estimated the frequency of heterozygote *ATM* mutation carriers in a series of 122 BC women with a family history of both BC and haematological malignancy and negatively tested for a *BRCA1/2* mutation. To perform the genetic screening, a new high throughput method, EMMA (Enhanced Mismatch Mutation Analysis) was applied.
Results: Twenty-eight different variants were evidenced. Amongst them we identified eight mutations in eight patients: two mutations leading to a putative truncated protein and six being likely deleterious mutations. One of the truncating mutations was initially interpreted as a missense mutation, p.Asp2597Tyr, but transcript analysis allowed to show that it was actually a splicing mutation (c.7789G>T/p.Asp2597_Lys2643>LysfsX3).
Conclusions: The estimated frequency of *ATM* heterozygote mutation carriers in our series was 6.56% (95% CI: 2.16-10.95), a significantly higher figure than that observed in the general population, estimated to be between 0.3 and 0.6%. Although a trend towards an increased frequency of *ATM* carriers was observed, it was not different from that observed in a population of familial BC women not selected for haematological malignancy as the frequency of *ATM* carriers was 2.70%, a value situated in the confidence interval of our study.

Poster session 8: Hereditary Breast and Ovarian Cancer

P51
SIMILAR FREQUENCIES OF ALDH1 POSITIVE CELLS IN THE MORPHOLOGICALLY NORMAL BREAST TISSUE OF BRCA1 MUTATION CARRIERS AND NON-CARRIERS.

M. Heerma van Voss[1], P. van der Groep[2], E. van der Wall[2], P. van Diest[1]
[1]*UMC Utrecht, Department of Pathology, Utrecht, The Netherlands*
[2]*UMC Utrecht, Department of Internal Medicine, Utrecht, The Netherlands*

Introduction: The BRCA1 protein has recently been reported to be essential in the differentiation process of mammary stem cells into mature luminal and myoepithelial cells. In cell line experiments, Wicha et al. showed that blocking BRCA1 activity impaired cells to become terminally differentiated oestrogen receptor (ER) positive cells and increased the aldeflour dehydrogenase 1 (ALDH1) positive population. ALDH1 is regarded as a marker for mammary stem cells. In addition they reported clusters of ALDH1 positive cells, to be solely present in the tissue of BRCA1 mutation carriers when compared to non-carriers. These results indicated that a BRCA1 mutation results in a differentiation block and that mammary stem cells are the potential cells of origin of BRCA1 related breast cancer. We set out to verify these in vitro experiments in a larger patient population.
Methods: Morphologically normal breast tissue of 28 prophylactic mastectomies of BRCA1 mutation carriers and 28 age-matched mammoplasties with no (family) history of breast cancer were immunohistochemically stained for ALDH1 and evaluated for the presence of clusters of ALDH1 positive cells. Stromal expression was rated from 0 (no expression) to 4 (strong expression).
Results: Clusters of ALDH1 positive cells were present in the normal breast tissue of 63% of BRCA1 mutation carriers compared to 80% of non-carriers (n.s.). The mean amount of ALDH1 clusters per slide was slightly higher in mutation carriers (10.18) compared to non-carriers (7.25), but this was not significant either. The median amount of cells in a cluster was similar in carriers (14.33) and non-carriers (14.20) (n.s.). All slides had at least some stromal ALDH1 expression. Strong expression was seen more often in carriers (42.9%) than in non-carriers (25.9%) but this was not significant.
Conclusion: Contradictory with earlier findings in a very small population, ALDH1 positive clusters seem to have similar frequencies in the normal breast tissue of BRCA1 mutation carriers and non-carriers. No difference in stromal expression could be found either. This indicates that the normal breast of BRCA1 mutation carriets does not have a clearly aberrant ALDH1 expressing cell population. This raises questions as to the validity of ALDH1 as a single stem cell marker in the human breast.

P52
FIBROTIC FOCUS: A HALLMARK OF BRCA1 RELATED BREAST CANCER?

M. Heerma van Voss[1], P. van der Groep[2], C. van Deurzen[1], J. Bart[3], E. van der Wall[2], P. van Diest[1]
[1]*UMC Utrecht, Department of Pathology, Utrecht, The Netherlands*
[2]*UMC Utrecht, Department of Internal Medicine, Utrecht, The Netherlands*
[3]*UMC Groningen, Department of Pathology, Groningen, The Netherlands*

Background: Germline mutations in the BRCA1 and BRCA2 genes predispose to the development of breast cancer, exhibiting a specific histological phenotype. Identification of possible hallmarks of these tumours serves several purposes. Biomarkers can contribute to the selection of high-risk patients for genetic screening, help to identify the pathogenicity of so called unclassified variant mutations, and provide insight in the carcinogenetic pathways allows to develop new targeted therapies.
A fibrotic focus (FF) is an area in the centre of a carcinoma of abundant tumour stroma formation. Several studies have put the presence of a fibrotic focus (FF) forward as a candidate histological marker for hypoxia and (lymph)angiogenesis. Since the formation of a FF is primarily a hypoxia driven process occurring in tumours with an expansive growth pattern, we hypothesized that BRCA1 related breast cancers are primed to form FFs. In addition, the presence of an FF and central necrosis is associated with a basal like gene expression profile and triple negative receptor status; subtypes that most BRCA1 cases belong to. The aim of this study was to compare the frequency of FFs in

BRCA related breast cancer to sporadic controls, as well as that of necrotic foci (NF) that may precede FFs.
Methods: A population of 68 BRCA1 and 10 BRCA2 related invasive breast cancers was evaluated for the presence of FFs and NFs by an experienced breast pathologist blinded to mutation status, and compared to a control group matched for age, grade and tumour type.
Results: Both FFs and necrosis were seen in similar frequencies in hereditary cases and controls. A FF was present in 20.3%, necrosis in 43.8%. NF was present in 24.2% of hereditary cases, compared to 21.9% of controls. FF were associated with NF (P=0.015, OR 2.59, 95% CI 1.18-5.63).
Conclusion: FF and necrosis seem to occur with similar frequencies in hereditary and matched sporadic controls. Since we found a strong association between FF and NF, we propose that NF could be a precursorstage of FF.

P53
LYMPHO-VASCULAR INVASION IN BRCA RELATED BREAST CANCER COMPARED TO SPORADIC CONTROLS

M. Heerma van Voss[1], P. van der Groep[2], J. Bart[3], E. van der Wall[2], P. van Diest[1]
[1]*UMC Utrecht, Department of Pathology, Utrecht, The Netherlands*
[2]*UMC Utrecht, Department of Internal Medicine, Utrecht,The Netherlands*
[3]*UMC Groningen, Department of Pathology, Groningen, The Netherlands*

Background: Germline mutations in the BRCA1 and BRCA2 genes predispose to the development of breast cancer, exhibiting a specific histological phenotype. Identification of possible hallmarks of these tumours serves several purposes. Biomarkers can contribute to the selection of high-risk patients for genetic screening, help to identify the pathogenicity of so called unclassified variant mutations, and provide insight in the carcinogenetic pathways allows to develop new targeted therapies. Since BRCA1-associated breast cancers have pushing borders that prevent them from easily reaching vessels and are often of the medullary (like) type that is known to have a low rate of lymphovascular invasion (LVI), we hypothesized that absence of LVI could characterize BRCA1 related breast cancer.
Methods: A population of 68 BRCA1 and 10 BRCA2 related invasive breast cancers was evaluated for LVI by an experienced breast pathologist blinded to mutation status, and compared to a control group matched for age, grade and tumour type.
Results: LVI was present in 25.0% of BRCA1 related cases, compared to 20.6% of controls (P=0.54, OR=1.29, CI 0.58-2.78). No significant differences were found for the BRCA2 group either (P=1).
Conclusion: LVI is frequent in BRCA1 germline mutation related breast cancers, but seems to occur as often in sporadic controls matched for age, grade and tumour type. Apparently, these hereditary cancers find their way to the blood and lymph vessels despite their well demarcation and often medullary differentiation, indicating that these cancers may have specific vasoinvasive properties.

P54
HIF-1ALPHA OVEREXPRESSION IN DUCTAL CARCINOMA IN SITU OF THE BREAST IN HEREDITARY PREDISPOSED WOMEN

P. van der Groep[1,2], P. van Diest[1], F. Menko[3], J. Bart[4], E. de Vries[5], E. van der Wall[2]
[1]*UMCUtrecht, Pathology, Utrecht, The Netherlands*
[2]*UMCUtrecht, Internal Medicine, Utrecht, The Netherlands*
[3]*VU medical cecnter Amsterdam, Clinical Genetics, Amsterdam, The Netherlands*
[4]*University Medical Center Groningen, Pathology, Groningen, The Netherlands*
[5]*University Medical Center Groningen, Internal Medicine, Groningen, The Netherlands*

Introduction: Recent studies have revealed that BRCA1/2 germline mutation related breast cancer show frequent overexpression of Hif1alpha, often next to necrosis, suggesting a role of hypoxia in carcinogenesis and progression. Little is known about the role of hypoxia in precursors of these hereditary cancers. Ductal carcinoma *in situ* (DCIS) is an established late precursor of sporadic invasive breast cancer and parallels its invasive counterpart with respect to molecular changes and immunophenotype. The aim of this study was to evaluate Hif1alpha expression in DCIS as a putative precursor lesion of BRCA1/2 related breast cancer, comparing Hif1alpha expression of these lesions with the invasive counterparts.
Methods: DCIS lesions of 19 proven BRCA1 and 6 BRCA2 germline mutation carriers and their accompanying invasive lesions were stained by immunohistochemistry for Hif1alpha, and for ER, PR, HER-2/*neu*, Ck5/6, Ck14, EGFR and Ki67

Results: 13/19 (68%) of the BRCA1 DCIS cases and 4/6 (67%) of the BRCA2 DCIS cases showed Hif1alpha overexpression. 7/16 (56%) of the BRCA1 DCIS cases showed only a diffuse and 6 a perinecrotic expression pattern17/19 (89%) and 2/6 (33%) of the invasive BRCA1 or BRCA2 counterparts showed Hif1alpha overexpression, respectively. Hif1alpha expression in DCIS matched that in invasive counterparts in 17/25 cases (68%).
Conclusion: BRCA1 and BRCA2 germline mutation related DCIS shows frequent Hif1alpha overexpression, usually similar to that of invasive counterparts. This suggests that hypoxia may already play a role in the DCIS stage of BRCA1/2 germline mutation related carcinogenesis.

Supported by an unrestricted educational grant from Aegon Inc.

Poster session 9: Transcription Factors, Tumor Suppressors and Oncogenes

P58
FUNCTIONAL STUDY OF SMAP1: A NEW GENE BETWEEN VESICULAR TRAFFIC AND ONCOGENESIS

F. Sanger[1], A.-S. Schreurs[1], O. Mauger[1], J. El-Bchiri[1], O. Buhard[1], D. Mercier[1], A. Chalastanis[1], A. Duval[1], F. Praz[1]
[1]*INSERM, INSERM UPMC UMR_S 938, PARIS, France*

Tumours displaying microsatellite instability (MSI) represent a frequent type of tumours in humans, due to the functional inactivation of the mismatch repair system (MMR). Microsatellites are repeats of 1-6 bp localized in the whole genome that are hotspots of insertion/deletion mutations. In absence of MMR, the mutations occurring during replication are left unrepaired, resulting in premature termination codon. Mutations that initiate and/or promote tumour cell development are thought to be selected; however, the oncogenic role has been demonstrated for only a few target genes, mostly genes involved in the TGFβ signalling pathway.
We have identified a gene containing a coding A10 repeat, whose mutations could be involved in the MSI oncogenesis pathway: SMAP1 (Small ArfGAP 1). SMAP1 is a member of the ArfGAP (ADP ribosylation factor-GTPase Activated Protein) family specific for Arf6, which is involved in the clathrin-dependent endocytosis of membrane receptors such as the transferrin receptor (TfnR). SMAP1 and Arf6-GTP are involved in E-cadherin internalization and actin depolymerisation, respectively, two major components of the adherens junctions, maintaining the epithelium integrity. We thus hypothesized that SMAP1 mutations could be responsible for junctions disassembly, the first step in tumour invasion.
In order to perform functional studies, we have constructed the expression vectors of the wild-type (wt) and mutated SMAP1 cDNA. In addition, we have produced an antibody, which recognizes both wt and mutated SMAP1 forms, but not SMAP2, a SMAP1 homolog. These tools will be used to determine whether truncated SMAP1 proteins are expressed or degraded, resulting in loss-of-function. If the truncated proteins are stably expressed, they may act as dominant negative or acquire new specificities. We will present the consequences of the inhibition of SMAP1 expression by specific siRNAs on cellular morphology, E-cadherin cellular distribution and Tfn internalization.

P59
PDGF-B ADDICTION IN A MURINE MODEL OF OLIGODENDROGLIOMA

I. Appolloni[1,2], F. Calzolari[1,2], P. Malatesta[1,2]
[1]*University of Genoa, DOBiG- Dip. di Oncologia, Biologia e Genetica, Genova, Italy*
[2]*IST-Istituto Nazionale per la Ricerca sul Cancro, Ricerca Traslazionale Oncologica, Genoa, Italy*

Gliomas are a heterogeneous group of cancers of the central nervous system. Partly due to lack of knowledge about their biological properties and the lack of animal models that allow testing novel therapeutic strategies, they are among the less treatable malignant tumors.
We recently established a new mouse model of gliomagenesis by the retroviral transduction of PDGF-B in embryonic neural progenitors. Our immunohistochemical and genome-wide expression analyses demonstrated that PDGF-B-induced tumors represent a surprisingly homogeneous class of oligodendrogliomas despite their induction by the infection of a highly heterogeneous population of progenitor cells, which normally produce all the cell types of the central nervous system. Importantly, we showed that this uniformity is partly due to the ability of PDGF-B to re-specify competent embryonic neural precursors toward the oligodendroglial lineage.

