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Abstracts

-OMICS AND PROGNOSTIC MARKERS

OM-01. NPAS3 IS A NEGATIVE PROGNOSTIC SURVIVAL MARKER IN GLIOBLASTOMAS

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BACKGROUND: Glioblastomas, the most common primary brain tumors in adults, still continue to have a predominantly fatal outcome. We previously cloned *NPAS3* (neuronal PAS 3), a gene which is among the largest genes in the human genome and encodes a transcription factor. We recently discovered NPAS3 drives the progression of malignant astrocytomas as a tumor suppressor, by modulating the cell cycle, proliferation, apoptosis, and cell migration/invasion, and has a further influence on angiogenesis. In human glioblastoma surgical specimens, up to 75% demonstrate aberrant NPAS3 protein expression. In this study, we evaluated the expression of NPAS3 in the overall survival of patients diagnosed with glioblastomas. METHODS AND RESULTS: We examined a panel of glioblastomas from 77 postoperative patients who had total resection of the tumor. Postoperative patients were treated by standard adjuvant radiation therapy (60 Gy, 6 to 7 weeks) combined with chemotherapy, with a study follow-up not exceeding 30 months. Among the glioblastomas, 28 had absent, 18 had elevated, and 31 had normal NPAS3 expression. There were 54 males and 23 females in this study, with a median age of 59 years. In this study, patients with glioblastomas who had absent NPAS3 expression were identified as having the poorest overall survival compared with patients with glioblastomas who had normal or elevated NPAS3 expression (log-rank P value <0.001). Such a trend is still maintained even when patients are stratified among different age groups (<60 and >60 years). However, no significant difference in overall survival among patients with glioblastomas who had either normal or elevated NPAS3 expression was noted (log-rank P value >0.05). Likewise, no significant difference in gender versus overall survival noted. CONCLUSION: Our findings are of clinical importance by demonstrating that NPAS3 is an informative negative prognostic survival marker in patients with glioblastomas.

OM-02. PRELIMINARY USE OF DIFFERENTIAL SCANNING CALORIMETRY OF CEREBROSPINAL FLUID, BRAIN TUMOR CELLS LINES, AND PATIENT TUMOR SAMPLES: CORRELATION WITH TUMOR HISTOLOGY AND PATIENT OUTCOME

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INTRODUCTION: Thermal stability signatures of complex molecule interaction in biological fluids can be measured using a new approach called differential scanning calorimetry (DSC). The thermal stability of plasma proteome has been described previously as a method of producing a disease-specific "signature," termed thermogram, in several neoplastic and autoimmune diseases. We describe the preliminary use of DSC performed on cerebrospinal fluid (CSF) brain tumor cell cultures and human brain tumor tissue samples to create unique thermograms for correlation with histological tumor classification. METHODS: Samples of CSF from 9 patients with confirmed glioblastoma multiforme (GBM) were evaluated using DSC, and the thermogram signatures were evaluated. These thermograms were compared with thermograms of CSF taken from patients with non-neoplastic conditions such as head trauma, hydrocephalus, or CSF leak. Further analysis was also performed on CSF from patients who had non-GBM neoplastic conditions such as carcinomatosis meningitis or central nervous system lymphoma or leukemia. With these established techniques, we then used the same technique to examine cell lines (3 GBM and 2 meningioma) and brain tumor tissue samples (10 GBM, 5 oligodendrogliomas, 5 low-grade astrocytomas, and 10 meningiomas) with confirmed histological diagnosis. RESULTS: The DSC thermograms of CSF of the patients with GBM were significantly different from the other neoplastic and non-neoplastic cases. The melting temperature of the major transition was shifted by 5°C, which makes it easily distinguishable from control cases. DSC profiles of glioma and meningioma cell lines are distinctly different. Brain tumor sample analysis correlates with the cell line work with distinct GBM, oligodendroglioma, low-grade astrocytoma, and meningioma "signatures." CONCLUSIONS: Our results are very preliminary, but it appears that the DSC of CSF has potential utility in diagnostics and monitoring disease progression in GBM patients. DSC of processed tumor samples seems to display unique "signatures" for different brain tumor histological classifications.

OM-03. USING NEUROFIBROMATOSIS-1 AS A MODEL SYSTEM TO DEFINE DISEASE HETEROGENEITY

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Advances in DNA sequencing technology and genomic profiling have positioned clinicians at the precipice of personalized medicine. However, approaches to individualizing health care by defining genotype-phenotype relationships in complex, multifactorial diseases must first be developed for heterogeneous single-gene disorders. Neurofibromatosis-1 (NF1) is a common inherited cancer predisposition syndrome caused by a germline mutation in the *NF1* tumor suppressor gene. NF1 can affect nearly every organ system in the body, with individuals developing a wide spectrum of clinical problems ranging from pigmentary abnormalities (e.g., café-au-lait macules) to central and peripheral nervous system tumors. To develop the necessary research infrastructure to address phenotypic heterogeneity in NF1, we have developed a clinically-annotated DNA biorepository. To date, we have collected blood samples and detailed clinical data from 56 individuals. Among significant clinical features observed in our cohort, 46% ($n = 26$) have food or environmental allergies, 39% ($n = 22$) have learning disabilities, 27% ($n = 15$) have plexiform neurofibromas, and 25% ($n = 14$) have brain tumors. Future sequencing and genomic analyses will involve collaborations with The Genome Institute at Washington University. By successfully establishing a DNA bank, clinical database, and multidisciplinary collaboration, we have used NF1 as a platform for future hypothesis generation as well as to evaluate specific genotype-phenotype relationships. The system we have developed may result in the stratification of individuals with NF1 into clinically and molecularly relevant subgroups, which will allow for predictive risk assessment and could potentially identify patients with high probabilities of responding to individualized therapies. If successful for a clinically heterogeneous single-gene disorder like NF1, this approach could be applied to other disorders in which clinical heterogeneity represents an obstacle to individualized patient management and therapy.

OM-04. MULTIDIMENSIONAL INTEGRATED MOLECULAR PROFILING OF GRADE II & III DIFFUSE ASTROCYTOMAS REVEAL DISTINCT SUBCLASSES CORRELATED WITH CLINICAL OUTCOME

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Diffuse astrocytomas exhibit a remarkable range of histopathological and biological behaviors. Whereas patients with glioblastoma (WHO grade IV) have uniformly dismal prognoses, survival of patients with low- and intermediate-grade astrocytomas (WHO grade II and III) is extremely variable. In this study, we performed a multidimensional, integrated molecular analysis on a large sample set ($n = 134$) of formalin-fixed paraffin-embedded WHO grade II-III diffuse astrocytomas in order to stratify patients into biologically and clinically relevant subtypes. Alterations of IDH 1/2, p53, PTEN, EGFR, and PDGFR genes were examined using Sequenom and focused sequencing analyses. Immunohistochemistry was employed for oncogenic pathway analysis. Additionally, using the Illumina DASL assay, whole-genome transcriptional profiling was performed on a subset of tumors ($n = 76$). Our results not only confirm that patients with IDH 1/2 mutations live significantly longer than patients without, but that IDH mutation status and survival are also significantly correlated with total PDGFR positivity by IHC and p53 mutation, while anticorrelated with the level of EGFR activation. Global transcriptional profiling revealed distinct patterns of gene expression (designated clusters A, B, and C) that are strongly associated with differential histological, genomic, proteomic, as well as clinical measures. Cluster A ($n = 14$) consisted mostly of grade II, IDH 1/2 mutants, p53 wild-type, PDGFR positive, p-EGFR negative tumors, with the most favorable relative prognoses. Cluster B ($n = 42$) was comprised of both grade II & III, largely IDH 1/2 mutants, p53 mutant, PDGFR positive, and p-EGFR negative tumors, with intermediate prognoses. Cluster C

(n = 20) was made up of mostly grade III, IDH 1/2 wild-type, p53 wild-type, PDGFR negative, p-EGFR positive tumors, with the worst relative prognoses. Our results demonstrate that transcriptional signatures with intrinsic prognostic value not only exist among low- and intermediate-grade diffuse astrocytomas, but also likely represent important differences in tumor biology and potential therapeutic response.

