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.

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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.

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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.

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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