We also demonstrated that PDGF-B-induced tumors undergo progression from low-grade toward highly malignant forms and that this progression needs additional molecular lesions, which however are not sufficient to release tumor cells from the need for PDGF-B overexpression. This is clearly demonstrated by transplantation assays showing that fully progressed tumors are addicted to PDGF-B since their tumor-propagating ability is lost whenever the PDGF-B transgene is silenced. Tumorigenicity is promptly reacquired after PDGF-B reactivation. Notably, we provide evidence that in this form of oncogene addiction PDGF-B is not necessary for its mitogenic activity but is rather required to overcome cell-cell contact inhibition and to confer in vivo infiltrating potential to tumor cells.

P60
Sp100 EXPRESSION IN HUMAN CANCER CELL LINES

W. Li[1,2], X. Jiang[1], K. Sun[2], W. Fu[2]
[1]*The First Affiated Hospital of China Medical University, ENT Department, Shenyang, China*
[2]*China Medical University, Genetics, Shenyang, China*

Background and Aims: Sp100 protein is a permanent ProMyelocytic Leukaemian nuclear bodies (PML NB) associated protein. The high-expression of Sp100 could inhibit both invasion and migration of the breast cancer cells and human umbilical vein endothelial cells. Our results from immunoprecipitation and MALDI/TOF MS (Matrix assisted laser desorption/ ionization time of flight mass spectrometry) in cancer cell in vitro, has revealed that Sp100 protein could interact with sh3gl2 protein, another tumor repressor gene whose frequent loss found by CGH and low expression proved in laryngeal cancer. This study was scheduled to investigate whether *Sp100* expression occurs in various cancer types.
Methods: Different cancer cell lines representing different tissue origins (cervical carcinoma, laryngeal carcinoma, gastric adenocarcinoma and hepatocellular carcinoma) and normal gastric epithelium cell line were studied. Detection of Sp100 mRNA was performed using reverse transcription PCR while protein expression was assessed by western blotting.
Results: Both transcriptional and translational levels of *Sp100* were down-regulated in cancer cell lines compared to normal epithelium cell lines ($p<0.05$). Thus, we offer evidence that Sp100 low expression characterizes cancer cells of various tissue origins.
Conclusions: The biological significance of these findings provides novel insight into the important role of *Sp100* in the development of cancer. Further studies are needed to address whether the down-regulation of *Sp100* is a cause or a consequence of the progression from normal epithelium to carcinoma.

P61
TARGETING TRANSCRIPTION FACTORS DNA BINDING PROPERTIES USING PHENYL-FURAN-BENZIMIDAZOLE DNA LIGANDS.

P. Peixoto[1], S. Depauw[1], M.-P. Hildebrand[1], R. Nhili[1], M.-H. David-Cordonnier[1]
[1]*Inserm U837, Team 4, IRCL, Lille, France*

Deregulation of gene transcription is associated with the initiation/development of numerous pathologies among which cancer. Targeting of transcription factors is an original therapeutic approach which could be developed though the direct targeting of the protein itself or its DNA binding function. We choose to develop this latter approach by using small sequence-selective DNA binding agents to target the DNA binding potency of specific transcription factors. To date, only a few sequence-specific DNA ligand families (distamycin derivatives, some alkylating agents and DNA intercalating drugs), have been developed/evidenced to competitively interfere with transcription factor binding.
The phenyl-furan-benzimidazole DB293 binds to AT-rich sites as monomers in the DNA minor groove and to the 5'-ATGA sequence as a dimer stacked in an expanded minor groove. DB293 inhibits the DNA binding potencies of the POU-domain factors Pit-1 and Brn-3 (Peixoto et al., 2008, Nucleic Acids Res, 36, 3341-53), implicated in pituitary and cervical/breast cancers, respectively. Both binding sites contain both an ATGA and AT-rich sites targeted by DB293.
Two series of structurally related derivatives were used, that differ from DB293 by the addition of a hydroxyl or methyl group in *meta-* or *ortho-* positions. DNase I footprinting experiments discriminate the ATGA binders from others. We found that Pit-1 and Brn-3 DNA binding inhibition occurs through direct interaction of DB293 and other ATGA binders to the respective consensus binding sites, thus competing for the correct positioning of the amino-acids of POU-H and POU-S sub-domains. By contrast, non-ATGA binders failed to affect protein/DNA interaction. O*rtho* modifications do not strongly affect the sequence selectivity for ATGA and transcription factor binding inhibition whereas the same *meta* modifications

abolish ATGA preference, leading to compounds that only interact with AT-rich sites and failed to compete with transcription factor binding to their target sequences.
In conclusion, DB293 and some heterocyclic dication derivatives are effective DNA binding inhibitors of Pit-1 and Brn-3 in relation with dimer recognition of ATGA sites in the DNA target sequence.

Acknowledgments:
MHDC thanks the LNCC, ALF and IRCL for grants and IRCL for a postdoctoral fellowship to PP.

P62
AROMATASE AND ESTROGEN ACTIVITY IN CARCINOGENESIS AND PROGRESSION OF HUMAN HEPATOCELLULAR CARCINOMA

G. Carruba[1], L. Cocciadiferro[1], V. Miceli[1], A. Piazza[1], G. Montalto[2], B. Agostara[3], O. Granata[1]
[1]*Experimental Oncology, Dept of Oncology, ARNAS-Civico, Palermo, Italy*
[2]*University of Palermo, Department of Clinical Medicine and Emerging Pathologies, Palermo, Italy*
[3]*Clinical Oncology, Dept of Oncology, ARNAS-Civico, Palermo, Italy*

In the present work we have investigated androgen metabolism and aromatase-driven estrogen formation in *nontumoral* and malignant human liver tissues and cells, also in relation to the expression of amphiregulin (AREG), a member of the epidermal growth factor family of EGFR ligands. This aiming to get insights into the potential role of estrogens and the underlying mechanism(s) in human hepatocellular carcinoma (HCC). Chromatographic and RT-PCR analyses were used to respectively assess activity and expression of androgen enzymes, including aromatase, and both *in vivo* and *in vitro*. Exon-specific RT-PCR and western blot analyses were also used to evaluate the expression of wild-type and variants of both estrogen receptor α (ERα) and ERβ, as like as AREG expression, *in vitro*. Following 24h and 72h incubation of tissue minces or hepatic cell lines, with either testosterone (T) or androstenedione (Ad) being used as tritiated androgen precursor, human HCC tissues and HepG2 hepatoma cells showed elevated aromatase activity, with estrogen formation rates respectively of 20% at 24h and >95% at 72h. By contrast, no aromatase activity could be detected in *nontumoral* hepatic tissues and HA22T cells at any incubation time. Cirrhotic samples exhibited intermediate enzyme activity, with average estrogen formation rates of nearly 4%, while Huh7 HCC cells gave rise to 34% estrogen formation by 72h. Markedly lower aromatase mRNA levels were observed in HA22T cells and *nontumoral* liver tissues, as compared to HepG2 cells and HCC samples. Cirrhotic specimens displayed variable transcript levels, comparable in turn to those observed in *nontumoral* or HCC tissues. Interestingly, patterns of AREG expression were consistently associated with those of aromatase, with ~3-fold and ~8-fold higher levels being seen in HepG2 cells than in Huh7 cells (P=0,002) and HA22T cells (P=0,0014), respectively. Furthermore, using exon-specific RT-PCR, ERα46 and ERα36 were the only ER variants expressed in all cell lines, while wild type ERα66 or ERβ could not be detected. Western blot analysis revealed a corresponding figure. This combined evidence suggests that AREG expression may be upregulated by estrogens in human HCC and that locally elevated aromatase activity may increase malignant cell proliferation also through AREG signaling.

Poster session 10: Cell Cycle Kinases in Cancer

P65
THE AURORA-A/TPX2 COMPLEX: REGULATION AND ROLES IN TUMORIGENESIS

I.A. Asteriti[1], M. Giubettini[1], W.M. Rensen[1], M. De Luca[2], J. Scrofani[1], C. Lindon[2], P. Lavia[1], G. Guarguaglini[1]
[1]*Institute of Molecular Biology and Pathology, CNR, c/o Dept. of Genetics and Molecular Biology, Sapienza University, Rome, Italy*
[2]*University of Cambridge, Genetics, Cambridge, UK*

The mitotic kinase Aurora-A is a key regulator of spindle assembly and function and is frequently overexpressed in tumors. Small molecule inhibitors targeting its catalytic domain are currently undergoing clinical trials as anti-cancer agents. The major activator of Aurora-A is the microtubule (MT)-binding protein TPX2. TPX2 binds Aurora-A, locking it in an active conformation, and mediates its binding to the spindle MTs. We are interested to understand the mitotic roles of Aurora-A and TPX2 and their contribution to cell transformation.
In recent work we have evidenced a previously unrecognised role of TPX2 in controlling Aurora-A stability. Aurora-A is normally degraded in late

mitosis. However, we have found that its abundance decreases in prometaphases interfered for TPX2. This decrease is proteasome- and Cdh1- (the activator of the APC/C ubiquitin ligase) dependent and it is counteracted by re-expressing, in TPX2-interfered cells, either full-length TPX2 or the Aurora-A binding region, but not a TPX2 mutant lacking the Aurora-A interaction domain. These results underscore a novel level of control of Aurora-A abundance and raise the possibility that concomitant deregulation of TPX2 may contribute to elicit the full oncogenic potential of Aurora-A in transformed cells. Indeed, in a data mining effort, we have obtained evidence that TPX2 is overexpressed in many tumor types and, moreover, that Aurora-A and TPX2 are frequently co-overexpressed. We therefore suggest that the complex, as a unit, may contribute to tumor formation.

P66
POTENTIAL MECHANISMS OF CELL CYCLE MODULATION BY THE IGF-1R INHIBITOR CYCLOGINAN PPP IN LIVER CANCER CELLS

E. Aleem[1,2], A. El-Waraky[1,2], O. Larsson[2]
[1]*Alexandria University, Faculty of Science, Division of Molecular Biology, Alexandria, Egypt*
[2]*Cancer Center Karolinska, Karolinska Institutet, Department of Oncology Pathology, Stockholm, Sweden*

The insulin-like growth factor-1 receptor (IGF-1R) is a cell surface receptor kinase being vastly expressed in malignant tissues and plays crucial roles in growth and survival of cancer cells. Furthermore, IGF-1R has been shown to be sumoylated and translocated to the nucleus, where it may affect transcription. Targeting IGF-1R is today a very attractive concept in cancer therapy. Upregultion of IGF-1R and its signalling pathway has been involved in many types of cancer including liver cancer. A small molecule inhibitor of IGF-1R, the cyclolignan PPP inhibits both phosphorylation of IGF-1R as well as it attenuates downstream signaling. Fortunately, PPP does not inhibit the highly homologous insulin receptor (IR). It has been previously shown that PPP affects the cell cycle in multiple myeloma cells. The goal of the present study is to investigate the mechanisms of cell cycle modulation by PPP using human hepatoma cell line HepG2, with the overall aim of developing improved targeting of the receptor for specific therapeutic purposes. *Methods:* In the present study we performed proliferation curves, analysis of cell cycle kinetics using Bromodeoxy uridine incorporation and propidium iodide staining followed by analysis with flow cytometry, analysis of apoptosis by TUNEL assay and protein expression by western blots combined with immunoprecipitations.
Results: PPP clearly inhibited the proliferation of HepG2 cells in a dose-dependent manner. Analysis of cell cycle kinetics demonstrated an initial increase in G1 cell population following PPP treatment at low doses. A high dose of PPP (0.5 uM) induced apoptosis as confirmed by TUNEL assay. To understand the potential molecular mechanisms underlying the increase in G1 cell population we studied the protein expression of cdks, cyclins and cdk inhibitors. We found that treatment of HepG2 cells with PPP increased the protein levels of p27, p21 and p53, while it reduced the levels of cyclin A and Cdk1 and did not affect the levels of Cdk2.
Conclusion: Our preliminary findings indicate that Cdk1 and cyclin A may be potential targets for IGF-1R inhibition by PPP. Currently, we are studying the different protein complexes associated with Cdk1 and cyclin A using immunopreciptations followed by western blot, as well as the kinase activities associated with cyclins A, E, B, Cdk1 and Cdk2. In view of the recent findings that IGF-1R may have a role in transcription, our future studies will be directed towards understanding the mechanisms by which PPP reduces the expression levels of Cdk1 and Cyclin A.