OM-05. A CPG ISLAND HYPERMETHYLATED PHENOTYPE (CIMP) IN ANAPLASTIC OLIGODENDROGLIAL BRAIN TUMORS IS A BETTER PREDICTOR OF SURVIVAL THAN MGMT METHYLATION. A REPORT OF EORTC STUDY 26951

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BACKGROUND. Surprisingly, in the randomized EORTC 26951 on PCV chemotherapy in anaplastic oligodendroglial tumors (AOT), MGMT promoter methylation was correlated to a superior progression-free survival (PFS) after radiotherapy (RT) alone. Because there is no biological explanation for this, we hypothesized MGMT promoter methylation is a marker of genome-wide methylation. MATERIAL AND METHODS. Methylation profiling (Illumina Human Methylation 27 bead chip) was performed on snap frozen tumor samples of 68 patients with oligodendroglial brain tumors and one normal brain. Fifty of these patients were treated as part of the EORTC 26951 study. The remaining patients received highly similar treatment paradigms. Hierarchical ordered partitioning and collapsing hybrid (Hopach) clustering was performed using the 2000 most variably methylated CpG loci. The forward stepwise analysis was performed to select for variables with independent prognostic value. RESULTS. Two main clusters were identified that were highly stable. These clusters could be separated by extensively methylated (CIMP) versus unmethylated CpG loci. Overall survival (OS) in the CIMP subgroup was markedly better than OS in the unmethylated subgroup (5. 62 vs. 1.24 years, $P < 0.0001$). CIMP status was strongly correlated to IDH1 mutational status and 1p19q LOH (both $P < 0.001$) and MGMT promoter methylation (for all $P < 0.001$). In a prognostic model that included all biological factors (IDH1 mutation, 1p/19q LOH, MGMT methylation and CIMP) and histology, CIMP status was the only factor selected ($P < 0.0001$).We validated our results on methylation profiling data from the cancer genome atlas (TCGA) by assigning TCGA samples to one of the molecular clusters identified in our data set. CONCLUSION. Genomic wide methylation profiling identifies 2 main subgroups of oligodendroglial brain tumors. CIMP is a strong prognostic factor. The correlation between CIMP and MGMT promoter methylation in grade III AOT suggests that MGMT promoter methylation is "only" an epiphenomenon of genomic wide methylation.

OM-06. MIR-181D: A PREDICTIVE GLIOBLASTOMA BIOMARKER THAT DOWNREGULATES MGMT EXPRESSION

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A major advance in glioblastoma therapy entailed the discovery that the DNA alkylating agent, temozolomide (TMZ), which confers survival benefit when combined with radiation therapy. Despite data suggesting that approximately 10% of the patients undergoing this therapy achieve 5-year survival, all glioblastoma patients undergo the therapy because it is currently impossible to predict which patients will experience a beneficial therapeutic response. Thus, a major challenge in neuro-oncology involves developing biomarkers that would facilitate such prediction. To identify such biomarkers, we conducted genome-wide microRNA (miRNA) profiling of 82 glioblastomas. We found that miR-181d inversely associated with patient survival after correcting for age, Karnofsky performance status (KPS), temozolomide (TMZ) treatment, and extent of resection. This association was observed using 3 distinct biostatistical methods of analysis. Furthermore, the association was validated using The Cancer Genome Atlas (TCGA) data set (n = 424) as well as another independent patient cohort (n = 35). The association was most evident in patients who underwent TMZ treatment. Bioinformatic analysis of potential genes regulated by miR-181d revealed methyl-guanine-methyl-transferase (MGMT) as a downstream target. Indeed, transfection of miR-181d downregulated MGMT mRNA and protein expression. Furthermore, luciferase reporter assays and coprecipitation studies revealed direct interactions between miR-181d and MGMT 3'UTR. Finally, MGMT expression inversely correlated with miR-181d expression in 2 independent glioblastoma collections. In the context of the known prognostic and predictive value of MGMT in glioblastoma patients, our results suggest that the observed correlations between miR-181d and glioblastoma patient survival are, at least in part, related to posttranscriptional regulation of MGMT. We propose miR-181d as a novel predictive biomarker for TMZ therapy.

OM-07. THE MGMT-DEPENDENT "METHYLOME" IN GLIOBLASTOMA AND ANAPLASTIC GLIOMA

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Results from the NOA-04 clinical trial on anaplastic gliomas revealed that silencing of the *MGMT* gene by promoter methylation was associated with prolonged progression-free survival in the chemotherapy but also in the radiotherapy arm. This contributes to the perception that loss of the MGMT protein does not solely explain the differential responsiveness to treatment, but that it is a surrogate marker for a set of epigenetically regulated genes (methylome) of biological and clinical relevance. We performed a genome-wide screen using an Infinium II Illumina Methylation Assay and identified a set of 39 genes that displayed a highly significant differential methylation signature depending on the *MGMT* promoter methylation status in 8 tissue specimens (4 *MGMT* promoter methylated; 4 *MGMT* unmethylated) derived from a prospective phase II glioblastoma trial (UKT-05; Weiler *et al.* 2008). Out of those, we validated 16 differentially methylated genes using MassARRAY in 34 tissue specimens from the UKT-05 trial. Statistical analysis revealed single CpG sites that showed significant differences in the methylation status between the 2 groups. Having ranked these single CpG sites according to the average methylation of all CpG sites of the amplicon, further experiments in independent datasets will focus on 6 of these genes. Furthermore, we analyzed the *MGMT*-dependent methylation patterns in anaplastic glioma specimens derived from the NOA-04 trial. To strengthen genome-wide DNA methylation assessment, we used 2 screening techniques, methyl-CpG immunoprecipitation ($n = 4$) and the Infinium II Illumina Methylation Assay ($n = 12$). Identification of differentially methylated regions was performed combining both approaches and revealed 47 highly significant candidate genes. These will also be validated by MassARRAY in the data set of the NOA-04 trial (Wick *et al.* 2009). Our work reveals novel candidate genes associated with the *MGMT* promoter methylation status that are of potential prognostic or predictive value for glioblastoma and anaplastic glioma.

OM-08. FINE MAPPING OF THE GERMLINE 8Q24 REGION ASSOCIATED WITH THE DEVELOPMENT OF OLIGODENDROGLIOMA

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Although germline polymorphisms mapped to chromosome region 8q24 near the CCDC26 gene have been associated with oligodendrogliomas and mixed oligoastrocytomas (MOA) with combined 1p/19q deletion (Jenkins et al., Cancer Genetics 204:13;2011), the causative germline alterations have not yet been elucidated. We have undertaken a combined custom single-nucleotide polymorphism (SNP) array, sequencing, and genotype imputation approach to identify candidate causative germline alterations. We first evaluated 89 SNPs within or near the CCDC26 gene in 390 oligodendroglioma and MOA cases and in 724 controls from the Mayo Clinic and UCSF Adult Glioma Study using a custom SNP array. With these data, we defined regions near the CCDC26 gene that are associated with these glioma subtypes. We then performed long-range PCR/pooled next-generation sequencing of this

region (in 4 pools) using 406 oligodendroglioma/MOA cases and 818 controls. With the custom SNP data, we also imputed genotypes using the 1000 Genomes data set. Using these approaches, we identified over 2100 variants with control or case allele frequencies greater than 1%. Over 400 candidate variants had nominally significant different ($p<0.05$) prevalences between cases and controls in one or more of the analyses. Twenty-eight variants have not been reported in publicly available data sets. Several variants have effect sizes >3.0 for oligodendroglioma/MOA in more than one analysis approach. We are currently reassessing the prevalence of the rare and novel variants in our cases and controls using a second custom SNP array. It is likely that several of the variants we have identified will be significantly associated with oligodendroglioma/MOA development and with further validation may become predictive markers. Functional analyses will be necessary to understand the biology that specifically links them to glioma development.

