mation, according to field cancerization concept, genetically altered but histologically normal appearing cells predate the development of neoplasia or coexist with malignant cells. Prostate cancer is often multifocal, and it is likely that multiple tumors arise from an organ which has been earlier genetically altered by a particular carcinogen. Aim of our study was to identify molecular signature of genetically changed but histologically normal prostate cells.

In this study we performed a comprehensive gene expression analysis on 36 human prostate biopsy samples including prostate cancer tissue, prostate tissue adjacent to tumor and benign prostatic hyperplasia, using U133 Plus 2.0 Affymetrix arrays.

In the first step of analysis genetic profiles of prostate cancer samples and benign prostatic hyperplasia samples were compared. We have found 279 genes which differentiate the groups, among them were genes found in other studies as changed in prostate cancer: AMACR, hepsin, EZH2, which demonstrates that microarray analysis of biopsy specimens gives similar results to the studies performed using prostatectomy specimens. In the next step we compared the genetic profiles of benign prostatic hyperplasia and normal-appearing prostate tissue adjacent to cancer. We obtained 98 probesets differentiating those two groups, and this difference was significant (p=0.054) according to the global test of difference. We also compared gene expression values of genes belonging to molecular pathways described in Biocarta database. This analysis revealed that pathway: “Chromatin Remodeling by hSWI/SNF ATP-dependent Complexes” seemed to be particularly down-regulated in prostate tissue adjacent to cancer (p<0.0001), with seven genes showing expression decrease (p<0.05). Genes identified by us has yet to be validated by RT-PCR and immunohistochemical analysis.

Molecular changes in prostate tissue adjacent to cancer found in our study appear to have potential utility as early diagnostic markers.

422 Poster

Clinical and biological significance of CDK4 amplification in well-differentiated and dedifferentiated liposarcomas

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BACKGROUND: MDM2 (12q15), HMGA2 (12q14.3) and CDK4 (12q14.1) are the main target genes of the 12q14-15 amplicon in well-differentiated and dedifferentiated liposarcomas (WDLPS/DDLPS). While MDM2 and HMGA2 are consistently amplified, CDK4 is not amplified in approximately 10% of WDLPS/DDLPS. Our aim was to determine whether the absence of CDK4 amplification was –i) associated with specific clinico-pathological features –ii) compensated by another genomic event involved in the p16-CDK4/cyclinD1-pRb pathway. MATERIAL AND METHODS: We compared the clinical characteristics of a series of 44 WDLPS/DDLPS with amplification of both MDM2 and CDK4 (MDM2+/CDK4+) to a series of 38 WDLPS/DDLPS with amplification of MDM2 but no CDK4 amplification (MDM2+/CDK4-). We have used fluorescence in situ hybridization (FISH) and real-time quantitative RT-PCR analysis to determine the status of the CDKN2A (9p21.3), RB1 (13q14.2) and CCND1 (CYCLIND1, 11q13.3) genes. RESULTS: A higher proportion of MDM2+/CDK4- WDLPS/DDLPS were low-grade lesions belonging to the lipoma-like subtype of WDLPS (58% versus 32%, p= 0.03). Moreover, MDM2+/CDK4- WDLPS/DDLPS were smaller in size than MDM2+/CDK4+ WDLPS/DDLPS (proportion of tumors ≥ 20 cm: 21% versus 45.5%, p=0.03) and occured almost exclusively in the deep soft tissues of the extremities. They were very rarely located in the retroperitoneum (10.5% versus 52%, p=0,0002). In order to determine whether CDKN2A or RB1 deletions or CCND1 amplification were alternative mechanisms for CDK4 amplification, we have analyzed by FISH the status of these 3 genes in 18 cases. We have detected neither CDKN2A and RB1 deletion nor CCND1 amplification. We have found a strong overexpression of CDKN2A in all the 8 cases analyzed by quantitative RT-PCR whereas the expression of RB1 was not significantly altered.. CONCLUSIONS: Although deletions of the CDKN2A locus is among the most frequent sites of genetic loss in human cancer, our results show that this aberration is not involved in the pathogenesis of WDLPS/DDLPS even in those lacking CDK4 amplification. A high level of CDKN2A mRNA has already been reported in several other tumor types and represents a well-known response of cells to oncogenic alterations such as impairment of the P53 pathway resulting from the amplification of MDM2. Altogether, our findings suggest that the absence of CDK4 amplification might not be counterbalanced by a genomic alteration of the p16-CDK4/cyclinD1-pRb pathway. CDK4 amplification could not be as indispensable as the amplification of MDM2 and HMGA2 in WDLPS/DDLPS and may only represent a secondary genomic aberration occurring more frequently in retroperitoneal lesions which have a prolonged evolution before clinical diagnosis.