Poster session 11: Translational Cancer Research

P71
TARGETED DELIVERY OF TRAIL TO THE CARCINOMA MARKER EPCAM BLOCKS EPCAM PRO-ONCOGENIC SIGNALLING AND SIMULTANEOUSLY ACTIVATES APOPTOSIS

E. Bremer[1], M. de Bruyn[1], D.F. Samplonius[1], G. van Dam[2], O. Gires[3], W. Helfrich[1]
[1]*UMCG, Surgery/Surgical Research Laboratory, Groningen, The Netherlands*
[2]*UMCG, Surgery, Groningen, The Netherlands*
[3]*University of Munich Hospital, Cooperation Group Molecular Oncology, Dept of Head and Neck Research, Munich, Germany*

Question: Novel insights into EpCAM biology highlight a possibly central role for EpCAM in the formation of metastases. In particular, it was recently uncovered that EpCAM can be proteolytically processed into an extra- and intra-cellular domain. This

intracellular domain assembles into a nuclear complex that induces pro-oncogenic signalling. Of note, this processing of EpCAM occurs in malignant, but not in normal colon epithelium tissue. Previously, we demonstrated that selective delivery of sTRAIL to EpCAM, using EpCAM-specific antibody fragment scFvC54, potently induces carcinoma-selective apoptotic activity. Based on these novel insights, we here addressed the question whether such EpCAM-selective delivery of sTRAIL might yield dual therapeutic activity comprised of **1.** inhibition of EpCAM pro-metastatic signalling and **2.** activation of TRAIL-apoptotic signalling.
Methods: The EpCAM-targeted TRAIL consists of sTRAIL genetically fused to EpCAM-specific antibody fragment scFvC54. EpCAM processing was visualized by confocal microscopy using among others EpCAM-YFP transfectant cells. Colony formation of carcinoma cell lines HT29 and HCT116 was performed by standard soft agar assay. Apoptosis induction in HT29 and HCT116 cells was determined by flow cytometric assessment of mitochondrial membrane potential.
Results: Our data indicate that scFvC54:sTRAIL blocks EpCAM-processing and pro-metastatic signalling. Furthermore, scFvC54:sTRAIL potently induced apoptosis and abrogated colony formation of carcinoma cells *in vitro*. In addition, a single treatment of tumor cells with scFvC54:sTRAIL not only strongly delayed tumor outgrowth *in vivo*, but also yielded a strong reduction in tumor size.
Conclusions: EpCAM-targeted delivery of sTRAIL simultaneously inhibits EpCAM processing and pro-metastatic signalling and selective induction of TRAIL-apoptotic signalling. Therefore, EpCAM-targeted TRAIL may be of therapeutic relevance for the treatment of carcinoma.

P72
PROGRESSION RISK OF COLUMNAR CELL LESIONS OF THE BREAST DIAGNOSED IN CORE NEEDLE BIOPSIES

A. Maes[1], P. van Diest[1]
[1]*UMC Utrecht, Pathology, Utrecht, The Netherlands*

Question: Columnar cell lesions (CCLs) of the breast are increasingly recognized as putative precursor lesions of invasive carcinoma. Although knowledge has been gained on the progression risk of CCLs, their management remains controversial. We therefore conducted a retrospective study on 4161 breast CCLs diagnosed in core needle biopsies (CNB) between 2002 and 2008.
Methods: 312 CNB beared a CCL as the most advanced lesion: 221 CCLs without atypia (including columnar cell change (CCC) and columnar cell hyperplasia (CCH) without atypia), 70 CCLs with atypia (CCL-A, including CCC/CCH with atypia) and 21 atypical ductal hyperplasias (ADHs) originating in a CCL (ADH-CCL). From all biopsies follow-up information was present through a search in the local hospital information systems, mammographies and the Dutch National Pathology Archive until November 2009. We discerned the immediate treatment group undergoing excision within 4 months after the CNB diagnosis of CCL (N=44) and the wait-and-see group followed up to 8 years (median 42 months, N=268). Relative risks (RR) of CCL-A and ADH-CCL to CCL were calculated.
Results: In 14 of 44 (32%) immediate treatment cases with a CNB diagnosis of CCL, surgical excision biopsies revealed ductal carcinoma in situ (DCIS, N=7) or invasive carcinoma (N=7), with no significant difference in frequency of events between CCL cases with and without atypia. Besides, In 2 of 6 (33%) ADH-CCL cases, in situ cancer was found on immediate surgery. In the wait-and-see group, 9 of 277 cases (3.2%) developed an invasive carcinoma (no further DCIS occurred). Progression risks of CCL, CCL-A and ADH-CCL were 2%, 14% and 17% during the follow-up interval of 8 years, respectively, with RRs of 16.7 for CCL-A and 41.2 for ADH-CCL compared to CCL without atypia.
Conclusions: A CNB diagnosis of CCL is associated with a high chance of DCIS or invasive carcinoma in the immediate surgical excision biopsy, especially in CCL with atypia or ADH-CCL. This may justify an excision biopsy for a CCL with atypia or ADH-CCL in a CNB. The long term progression risks for CCLs with atypia and ADH-CCL are also significant with RRs of 16.7 and 41.2, in contrast with CCLs without atypia which seem to have a low progression risk.

P73
DECIPHERING THE ROLE OF NOTCH SIGNALLING IN NORMAL BREAST AND BREAST CANCER DEVELOPMENT

C. Ercan[1], P. van der Groep[1], E. van der Wall[2], P. Derksen[3], P. van Diest[1], M. Vooijs[1]
[1]*University Medical Center of Utrecht, Pathology, Utrecht, The Netherlands*
[2]*University Medical Center of Utrecht, Internal Medicine, Utrecht, The Netherlands*
[3]*University Medical Center of Utrecht, Medical Oncology, Utrecht, The Netherlands*

Background and Research Question: Breast cancer is one of the most frequent malignancies in women with a risk of developing the disease as high as one in every nine women. There is a strong need for a better understanding of this malignancy to improve treatment. The mammary gland is a dynamic organ that goes through significant developmental changes during pregnancy, lactation, and involution: a process driven by mammary stem cells that undergoes self-renewal and differentiation. Alterations in signalling pathways involved in cell-fate and differentiation decisions are likely to be the underlying reason of the malignant transformation of breast epithelium. Notch signalling appears to be one of these pathways. Germ line mutations in the Notch pathway cause several hereditary diseases and somatic mutations found in cancer. The main objective of this study is to investigate the role of Notch1 signalling in normal breast and breast cancer development and to address its clinical significance.
Methods and Results: One of our primary aims has been to evaluate the activity of Notch1 in human clinical breast cancer specimens to investigate the clinical significance of Notch1 activity in breast cancer. Immunohistochemical analysis of tissue microarrays of well-defined patient groups revealed high Notch1 activity in both ductal and lobular carcinomas. Furthermore, we investigated the effect of inhibition of Notch signaling on 2D cultures of human breast cancer cell lines of different origin by using a pharmacological inhibitor of the pathway. Proliferation of drug treated cells was dramatically affected by Notch inhibition. High Notch1 activity has been observed before in several other cancer types and it has been demonstrated that mutations (such as in the case of T-ALL) are the cause of this aberrant activity. Therefore we investigated whether similar activating mutations occurred in Notch1 in 36 different breast cancer cell lines derived from different breast cancer subtypes. However we did not observe any published or novel mutations except several synonymous SNPs.
Conclusion: Although mutations of Notch1 may not be playing an important role in breast cancer, our data indicates the importance of Notch1 activation in this malignancy.

P74

ANTITUMOR AND ANTIANGIOGENIC ACTIVITY OF DOCETAXEL AND SORAFENIB COMBINATION IN HUMAN PANCREATIC CANCER MODELS

P. Ulivi[1], C. Arienti[1], W. Zoli[1], S. Carloni[1], E. Chiadini[1], A. Orlandi[2], M. Scarsella[3], F. Fabbri[1], A. Tesei[1], D. Passeri[2], G. Zupi[3], R. Silvestrini[1], D. Amadori[1], C. Leonetti[3]
[1]*Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (I.R.S.T.), Biosciences Laboratory, Meldola, Italy*
[2]*Anatomic Pathology Institute, Tor Vergata University, Department of Biopathology and Image Diagnostics, Rome, Italy*
[3]*Regina Elena Cancer Institute, Experimental Chemotherapy Laboratory, Rome, Italy*

Background: The response of pancreatic cancer to treatment remains highly unsatisfactory, highlighting the need for more effective therapeutic regimens. Sorafenib, an orally available multikinase inhibitor, is active against different tumors, including pancreatic cancer. We studied the antitumor efficacy of sorafenib in combination with different antitumor drugs currently used in clinical practice in in vitro and in vivo experimental models of human pancreatic cancer.
Methods: The cytotoxic effect of sorafenib and conventional antitumor drug combinations was evaluated in vitro in human pancreatic cancer cell lines and the most active combination was then tested on tumor-bearing mice. Flow cytometric, western blot and immunohistochemistry analyses were performed to investigate the mechanisms involved in the activity of single drugs and of their interaction when used in combination.
Results: Sorafenib showed a strong sequence-dependent synergistic interaction in vitro with docetaxel. In vivo, the treatment of human pancreatic cancer-xenografted mice with docetaxel followed by sorafenib reduced and delayed tumor growth, with complete tumor regression observed in half of the mice. This marked antitumor effect resulted in an overall 70% increase in mouse survival and a complete cure in 3 of the 8 mice treated. This impressive antitumor effect was accompanied by marked apoptosis induction, inhibition of tumor angiogenesis and downregulation of MAPK signalling.
Conclusions: Our results show that the combination of docetaxel and sorafenib exerts high therapeutic efficacy in experimental models of human pancreatic

cancer, thus identifying a potentially effective antitumor strategy for clinical use.

P75
DECREASED EXPRESSION OF Sp100 IS ASSOCIATED WITH MALIGNANT TRANSFORMATION AND HPV-INFECTION OF SINONASAL INVERTED PAPILLOMA

W. Li[1,2], X. Jiang[1], K. Sun[2], W. Fu[2]
[1]*The First Affiated Hospital of China Medical University, ENT Department, Shenyang, China*
[2]*China Medical University, Genetics, Shenyang, China*

Background and objectives: Inverted papilloma (IP) is locally aggressive tumors of the sinonasal epithelium, which is characterized by a high rate of recurrence and a potential of transformation to squamous cell carcinoma. Involvement of HPV infection, especially the high risk HPV types, has been reported to be associated with malignant transformation of IP. Sp100 protein is a permanent ProMyelocytic Leukaemian nuclear bodies (PML NBs) associated protein. L2, the minor capsid protein of HPV, could induce the dislocation of Sp100 from PML NBs and further degradation. Furthermore, our previously study showed low expression of Sp100 in both cancer cell lines and laryngeal cancer tissues. In this study, we aim to indentify *Sp100* expression in IP grouped by HPV infection and its correlation with malignant transformation and HPV infection.
Methods: Paraffin-embedded biopsies of IP previously tested for HPV-DNA by PCR underwent immunohistochemical evaluation. The results were analyzed using quantitative immunohistochemical analysis.
Results: Based on immunohistochemical evaluation, significantly low level of Sp100 expression was observed in IP and IP with carcinoma compared to control nasal mucosa. Moreover, IP with HPV DNA positive showed significant decrease of Sp100 compared to IP without HPV infection, and no correlation with specific HPV genotypes was revealed.
Conclusions: Precancerous lesions of IP exhibited down-regulated levels of Sp100, which support the hypothesis that *Sp100* might play an important role in the HPV infection, and also the early event of a multistep process of IP carcinogenesis.

Poster session 12: Tumor Tissue Banking

P81
THE TISSUE MICROARRAY DATA EXCHANGE SPECIFICATION: A DATABASE FRAMEWORK FOR VIRTUAL SLIDES MANAGEMENT

Y. Wang[1], R. Xavier[1], D. McCleary[2], P. Hamilton[1]
[1]*Queen's University Belfast, Medical Bioinformatics, Belfast, UK*
[2]*i-Path Diagnostics Ltd., Belfast, UK*

Tissue Microarrays (TMAs) provide a platform for high-throughput analysis of tissue biomarkers on numerous patients but in a single assay. With the development of digital pathology, a TMA glass slide can be scanned in the form of a virtual slide. The structure and complexity of TMA data demands the developments of standards which support interoperability between laboratories and different systems, as well as the easy access of all relevant information. We propose a TMA data exchange specification which supports not only basic biomarker evaluation, but also the rapid analysis of TMAs using image processing algorithms.
Our TMA data exchange specification design is based on the Common Data Elements (CDEs) from the Association for Pathology Informatics (API) containing fundamental clinical TMA information. In addition, quantitative TMA core features are included to cover a range of features that could be extracted from each core; namely geometric, textural and densitometric features. These allow for the inclusion of numerical data associated with tissue pattern, cell morphology and biomarker density. These features and their analytic results are structured as separate entities interacting with TMA core images. Disease specific information is utilised with the national minimum data for the reporting of cancer pathology investigations. For the purpose of cross-reference, the whole slide scan for each TMA core is also integrated seamlessly in the database.
To facilitate data exchange for researchers across different centres, frequently used SQL queries are summed up in the form of common tasks, such as two-way record exchange with standard XML metadata. These metadata are encapsulated in the level of recipient block, TMA slide or core. For the rapid analysis and cross-reference of TMA data, another set of common tasks are also implemented so that

information related to TMA cores taken from a same recipient block, and TMA cores with their original whole slide scan can be easily accessed using one button click.
This TMA specification now supports the ongoing biomarker research within our organisation and has been assessed across a range of TMA types and biomarkers. Initial evaluation suggests the proposed database architecture is valid, the proposed common tasks are robust and facilitating the discovery of biomarkers.