OM-09. CANCER PATHWAY DISCOVERY IN A MOUSE MODEL OF MEDULLOBLASTOMA

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OBJECTIVE: Aberrant Hedgehog (Hh) target-gene activation causes a significant percentage of medulloblastomas (MB), the most common pediatric brain tumor. In our ptch +/−;Math1-GFP mouse model of Hh-dependent MB, 15% of mice develop MB; however, >90% develop pretumor lesions that do not progress to MB. This is a unique pretumor model that can be used to identify additional genetic changes contributing to tumorigenesis. MATERIALS AND METHODS: DNA was prepared from MB and normal surrounding cerebellar tissue from ptch +/−;Math1-GFP mice and was used for whole-genome sequencing. The data were analyzed, aligning the mate-pair reads to the reference mouse genome (NCBI Build 37, mm9). Chromosomal structural variants (insertions, deletions, duplications, inversions, and translocations) were detected in each sample separately. Variants that appeared in the tumor samples but not in the corresponding normal samples were selected, as tumor-specific variants are likely to be relevant to MB development. These data are being compared with human and mouse MB gene expression data to derive new hypotheses about genes contributing to the development of MB. RESULTS: We identified 15 distinct genetic mutations that occur in MBs but not in normal surrounding cerebella. CONCLUSION: The identification of combinations of genetic lesions that can trigger or sustain MB will guide hypotheses about tumor development, paving the way for new and more effective medical therapies. We will proceed to test these hypotheses in our mouse model, ptch +/−; Math1-GFP, which most accurately represents the human pediatric disease.

OM-10. USING PRECISION PROTEOMICS TO ANALYZE OF HISTONE POSTTRANSLATIONAL MODIFICATIONS IN GLIOMAS

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Malignant gliomas represent the most common and lethal brain tumors involving the cerebral hemispheres of adults. Previous research clearly demonstrates that chromosomal aberrations and epigenetics can drive gliomagenesis. Our research seeks to identify histone modifications via mass spectrometry that impact chromatin regulation in gliomas. To this primary endpoint, frozen tissue samples and cell lines derived from tissue samples were utilized for histone isolation followed by mass spectrometry. Following analysis of histone H3, a source of site-specific, posttranslational modifications known to directly impact gene regulation, we profiled the differences in the methylation and acetylation states of K4 (regulator of transcriptional activation), K9, K14, K27 (regulator of transcriptional repression), K36, and K79. Preliminary data includes analysis of histones from HeLa S3 and other primary glioma cell lines. Currently, technical triplicates of each sample have a coefficient of variation (CV) of 2-20%. The CV is currently dependent on the abundance of the measured mark, i.e. trimethylation of K4 and K79 have low abundance and thus higher CVs. The above approach may define the variation in methylation and acetylation that occurs in high-grade gliomas. Our mass spectrometric-based approach allows for rapid analysis of relevant histone modifications of cells and tissue samples and may ultimately lead to precision proteomics being involved in making therapeutic decisions.

OM-11. ^{18}F-FLT-PET UPTAKE DYNAMICS AND OVERALL SURVIVAL PREDICTIONS FOR PATIENTS WITH RECURRENT HIGH-GRADE GLIOMA ON BEVACIZUMAB THERAPY

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OBJECTIVE: Recent successes of anti-angiogenic drugs in the treatment of malignant glioma patients has led to a widespread incorporation of bevacizumab in the treatment modalities of these devastating tumors. Although not effective in all patients, it has been shown to significantly prolong life in responding patients. In an effort to stratify responders versus nonresponders, we performed a series of ^{18}F-FLT-PET scans in 30 patients on bevacizumab treatment. This study aims at correlating ^{18}F-FLT uptake with overall patient survival. METHODS: Thirty patients with recurrent high-grade glioma underwent ^{18}F-FLT-PET scans prior to the beginning of bevacizumab treatment and at 2 and 6 weeks. ^{18}F-FLT responses at different time points were compared with MRI response and correlated with progression-free survival and overall survival using Kaplan-Meier analysis. RESULTS: ^{18}F-FLT-PET uptake changes were more predictive for overall survival ($P = 0.0001$) as well as progression-free survival ($P = 0.0006$) than was the MRI study. Sixteen responders and 14 nonresponders were identified with the median overall survival of 12.5 months for responders versus 3.8 months for nonresponders. An initial drop of mean ^{18}F-FLT uptake was observed for all patients at 2 weeks. At 6 weeks, those with more than 12-month survival maintained the mean SUV decrease of 47% from baseline, whereas the group with less than 12-month survival had a mean SUV rebound to the baseline value. CONCLUSION: ^{18}F-FLT uptake changes during bevacizumab treatment of recurrent high-grade gliomas are predictive of patient survival. The observed effects of bevacizumab treatment on ^{18}F-FLT SUV, the initial decrease and subsequent divergent behavior of uptake values, may be indicative of the complexity of bevacizumab 's antineoplastic effects.

OM-12. ABSOLUTE QUANTITATIVE PROTEOMICS-BASED TAILORED MOLECULAR TARGETED THERAPY FOR GLIOBLASTOMA

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INTRODUCTION: Tailored chemotherapy based on gene diagnosis is ideal for human malignant tumors. Molecular-targeted drugs that act on growth-factor receptors are being tested for human glioblastomas (GBMs). However, tailored molecular-targeted therapy is not established for GBM. Protein expression levels of target molecules in the tumor may be a suitable indicator for the efficacy of molecular-targeted drugs. The purpose of this study is to clarify the absolute protein expression levels of membrane proteins that are the targets of molecular-targeted drugs in GBM. MATERIALS AND METHODS: We analyzed tumor samples resected during surgery with absolute quantitative target proteomics. The mass spectrometer was set up to run a multichannel reaction monitoring for 13 target membrane proteins for which molecular-targeted drugs are available. Our absolute quantitative proteomic analysis is able to quantify the protein expression levels of targets with high accuracy (coefficient of variation, <20%). RESULTS: Absolute protein levels allowed us to compare the expression levels among different target molecules and among different patients. The data showed that GBM ($n = 27$) could be classified into 4 groups based on the dominant expression of target molecules: 1) EGFR, 2) ERBB2, 3) PDGFRalpha, and 4) others. The patients with high expression of VEGFR ($n = 5$) were involved in EGFR group. Bevacizumab, which binds to and inhibits VEGFA, was administered to the patient with high expression of VEGFR and showed a remarkable effect. CONCLUSIONS: This result suggests that absolute quantitative proteomics is useful for identifying target molecules. Quantitative proteomics-based tailored molecular-targeted therapy might be ideal for the treatment of GBM patients.

OM-13. ELUCIDATION OF RESISTANCE TO TEMOZOLOMIDE IN GLIOBLASTOMA MULTIFORME

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Combined therapy of surgery, radiotherapy, and temozolomide (TMZ) is the current standard-of-care for patients with glioblastoma multiforme

(GBM). Despite this multimodality treatment, prognosis for these patients is still dismal. While adding TMZ to radiotherapy resulted in an overall increase in the life expectancy of GBM patients, in some patients the tumors do not seem to be sensitive to TMZ (intrinsic resistance). However, a substantial percentage of patients initially show a favorable response to TMZ but later present with recurrent tumor growth that has developed resistance to TMZ. In this study, we investigated the mechanisms involved in acquired resistance to TMZ in GBMs to identify a gene expression/genome profile predictive for TMZ resistance. We created 6 TMZ resistant clones of 3 different human GBM cell lines. We used genomic tools (gene-expression analysis, CGH analysis) to identify factors involved in TMZ resistance within these resistant GBM subclones. Data analysis revealed a subset of transcripts consistently overexpressed in the resistant subclones versus the parental cell lines. The overexpression of a number of these transcripts was confirmed by qRT-PCR. Subsequently, these transcripts were validated by in silico gene-expression data analysis in publically available data sets of GBM samples and in available primary GBM samples. The genes overexpressed in all 6 TMZ resistant subclones were implemented in a prediction model to assess the ability of these genes to predict outcome in a cohort of GBM patients. This prediction model has the capacity to classify patients into outcome groups in which the patients with overexpression of these genes have a shorter overall survival compared with the patients with moderate expression of these genes. Here, we show that a specific subset of deregulated genes could be used to predict TMZ resistance in GBMs and this could potentially lead to patient stratification for therapy selection.