423 Poster

Temozolomide and radiotherapy antitumor efficacy evaluation with magnetic resonance imaging and proton magnetic resonance spectroscopy in human glioma models in nude rats

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Malignant glioblastoma remain uniformly fatal despite aggressive therapeutic protocols. Validation of more predictive biomarkers of treatment efficacy in experimental human glioblastoma models would greatly benefit from the establishment of additional quantitative endpoints. The aim of this study was to validate proton Magnetic Resonance Spectroscopy (1H-MRS) and Diffusion-weighted MR Imaging (DwMRI) to evaluate the anti-tumor activity of Temozolomide (TMZ) and radiotherapy (RT) in 2 human glioblastoma models.

CGL9 and U87-MG glioma cells were inoculated at D0 by stereotactic injection in the right caudate nucleus of 2 groups of 22 nude rats. Tumor-bearing rats were ranked according to body weight and randomized at D12 (U87-MG) or at D19 (CGL9) to receive either 5 administrations of 16.5 mg/kg TMZ per os daily or 5 tumor-localized irradiations of 2Gy daily (D12-D16 and D19-D23 for U87-MG and CGL9 respectively), or no treatment (CTL). Imaging was performed on a Bruker Pharmascan 4.7 T at D12, D13, D16, D19, D23 (U87-MG) and D19, D20, D23, D26, D33 (CGL9). Tumor volume was measured using T2-weighted images (U87-MG) or T1-weighted, contrast-enhanced images (CGL9). DwMRI and 1H-MRS were performed at the same timepoints.

Apparent Diffusion Coefficient (ADC) maps were computed from DwMRI volumes, and distributions of ADC analyzed within regions of interest within the tumor and the contralateral lesion-free tissue. Spectroscopic data were acquired using a SVS PRESS sequence, with voxel sizes adapted to the dimensions of the glioma in order to avoid partial volume effects with normal cerebral tissue. A spectrum was also acquired on the contralateral tissue. Spectral data were analyzed using LC-Model.

TMZ increased the life span of both U87-MG and CGL9 tumor-bearing rats (ILS = 126% and >200% for U87-MG and CGL9 respectively). The TMZ-treated to CTL tumor volume ratios (T/C%) were 8 and 2% for U87-MG and CGL9 at the last imaging timepoint, respectively. Radiotherapy (RT) increased the life span of 27% of U87-MG and 10% of CGL-9 tumor-bearing rats

ADC was increased by 34% in TMZ compared to CTL group for U87-MG tumors, whereas ADC was not modified by TMZ in CGL9 tumors.

In the U87-MG CTL group, a progressive reduction in NAA and creatine was observed during the study period. The ratio of total choline and total creatine increased from 0.5±0.2 to 2.5±0.3 in the CTL group, while it decreased from 0.8±0.3 to 0.3±0.2 in the TMZ group. Analysis of MRS data on the CGL9 model and on RT group is pending.

Using MRI, we observed a strong inhibition of tumor growth by TMZ treatment on both models, together with increased survival. ADC is a sensitive parameter to the effect of TMZ on U87-MG, but not on CGL9 tumors. Monitoring tumor metabolism using 1H-MRS is well suited to follow the growth of U87-MG tumors and allows quantification of the antitumor effect of TMZ with choline being the most obvious candidate as a pertinent biomarker.