P82
THE WALES CANCER BANK : TISSUE BANKING ON A NATIONAL SCALE

G. Thomas[1], V. Woods[2], A. Parry-Jones[2], M. Mason[1]
[1]*Velindre Hospital, Oncology, Cardiff, UK*
[2]*Wales Cancer Bank, Cardiff, UK*

The development of targeted therapy for cancer requires access to large collections of quality assured human tissue with detailed clinical and pathological annotation. In addition, the provision of biological specimens in different formats from the same patient will permit a systems biology approach to be developed for identification of key target pathways in cancer. The Wales Cancer Bank (WCB) is a Welsh Assembly Government and Cancer Research Wales funded initiative that aims to collect blood, serum and frozen and FFPE tissue from all patients undergoing treatment for cancer in Wales. Established in 2004, the WCB has consented over 3202 patients. The current collection includes 17 tissue sites, the largest collections being breast, colorectal, prostate, bladder, renal, head and neck and ovarian. Data is linked-anonymised via the all-Wales electronic clinical database (CanISC), and consent permits future access to clinical outcome data. WCB is licensed by the Human Tissue Authority and approved by the UK National Research Ethics Service as a Research Tissue Bank, obviating the need for researchers using material from the bank to obtain Ethical approval of their research projects. Currently, WCB holds or has access to tissue from 3000 patients, and has received applications from 44 research projects from within and outside the UK. Input from pathologists in Wales was crucial to the development of the bank and much of the funds provided for the bank supports staff and resources in Histopathology departments in Wales. To maximise potential, RNA and DNA are extracted from the same frozen blocks and quality assured materials are provided to researchers. Tissue microarrays are currently being prepared from the breast cancer collection, and microdissected tissue from FFPE and frozen can also be supplied. Researchers are asked to provide their raw data back to the bank to provide a future data warehouse for research data generated from the samples supplied by the WCB.

Poster session 13: Cancer Epigenetics

P85
HUMAN CRYPT STEM CELL DYNAMICS IN FAMILIAL ADENOMATOUS POLYPOSIS

D. Langeveld[1], G.J.A. Offerhaus[1], W.W.J. de Leng[1]
[1]*UMC Utrecht, Pathology, Utrecht, The Netherlands*

Stem cells in the intestinal crypt are believed to be the target cells for the initiation of tumourigenesis and adenoma development. Macroscopic and histological normal appearing colorectal mucosa of patients with symptomatic familial adenomatous polyposis (FAP) harbour cell kinetic and stem cell abnormalities suggesting the existence of pre-tumour progression.
Pre-tumour progression can be made visible by studying methylation patterns, or tags, in stem cells of the crypt, where heterogeneity among these tags is a representation of genetic drift and stem cell survival. Increased stem cell survival may be a predictive marker of an increased risk of neoplastic outgrowth. The objective of this study is to investigate potential biomarkers predictive of adenoma development; the focus lies on alterations in stem/progenitor cell kinetics and stem cell survival.
Immunohistochemistry (IHC) was conducted in order to assess cell kinetic alterations using proliferation (Ki-67) marker.
DNA was isolated from laser-microdissected crypts of normal colonic FAP tissue and age matched controls. DNA was bisulphite converted and methylation tags were created by amplifying the CpG islands in the *CSX* gene. Sequence analysis of this pool of methylation tags allows characterisation of the heterogeneity among the different tags recovered from multiple stem cell pools.
Although Ki67 IHC showed a significant increase in length of the proliferative compartment in FAP patients compared to non-FAP, the coinciding increase in overall crypt length in FAP patients rendered the labelling index unchanged. This is consistent with increased stem cell survival. The bisulphite treatment,

with subsequent sequencing, showed a significantly (p=0.030) greater number of unique methylation tags for FAP patients (mean number of unique methylation tags is 2.8) compared to control patients (mean number of unique methylation tags is 2.1) which indicates enhanced stem cell survival. This, in turn, supports the idea of pre-tumour progression since longer lived stem cells have the potential to gain more mutations with higher risk to develop into a carcinoma.

P86
METHYLATION-SPECIFIC MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION (MS-MLPA) IN COLUMNAR CELL LESIONS OF THE BREAST

A. Maes[1], P. van Diest[1]
[1]*UMC Utrecht, The Netherlands, Pathology, Utrecht, The Netherlands*

Question: Promotor methylation contributes to inactivation of tumor suppressor genes and is a common epigenetic mechanism in the development of breast cancer. Over the last years, columnar cell lesions (CCLs), especially those with atypia, are proved to be precursors of invasive breast cancer. In this study, we investigated whether CCLs show methylation patterns similar to DCIS and/or invasive carcinoma and which genes are most often involved.
Methods: Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) of 24 tumor suppressor genes was carried out on 23 CCLs (16 with atypia), which were related to DCIS (N=7) and/or invasive carcinomas (N=8) in a proportion of the cases.
Results: The cumulative methylation index of CCLs (13.9) was in the same range as DCIS (12) and invasive carcinoma (13.5). The most prevalent methylated genes in CCLs were RASSF1 (83%), APC (48%), CDH13 (48%), GSTP1 (30%) and CDKN2B (13%). Invasive carcinoma and DCIS showed almost the same most frequently methylated genes: RASSF1 (75% resp. 75%), APC (50% resp. 25%), CDH13 (38% resp. 38%), GSTP1 (25% resp. 25%), CDKN2A (25% resp. 25%) and FHIT (13% resp. 25%).
Conclusions: These (preliminary) data indicate that CCLs have a similar pattern of methylation as DCIS or invasive carcinoma, providing more evidence for the precursor role of CCLs.

P87
IDENTIFICATION OF PROGNOSTIC MOLECULAR BIOMARKERS IN EARLY-STAGE BREAST CANCER

S. Jarmalaite[1], A. Laurinaviciene[2,3], J. Tverkuviene[1], K. Daniunaite[1], A. Scesnaite[1], V. Ostapenko[2], J. Lazutka[1]
[1]*Vilnius University, Faculty of Natural Sciences, Vilnius, Lithuania*
[2]*Vilnius University, Institute of Oncology, Vilnius, Lithuania*
[3]*Nacional Centre of Pathology, Vilnius, Lithuania*

Breast cancer is the most prevalent malignancy of women. Despite marked improvement in early detection and treatment of disease, breast cancer still remains one of the most deadly tumours world-wide. Identification of novel molecular markers for reliable selection of patients with high risk of disease progression might favour individualization of therapy and improve survival rates. In 119 breast carcinomas of early stage (pT1-pT2) epigenetic and genetic changes in a wide panel of cancer-related genes was analysed in addition to current prognostic markers of breast cancer, such us oestrogen and progesterone receptor (ER and PR) status, amplification of HER2, and expression of cancer-related proteins. Aberrant DNA methylation in promoter regions of cancer-related genes was analysed by means of methylation-specific PCR. Automated single strand conformation polymorphism analysis was used for the detection of *TP53* gene mutations. Tissue array based immunohistochemistry was used for expression analysis of six breast cancer-related proteins ER, PR, HER2, Ki-67, p16, and p53. DNA methylation changes in at least one of 12 analysed promoters were detected in 115 out of 119 tumours (97%). The most informative epigenetic biomarkers of early-stage breast cancer were hypermethylated genes *ESR1* and *RASSF1*. Gene *TP53* mutations were detected in 32% of breast carcinomas. Logistic regression analysis revealed statistically significant associations between higher grade of tumour and loss ER expression, hypermethylation of the *GSTP1* gene and *TP53* mutation. Mutations of the *TP53* gene were also predominant in ER negative and triple negative (ER, PR, and HER2) tumours, characterised with poor prognosis. Progression of disease was related to increased frequency of gene *RARB* hypermethylation and hypomethylation of *hTERT* promoter. Preliminary results of our study show significant value of DNA methylation biomarkers for prediction of increased risk of diseases progression in early-stage breast cancer.

P88
IGFBP7 IS A p53 TARGET GENE INACTIVATED IN HUMAN LUNG CANCER BY DNA HYPERMETHYLATION

Y. Chen[1], T. Cui[1], T. Knoesel[1], L. Yang[1], K. Zoeller[1], I. Petersen[1]
[1]*University Hospital Jena, Institute of Pathology, Jena, Germany*

Aims: Insulin-like growth factor binding protein 7 (IGFBP7) was considered a tumor suppressor gene in lung cancer. However, the mechanisms responsible for the inactivation and regulation of IGFBP7 have not yet been fully understood.
Methods: The expression of IGFBP7 was analysed by real-time RT-PCR and immunohistohemistry in lung cancer cell lines and primary lung tumors. The methylation status of IGFBP7 was evaluated by bisulfite sequencing (BS) and methylation-specific-PCR (MSP). Additionally, the role of tumor suppressor p53 in regulation of IGFBP7 was investigated by transfection with a p53 expression vector, and treatment of lung cancer cells alone with p53 inducer adriamycin (ARD) or in combination with demethylation reagent 5-aza-2'-deoxycytidine (DAC).
Results: We found that 14 out of 16 lung cancer cell lines showed decreased expression of IGFBP7 compared to control cells by real-time RT-PCR, and 27 out of 60 patients (45%) with primary lung tumor exhibited negative staining of IGFBP7 by immunohistochemistry analysis. The IGFBP7 expression could be restored by demethylatioon agent DAC in 7 cancer cell lines. Bisulfite sequencing (BS) and methylation-specific-PCR (MSP) showed that low expression of IGFBP7 was associated with DNA methylation in both lung cancer cell lines and primary lung tumors. However, the methylation status in primary lung tumor is neither significantly related to clinicopathological data nor to clinical outcome. Transfection with a wild type p53 expression vector into lung cancer cell line H1299 and H82 increased IGFBP7 expression only in H82 harbouring no IGFBP7 methylation, while tranfection of p53 in combination with DAC induced the expression of IGFBP7 in H1299, in which IGFBP7 was highly methylated. Furthermore, treatment with p53 inducer adriamycin (ADR) alone or in combination with DAC increased the expression of IGFBP7 in 4 lung cancer cell lines.
Conclusions: Our data suggest that IGFBP7 is inactivated in human lung cancer by DNA hypermethylation, and IGFBP7 might be regulated by p53 in lung cancer cells.

Poster session 14: Tumor Microenvironment

P91
DIFFERENTIAL VASCULAR EXPRESSION OF ONCOFETAL FIBRONECTIN AND TENASCIN-C IN RENAL CELL CARCINOMA (RCC) – IMPLICATIONS FOR ANTIBODY BASED TARGETED PHARMACODELIVERY

A. Berndt[1], K. Galler[1], R. Köllner[1], P. Richter[1], M. Franz[2], M. Gajda[1], A. Göhlert[1], K. Junker[3], D. Neri[4], H. Kosmehl[5]
[1]*University Hospital Jena, Institute of Pathology, Jena, Germany*
[2]*University Hospital Jena, Department of Internal Medicine I, Jena, Germany*
[3]*University Hospital Jena, Department of Urology, Jena, Germany*
[4]*ETH, Institute of Pharmaceutical Sciences, Zurich, Switzerland*
[5]*HELIOS Klinikum Erfurt, Institute of Pathology, Erfurt, Germany*

Aims: Angiogenesis in renal cell carcinoma is associated with the occurrence of oncofetal fibronectin (oncFn) and tenascin-C (oncTn-C) variants which are putative structures for an antibody-mediated targeted drug delivery. Recombinant antibodies are available which have already progressed to clinical studies. Knowledge on the distribution and structural organization of these proteins in RCC subtypes as well as metastases is crucial for understanding tumour vessel formation and for therapy planning.
Methods: Human SIP antibodies against the oncFn ED-A, oncFn ED-B as well as the oncTn-C A and C splicing domains were used for immunofluorescence. Vascular staining was semiquantitatively assessed in 10 clear cell (ccRCC), 10 papillary (papRCC), and 10 chromphobe (chrRCC) renal cell carcinoma. Furthermore, vascular expression was compared in primary and metastases of ccRCC. The vascular heterogeneity and structural organization in relation to vascular basement membrane (vBM) was analysed in ccRCC combining SIP´s with CD31 and laminin alpha4 chain antibodies.
Results: 1) RCC subtypes showed a differential oncFn and oncTn-C positivity of carcinoma vessels: While

ccRCC is characterized by a positivity for ED-A+ Fn, ED-B+ Fn and A+ Tn-C, the papRCC showed a preferential vascular staining for A+ Tn-C and the chrRCC for ED-A+ Fn. 2) Primary tumours and metastases of ccRCC showed the same expression pattern. 3) In ccRCC, there are different types of vessels concerning the incorporation of oncFn and oncTn-C variants. Furthermore, oncFn is localized luminal and oncTn-C extraluminal of the vBM.
Conclusions: Composition and spatial reorganization of oncFn and oncTn-C in tumour vessels depend on RCC subtype and may reflect an individual tumour stroma interaction or different stages of vessel development. Concerning RCC therapy, oncFn or oncTn-C based targeting must be adapted to the individual patient. Combinations of oncFn and oncTn-C antibodies may enhance the therapeutic effect.

Funded by European Community Seventh Framework Programme (FP7/2007-2013) under grant agreement n° Health-F2-2008-201342 (ADAMANT).