OM-14. GENOME-WIDE ANALYSIS OF CLINICALLY SIGNIFICANT CPG METHYLATION SITES IN GLIOBLASTOMA MULTIFORME

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INTRODUCTION: Recent studies, such as the description of the glioma CpG island methylator phenotype, highlight the role of epigenetic modification in gliomagenesis. Prior GBM methylome studies have been limited in the breadth and depth of their sets (<70 glioma, <10 controls, <2000 CpG sites). This restricted our analysis to genome-wide descriptions and supervised clustering of limited methylation sites. We used our set of control brains, the largest high-resolution set described in literature, to supplement the samples from The Cancer Genome Atlas (TCGA: 275 GBM, 1 control). We used unsupervised clustering to define subtypes of GBM based on methylation at individual CpG sites and to investigate associations between these subtypes and overall survival. METHODS: We used CpG methylation microarrays (25,014 CpG sites) of 275 TCGA GBM specimens and of our group's 66 age-range matched perimortem brain specimens from patients with no history of neurological disorders. We selected 1% of the assayed CpG sites based on highest variance in methylation. Unsupervised clustering was performed across the CpG sites and across the specimens. While controlling for MGMT methylation status, overall survival of each subtype was compared using Kaplan-Meier survival curves. The biological relevance of these sites was assessed using gene ontology term-enrichment analysis. RESULTS: Unsupervised clustering, based on the 249 highest variation methylation sites, successfully distinguished control from GBM specimens. Furthermore, it differentiated the GBM specimens into 4 subtypes. Of these subtypes, 2 have longer survival and 2 have similarly shorter survival. In gene ontology analysis, the GBM hypermethylated genes were pro-apoptotic and cell differentiating, while GBM hypomethylated genes were pro-cell proliferation and inflammation modulating. CONCLUSION: In this study, using unsupervised clustering on the largest set of GBM and control specimens and a high resolution CpG methylation array, we identified 249 CpG sites that defined 4 subtypes of GBM that have distinct prognoses.

OM-15. DICKKOPF-3 (DKK-3) PROTEIN IN CEREBROSPINAL FLUID (CSF): A POTENTIAL BIOMARKER FOR NEOPLASTIC MENINGITIS

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BACKGROUND. New markers in CSF are needed to improve the diagnostic accuracy for neoplastic meningitis (NM). Here, we determined the value of Dkk-3-glycoprotein in CSF and serum as a potential biomarker for NM. METHODS. Dkk-3 concentrations were measured by ELISA in matched samples of CSF and serum from 156 patients (pts): meningeosis carcinomatosa (MC, n = 33), meningeosis leukemica/lymphomatosa (ML, n = 13), pts with cancer with or without brain metastases but in the absence of concomitant NM (non-NM, n = 25), bacterial (BM, n = 25) and viral (VM, n = 20) meningitis, multiple sclerosis (MS, n = 10), and pts with tension-type headache (HA, n = 30). RESULTS. There were significant differences of CSF-Dkk-3 levels (mean ± SEM) between the following groups ($p = 0.0001$, Kruskal-Wallis): MC 883 ng/mL ± 406, ML 1499 ng/mL ± 1579, non-NM 650 ng/mL ± 278, BM 683 ng/mL ± 290, VM 901 ng/mL ± 370, MS 587 ng/mL ± 226, and HA 579 ng/mL ± 320. In all groups significantly higher amounts of CSF-Dkk-3 compared with serum were found (HA mean 40 ng/mL ± 22, 14.6 times higher). CSF-Dkk-3 levels were higher in ML than in MC pts ($p = 0.041$, t-test). CSF-Dkk3 significantly distinguishes MC/ML from non-NM ($p = 0.017$/$p = 0.012$), BM ($p = 0.041$/$p = 0.016$), MS ($p = 0.034$/$p = 0.036$), and HA ($p = 0.001$/$p = 0.003$) pts. VM pts also showed high CSF-Dkk-3 levels, but levels were not discriminative between NM or VM. In VM pts serum-Dkk-3 levels were significantly higher compared with all other groups (VM 53 ng/mL ± 18 vs. HA 40 ng/mL ± 22, $p = 0.001$). During intrathecal treatment, CSF-Dkk-3 levels correlated to treatment response. CONCLUSIONS. In pts with NM and VM, significantly higher amounts of Dkk-3 are released into the CSF. Therefore, Dkk-3 does not represent a specific biomarker for NM. After exclusion of VM, however, Dkk-3 may be useful for both the diagnosis and treatment response evaluation of NM.

OM-16. GENE EXPRESSION PROFILING OF GLIOBLASTOMAS IS MORE ACCURATE THAN RECURSIVE PARTITIONING ANALYSIS AND MGMT METHYLATION FOR PREDICTING TEMOZOLOMIDE RESPONSIVENESS

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Glioblastoma (GBM) is an aggressive cancer with median overall survival of 15 months following today's standard-of-care treatment of tumor resection followed by radiation therapy with concomitant and maintenance temozolomide (TMZ). GBM patients benefit from a combination of radiation therapy and chemotherapy compared with radiation therapy alone, but not all patients respond favorably to TMZ. Within the TMZ-treated population, 2 groups emerge: short-term (< 2 years survival) and long-term survivors. There are several predictors of TMZ response, including recursive partitioning analysis (RPA), MGMT methylation, and a clinically available gene expression profile (GEP) test. While RPA class assignment takes into account several clinical characteristics of the patient, MGMT methylation analysis was developed under the directed hypothesis that silencing this DNA-repair gene enhances the activity of the alkylating agent TMZ, and GEP techniques have identified tumor cell genetic differences that distinguish short-term from long-term survivors. The power to predict survival has been compared between RPA and MGMT and between MGMT and GEP, but it has not been compared between RPA and GEP. We report a cross-study analysis evaluating TMZ responder data from the EORTC (EORTC 26981/22981-NCIC CE3), a multicenter TMZ registration study, and two resection sequential validation studies performed at The University of Texas MD Anderson Cancer Center. The results of the cross-study indicate that median overall survival is consistent between RPA and GEP (15 and 16 months, respectively), but that TMZ treatment response for long-term survivors was significantly higher when stratifying patients according to GEP class (differential of 74 months) compared with RPA class (differential of 11 months) or MGMT methylation status (differential of 23 months). The results reflect greater accuracy of GEP over RPA and MGMT methylation for predicting TMZ treatment response and suggest that GEP testing can provide patients with the best information regarding disease status and options for personalized care.

OM-17. ABERRANT EXPRESSION OF LOW-MOLECULAR-WEIGHT PROTEINS ASSOCIATED WITH RESPONSE TO CONVENTIONAL CONCURRENT CHEMORADIOTHERAPY TREATMENT

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Glioblastoma (GBM) is highly refractory to treatment. The discovery of biomarkers with utility to identify patients who are most likely to benefit from chemotherapy is critical. Aberrant protein expression in the surrounding tumor microenvironment is thought to play a significant role in mediating this treatment resistance. We used SELDI-TOF-MS to identify new

low-molecular-weight proteins associated with poor response to concurrent chemoradiotherapy. Patients with newly diagnosed GBM were recruited from Royal North Shore Hospital (Sydney, Australia). For uniformity, only patients who received conventional concurrent chemoradiotherapy were included in this study. To categorize patients into responders and non-responders to treatment, overall survival (OS) ($\leq$ 2 years) and 6-month progression-free survival (PFS6) were used. PFS6 was necessary because all patients received variable salvage therapies diluting the use of OS as a valid endpoint. MGMT promoter methylation and protein expression were also measured in all patients. Proteins extracted from 42 fresh-frozen GBM tumor samples were analyzed on four different chip surfaces: H50 (hydrophobic), IMAC (metal-affinity), Q10 (anion-exchange), and CM10 (cation-exchange). Data were available for 27 patients: PFS $<$ 6 months ($n = 12$) and PFS $\geq$ 6 months ($n = 15$). Pooling the data from all chip surfaces, 53 significantly differentially expressed proteins were detected between the two PFS groups. These proteins were evaluated individually and in combination using binary logistic regression (BLR) analysis. Combined analysis of three upregulated proteins in PFS $<$ 6 months group showed a stronger area under the curve (AUC = 1.000) when compared with their individual AUCs. Joint BLR analysis of six significantly upregulated proteins in PFS $\geq$ 6 months group demonstrated a powerful predictability (AUC = 1.000). Identification and validation of these peptides will provide a new tool for predicting treatment response to concurrent chemoradiotherapy as well as provide new targets for treatment development and offer insight into understanding the role of tumor and/or tumor microenvironment proteins associated with this treatment response.

