CONCLUSIONS: The present findings show that molecules as morphine are able to interfere with molecules normally unable to cross the BBB. This mechanism could be used for new approaches in therapy of refractory CNS tumors as glioblastoma.

P06 BIOMARKERS

P06.01 CORRELATING FLUORESCENCE AND CONVENTIONAL MRI WITH PROLIFERATION MARKERS IN GLOBLASTOMA

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BACKGROUND: Glioblastoma is the commonest primary brain cancer and is associated with a devastating prognosis. Median survival is just 14 months with surgery, chemoradiotherapy. Tumour heterogeneity likely underlies poor prognosis and treatment resistance. We sought to correlate imaging and biological features of tumour heterogeneity.

METHODS: We used contrast enhancement on conventional MRI and fluorescence as markers for proliferation in glioblastoma. We quantified the expression of three cell surface receptors CD133, EGFR and NGF in fluorescent positive and fluorescent negative areas using flow cytometry. We also quantified the expression of these three cell surface receptors according to their contrast enhancement status (positive or negative).

RESULTS: Eight patients with histologically confirmed glioblastoma studied. Nineteen tumour samples were assayed. We found a statistically significant correlation between fluorescence and expression of all three cell surface receptors (CD 133 p-value = 0.015 (p <0.05), EGFR p-value +0.035, NG2 p-value 0.015). We observed a statistically non-significant decrease in expression of all three cell surface receptors in contrast enhancing areas of tumour.

CONCLUSIONS: Fluorescence may be used as a guide to identify areas of high proliferation in glioblastoma. Contrast enhancement does not identify areas of high proliferation. Further work is required to confirm these findings.
Abstracts

P06.03 ANAPLASTIC OLIGODENDROGLIOMA AND OLGIOASTROCYTOMA WITHOUT 1P-1Q CO-DELETION: A MONO-INSTITUTIONAL RETROSPECTIVE STUDY
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Allelic losses of chromosomes 1p and 19q in patients with anaplastic oligodendroglioma(AOD) and oligoastrocytoma(AOA) represent a strong prognostic factor and a powerful predictor of survival. This study compares the patients benefit from the addition of chemotherapy(CT) to radiotherapy(RT) with a significant improvement of overall survival (OS) and progression-free survival (PFS). In patients without 1p19q co-deletion, there are no standard-of-care and conflicting opinions impose to review the possible therapeutic approach. A total of 214 patients with histological proven of AOD and AOA treated in our Institution over the last 10 years were evaluated. Eighty patients were eligible for loss-of-heterozygosity (LOH) evaluation. The absence of 1p19q co-deletion was confirmed in 33 patients. Clinical and genetic data, treatments and survival assessment were described and discussed. In non co-deleted group, m-OS and m-PFS were respectively 38.4 months (95% CI 21.99-59.77) (p=0.018) and 16 months (95% CI 13.0-20.0). On the other hand, m-OS of the whole group was 59.7 months. Concerning treatments, therapeutic approach has been varied with respect to percentage in RT and concomitant temozolomide in the past two years. In 6% of patients CT was considered as first-line treatment after surgery. Data confirmed the prognostic role of LOH, with the most advantages for patients with co-deletion. Regarding therapeutic approaches, results should be quite aggressive in our clinical practice. The choice between RT or CT as first line treatment after surgery do not seem to influence the prognosis.

P06.04 MUTATION ANALYSIS OF THE ISOCITRATE DEHYDROGENASE 1(IDH) GENE USING CELL FREE DNA FROM THE CEREBROSPINAL FLUID (CSF) OF GLIOMA PATIENTS
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BACKGROUND: Mutation in the IDH1 gene is a genetic defect exclusively found in glioma as a mutation in solid tumors, and has been established as a prognostic factor for WHO Grade 2-4 glioma. Therefore, detecting this mutation is clinically important. In this study, we report the results of analyzing defects in the IDH1 gene by detecting circulating cell free (ccf) DNA in the CSF derived from the tumor tissue of glioma patients.

METHODS: Lumbar puncture was performed to obtain CSF from 7 patients with glioma. ccfDNA was extracted from 1ml of CSF using the Maxwell rapid sample concentrator system. Subsequently, the presence of point mutation of the IDH1 gene at codon 132 was screened by real-time PCR/high-resolution melting curve analysis, and the mutation was confirmed on the basis of DNA sequencing results. Status in the IDH1 gene was also analyzed using the same assay technique for the DNA extracted from the excised fresh tumor tissue, to compare the results with that of ccf-derived ccfDNA. In addition, ccfDNA was also extracted from the plasma in 3 patients to additionally examine the presence of the IDH1 gene mutation.