P92
RELATIONSHIP BETWEEN NHERF1 EXPRESSION AND PROGRESSION MARKERS IN FAMILIAL BREAST CANCER

A. Malfettone[1], A. Bellizzi[1], A. Paradiso[1], A. Mangia[1]
[1]*National Cancer Centre "Giovanni Paolo II", Clinical and Experimental Oncology Laboratory, Bari, Italy*

Background: Na+/H+ exchanger regulatory factor 1 is a scaffolding protein that recruits membrane and cytoplasmic proteins into functional complexes. We have recently demonstrated a different physio-pathological role of NHERF1, depending on its subcellular localization. NHERF1 is strongly associated with a poorer prognosis and with increasing levels of the tumor microenvironment marker HIF-1α in cultured breast cancer cells and in human breast tumor biopsies. The hypoxia-inducible factor-1 (HIF-1α), mediating transcriptional activation of vascular endothelial growth factor (VEGF) gene, is considered a key regulator in the survival of proliferating tumor cells in a hypoxic microenvironment. Our aim was to analyse NHERF1 expression in familial and sporadic breast cancer patients and determine its relationship with these progression tumour markers, HIF-1α and VEGFR1.
Methods: This study was performed utilizing a tissue microarray, composed by 94 familial and 93 sporadic breast cancer patients, with immunohistochemical analyses of NHERF1,VEGFR1 and HIF-1α.
Results: Apical membranous NHERF1 expression was significantly higher in sporadic than familial tumours (p=0.000). Higher levels of cytoplasmic NHERF1 expression no statistically significant were observed in tumour cells from familial patients compared with tumour cells from sporadic patients. Furthermore, in familial patients high levels of nuclear NHERF1 were associated with positive HIF-1α tumours (p=0.003); moreover, cytoplasmic NHERF1 overexpression was associated with VEGFR1 positivity, (p=0.009). In sporadic patients, nuclear NHERF1 immuoreactivity is significantly correlated with HIF-1α expression (p=0.019); but, any significant association between cytoplasmic NHERF1 and both HIF-1α and VEGFR1 was found. This is in agreement with a pivotal role of NHERF1 in the more aggressive tumours such as familiar breast cancers.
Conclusion: In familial cases elevated levels of cytoplasmic NHERF1 are present in the more aggressive and proliferating tumors. NHERF1 resulted strongly related with HIF-1α protein and more importantly with VEGFR1. In this context, we suggest an emerging role of NHERF1 in tumor progression and survival.

P93
TUMOR-DERIVED CANCER STEM CELLS THERAPY: NICHE MODULATION VERSUS MANIPULATION OF CANCER STEM CELLS THEMSELVES

H.R. Mirzaei[1], M. Mirzaei[2]
[1]*Isfahan University of Medical Sciences, Immunology, Isfahan, Islamic Republic of Iran*
[2]*University of Arak, Biology, Arak, Islamic Republic of Iran*

A great deal of studies have been shown that a growing tumor is a heterogeneous mix of mostly differentiated cancer cells and cancer stem cells (CSCs), a rare population that have stem-like properties and having capability of self-renewal and multi-potency of differentiation . The differentiated cancer cells exhibit the characteristic rapid proliferation, while the CSCs are much slower at dividing, making them resistant to conventional therapy. To date, current concepts for treatment and/or cure of tumor-cancer stem cells are based on differentiation therapy and/or eradication of neoplastic stem cells as the root of cancers. It seems that the role of CSCs niche in tumor-derived cancer stem cells therapy is underestimated since all of the supposed therapies are only based CSCs themselves. It would be speculated that modulation of CSCs niche is

as pivotal as or more important than manipulation of CSCs themselves since current and/or future therapies may eradicate or differentiate CSCs, but recurrence of cancers is inevitable because although the y may manipulate CSCs but the cancer niche is in the original state (cancerous state), in turn, normal tissue-resident stem cells or progenitors will be exposed to already existing transformating cues and maybe leading to new CSCs formation and finally cancer relapse. Therefore, it seems that new therapies should be directed against CSCs niche and CSCs themselves and/or at least CSCs niche. The concept of the role of CSCs niche and its modulation appear to be a pivotal approach for treatment and/or cure of cancers. Ultimately, an understanding of the CSCs niche in addition to the CSCs themselves lead to a better understanding of the therapeutic approaches for tumor-derived cancer stem cells therapy.

P94
QUANTITATIVE IMAGING OF THE VASCULARIZATION OF A HUMAN TUMOR XENOGRAFT MODEL, WHICH IS MODIFIED BY CO-TRANSPLANTATION OF ENDOTHELIAL CELLS

M. Mirus[1], S. Tokalov[1], A. Koch[1], N. Abolmaali[1]
[1]TU Dresden, OncoRay - Center for Radiation Research in Oncology, Dresden, Germany

Introduction: Gaining well vascularizated stroma is a major step in the development of solid tumors in all species (angiogenic switch). High vascularization in tumors can be linked with high expansion, metastasis and bad prognosis. Therefore detecting vascularization in tumors is essential for both diagnosis and prognosis as well as choosing and observing therapy strategies. The aim of this study was to establish a human tumor xenograft model of one tumor cell type with different vascularization to define in vivo imaging markers for the evaluation of tumor vascularization using clinical PET, CT and MRI.
Materials and methods: Tumor xenografts were established in 71 nude rats by subcutaneous transplantation of non small cell lung cancer (NSCLC) cells (A549 or H1299) alone or together with rat glomerular endothelial (RGE) cells. Differences between tumors were quantified in vivo by contrast enhanced computed tomography (CE-CT), 18-fluorodesoxy-D-glucose positron emission tomography (FDG-PET) and diffusion weighted magnetic resonance imaging (DWI-MRI) and ex-vivo by CD31 staining.
Results: Expansion rates (tumor diameter: D = 2 cm in 21.5 vs. 54.5 days), contrast enhancement in CE-CT (328 vs. 256 %, $p<0.05$), FDG uptake in FDG-PET (1.8 vs. 1.0 SUVmean, $p<0.05$) and perfusion values in DWI-MRI (722 vs. 585 $\mu m^2/s$, $p<0.05$) were higher in co-transplantated tumors as compared to control tumors. These findings correlated with higher vascularization as evaluated by CD31 staining.
Conclusions: The increased expansion rate of co-transplanted tumors is assumed to be a result of good supply of oxygen and nutrients due to better vascularization. Clinical DWI-MRI, CE-CT and PET allow the in vivo assessment of vascularization in tumors and might be useful for preclinical investigations of antiangiogenic drugs.

This research and the authors are supported by the Federal Ministry of Education and Research, Germany, BMBF Contract 03ZIK042.

P95
INVOLVEMENT OF ENDOTHELIAL CELLS IN THE TUMOR BED EFFECT.

C. Glauert[1], S. Tokalov[1], A. Koch[1], G. Wolf[1], N. Abolmaali[1]
[1]TU Dresden, OncoRay - Center for Radiation Research in Oncology, Dresden, Germany

Introduction: Tumor cells transplanted to preliminary irradiated host tissue usually show a delayed and overall reduced growth compared to tumor cells transplanted to an unaffected microenvironment. This phenomenon, known as a tumor bed effect, is considered to be a result from the reduced ability of the host tissue to form new vessels supporting the tumor. So the administration of donor endothelial cells (EC) in tumor bed might influence the results. The aim of this study was to evaluate the role of EC in tumor bed effect using a human tumor xenografts.
Material & Methods: After whole body irradiation (4 Gy) and TBI (15 Gy) animals received subcutaneously implanted non small cell lung cancer cells (H1299) alone (12 rats) or co-tranplanted together with EC, VEGF and FGF (12 rats). Tumor growth was followed over 6 weeks. Subsequently, tumors were analyzed by diffusion weighted magnetic resonance imaging (DW-MRI) using a clinical MRI scanner (Magnetom Avanto, Siemens). Apparent diffusion coefficient (ADC) values were calculated investigating both diffusion and perfusion parameters of tumor tissue. Regions of interest were selected excluding necrotic parts.

Results: It was found, that it took 46 days to develop a tumor of 2 cm diameter after implantation with H1299 cells alone and 28 days after co-transplantion with EPC, VEGF and FGF. After implantation with H1299 cells alone tumors showed average ADC diffusion values (ADC_{diff}) of 815 mm^2/s and ADC perfusion values (ADC_{perf}) of 550 mm^2/s. Co-transplanted tumors of the same size showed ADC_{diff} of 733 mm^2/s and ADC_{perf} of 817 mm^2/s.
Discussion: Cotransplantation with EC, VEGF and FGF improved the tumor blood supply and influenced tumor bed effect by forming new vessels resulting in a better perfusion in the tumor tissue. Thus, tumors with a higher level of vascularization could be created.

This research and the authors are supported by the Federal Ministry of Education and Research, Germany, BMBF Contract 03ZIK042.

Poster session 15: Metastasis Formation

P101
THE DYNAMICS OF THE FORMATION OF EXTRANODAL LEUKEMIA METASTASIS IN THE LIVER LEADING TO ACUTE LIVER FAILURE IN MICE.

A. Bigildeev[1], I. Shipounova[1], D. Svinareva[1], N. Drize[1]
[1]*National Hematology Research Centre, RAMS, Lab. of Physiology of Hematopoiesis, Moscow, Russian Federation*

The colonization of liver by leukemic cells was studied in dynamics by means of the model of transplantable MPD-like myeloid leukemia. (CBA x C57Black) F1 mice were injected i.v. with 10^6 leukemic bone marrow cells of syngeneic mice. Every day after cells injection from day 1 until death of animals 2-3 mice were sacrificed, the liver was isolated and CD45+ cells were sorted using magnetic columns. The expression of genes in isolated populations was studied using traditional and real-time PCR. The expression of chemokines secreted by liver and their receptors was compared in unsorted liver of terminally sick animals and normal control animals. The concentration of leukemia stem cells (LSC) was calculated using Poisson statistics and i.v. injection of limiting dilutions of leukemic liver cells. The surface phenotype of LSCs was assessed by i.v. injection of magnetically sorted c-kit^-CD45^-, c-kit^+, c-kit^-CD45^+, $Ter119^+$ and $Ter119^-$ cells.
The increase in number of CD45+ cells in the liver took place starting from day 9 after transplantation of leukemic cells. CD45+ cells invading liver used hepatic tissue as maintaining microenvironment partly due to increased expression of receptors for chemokines secreted by liver. The possibility of LSC to use hepatic niches was observed. The frequency of LSCs among leukemic cells invading liver was 3 orders higher than in bone marrow. It means that the amount of niches maintaining LSCs in liver is increased comparing with bone marrow. Thus the unique case of extranodal localization of myeloid leukemia is described. The heterogeneity of LSCs in this model was observed by the ability to use hepatic niches and thus invade liver by cells not displaying the surface markers of stem cells. The 40-50 fold increase in the expression of genes responsible for self-renewal in those cells indicates the differences in regulatory signals of hepatic and bone marrow niches. The expression of house-keeping genes in CD45+ cells invading liver was increased 30-100 fold. These data together with 20-times elevated amount of ribosomal RNA in those cells indicate tremendous protein synthesis and rapid proliferation of leukemic cells.
The studied leukemia model opens a prospect for the investigation of the regulatory mechanisms and properties of LSCs that could be important for treatment of myeloid leukemia.

P102
RECEPTOR CONVERSION IN DISTANT BREAST CANCER METASTASES

L. Hoefnagel[1], M. van de Vijver[2], P. van Diest[1]
[1]*UMC Utrecht, Department of Pathology, Utrecht, The Netherlands*
[2]*Academic Medical Center, Department of Pathology, Amsterdam, The Netherlands*

Background: When breast cancer patients develop distant metastases, the choice of systemic treatment is usually based on tissue characteristics of the primary tumor as determined by IHC. Several previous studies have shown that the immunophenotype of distant breast cancer metastases may be different from that of the primary tumor (receptor conversion), leading to inappropriate systemic treatment. The studies published so far are however small and/or methodologically suboptimal. Therefore, definite conclusions that may change clinical practice could not be drawn. The aim of the present study was to study

receptor conversion for ERα, PR, and HER2 in a large group of distant (non-bone) breast cancer metastases by re-staining all primary tumors and metastases with current optimal immunohistochemical and ISH methods on full sections.
Methods: A series of 233 distant breast cancer metastases from different sites (76 skin, 63 liver, 43 lung, 44 brain and 7 gastro-intestinal) was IHC stained for ERα, PR and HER2, and receptor expression was compared to that of the primary tumor. HER2 ISH was done in cases of IHC conversion or when primary tumors or metastases showed an IHC 2+ result.
Results: Receptor conversion by IHC for ERα, PR and HER2 occurred in 10.3%, 30.0% and 5.2% of patients, respectively. Of the 12 cases that showed HER2 conversion by IHC, 5 showed also conversion by ISH. One further case showed conversion by ISH, but not by IHC. Conversion was mainly from positive in the primary tumor to negative in the metastases for ERα and PR, while HER2 conversion occurred equally both ways. Receptor conversion seemed to occur mostly in liver and brain metastases for ERα and PR, and in liver metastases for HER2. In 10.7% of the patients, conversion from ER+ or PR+ to ER-/PR- and in 3.4% from ER-/PR- to ER+ or PR+ was found.
Conclusion: Receptor conversion by immunohistochemistry in (non-bone) distant breast cancer metastases does occur, is relatively uncommon for ERα and HER2, more frequent for PR, and relatively frequent in brain and liver metastases.

P103
ANALYSIS OF SINGLE CIRCULATING TUMOUR CELLS ISOLATED FROM GASTRO-INTESTINAL CANCER PATIENTS

K. Goerner[1], M. Schiemann[2], J. Bachmann[2], C. Holzhauer[1], R. Kirchner[1], P. Hartmann[1], M. Alunni-Fabbroni[1], M. Martignoni[2]
[1]*Beckman Coulter Biomedical GmbH, Munich, Germany*
[2]*Technische Universität München, Munich, Germany*

The gastro-intestinal (oesophageal, gastric, liver and pancreatic) tumours, with generally short life expectation after diagnosis, are among the most aggressive cancers known so far, showing one of the highest mortality rates. The discovery of the tumor is possible at the moment only through standard imaging analysis such as CT, MRT, (Endo)-sonography, ERCP and PET with early diagnosis and surgery the only possibility of recovery. In recent years, Circulating Tumour Cells (CTCs) came more and more into focus because of their association with tumour growth and metastasis. It became clear very soon that early diagnosis and prognosis of the disease could be based on their detection and genetic analysis. Despite CTCs may play a key role in tumour development, little is known about their gene expression profile and interrogation of these cells on a molecular basis is still in an early phase. In the present study, spiked tumour cells and circulating tumour cells isolated directly from gastrointestinal cancer patients blood, were deposited by Flow Cytometry Cell Sorting on AmpliGrid slides to analyse CTCs for RNA expression of marker genes. Tumour cells were defined as positive for the Epithelial Cell Adhesion Molecule (EpCAM) and negative for CD45 pan-leukocyte antigen and Propidium Iodide (PI) staining. Aim of this study was to establish a reliable assay for CTC detection and isolation followed by genetic characterization. The identification of changes in CTCs gene expression might represent a powerful diagnostic tool leading to a more targeted and personalized therapy. Furthermore, in understanding the regulation of CTCs we might gain key insights in understanding metastatic disease and tumour progression.