OM-18. DETECTION OF 1P/19Q DELETION IN GLIOMAS BY REAL-TIME COMPARATIVE QUANTITATIVE PCR

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Chromosome 1p/19q deletion status is considered a prognostic and a predictive marker to radiation therapy or chemotherapy for patients with oligodendroglial tumors (OT). Detection of 1p/19q deletion had been carried out mainly by loss of heterozygosity (LOH) and florescence in situ hybridization (FISH). LOH assay requires a paired DNA sample from patient's blood, while FISH-detection of 1p/19q for OT is relatively recent and costly. Considering low incidence rate of OT (either WHO grade II or III together comprise about 10% of gliomas) and relatively longer overall survival, it is vital in a prognosis study to include all the preserved OT samples and the associated overall survival information. Thus, we developed real-time comparative quantitative PCR (CQ-PCR) to detect copy number variations on single-copy gene loci in tumor DNA samples. We applied it to extract 1p/19q deletion information from 44 OT and compared with available FISH data. Forty-four OT and 9 paired-blood DNA samples were subjected to CQ-PCR quantification of three marker genes (*CAMTA1*, *E2F2*, and *NOTCH*) located in 1p, one marker gene (*PLAUR*) in 19q, and three reference genes (*ERC2*, *SPAG16*, and *SPOCK1*) located in chromosomes 3p, 2q, and 5q, respectively. The mean value of marker and reference ratio was taken for determination of deletion ($<$0.8) or amplification ($>$1.2), with consideration of 20% variation inherited with real-time PCR. The result showed 1p/19 deletion in 5 of 9 OT DNA, but none in the paired blood DNA samples. Comparing the 1p/19 deletion status detected by FISH and CQ-PCR, the concordance rate was 67%. Consistent with earlier reports, our study showed common existences of 1p/19q deletions in OT (61% OT had 1p/19q deletion by CQ PCR), suggesting CQ-PCR could be used to obtain 1p/19q deletion status based on tumor DNA samples. CQ-PCR is quantitative, which avoids pitfalls associated with FISH.

OM-19. LACK OF CONCORDANCE BETWEEN IDH1 MUTATION AND 1P19Q DELETION IN GLIOMAS

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Both 1p/19q (1p and/or 19q) chromosomal deletions and *IDH1* mutation are considered independent prognostic markers for overall survival of patients with low-grade gliomas, with 1p/19q deletion predicting positive response to either radiation therapy or chemotherapy. Recognizing the strong association between *IDH* mutations and 1p/19q deletion in some earlier reports, we specifically explored their potential concordance in a large sample of primary human gliomas. Using real-time comparative quantitative PCR (CQ-PCR), we analyzed DNA samples from 47 grade II or grade III oligodendroglial tumor (OT) and 26 glioblastoma multiforme (GBM) for copy number variations of three genes (*CAMTA1*, *E2F2*, and *NOTCH*) in 1p and one gene (*PLAUR*) in 19q13, relative to 2-3 internal reference genes (*ERC2*, *SPAG16*, and *SPOCK1*). We designed a pair of PCR primers to amplify a 330-bp DNA fragment corresponding to the entire exon 4 and 5' of intron 4 of *IDH1* and used direct sequencing of PCR-amplified DNA fragments to identify the reported *IDH1* mutation R132H. No *IDH1* mutation was identified in any of the 26 GBM samples analyzed. The *IDH1* mutation R132H was found in 12 of 47 (26%) of OT. Our CQ-PCR detected neither 1p19q codeletion nor 19q deletion at the selected loci in GBM, but 57% of the GBM tested had deletions in 1p. In OT, 27 of 44 (61%) had 1p/19q deletions, with codeletions seen in 17 of 27 (63%). About 48% of the OT exhibited 1p/19q deletion without *IDH1* mutation; only 14% exhibited simultaneous *IDH1* mutation along with either 1p/19 q deletion, while 11% had *IDH1* mutation without 1p/19q deletion, and 27% had neither. Our study confirms the lack of *IDH1* and 1p19q codeletion in GBM as well as the common presence of *IDH1* mutation and/or 1p/19q deletions in OT. However, the presence of *IDH1* mutation in OT appears to be independent of 1p/19q deletion status.

OM-20. OVEREXPRESSION OF THE STEM CELL SPECIFIC MIRNA CLUSTER 302-367 DISTINGUISHES GLIOBLASTOMA PATIENTS WHO ARE SHORT-TERM SURVIVORS FROM THOSE WITH LONG-TERM SURVIVAL

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BACKGROUND: Our previously published results demonstrated that the stem cell (SC)-specific miRNA cluster 302-367, comprising miR-302a-d and mir-367, is overexpressed in both mouse and human gliomas. We also showed that these miRNAs have the highest expression level in embryonic SCs, while their expression diminishes gradually during neural differentiation. An induced overexpression of miRNA 302-367 was reported to increase the efficiency of pluripotent SC generation (reprogramming) in fibroblasts. Therefore, we assumed that this miRNA cluster contributes to stemness and invasiveness of gliomas and thus may be associated with tumor progression. AIM: Our purpose was to evaluate whether the expression of miRNA cluster 302-367 differentiates between GBM patients who are either short-term survivors (STS) or long-term survivors (LTS). METHODS: The expression of miRNAs from cluster 302-367 was assessed by real-time RT PCR on fresh-frozen GBM tissues obtained from either STS (4-9 months, $N = 13$) or LTS (26-34 months, $n = 15$). A commercial mix of normal brain and human neural progenitor cells (NPCs) served as reference. RESULTS: All miRNAs from this cluster were overexpressed in NPCs when compared to normal brain with a maximum expression ratio of 495.8 in mir-302d. As predicted, all these miRNAs were also overexpressed in GBM samples. However, when the expression level in tumors of STS was compared to LTS, a ratio ranging from 1.3 in mir-302d to 11.9 in mir-302c was found. The differential expression between STS and LTS of mir-302c and 367 was statistically significant ($P < 0.05$). DISCUSSION: Our results suggest that the higher expression level of miRNA cluster 302-367 in STS of GBM contributes to stemness and invasiveness of these tumor cells. It also may imply that worse prognosis in GBM is associated with less differentiated cells of origin. Therefore, this cluster, and particularly mir-302c and 367, holds potential value as prognostic markers and future therapeutic targets.

OM-21. C/EBP-B AND C/EBP-DELTA, MASTER REGULATORS OF THE MESENCHYMAL TRANSITION IN GLIOBLASTOMA, ARE HIGHLY CORRELATED WITH EXTENT OF NECROSIS AND STRONGLY EXPRESSED BY HYPOXIC PSEUDOPALISADING CELLS

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We analyzed gene expression correlates of angiogenesis and necrosis in glioblastoma (GBM) using The Cancer Genome Atlas (TCGA) data and extended findings using IHC on institutional GBMs. Digital images of frozen sections from 88 TCGA GBMs were marked to quantitate regions of necrosis and angiogenesis as a percentage of total tissue using a human-computer interface. Associated gene expression from the HT-HGU133A platform was analyzed using significance analysis of microarrays (SAM) and cox proportional hazards modeling to identify mRNAs associated with necrosis and/or angiogenesis. The mesenchymal subtype of GBM was enriched with high-necrosis samples (29% with $>$ 25% necrosis) and had a higher mean percentage of necrosis ($p = 0.035$) than the other 3 subtypes (3% with $>$ 25% necrosis). Extent of angiogenesis was not significantly associated with GBM subtype, yet all extreme outliers (angiogenesis $>$

8%) were mesenchymal. SAM analysis identified 2184 genes correlated with the extent of necrosis. Among the most strongly correlated were transcription factors identified as master regulators of the mesenchymal subtype, including C/EBP-beta, C/EBP-delta, STAT3, bHLH-B2, FOSL2, and RUNX1 (Carro MS, et al. Nature 263: 318-25, 2010). None of the 188 genes correlated with angiogenesis by SAM were mesenchymal master regulators. Ingenuity pathway analysis (IPA) of genes correlated with necrosis identified the following enriched canonical pathways: HIF-1alpha, NFkappaB, IL-6, FGF, ERK/MAPK, protein kinase A, thrombin, and HGF signaling. IPA analysis of genes correlated with angiogenesis identified HIF-1alpha signaling. IHC of 10 human GBMs selected to include normal brain, infiltrating tumor, and high-grade regions with necrosis revealed that C/EBP-beta and C/EBP-delta were strongly expressed by hypoxic perinecrotic "pseudopalisading" cells and weakly by other tumor cells. STAT3 and pSTAT3 were strongly and uniformly expressed in neoplastic nuclei across the specimen and also in macrophages and vascular endothelial cells. Thus, the GBM microenvironment influences the expression of transcription factors associated with the mesenchymal transition.