424 Poster

Identification of the best molecular markers for early detection of melanoma metastases

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Melanoma, the deadliest of skin cancers, is typified by its high propensity to metastasize and its refractoriness to treatments thereafter. Metastasis occurs mostly through the lymphatic system, and the extent of lymph node metastasis involvement is considered as the best prognostic indicator. Unfortunately, the lymphatic metastatic process is still poorly understood, and the present immunohistological analyses underestimate the number of

sentinel node metastases (micrometastases). The aim of this study was to characterize the early metastatic cells molecularly and to find the best diagnostic markers for metastatic melanomas by genome-wide gene expression analyses of melanoma lymph node micrometastases and macrometastases, and of primary melanomas and benign nevi. Significance analysis of microarrays with a false discovery rate of 0.93% identified 22 over- and 5 under-expressed genes with >4-fold changes in the micrometastases. Of these genes, melan-A (MLANA), tyrosinase (TYR), melanoma inhibitory activity (MIA), v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (ERBB3), preferentially expressed antigen in melanoma (PRAME), and secreted phosphoprotein 1 (SPP1) were tested as potential markers in RT-PCR and immunohistochemistry. In a prospective study of 160 patients, graded MLANA- and TYR-RT-PCR analyses could disclose clinically significant metastases and stratify patients (in regard to tumour burden) into distinct risk groups for recurrence better than did histological and immunohistochemical examinations. In the light of these data, quantifiable RT-PCR assays should be implemented in clinical use to confirm and complement pathological examination of sentinel node metastases. In addition, SPP1 and PRAME proved valuable as melanoma-specific markers capable of differentiating melanoma cells from benign nevocytes occasionally present in the sentinel lymph nodes. Most molecular traits of the micrometastases were already present in the primary tumors, suggesting that micrometastasis to lymph nodes is a fairly non-selective process. Taken together, these findings offer clues to the development of melanoma micrometastases and provide biomarkers for more accurate and earlier detection of significant metastases as well as rational targets for therapy.

425 Poster

Loss of PTEN expression in colorectal cancer (CRC) metastases (mets) but not in primary tumors predicts lack of activity of cetuximab plus irinotecan treatment

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Introduction: PTEN is a key tumor suppressor that inactivates PI3K, a downstream effector of the EGFR cascade. Mutations resulting in PTEN loss lead to uncontrolled activation of PI3K/AKT signalling pathway that may result in resistance to EGFR-blockade. Methods: We retrospectively investigated the role of PTEN immunoreactivity loss (anti-PTEN antibody clone 17.A, Immunomarkers) both on primary CRC and related mets in predicting the activity of cetuximab plus irinotecan combination treatment in EGFR-positive irinotecan-refractory metastatic CRC patients (pts). Results: As of today 102 pts have been included. M/F=60/42, median age=62 (38-78), median number of previous lines of chemotherapy=2 (1-5). Among the 100 pts evaluable for response we observed a partial (PR) or a complete response (CR) in 13 and 1 cases respectively for an overall response rate of 14%. PTEN immunostaining resulted positive (+), negative (-) or unconclusive (NE) in respectively 48, 36, 11 out 98 primary tumors. On 57 mets PTEN analysis was +, - or NE in 31, 22, 4 cases respectively. PTEN positivity or negativity on primaries was confirmed on 45 related mts in 27 cases (60%) while 7 (16%) + and 11 (24%) - primaries resulted respectively - and + on mets. PTEN status tested on primary tumor was not significantly predictive of response nor PFS. Defining as responders those pts obtaining a PR or CR (RECIST) or SD lasting >6 mos and clearly progressed on previous irinotecan-based regimen with a TTP<3 mos (5 pts), analysis of PTEN on mets showed: 1- vs 12+ responders and 21- vs 19+ non responders (p=0.008). Median PFS in pts with PTEN+ mets was 4.8 vs 3.3 mos in PTEN- (p= 0.009, HR=0.50, 95% CI 0.23-0.81). Conclusions: Loss of PTEN immunoreactivity tested on mets may predict the activity of cetuximab plus irinotecan combination treatment. Further analysis on KRAS mutational status and p-AKT immunostaining are ongoing. Final data will be presented at the meeting. Supported by A.R.C.O. Foundation.