Poster session 16: MicroRNA in Cancer

P106
DIFFERENTIALLY EXPRESSED MICRORNAS LOCATED ON 20Q INVOLVED IN COLORECTAL ADENOMA TO CARCINOMA PROGRESSION

L. Timmer[1], A. Bolijn[1], E. Cuppen[2], G. Meijer[1], B. Diosdado[1]
[1]*VU University medical centre, Pathology, Amsterdam, The Netherlands*
[2]*Hubrecht Institute, Utrecht, The Netherlands*

Background: Colorectal cancer (CRC) is the second leading cause of cancer death in the Western world and it is caused by the gradual accumulation of mutations, chromosomal aberrations, methylation changes and altered expression of microRNAs (miRNAs). The most frequent chromosomal aberration associated with adenoma to carcinoma progression involves gain of 20q, which occurs in more than 65% of the colorectal tumours.
MiRNAs, a class of small non-coding RNAs, have been shown to regulate central mechanisms of tumorigenesis. MiRNAs are involved in the initiation

and progression of human cancers due to their altered expression. The causes of this altered expression can be related to DNA copy number changes, epigenetic changes or to direct transcriptional activation of oncogenes or tumour suppressor genes. MiRNAs have been previously identified to be differentially expressed in CRC, but the gene dosage effect on the miRNAs located on the amplicon 20q has not been investigated.
The aim is to study the effect of DNA copy number gain of 20q on the expression of the miRNAs located on this chromosomal arm during adenoma to carcinoma progression.
Materials and Methods: Presence of DNA copy number gain on 20q will be characterized in 40 colorectal adenomas and 40 colorectal carcinomas by Multiplex Ligation-dependent Probe Amplification (MLPA). The expression of 18 miRNAs located on 20q will be determined by quantitative RT-PCR.
Results: MLPA experiments have been carried out in 80 colorectal tumours. Expression levels of miRNAs located on 20q are being analyzed. MiRNAs contributing to CRC will be determined by comparing their expression levels in colorectal tumours to 10 healthy controls. Further, to establish gene dosage effect of 20q on these miRNAs, the expression of the differentially expressed miRNAs between colorectal tumours with and without 20q gain will be determined.
Conclusion: The integration of miRNA expression and DNA copy number gain of 20q will lead us to the identification of miRNAs involved in colorectal adenoma to carcinoma progression.

P107
Let-7 DOWNREGULATES *Aurora-B* AND AFFECTS *G2/M* TRANSITION

A. Belur Nagaraj[1]
[1]*Uniklinikum Dresden, MK1-L11, Stem cell lab2, Dresden, Germany*

The let-7 family of miRNAs has a known conserved function to regulate cell differentiation and proliferation. Microarray studies suggest that exogenous addition of *let-7* downregulates expression of a variety of cell cycle regulators in human cancer cell lines. Our screening microarray experiments done in A549 and HepG2 cells demonstrated *Aurora-B* mRNA is a likely target of *let-7* activity. We show here that *let-7* indeed downregulates *Aurora-B* on both mRNA as well as protein level in HeLa cells. Reporter gene assays demonstrated a direct influence of *let-7* on the 3`UTR of *Aurora-B*. In order to prove the functional influence of *let-7* on *Aurora-B* mediated cell cycle changes, we explored the effect of *let-7c* overexpression on cell cycle and we observed an increase in cells in G2/M phase. Next, we studied the effects of *let-7* overexpression on nocodazole induced mitotic events. *Aurora-B* protein levels were strongly reduced in HeLa cells treated with nocodazole after *let-7* overexpression. In our study we have characterized *Aurora-B* as a novel functional target of *let-7* and studied the effect of *let-7* on downstream events of Aurora-B kinase activity. Downregulation of the potential oncogene *Aurora-B* by *let-7* further strengthens the fact that *let-7* functions as a tumor suppressor.

Poster session 17: Molecular Mechanisms of Resistance to Therapy

P111
RNAi-MEDIATED SILENCING OF TCF7L2 SENSITIZES COLORECTAL CANCER CELLS TO RADIATION

E. Kendziorra[1], M. Spitzner[1], G. Emons[1], K. Ahlborn[1], J. Gaedcke[1], M. Rave-Fränk[2], T. Beißbarth[3], H. Becker[1], B.M. Ghadimi[1], T. Ried[4], M. Grade[1]
[1]*University Medical Center, General and Visceral Surgery, Göttingen, Germany*
[2]*University Medical Center, Radiotherapy and Radiooncology, Göttingen, Germany*
[3]*University Medical Center, Medical Statistics, Göttingen, Germany*
[4]*Genetics Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA*

Question: The clinical response of locally advanced rectal cancers to preoperative chemoradiotherapy is very heterogeneous. To determine the molecular characteristics associated with this heterogeneity, we recently profiled a series of responsive and resistant rectal adenocarcinomas using gene expression microarrays, and identified a set of differentially expressed genes. One gene that was significantly overexpressed in the resistant tumors was TCF7L2, the main downstream effector of the Wnt signaling pathway. The aim of this study was to evaluate if RNAi-mediated silencing of TCF7L2 sensitizes tumor cells to radiation therapy.

Methods: We transfected the colorectal cancer cell line SW480, which expresses TCF7L2, with two different shRNA-vectors targeting TCF7L2 and a non-silencing control, and subsequently established stable single cell clones. Protein levels after RNAi-mediated gene silencing were analyzed by Western blotting. For each vector, single cell clones were plated and irradiated at 2, 4, 6 and 8 Gy. Colonies were counted after 10 days, and survival fractions were subsequently calculated.
Results: The TCF7L2 protein levels were reduced to ~ 34% (shRNA_1) and ~ 29% (shRNA_2) of the non-silencing control. RNAi-mediated silencing of TCF7L2 led to a ~ 1.6-fold growth inhibition (plating efficiency) compared to the non-silencing control, and, importantly, significantly reduced colony-formation after radiation: We observed dose reduction factors of ~ 1.45 and ~ 1.65 at 37% survival for shRNA_1 and shRNA_2, respectively.
Conclusions: TCF7L2 was found to be overexpressed in resistant rectal carcinomas, and its RNAi-mediated silencing caused a significant growth inhibition and radiosensitization of colorectal cancer cells. Thus, TCF7L2 might represent a potential molecular target to sensitize a priori resistant tumor cells.

P112
HeLa CELL LINES MICROCELL VISUALIZATION USING pGFP2-N3 PLASMID TRANSFECTION.

T. Freivalds[1], D. Bema[1], D. Pjanova[2], R. Petrovska[2], L. Harju[1], I. Buikis[1]
[1]*University of Latvia IECM, Institute of Experimental and Clinical Medicine, Riga, Latvia*
[2]*Latvian Biomedical Research Centre, Riga, Latvia*

Tumour treatment can be improved exploring resistant cancer cell unique properties. We suggested that microcells are the source of resistance to anticancer therapy. One of microcell significant property is endocytosis. The aim of our study was to investigate possibility to mark these resistant microcells using GFP gene cloned into plasmid. The HeLa cell line was maintained at 37 degrees in DMEM medium for 3-4 days. Development of microcells was induced with Hg UV lamp irradiation behind UFC-1 filter. Plasmids (pGFP2-N3) containing GFP gene were mixed with TurboFect^{TM} *in vitro* transfection reagent (Fermentas, Lithuania) according to the manufacturer's guidelines or with zymosan - polysaccharide from (*Saccharomyces cerevisiae*) also plasmids was added to cells without any transfection reagent. After transfection HeLa cells were fixed in 4% paraformaldehide, nuclei were stained with DAPI and cells with WGA (wheat germ agglutinin) conjugated with Alexa Fluor 594. Pictures from samples were taken using Leica DM6000B microscope connected with DFC 490 digital camera. We found that HeLa cells do not uptake plasmids alone or mixed with zymosan. It is still unknown whether zymosan alone can get inside cells. Some of the HeLa cancer cells enclosed plasmids mixed with turbofect. 24 hours after transfection GFP expression can be seen in developing microcells cells. After 48 and 72 hour period GFP expression can be seen in microcells and in some matured HeLa cancer cells. We conclude that TurboFect^{TM} transfection reagent can be used as agent that helps to get plasmids (with necessary genes) inside cancer microcells. So it can further be used in tumor treatment work out process.

P113
HEAD NECK SQUAMOUS CELL CARCINOMA EXPRESSES HEME OXYGENASE-1: POSSIBLE MARKER OF SENSITIVITY TO RADIOTHERAPY

L. Shi[1], W. Li[1]
[1]*The First Affiated Hospital of China Medical University, ENT Department, Shenyang, China*

Heme oxygenase catalyzes the rate-limiting step of heme degradation, leading to formation of biliverdin, carbon monoxide, and free iron. HO-1, one isoform of heme oxygenase, is a member of the heat shock protein family, which plays an important role in rapid tumor growth by its anti-oxidative and anti-apoptotic effects. Recently, high level of HO-1 expression has been reported in renal cell carcinoma and prostate tumors. The aim of this study is to investigate the relationship between HO-1 and head neck squamous cell carcinoma (HNSCC), especially its involvement in the response of HNSCC to radiotherapy.
Fifty seven HNSCC patients were enrolled in this study. Tissue samples were semi-quantitatively analyzed by RT-PCR, and the expression of HO-1 was correlated with the consequence after novel radiotherapy (70Gy in total).
Among 57 HNSCC patients, high expression of HO-1 was found in 34 samples, in which 11 patients showed no response to radiotherapy (the reduction rate of tumor size less than 50%); interestingly, in 23 patients with negative expression of HO-1, radiotherapy exhibited to be responsive in 11 patients (the reduction rate of tumor size more than 50%) or effective in 12 patients (tumor totally vanished). Thus, high

expression of HO-1 induced a great resistance to radiotherapy ($P < 0.05$ vs HO-1 negative group).
In this study, we demonstrated the expression of HO-1 in HNSCCs, and HO-1 seems to be a useful index in identifying patients with well response to radiotherapy. These data indicate a new therapeutic for HNSCCs by inhibiting HO-1 activity, which warrants further investigation.

Poster session 18: Tumor Biology and Response to Therapy

P116
SPECIFIC MICROCELL DEVELOPING MECHANISM IN HeLa CANCER CELL LINE

D. Bema[1], V. Skele[1], T. Freivalds[1], I. Buikis[1], L. Harju[1]
[1]*University of Latvia, Institute of Experimental and Clinical Medicine, Laboratory of Morphology, Riga, Latvia*

Carefully explored cancer stem cell developing mechanism could show the way how to treat cancer effectively. The aim of our study was to investigate specific cells (called microcells) properties and development in HeLa cancer cells. The HeLa cells were grown in DMEM medium at 37 degrees for 3-4 days and irradiated with Hg UV lamp behind UFC-1 filter. The cell samples were supravitaly stained with acridine orange, water soluble CdSe/ZnS nanoparticles, Indian ink, carmine red. Expression of embryonic stem cell antigens (SSEA-1, SSEA-3, SSEA-4, Nanog, Oct-4) in HeLa cells and mesenchymal stem cell antigens (CD29, CD54) in HeLa and human bone marrow samples were initiated using "Millipore" monoclonal antibodies. Snapshots of cell samples were taken using Leica DM6000B microscope connected with DFC 490 digital camera. Microscope images were analyzed and measured with "Media Cybernetics" image analyzing program IPP 5.0. We found that different markers accumulate more in specific morphological states of cancer cells called microcells. High mean fluorescence intensity indicates more intense uptake and higher endocytosis capability in early microcell development stages. All used supravital staining methods allow detecting increased endocytosis ability in young cancer cells. We noticed that after UV irradiation in early microcell development phases (while microcells are still in mother cells) stem cell markers SSEA-1, SSEA-4 and OCT4b are expressed more, but mesenchymal cell markers CD29 and CD54 were expressed later in already detached young cancer cells. In conclusion we suggested that microcell developing mechanism is cancer stem cell arousing source via selection process.