OM-22. HIGH-THROUGHPUT LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY MEASUREMENTS OF BIOACTIVE LIPIDS INTEGRATED WITH GENE EXPRESSION DATA IN GLIOBLASTOMA

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BACKGROUND: Sphingolipids were originally thought to be limited to maintaining the cellular bilayer membrane in a mechanical and chemical manner. There has been growing evidence that these metabolites, specifically sphingosine-1-phosphate (S1P), are bioactive signaling molecules involved in cell survival, motility, angiogenesis, and lymphocyte trafficking. Higher expression of the S1P forming enzyme sphingosine kinase 1 (SphK1) has been shown previously to correlate with a poorer prognosis in glioblastoma. Increases in SphK1 have also been associated with increased progression in grade II and III astrocytomas. Studies of sphingolipid metabolism have historically focused solely on the enzymes and receptors in the pathway, with very little focus on the actual lipid metabolites. METHODS: Using liquid chromatography-mass spectrometry (LC-MS), we employed single-reaction monitoring (SRM) to scan for sphingolipids and evaluated the correlation of levels in commercial glioma cell lines ($n = 9$), alongside real-time qPCR measurements for the SphK1 enzyme. RESULTS: The DBTRG recurrent cell line showed significantly higher levels of S1P (adjusted to cell count) than the primary commercial cell lines ($p < 0.01$), with the exception of U87MG and A172 showing only a trend increase. The SphK1 data were not concordant with the S1P levels. CONCLUSION: The basal level of S1P, as a direct effecter of cell survival and tumorigenesis in the setting of cancer, is informative as to the innate aggressiveness of the tumor. Further examination of this dynamic pathway is ongoing and will include measuring sphingolipid levels in glioma clinical samples at various grades, matching it with the enzymatic gene expression levels.

OM-23. ABERRANT TRANSCRIPTIONAL ACTIVATION IS ASSOCIATED WITH GENE BODY HYPOMETHYLATION IN GLIOBLASTOMA

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DNA methylation alterations play an important role in the growth rate and therapeutic response of GBM, but the full genomic distribution and functional impact of aberrant methylation remain poorly understood. Hypermethylation of CpG islands (CGIs) at the 5' promoters of tumor suppressor genes has been reported in most cancer types. We performed sequencing-based genome-wide methylation profiling to determine common and functionally relevant targets of hyper- and hypomethylation in each of five non-G-CIMP primary GBMs and integrated this data with genome-wide gene expression and copy number analysis. Recurrent methylation changes were confirmed by analysis of TCGA infinium methylation data from 288 GBMs. We found that in contrast to current dogma, classic tumor suppressor genes in primary GBM are rarely hypermethylated. When these CGI do exhibit aberrant methylation, the methylation level is quite low, suggesting only a minor cell subpopulation has the aberrant methylation. However, recurrent and dense 5' CGI hypermethylation was detected at a different set of gene promoters and is consistently associated with decreased mRNA expression for some of the hypermethylated genes. In contrast, DNA hypomethylation occurs within many gene bodies and some normally methylated intragenic CGIs are partially hypomethylated in GBM. We identified two loci where a hypomethylated intragenic CGI overlaps an annotated alternate promoter, and we provide evidence that aberrant gene body hypomethylation activates the expression of alternate transcript isoforms that are not expressed in normal brain. We also show that DNA hypomethylation of intragenic CGIs can occur concurrently with 5' promoter CGI hypermethylation in the same gene and in the same tumor. Together, the data suggest that GBM hypermethylation targets pathways different from those targeted by genetic alterations in tumor suppressor genes and that aberrant gene body hypomethylation could be a mechanism for activation of potentially oncogenic transcript isoforms in GBM.

OM-24. GLIOMAS WITH PARTIAL 1P19Q DELETIONS: A DIFFERENT SUBSET OF TUMORS

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Codeletion of chromosome arms 1p and 19q is a genetic hallmark of oligodendroglial tumors and predicts a favorable prognosis and better response to treatment. Some gliomas with a 1p19q codeletion, however, are associated with a much worse prognosis as expected. This could be owing to the fact that partial 1p and/or 19q deletion rather than complete loss of the chromosome arms occurred. The techniques used for detection of these aberrations differs among institutions, and when only regions 1p36 and 19q13.3 are analyzed by LOH or FISH, it is impossible to distinguish between complete or partial losses. As our previous research showed clinical discrepancies between the different types of 1p/19q losses, we extensively studied their clinical implications in 445 low-grade and high-grade diffuse gliomas. The presence of 1p and 19q aberrations was analyzed with multiplex ligation-dependent probe amplification (MLPA), a technique that screens multiple loci on both chromosome arms, allowing a distinction between partial and complete losses as well as codeletions. Isolated partial 1p loss or isolated partial 19q loss is mainly present in glioblastoma, while a partial deletion in both 1p and 19q is found relatively often in diffuse astrocytomas. The presence of partial deletions of 1p and/or 19q was significantly correlated with shorter survival compared with isolated complete loss of either 1p or 19q or combined complete codeletion of 1p19q.This latter codeletion is known to occur in early (oligodendro)glioma development. In contrast, the partial deletions seem to occur at a later stage of gliomagenesis and to be indicative of malignant progression. In conclusion, our study shows that gliomas with partial deletions of 1p and 19q are a different subset of tumors and have a worse prognosis than gliomas with complete 1p19q codeletion. It underlines the importance of applying a technique for molecular diagnostics that analyzes the complete chromosome arms 1p and 19q.

OM-25. ALDH1A1 AS A NEW PROGNOSTIC MOLECULAR MARKER IN GLIOBLASTOMAS

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Note: The first two authors contributed equally to this study.

Aldehyde dehydrogenase 1A1 (ALDH1A1) has been identified in a variety of human cancers to be expressed by tumor cells and to serve as a reliable marker to predict patients clinical outcome. However, little is known thus far about the function of ALDH1A1 in malignant brain tumors. We analyzed the expression of ALDH1A1 protein in developing and mature cerebral and cerebellar tissue as well as in a series of 93 cases of primary glioblastoma. Tissue samples for histopathological diagnosis and molecular genetic analysis were acquired either via microsurgical tumor resection ($n = 56$) or stereotactical serial biopsy ($n = 37$). Tumor diagnoses were established based on the criteria of the latest WHO brain tumor classification. Patients were treated mainly with radiochemotherapy followed by adjuvant temozolomide according to the EORTC/NCIC protocol. In cases of tumor recurrence, salvage treatment was rendered by our institutional guidelines and on a

personalized cancer therapy basis. While ALDH1A1 was absent in the stem cell niches at varying stages of CNS development, strong ALDH1A1 expression was observed in mature astrocytes coexpressing GFAP and S100. Ninety-two of the 93 (99%) examined glioblastoma cases showed ALDH1A1 expression in up to 49% of tumor cells. The majority of these cells coexpressed GFAP but not stem cell markers, such as nestin, OLIG2, or SOX2. Finally, strong expression of ALDH1A1 detected by immunohistochemistry correlated with a significantly better overall survival of the patients and proved to be an independent prognostic marker ($p < 0.01$). We suggest ALDH1A1 as a marker of astrocytic differentiation and of better prognosis in patients with glioblastoma.