RESULTS: Ccf-derived ccfDNA was successfully extracted from all patients and analyzed using real-time PCR/high-resolution melting curve analysis. IDH1 gene mutation was detected in 3 of the 7 glioma patients. The results of the IDH1 gene analysis of ccf-derived ccfDNA and that of the DNA extracted from the surgically excised tumor tissue were consistent in all patients. However, IDH1 gene mutations was not detected all in plasma-derived ccfDNA.

CONCLUSION: Therefore, gene analysis of ccfDNA from the CSF, not from the plasma, enabled the evaluation of IDH1 gene defect in glioma patients less invasively, without directly obtaining any tumor tissue. Moreover, this technique can be applied to analyze molecular markers other than the IDH1 gene, such as MGMT gene promoter methylation.

P06.05 MYOINOSITOL AS A PREDICTIVE BASELINE BIOMARKER FOR OVERALL SURVIVAL OF PATIENTS WITH RECURRENT GLOBLASTOMA TREATED WITH BEVACIZUMAB: A 1H-MAGNETIC RESONANCE SPECTROSCOPY STUDY
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BACKGROUND: Antiangiogenic treatment of glioblastoma with Bevacizumab (BVZ) lacks predictive markers. Myo-inositol (MI) is an organic osmolyte, with intracellular concentration changes depending on the extra-cellular osmolality. Since BVZ markedly reduces tumor edema, we asked whether the MI concentration in the tumor changes during therapy.

METHODS: We used 1H-MR spectroscopy to measure the MI concentration in the tumor and contralateral control tissue of 39 prospectively recruited patients with recurrent glioblastomas before and 8–12 weeks after starting therapy. 30 patients received BVZ and 9 patients were treated with CCNU/VM26 as control. We performed a survival analysis to evaluate MI as a predictive biomarker for BVZ therapy.

RESULTS: MI concentrations increased significantly during BVZ therapy in tumor (p<.001) and control tissue (p<.001), but not during CCNU/VM26 treatment. For the BVZ cohort, higher MI concentrations in the control tissue at baseline (p<.01) and higher MI concentrations in the tumor (delta MI, p<.011) were associated with longer survival. A Kaplan-Meier analysis showed a median OS of 164 days for patients with deltaMI < 1,817 mmol/l and 273 days for patients with a deltaMI > 1,817 mmol/l. No differences were observed for the relative changes or the post treatment concentrations.

CONCLUSION: Pre-therapeutic MI concentrations are predictive of overall survival in patients with recurrent glioblastoma treated with BVZ. Our data confirm that recurrent glioblastoma shows a strong metabolic reaction to BVZ. Further, our results support the hypothesis that MI might be a marker for early tumor cell invasion.

P06.06 IS ACTIVATING TRANSCRIPTION FACTOR 5 (ATF5) A NEW THERAPEUTIC TARGET IN ASTROCYTOMAS OF DIFFERENT WHO GRADES AND VARYING BIOLOGICAL BEHAVIOR? AN EXPRESSION ANALYSIS
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OBJECTIVE: Activating transcription factor 5 (ATF5), a widely expressed basic leucine zipper protein, suppresses differentiation of neuroprogenitor cells into glia or neurons in the normal brain (NB). It has been reported to be overexpressed in glioblastoma (GBM). A reduction of ATF5 expression or activity in GBM cells leads to p53-independent apoptotic cell death, therefore suggesting that it might serve as a novel therapeutic target. However, to our knowledge, no data on ATF5 expression in low grade astrocytomas (LGA) or recurrent GBM has been published so far. Therefore, we aimed to examine the ATF5 expression in patients’ astrocytoma samples of different WHO grades and varying biological behavior.

METHODS: ATF5 mRNA expression was measured by quantitative PCR (qPCR). The mRNA was extracted from cell lines U87, GaMG, U343, DKMG, SNB19, and U138 and from frozen samples of patients’ GBM (n=15), LGA (n=12) and NB (n=4). In addition, an independent panel of GBM with varying histological behavior, i.e. GBM with local growth and local relapse (local primary tumor (LP): n=26; local relapse (LR): n=8), GBM with local growth and multifocal relapse (primary tumor (PMR): n=12; relapse (MRM): n=3), and GBM with a multifocal primary growth (MPP) (n=9) was examined. Data were analyzed using unpaired two-sided t-tests.

RESULTS: A nearly 12 times overexpression of ATF5 mRNA was observed in LGA (p<0.004), as well as GBM (p=0.001) compared to NB. The subgroup analysis revealed a significant increase of ATF5 expression in LP (p=0.015), LR (p=0.041), PMR (p=0.018), and MP (p=0.012). However, no difference could be detected between primary tumors and local (p=0.572/multifocal relapse (p=0.577). Primary tumors LP, PMR and MP displayed a similar ATF5 expression (p=0.035). Among the analyzed GBM cell lines U343 cells showed the strongest expression.