P117
CIGLITAZONE MODULATES LEPTIN EXPRESSION IN BREAST CANCER CELLS

M. Terrasi[1], L. La Paglia[1], V. Amodeo[1], L.R. Corsini[1], D. Fanale[1], L. Insalaco[1], M. Perez[1], V. Bazan[1], E. Surmacz[2], A. Russo[1]
[1]*University of Palermo, Oncology, Palermo, Italy*
[2]*Temple University, Biochemistry School of Medicine, Philadelphia, USA*

Background: The obesity hormone leptin, initially discovered as a cytokine controlling food intake and energy balance, has recently emerged as a potent regulator of different physiological and pathological processes, including cancer development and progression. The importance of leptin signaling in breast tumorigenesis has been confirmed by the fact that breast tumors overexpress both leptin and its receptor, both of which correlate with higher tumor grade and worse prognosis. In vitro studies demonstrated that breast cancer cells are able to synthesize leptin in response to obesity-related stimuli, like hyperinsulinemia and hypoxia. This process is mediated through interactions of Sp-1, a nuclear factor that mediates the effects of insulin and/or HIF-1, the master transcription factor in cellular response to oxygen deficiency, with specific motifs within the leptin gene promoter. Considering that in adipocytes leptin promoter is regulated by the activation of peroxisome proliferator activated receptor (PPAR) γ, we studied whether or not ciglitazone, a PPAR-γ ligand, used for treatment of patients with diabetes and obesity and a potential anti-neoplastic agent, can modulate the expression of leptin mRNA in breast cancer cells.
Methods and results: Using chromatin immunoprecipitation (ChIP), we found that treatment of MCF-7 and MDA-MB-231 breast cancer cells with submolar concentrations of ciglitazone induced binding of PPAR-γ to the proximal portion of the leptin promoter, while it decreased the association of Sp-1 with this DNA region. Results obtained with Real Time PCR, Western blotting as well as growth experiments confirmed that these effects coincided with elevated leptin mRNA expression, protein synthesis and increased cell proliferation. The

mitogenic effects of ciglitazone were not observed when higher doses of the drug were used.
Conclusions: These data suggest that one of the mechanisms of leptin overexpression in breast tumors might involve activation of PPAR-γ with submolar concentrations of ciglitazone.

P118
QUANTITATIVE ANALYSIS OF EARLY EFFECTS OF RADIOTHERAPY IN A HUMAN TUMOUR XENOGRAFT MODEL

J. Heinold[1], S. Tokalov[1], A. Koch[1], G. Wolf[1], N. Abolmaali[1]
[1]*TU Dresden, OncoRay - Center for Radiation Research in Oncology, Dresden, Germany*

Introduction: Exploring early response to radiotherapy (RT) is thought to be important to reach more efficient treatment schemes and fewer side effects. The aim of this study was to quantify in vivo changes in water diffusion and perfusion effects in human tumor xenografts before and after RT.
Method: 27 nude rats were co-transplanted subcutaneously with non small cell lung cancer cells (A549) and rat glomerular endothelial (RGE) cells. Tumors were examined 24 h before and 36 h after RT (15 Gy, 14 rats) or without RT (13 rats, control) using a 1.5T clinical MRI scanner. Tumor volume was analyzed in T2- weighted magnetic resonance imaging (MRI). Apparent diffusion coefficient (ADCdiff) and perfusion-influenced ADCperf were calculated from high and low b-value diffusion-weighted (DW) MRI, respectively. Two minutes after intravenous injection of 2.5 µM fluorescent beads, rats were sacrificed; tumors and organs were collected and analyzed by fluorescent microscopy.
Results: It was found that 36 h after RT, tumor volume increased 2.1 times in comparison to the control group. ADCdiff increased by 70% and ADCperf decreased by 6.4% (vs. 0.1% in the control group) in irradiated tumors. Fluorescent imaging evaluated areas of high vascularity and vessel permeability suitable for fusion with MRI findings.
Discussion: RT damages endothelial cells in small tumor vessels, leading to an increased permeability and increased diffusion of free water quantifiable by DW-MRI. Destroyed vessels may also lead to reduced perfusion causing hypoxia, cell swelling and enlargement of the extracellular space in the tumor represented by increased tumor volume. These changes in the tumors vasculature happen during the first days after irradiation and might be useful for the evaluation early response to RT.

This research and the authors are supported by the Federal Ministry of Education and Research, Germany, BMBF Contract 03ZIK042.

Poster session 19: Experimental Therapy

P121
CANCER STEM CELLS PLASTICITY: A TWO EDGED SWORD

H.R. Mirzaei[1], M. Mirzaei[2]
[1]*Isfahan University of Medical Sciences, Immunology, Isfahan, Islamic Republic of Iran*
[2]*University of Arak, Biology, Arak, Islamic Republic of Iran*

To date, one of the most contentious issues in biology is the existence of stem cell (SC) plasticity. The term plasticity refers to the capacity of tissue derived SCs to exhibit a phenotypic potential that extends beyond the differentiated cell phenotypes of their resident tissue. Although the classic definition of cell plasticity taken from stem cell biology implies the ability of SCs to differentiate into various cell lineages, the term is also currently applied to the ability of a given cell type to reciprocally dedifferentiate, redifferentiate, and/or transdifferentiate in response to specific stimulation. Furthermore, a great deal of studies have been shown that a growing tumor is a heterogeneous mix of mostly differentiated cancer cells and cancer stem cells (CSCs), a rare population that have stem-like properties and having capability of self-renewal and multi-potency of differentiation . The differentiated cancer cells exhibit the characteristic rapid proliferation, while the CSCs are much slower at dividing, making them resistant to conventional therapy. Since CSCs have stem like properties as major features of plasticity, hence, on the one hand, plasticity potential of CSCs can considers as a strategy for tumor-derived CSCs therapy (e.g. differentiation therapy) and on the other hand it can be considered as a pitfall since CSCs may transdifferentiate to endothelial-like cells causing resistance to current anti-angiogenic therapies. In this respect, cancer stem cells plasticity might be seen as a two-edge sword, its bright side being represented by new strategies for cancer therapies (i.e. differentiation therapy), its dark side by

resistance to current cancer therapies. A better understanding of CSCs biology will help fulfill the promise of cancer therapy aimed at fighting and curing cancer from its root.

P122
IMMUNOTHERAPY AS A MOST WISE THERAPEUTIC APPROACH IN TUMOUR-DERIVED CANCER STEM CELL THERAPY

H.R. Mirzaei[1], M. Mirzaei[2]
[1]*Isfahan University of Medical Sciences, Immunology, Isfahan, Islamic Republic of Iran*
[2]*University of Arak, Biology, Arak, Islamic Republic of Iran*

A growing tumour is a heterogeneous mix of mostly differentiated cancer cells and cancer stem cells (CSCs), a rare population that have stem-like properties and having capability of self-renewal and multi-potency of differentiation . The differentiated cancer cells exhibit the characteristic rapid proliferation, while the cancer stem cells are much slower at dividing, making them resistant to conventional therapy. Albeit, following conventional treatment, such as chemotherapy and radiation therapy, the majority of the tumour is eliminated; however, the cancer stem cells survive, differentiate, and cause tumour relapse. Immunotherapy that targets antigen(s) expressed by all cancer cells, including stem cells, could result in complete eradication of the tumour through immune effector mechanisms and prevention of tumour recurrence. Elimination of cancer stem cells early in disease may have the added benefit of preventing metastasis because migration of these cancer stem cells may be the mechanism by which tumours spread from the primary site and establish themselves at distant sites. Indeed, even immunotherapy does not cure but rather converts cancer to a long-term subclinical condition can represent a resounding success. Therefore, Immunotherapy may turn out to be the only therapy that is effective against cancer stem cells. Herein, we will not only discuss the significance of the cancer stem cell model for understanding cancer stem cell model but also possible strategies for controlling the viability and tumourigenecity of cancer stem cells, and extend our discussion to immunologic strategies approaching the prevention and/ or eradicating tumour-derived cancer stem cells.

P123
INITIATING ADMINISTRATION OF CYTOSTATIC COMPOUNDS IMPROVES RADIATION SENSITIZATION OF P53-DEFICIENT LUNG CANCER CELLS.

S. Tokalov[1], N. Abolmaali[1]
[1]*University of Technology Dresden, OncoRay - Center for Radiation Research in Oncology, Dresden, Germany*

Non-small cell lung cancer (NSCLC) is one of the most frequent causes of cancer-related death. Radiotherapy has been the standard therapy for locally advanced inoperable NSCLC. Although radiotherapy results in a modest improvement in patient survival most patients will suffer from a relapse. One of the strategies to improve local disease control is radiation sensitization. Development of NSCLC involves mutations in the tumour suppressor gene p53 with a mutations frequency of 50% in advanced diseases. This might be exploited for therapeutic advantage by selective synchronization of p53 deficient (p53-/-) cancer cells in radiosensitive phase of the cell cycle and by protecting surrounding p53 wild type cells (p53wt) using a sequence of antagonistic drugs. This study specifies a strategy to improve radiotherapy by partial synchronization of p53-deficient lung cancer cells (H1299) in mitosis using taxol and to protect p53 wild type cells (A549) by the prior administration of doxorubicin. Method: Cytotoxic and cytostatic effects of ionizing radiation, doxorubicin and taxol administrated alone or in combination were investigated in vitro by flow cytometry. Results: A protective effect of doxorubicin was found after administration of a triple sequence of ionizing radiation, taxol and doxorubicin. It was found that an initiating administration of doxorubicin induced growth arrest and protected A549 cells from the taxol / radiation treatment, simultaneously killing H1299 cells. Conclusions: These data provide a cellular basis for the synergistic actions of radiation and chemotherapy and may be useful for further preclinical studies using human tumour xenografts and clinical investigation of combined therapy for lung cancer.

This research and the authors are supported by the Federal Ministry of Education and Research, Germany, BMBF Contract 03ZIK042.

P124
DEMETHYL FRUCTICULIN A (SCO-1) INDUCES APOPTOSIS IN HUMAN CANCER CELLS BY INDUCING REACTIVE OXYGEN SPECIES IN MITOCHONDRIA.

M. Monticone[1], A. Bisio[2], A. Daga[1], P. Giannoni[3], W. Giaretti[1], M. Maffei[1], U. Pfeffer[1], F. Romeo[1], R. Quarto[4], G. Romussi[2], G. Corte[5], P. Castagnola[1]
[1]*Istituto Naz. per la Ricerca sul Cancro, Ricerca Traslazionale Oncologica, Genova, Italy*
[2]*Università, Chimica e Tecnologie Farmaceutiche ed Alimentare, Genova, Italy*
[3]*Centro Biotecnologie Avanzate, Genova, Italy*
[4]*Università, Dip. Medicina Sperimentale, Genova, Italy*
[5]*Università, DOBIG, Genova, Italy*

Question: Demethyl-fructiculin A is a component of the exudates from Salvia corrugata (SCO-1) leaves, which was recently reported to induce anoikis in mammalian cells via a mechanism involving the presence of the membrane scavenging receptor CD36. However, experiments performed with cells lacking CD36, showed that SCO-1 was able to induce apoptosis also via alternate pathways. The aim of the study was to get an insight on the biological processes and pathways elicited by this compound.
Methods: To reveal these alternate pathways we decided to undertake an unbiased pharmacogenomic approach by determining changes in the gene expression profile induced by SCO-1 in glioblastoma tumor initiating cells (GBM TICs). To investigate the extent of cell killing, we took advantage of a multi-color labeling system able to detect by Flow Cytometry (FCM) apoptosis and necrosis at the same time. To determine superoxide anion generation in mitochondria, cells were labeled with MitoSOX Red and subjected to FCM analysis. The cell cycle was studied by nuclear staining with DAPI and high resolution FCM.
Results: Our results clearly indicated that the most affected genes by SCO-1 were first those belonging to the glutathione pathway and later those required to accomplish the different steps of the cell cycle, together with an increased expression of genes able to actively inhibit these processes and in particular those belonging to the p53 signaling pathway. Accordingly, we found that GBM TICs challenged with SCO-1 underwent to apoptosis and to a G2-M cell cycle arrest. On this bases we hypothesized that the SCO-1 killing effect could be due to the induction of reactive oxygen species (ROS) production in the mitochondria. This hypothesis was confirmed by using MitoSOX, a mitochondria-selective fluorescent reporter of ROS, and by the ability of N-acetyl cystine (NAC) to inhibit apoptosis when co-administered to the GBM TICs. Furthermore, we showed that NAC protects from apoptosis also other cell types such as the HeLa and the MG-63 human cell lines.
Conclusions: We propose that ROS production and subsequent apoptosis are the major toxic effects induced by SCO-1 in human cancer cells and we suggest that SCO-1 as such, or after chemical modifications, may have a potential therapeutic value deserving further investigation in animal models.

P125
z-LEUCINYL-LEUCINYL-NORLEUCINAL INDUCES APOPTOSIS OF HUMAN GLIOBLASTOMA TUMOR-INITIATING CELLS BY PROTEASOME INHIBITION AND MITOTIC ARREST RESPONSE

M. Monticone[1], E. Biollo[2], M. Fabbi[3], A. Daga[3], F. Romeo[3], M. Maffei[3], A. Melotti[3], W. Giaretti[3], G. Corte[3,4], P. Castagnola[3]
[1]*Centro Biotecnologie Avanzate, Genova, Italy*
[2]*Università, Chimica e Tecnologie Farmaceutiche ed Alimentare, Genova, Italy*
[3]*Istituto Naz. per la Ricerca sul Cancro, Ricerca Traslazionale Oncologica, Genova, Italy*
[4]*Università, Genova, Italy*

Question: Gamma-secretase inhibitors have been proposed as drugs able to kill cancer cells by targeting the *NOTCH* pathway. Here we investigated two of such inhibitors, LLNle and DAPT, in order to assess whether they were effective in killing human glioblastoma tumor initiating cells (GBM TICs) in vitro.
Methods: To reveal the effects of the above compounds in GBM TICs, we decided to undertake an unbiased pharmacogenomic approach by determining changes in the gene expression profile induced in these cells. To investigate the extent of cell killing, we took advantage of a multi-color labeling system able to detect by Flow Cytometry apoptosis and necrosis at the same time. The cell cycle was studied by nuclear staining with DAPI and high resolution FCM.
Results: Our results indicate that LLNle is indeed able to induce death by apoptosis in human GBM TICs, which undergo a G2-M cell cycle arrest which is already detectable after 24 hours of treatment and more evident after 48 hours. On the other hand, treatment with DAPT, one of the most selective Notch inhibitors,

yielded little or no effective GBM stem cell killing and inhibition of the Notch1 processing.
Genome wide expression analysis indicated that the apoptotic process induced by LLNle is most likely related to the inhibition of the proteasome. This is then followed by a reduced expression of a number of genes required for cell proliferation together with an increased expression of genes able to actively inhibit these processes and those belonging to the p53 pathway. The central role of proteasome inhibition in the activity of LLNle was further supported by our observation of accumulation of poly-ubiquitinylated proteins and of induction of genes related to the unfolded protein response and endoplasmic reticulum stress (including *HSPA1B*).
We also showed that lactacystin, a pure proteasome inhibitor, inhibited GBM TICs viability as expected from the results obtained with LLNle.
Conclusions: We showed in vitro killing activity by LLNle on GBM TICs via apoptotic cell death following gamma-secretase and proteasome inhibition. Future studies in animal models bearing human GBM orthotopic transplant may be foreseen with the goal to assess the efficacy of drugs targeting both the proteasome and gamma-secretase, either alone or in combination with other drugs, in the therapy of this disease.