OM-26. METABOLIC TUMOR VOLUME CHANGES BY AMINO ACID PET TRACER FDOPA IS PREDICTIVE OF TREATMENT RESPONSE IN PATIENTS WITH RECURRENT HIGH-GRADE GLIOMAS ON BEVACIZUMAB THERAPY

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OBJECTIVE: The purpose of our study was to evaluate the predictive power of amino acid analog [^{18}F]fluoro-L-dihydroxyphenylalanine (FDOPA) positron-emission tomography (PET) in patients with recurrent malignant gliomas on bevacizumab therapy. METHODS: Twenty-nine patients with recurrent malignant gliomas were prospectively studied with FDOPA-PET at baseline, at 2 weeks, and at 6 weeks after starting treatment. FDOPA scans were analyzed in two ways. First, standardized uptake values (SUV) were determined by taking a region of interest of standard size over the tumor with the highest uptake values. Second, tumor volumes were derived by applying a threshold method in a three-dimensional reconstruction. FDOPA PET uptake changes as well as tumor volume (FDOPAvol) changes were evaluated with regard to progression-free survival and overall survival using Kaplan-Meier analysis. RESULTS: Following therapy, no significant declines of SUVs were seen (2 weeks: 1.4%; 6 weeks: 1.3%, $P = 0.90$). Significant decreases in FDOPAvol were seen at 2 weeks (-38.6%) and 6 weeks (-43.8%, $P = 0.03$). Receiver operating characteristic curve analysis identified a 35% decrease of FDOPAvol as the best criteria for metabolic response. FDOPAvol changes at 2 weeks after starting treatment were predictive of tumor progression ($P = 0.0001$) as well as of overall survival ($P = 0.02$). Metabolic responders ($n = 20$) had significantly longer median time to progression (6.6 vs. 2.1 months) and overall survival time (11.3 vs. 7.6 months) than nonresponders ($n = 9$). FDOPAvol changes at 6 weeks were also predictive of progression-free survival ($P = 0.008$). In contrast, SUV changes were not predictive of progression-free survival or overall survival. CONCLUSION: Tumor volume changes but not SUV changes by FDOPA-PET at 2 weeks after starting therapy are predictive of tumor progression and overall survival for patients with recurrent malignant gliomas on bevacizumab therapy.

OM-27. THE EFFECT OF TUMOR SUBTYPE ON SURVIVAL AND THE GRADED PROGNOSTIC ASSESSMENT (GPA) FOR PATIENTS WITH BREAST CANCER AND BRAIN METASTASES

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BACKGROUND: The diagnosis-specific graded prognostic assessment (GPA) was published to clarify prognosis for patients with brain metastases. This study refines the existing breast-GPA by analyzing a larger cohort and tumor subtype. METHODS: A multi-institutional retrospective database of 400 breast cancer patients treated for newly diagnosed brain metastases was generated. Prognostic factors significant for survival were analyzed by multivariate Cox regression (MCR) and recursive partitioning analysis (RPA). Factors were weighted by the magnitude of their regression coefficients to define the GPA index. RESULTS: Significant prognostic factors by MCR and RPA were Karnofsky Performance Status (KPS), HER2, ER/PR status, and the interaction between ER/PR and HER2. RPA showed age was significant for patients with KPS scores of 60-80. The median survival time (MST) overall was 13.8 months, and for GPA scores of 0-1.0, 1.5-2.0, 2.5-3.0, and 3.5-4.0 was 3.4 ($n = 23$), 7.7 ($n = 104$), 15.1 ($n = 140$), and 25.3 ($n = 133$) months, respectively ($p < 0.0001$). Among HER2-negative patients, being ER/PR-positive improved MST from 6.4 to 9.7 months whereas in HER2-positive patients, being ER/PR-positive improved MST from 17.9 to 20.7 months. The log-rank statistic (predictive power) was 110 for the breast-GPA versus 55 for tumor subtype. CONCLUSIONS: The breast-GPA documents wide variation in prognosis and shows clear separation between subgroups of patients with breast cancer and brain metastases. This tool will aid clinical decision-making and stratification of clinical trials. These data confirm the effect of tumor subtype on survival and show the breast-GPA offers significantly more predictive power than the tumor subtype alone.

OM-28. TRUNCATED NEUROKININ 1 RECEPTOR: EXPRESSION IN PRIMARY GLIOBLASTOMAS AND RELATIONSHIP WITH PATIENT SURVIVAL

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The neurokinin 1 receptor (NK1R), a G protein-coupled receptor that mediates the effects of substance P, is consistently detected in glioblastoma (GBM). Delineation of signal transduction pathways utilized by NK1R in GBM cells indicates that this receptor activates at least seven targets (EGFR, IGF-1R, Src, Akt, NF-kB, IL-6, and VEGF) previously identified as playing key roles in GBM growth. Furthermore, blockade of NK1R inhibits GBM growth. These and other findings suggest that NK1R may function as the conductor of the molecular orchestra involved in GBM growth. The notion that NK1R may be a good target for blocking the growth of GBM is further supported by our finding that GBM cells express a constitutively active form of NK1R that produces elevated levels of phospho-Akt. This constitutive activity arises from the truncated form of NK1R. It is already known that truncated NK1R is oncogenic; its expression, but not the expression of full-length NK1R, in normal breast cells results in a transformed phenotype. In this study, we examined the distribution of the truncated form of NK1R in primary glioblastomas and assessed its relationship with patient survival. Using PCR primers specific for full-length and truncated NK1R, we examined 17 primary GBM tumors. Of these, 12 tumors expressed mainly the truncated from of NK1R, and 5 tumors expressed both forms. Patients whose tumors expressed mainly the truncated form had a median survival of 63.9 weeks, while patients with tumors expressing both forms had a median survival of 93.3 weeks. These preliminary data suggest that the expression of the truncated form may predict poor survival. A larger study that will measure the expression of the full length and the truncated forms of NK1R more precisely using real-time quantitative PCR is underway.

OM-29. THE PROGNOSTIC VALUE OF IDH1 MUTATIONS IN GLIOMAS

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Gliomas are the most common and lethal types of brain tumors and within this group of brain tumors, new diagnostic and prognostic markers are highly needed. Recently, it was reported that the R132H mutation of isocitrate dehydrogenase 1 (IDH1) has both diagnostic and prognostic potential in gliomas. However, further validation is needed. The aim of this study was to investigate the frequency of IDH1 R132H mutated tumors and the prognostic value of this mutation in patients with primary gliomas. Tumor tissue from 243 patients with astrocytic and oligodendroglial tumors (WHO grade II [25], III [30], and IV [188]) was collected between January 1, 2005, and December 31,2009, in the region of southern Denmark. The tissue was stained by immunohistochemistry using a recently developed antibody

(anti-IDH1 R132H, clone H09, Dianova), identifying tumors with the IDH1 R132H mutation. Data were evaluated by univariate and multivariate analyses. Mutated IDH1 (mIDH1) was detected in 12.0% of the gliomas (30/243 gliomas), especially in grade II-III tumors: 62.5% of diffuse astrocytomas (10/16), 80.0% of oligo-astrocytomas (4/5), 50.0% of oligodendrogliomas (3/6), 15.0% of anaplastic astrocytomas (3/20), 75.0% of anaplastic oligo-astrocytomas (3/4), and 50.0% of anaplastic oligodendrogliomas (3/6). Only 2% (4/188) of grade IV tumors were positive. In a univariate analysis, mIDH1 was significantly correlated to survival ($p < 0.0001$). Multivariate analysis carried out for patients with grade II and III tumors showed a strong trend suggesting that mIDH1-positive patients had a better survival than the negative patients (HR 0.43, $p = 0.06$, 95% CI 0.17-1.04), when adjusted for the effect of performance status, age, and tumor grade. In conclusion, mIDH1 was identified with highest frequency in grade II and III gliomas, but was identified in only a few glioblastomas. There was a strong trend toward a better survival in patients with grade II-III gliomas carrying the IDH1 mutation, which is in accordance with previous results.