426 Poster

Expression of the HER4 isoform JM-a/CYT2 correlates to improved survival in bladder cancer patients lacking Estrogen receptor alpha

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The epidermal growth factor receptor HER4 consists of several isoforms, generated by alternative mRNA splicing. Two isoforms differ in the juxta-membranous domain (JM-a and JM-b) and two in the cytoplasmic domain (CYT1 and CYT2). The HER4 isoforms JM-a/CYT1 and JM-a/CYT2 can undergo intracellular cleavage and the released peptide (4ICD) acts as a transcription factor when complexed with the Estrogen receptor α (ER-α). When 4ICD is not complexed with ER-α, 4ICD can induce apoptosis in cancer cells in vitro. Previous studies indicate an improved survival in bladder cancer patients expressing high HER4 levels, but the isoform composition was not examined. In the present study we examine the expression of the individual isoforms of HER4 and the expression of ER-α in biopsies from patients with bladder cancer.

Quantitative mRNA assays specific for HER4 isoforms JM-a, JM-b, CYT1, and CYT2 as well as ER-α were established and used to analyse tumour samples from 86 bladder cancer patients. Expression of the isoforms was compared to overall survival with a median follow up time of 39.2 months.

No HER4 expression was identified in 58% (n=50) of the bladder cancer samples. HER4 positive samples (n=36) all expressed JM-a/CYT2. In addition the CYT1 isoform was co-expressed in half of these samples. As previously described the expression of HER4 (n=36) was associated to improved survival (P=0.008) and the expression correlated inversely to tumour stage, grade, and type (all P<0.05). ER-α was expressed in 37% (n=32) of the samples while 63% (n=54) were ER-α negative. A survival benefit was observed only for patients expressing HER4 JM-a/CYT2 but no ER-α (n=17, P=0.007). When HER4 JM-a/CYT2 and ER-α was co-expressed no difference in survival was observed (n=19, P=0.347).

We show that the HER4 isoform JM-a/CYT2 or a combination of JM-a/CYT1 and JM-a/CYT2 are expressed in bladder cancer biopsies. Interestingly, expression of HER4 JM-a/CYT2 is related with a favourable prognosis only in patients with no expression of ER-α.

427 Poster

API-2/Triciribine functions as chemoradio-sensitizer in human cancer by specific inhibition of constitutively active AKT including AKT1-E17K signaling

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The serine/threonine kinase Akt/PKB is frequently hyperactivated in human cancer and functions as a cardinal nodal point for transducing extracellular and intracellular oncogenic signals. In addition, mounting studies showed that activation of AKT is closely associated with chemo-, radio- and TKI (tyrosine kinase inhibitor)-resistance. Thus, AKT presents an exciting target for molecular therapeutics. We previously identified an AKT inhibitor, API-2/triciribine. Recent phase I clinical trials showed promising results of API-2/triciribine as single agent in solid tumors and advanced hematological malignancies. Here, we reported that API-2/triciribine sensitizes cancer cells to apoptosis and growth arrest induced by radiation, mTOR inhibitor and conventional chemotherapeutic agents, which include cisplatin and taxol in human ovarian and lung cancer, velcade in multiple myeloma and temazolamide in glioblastoma. In addition, API-2/triciribine overcame cisplatin-, taxol- and gefitinib-resistance in ovarian and lung cancer. We further demonstrated that API-2//triciribine inhibits constitutively activated Akt kinase activity, including myr-Akt, Akt1-E40K and naturally occurring mutation AKT1-E17K which is insensitive to allosteric Akt kinase inhibitor. AKT plasma membrane translocation induced by growth factor was also blocked by API2//triciribine. Notably, API-2//triciribine inhibits mTOR inhibitor feed back activated AKT and synergizes with RAD001 to induce cell cycle arrest and apoptosis. We also revealed a novel molecular mechanism by which RAD001 and rapamycin activate AKT pathway. These findings indicate that API-2//triciribine is a chemoradio-sensitizer and overrides chemoresistance by directly targeting the AKT pathway, and thus lay the foundation for clinical trial using API-2//triciribine combined with conventional chemotherapeutic agents, TKI and radiation to treat human malignancy.

428 Poster

Incidence and the clinical outcomes of epidermal growth factor receptor (EGFR) mutations in male smokers with squamous cell carcinoma of lung

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Background: EGFR mutations in non-small cell lung cancer (NSCLC) have been reported to be related to certain clinical characteristics (i.e., female, non-smokers with adenocarcinoma) and gefitinib responsiveness.