Poster session 20: Molecular, Cellular and 3-D Imaging

P131
NEW COMPUTER VISION APPROACH IN THE CLASSIFICATION OF ADENOCARCINOMA AND SQUAMOUS NSCLC USING H&E TISSUE IMAGES

C.-W. Wang[1], D. Fennell[1], P. Kelly[2], J. James[1,2], P. Hamilton[1]
[1]*Queen's University Belfast, Centre for Cancer Research & Cell Biology, Belfast, UK*
[2]*Belfast Health and Social Care Trust, Tissue Pathology, Belfast, UK*

Lung cancer is the leading cause of cancer-related death worldwide. The majority of patients with non-small cell lung carcioma (NSCLC) present with locally advanced or metastatic disease for which systemic treatment with chemotherapy is the standard of care. An important development in the treatment of metastatic NSCLC has been the tailoring of therapy for patients with NSCLC on the basis of histology. In the recent JMRB phase III clinical trial, pemetrexed with cisplatin has been shown to improve survival in patients with adenocarcinoma (AC) but has adverse effects on squamous cell cancer (SC). In contrast, squamous cell histology patients fared better with the gemcitabine combination. For SC patients, bevacizumab is contraindicated, but cetuximab in combination with chemotherapy is effective. Hence, a pathology diagnosis of non-specified NSCLC is no longer routinely acceptable, and a robust and efficient approach for classification of AC and SC carcinoma histotypes is needed for optimizing therapy for NSCLC patients.
We have developed a new computer vision approach for real time classification of AC and SC based on morphological tissue pattern of H&E images alone. Tissue microarrays (TMAs) containing 369 tissue samples, routinely stained with H&E, from 114 patients with NSCLC were used in this study. This resulted in 272 cores showing SC and 97 cores showing AC, as defined using experienced pathologist opinion. A new image processing approach of H&E colour space, together with tissue morphological pattern extraction and machine learning algorithms, a robust computer vision approach was developed, which achieves 92.41% classification accuracy using 10 fold cross-validation.
Since visual classification of squamous and non-squamous NSCLC can at times be poorly reproducible, particularly in small samples of poorly differentiated tumours, this approach offers a valuable adjunct to conventional pathological investigation. Pattern classification on small sample sizes could be implemented as part of routine diagnostic evaluation with classification of NSCLC samples and subsequent therapy selection. The approach also benefits subsequent biomarker evaluation in NSCLC TMAs where diagnostic classification of sequential sections can be automatically evaluated using the presented method.

P132
STRETCHING CHROMATINE FIBERS TO AUGMENT FISH SIGNALS RESOLUTION; APPLICATION IN HAEMATOLOGICAL MALIGNANCIES

M. Elkhoury[1], D. Chassoux[2], F. Viguie[1], A. Aboura[1,3]
[1]*AP-HP, Hopital Cochin-Hotel-Dieu, Paris, France*
[2]*Inserm, RDDM, MNHN, Paris, France*
[3]*AP-HP, Hopital Robert Debre, Medical Genetics, Paris, France*

The aim was to make use of standard cytogenetic cell pellets after methanol/acetic acid fixation in attempt to stretch chromatine and thus augment FISH resolution. This may be of use in the detection of duplications for instance and more generally to describe structural rearrangements. In addition to existing methods, it would provide a relatively simple way to describe genomic alterations.
As an example, a Cy3 labeled-DNA probe covering 185 kb in the 12p12.1 sub-band was hybridized to bone marrow cells submitted to chromatine stretch. For a given nucleus, a linear signal was detected indicative of a maximum elongation factor of 4kb/micrometer . In contrast, bone marrow cells from a patient presenting an AML with a karyotype showing a large segmental duplication for that region, displayed numerous linear signals per nucleus.
Moreover, in control cells normal for the MLL/11q23 locus, a dual colour MLL split probe showed linear contiguous red-green signals.
The stretch of chromatine appears different for some nuclei in the bone marrow cell population, possibly reflecting various differentiation states. This point in under investigation.

P133
QUANTITATIVE IMMUNOHISTOCHEMISTRY THROUGH HYPERSPECTRAL IMAGING

M. Khojasteh[1], C. MacAulay[1]
[1]*BC Cancer Research Center, Cancer Imaging, Vancouver, Canada*

Immunohistochemistry is widely used to elucidate the distribution and localization of biomarkers in different parts of a tissue. Interpretation of immunohistochemistry has been traditionally performed according to a semi-quantitative grading of the slides based on the visual estimate of the percentage and intensity of the positively stained cells. Computerized quantification through image analysis particularly if automated, holds the promise of improving the accuracy and reproducibility of immunohistochemistry assays.
The purpose of this work is to develop an objective and stable computerized method for quantification of enzyme labeled immunohistochemistry assays.
Image analysis is increasingly being used to quantify immunostains. The three broad bands of an RGB camera or one or two narrow bands filters may not be the optimal imaging channel for separating the positively stained components from the negatively stained and background. This becomes particularly important when multiple stains are used and the stains are both spatially and spectrally overlapping which results in muddy mixtures of color.
Our approach is based on hyper-spectral imaging. We use a spectrally programmable light source, OneLight Spectra (OneLight Corp., Vancouver, BC, Canada) to create images of the immunostained tissue section in many narrow-band channels. This information rich dataset encourages the application of numerous powerful analytical techniques. We applied linear unmixing to several image-sets (nuclear, cytoplasmic and membrane bound examples) to estimate the relative concentrations of the stains at every pixel of the image. The concentration map of the counterstain, which is typically used to label the nuclei, was then used to identify the nuclei through automated segmentation. Once the nuclei were located, the concentration map of the immunostain was used to quantify the immunostain on a cell-by-cell basis. The results on three different immunohistochemical stains, nuclear, cytoplasmic, and membrane will be reported.
In conclusion, the accuracy of the proposed method of imaging the immunostained tissue sections, and automated nuclei identification and cell-based immunostain quantification was validated, and provides a useful tool for the quantitative interpretation of immunohistochemistry assays.

P134
EVALUATION OF CELLULAR METABOLISM IN SMALL ANIMALS BY POSITRON EMISSION TOMOGRAPHY USING CLINICAL SCANNER

S. Tokalov[1], J. Heinold[1], A. Koch[1], G. Kotzerke[1], N. Abolmaali[1]
[1]*TU Dresden, OncoRay - Center for Radiation Research in Oncology, Dresden, Germany*

Introduction: An imaging of small animals presents a potential issue for ^{18}F-FDG PET handling, because

anaesthetic agents have been found to affect glucose turnover rates altering ^{18}F-FDG PET uptake and metabolism, for example, in the brain. The aim of this study was evaluation of the effects of the ketamine and xylazine (Ke/Xy), fasting and method of ^{18}F-FDG injection on the brain ^{18}F-FDG uptake in the rats.
Material and methods: ^{18}F-FDG uptake was analysed after intraperitoneal (i.p.), subcutaneous (s.c.), per oral (p.o.) and intravenues (i.v.) injection of 20 Mega Becquerel (MBq) in the intact rats and animals fasting for 20 h. Injections were done 20 min before or 20 min after Ke/Xy injection. Uptake distributions of ^{18}F-FDG in the body of the animals (10 per group) were investigated 30 min after ^{18}F-FDG administration using a clinical scanner (Biograph 16, Siemens).
Results: It was shown that both Ke/Xy narcosis and fasting resulted in marked differences of ^{18}F-FDG uptake. Administration of Ke/Xy reduced ^{18}F-FDG uptake by the brain of fasting intact rats from 6.4±0.1% to 4.1±0.1% ($p<0.01$) and of control rats from 5.7±0.1% to 2.2±0.1% ($p<0.001$). Fasting induced ^{18}F-FDG uptake by brain of the anaesthetised intact rats from 2.2±0.1% to 4.1±0.1% to ($p<0.05$). The same 18F-FDG uptake by brain was registered in fasting rats after i.p. (4.1±0.1%), i.v. (3.7±0.1%), p.o. (3.5±0.1%) and s.c. (4.5±0.1) injection under narcosis.
Conclusions: The choice of the narcotic, the duration of the fasting and method of ^{18}F-FDG injection are important factors influencing the in-vivo ^{18}F-FDG distribution and should be taken into account in evaluation of cellular metabolism in vivo using PET scanners.

This research and the authors are supported by the Federal Ministry of Education and Research, Germany, BMBF Contract 03ZIK042.

P135
MESENCHYMAL PROGENITOR CELLS VISUALIZED IN A CLINICAL 1.5 TESLA MRI

S. Schindler[1], S. Tokalov[1], G. Wolf[1], S. Schindler[1], A. Koch[1], N. Abolmaali[1]
[1]*TU Dresden, OncoRay - Center for Radiation Research in Oncology, Dresden, Germany*

Introduction: Mesenchymal progenitor cells (MPC) derived from bone marrow (BM) are pluripotent precursor cells which have a high proliferation and differentiation capacity. They are regarded as a particularly attractive cell type for cell-based diagnostic and therapeutic strategies. MPC selectively home to tumors, where they contribute to the formation of tumor-associated stroma. The aim of this study was to non-invasively quantify the in vivo migration of BM derived MPC into human tumor xenografts using a clinical 1.5 Tesla MRI.
Material & Methods: MPC were isolated und their phenotype and growth properties and capability to adipogenic, osteogenic and endothelial differentiation was proven in vitro. They were labeled with Resovist (Fe-MPC) and showed no change in survival or proliferation rates (using MTT-Assay and Annexin Apoptosis kit) during 1 week of culture. Distribution of Fe-MPC in tumor-bearing (H1299) nude rats was analyzed with T2* maps using a 1.5 Tesla MRI scanner (Magnetom Avanto, Siemens) in comparison with corresponding histological slides of the same areas stained with Prussian blue.
Results: Iron positive areas in tumors were revealed after intraperitoneal injection of Fe-MPC in tumor-bearing rats. Total level of Fe-MPC uptake in tumors was significantly.
Discussion: The easy isolation and the ability of MPC to differentiate into multiple tissue and mainly the "homing" in tumor areas describes a high application potential of MPC for further utilization as transporters for chemotherapeutics or the integration of vectors for production of tumor damaging substances.

This research and the authors are supported by the Federal Ministry of Education and Research, Germany, BMBF Contract 03ZIK042.

Poster session 21: Image Cytometry

P141
PATHTOOL - A SYSTEM FOR AUTOMATIC GLEASON GRADING

T.S. Hveem[1,2,3], J. Maddison[1], J.A. Nesheim[1,3], B. Risberg[1,4], H. Danielsen[1,2,3]
[1]*Oslo University Hospital, Institute for Medical Informatics, Oslo, Norway*
[2]*University of Oslo, Institute for Informatics, Oslo, Norway*
[3]*University of Oslo, Centre for Cancer Biomedicine, Oslo, Norway*
[4]*Oslo University Hospital, Department of Pathology, Oslo, Norway*

Background: Gleason grading is an important prognostic marker for prostate cancers. The Gleason scoring method is based upon the degree of loss of normal glandular tissue architecture. A pathologist grades the most common tumour pattern and the second most common tumour pattern. These two grades are added together to get a Gleason score. Gleason grading strongly influences decisions regarding options for therapy. However, studies indicate that the inter observer reproducibility of Gleason grading is relatively poor and that the scoring is inaccurate when performed by non specialist pathologists infrequently [1]. We have developed a system for performing automatic Gleason grading. H&E stained tissue sections are scanned on a high resolution scanner. An algorithm is used to extract morphological features as well as methods based on graph theory to describe nuclei arrangement. The metrics are used together in a classifier which predicts Gleason grade for the section.
Results: Preliminary results from training on 38 sections from 38 separate cases indicate that the method is promising. 508 fields of view from the 38. scans were identified as homogenous and assigned a Gleason grade (2-5) or classified as benign. Our system processed the images, extracting 32 features from each image Feature selection was performed among these and the resulting 11 features combined into a classifier using discriminant analysis. The classifier classified the 508 fields of view in the training set with a correct classification rate of 72.4%. 79.3% of the errors were between neighbouring grades (e.g. predicting grade 3 for a grade 4 sample).
Discussion: The limited initial preliminary results are promising and we are extending the study. A web based system where specialist pathologists manually identify and grade homogeneous regions in prostate tissue samples has been developed. This web system will allow more samples to be included efficiently and will provide more training and a separate test data set to enable the method to be validated.

References:
[1] Interobserver reproducibility of Gleason grading of prostatic carcinoma: general pathologist, Human pathology, 01.02.2001; 32(1):81-8