OM-30. A COMBINED MOLECULAR CLINICAL PREDICTOR OF SURVIVAL VALIDATED WITH THE RTOG-0525 COHORT

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For glioblastoma (GBM), survival classification has primarily relied on clinical criteria, exemplified by the Radiation Therapy Oncology Group (RTOG) recursive partitioning analysis (RPA). We sought to improve tumor classification by combining tumor biomarkers with the clinical RPA data. To accomplish this, we first developed 4 molecular biomarkers derived from gene expression profiling, a glioma CpG island methylator phenotype, a novel MGMT promoter methylation assay, and IDH1 mutations. A molecular predictor (MP) model was created with these 4 biomarkers on a training set of 220 retrospectively collected archival GBM tumors. This MP was further combined with RPA classification to develop a molecular-clinical predictor (MCP). The median survivals for the combined, 4-class MCP were 65 months, 31 months, 13 months, and 9 months, which was significantly improved when compared with the RPA alone. The MCP was then applied to 725 samples from the RTOG-0525 cohort, showing median survival for each risk group of NR, 26 months, 16 months, and 11 months. The MCP was significantly improved over the RPA at outcome prediction in the RTOG 0525 cohort with a 33% increase in explained variation with respect to survival, validating the result obtained in the training set. To illustrate the benefit of the MCP for patient stratification, we examined progression-free survival (PFS) for patients receiving standard-dose temozolomide (SD-TMZ) vs. dose-dense TMZ (DD-TMZ) in RPA and MCP risk groups. A significant difference between DD-TMZ and SD-TMZ was observed in the poorest surviving MCP risk group with a median PFS of 6 months vs. 3 months ($p = 0.048$, log-rank test). This difference was not seen using the RPA classification alone. In summary, we have developed a combined molecular-clinical predictor that appears to improve outcome prediction when compared with clinical variables alone. This MCP may serve to better identify patients requiring intensive treatments beyond the standard of care.

OM-31. DETECTION OF O6- AND N7-METHYLGUANINE ADDUCTS IN BRAIN TUMOR TISSUES OBTAINED FROM PATIENTS TREATED WITH TEMOZOLOMIDE

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The current standard of care for glioblastoma includes surgery, radiotherapy, and chemotherapeutic agents such as temozolomide (TMZ), a DNA methylating compound. The cytotoxic effects of TMZ have been linked to guanine methylation at the N7 and O6 positions. These adducts are not currently used as markers of TMZ efficacy. Using liquid chromatography/mass spectrometry (LC/MS), we have established a sensitive analytical assay to directly detect both N7- and O6-methylguanine adducts from DNA following TMZ treatment. A limit of detection below 1 fmol was observed for O6-methylguanine, while N7-methylguanine was observed below 5 fmol. O6- and N7-methylguanine were successfully detected by LC/MS in tumor and normal brain tissue samples from patients treated with a neoadjuvant TMZ regimen for 14 days (75 mg/m^2). Variations in levels of both methylated guanines were detected between patients as well as within different locations of the same tumor sample. This technique provides a direct detection of the damage inflicted by TMZ. This could potentially indicate the efficacy of the drug, allowing for prompt analysis and response. It also holds potential for determining efficacy of treatment dose, schedule, and possible concomitant drugs.

OM-32. PREDICTING OUTCOMES FOLLOWING THERAPY FOR GLIOBLASTOMA USING RESPONSE METRICS FROM PATIENT-SPECIFIC 3-DIMENSIONAL TUMOR MODELS

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Current metrics for assessing patient response to treatment for glioblastoma multiforme (GBM) do not fully account for the wide variability in the dynamics of these brain tumors and are limited in predicting outcomes. We have developed a method for predicting survival following cytotoxic therapy using 3D computational models that account for each tumor's unique growth dynamics and geometry. As virtual controls, these simulated, untreated tumors can be compared directly to a patient's actual, treated tumor to quantify the patient's therapy response. Our method provides a host of novel metrics for assessing this response, including a "days gained" score that estimates the number of days the therapy delayed tumor progression. We created virtual controls for 27 patients with grade IV GBM and compared their days gained score to their actual survival following therapy. Patients ranged in age from 40-89 years (mean, 58 years) and had RTOG RPA classifications of III ($n = 5$), IV ($n = 12$), or V ($n = 10$). We found that with few exceptions, patients with a days gained score above 98 survived longer than 380 days following therapy, and those with lower scores did not (Fisher exact test, $p = 0.0001$). Our results illustrate the capability of our GBM models to predict survival following therapy using a novel response metric in a 3D setting.

OM-33. THE IMPACT OF IDH 1 MUTATION ON CLINICAL OUTCOME IN PATIENTS WITH GRADE 3 ASTROCYTOMAS WITHOUT 1P19Q CHROMOSOMAL DELETIONS

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INTRODUCTION: The optimal treatment for nondeleted grade 3 astrocytoma is uncertain because of the wide variation of natural history that occurs with tumors diagnosed under the WHO grade III classification subgroup. Isocitrate dehydrogenase 1 (IDH1) is emerging as a potential biomarker to assist in determining which patients should be managed with more treatment intensification. METHODS: Patients managed with a WHO grade III astrocytoma and absence of 1p19q co-deletion were identified from the neuro-oncology unit database and assessed for clinical characteristics and outcome. IDH1 mutation (IDHmt) was initially tested using an IHC antibody, and those with negative tumors (IDHwt) were subsequently confirmed by sequencing of DNA extracted from formalin-fixed paraffin-embedded tissue. Overall survival (OS) and progression-free survival (PFS) were calculated and the interaction of clinical, radiological, and pathological factors on outcome was determined. RESULTS: Forty patients with nondeleted grade III pathology were identified and included for analysis. The median age at diagnosis was 38 years, and MRI Gd enhancement was present in 12 patients. Thirty-five patients had a debulking surgical procedure, and all received adjuvant radiation therapy. Sixteen (40%) patients received adjuvant temozolomide (14) or BCNU (2). IDHmt was present in 24 (60%). At a median follow-up of 47 months (range, 4-184 months), 17 patients had relapsed with median PFS of 87 months (range, 20-154 months) and a median OS of 120 months (range, 87-154 months). IDHmt was strongly associated with PFS ($p < 0.001$) and OS ($p < 0.008$), while age, extent of surgery, Gd enhancement, adjuvant chemotherapy, and Ki67 index were not significantly associated. IDHmt patients had median PFS and OS of 174 months and 184 months compared with IDHwt patients of 30 months and 60 months. CONCLUSION: These data suggest patients without IDH1 mutations diagnosed with grade 3 non-1p19q deleted astrocytomas have a worse prognosis and should be considered for more intense adjuvant therapy.

OM-34. MAGNETIC RESONANCE OF 2-HYDROXYGLUTARATE IN IDH1-MUTATED LOW-GRADE GLIOMA

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Infiltrating gliomas are heterogeneous tumors of the central nervous system that include astrocytomas, oligodendrogliomas, and mixed oligoastrocytomas. Recent studies have indicated that a significant survival advantage and favorable response to temozolomide is conferred to glioma patients whose lesions harbor mutations in the isocitrate dehydrogenase 1 and 2 (IDH1/2) genes (Yan et al., 2009; Houillier et al., 2010). Interestingly, these mutations result in the aberrant production of the potential oncometabolite d--2-hydroxyglutarate (2HG) (Dang et al., 2009). Here, we report the ex vivo detection of 2HG in pathologically confirmed IDH1-mutated tissue samples from 53 recurrent low-grade glioma patients using the nuclear magnetic resonance (NMR) technique of high-resolution magic-angle spinning (1H HR-MAS) spectroscopy. Relative 2HG levels and mutant IDH1 cells demonstrated correlations with various histopathology parameters including mitotic activity, vascular neoplasia, axonal disruption, relative tumor content, and increased cellularity. Of interest is that ex vivo spectroscopic measures of choline-containing species were also correlated with 2HG levels, as well as in vivo magnetic resonance measures of the apparent diffusion coefficient (ADC). These data provide a novel and extensive characterization of mutant IDH1 lesions, while confirming the potential diagnostic value of 2HG and related imaging parameters as markers of clinically relevant tumor characteristics. Considering the survival benefits associated with IDH1/2 mutations, the prognostic value for patients with low-grade glioma is significant. This information may augment the ability of clinicians to monitor therapeutic response in patient studies and provide criteria for stratifying patients to specific treatment regimens. If 2HG is confirmed to be a tumor supporting oncometabolite, additional monitoring of 2HG levels may become important, especially for therapies targeting the IDH1 pathway. The magnetic resonance of 2HG and its correlates may assist in the development of clinical in vivo sequences for IDH-mutant glioma.

OM-35. CLINICAL UTILITY OF G-CIMP AND IDH1 STATUS AS DUAL PROGNOSTIC MARKERS IN GLIOBLASTOMA

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The glioma CpG island methylator phenotype (G-CIMP) has been shown to be highly correlated with prognosis and was noted to be highly concordant with IDH1 mutation in malignant glioma in the limited number of samples analyzed. To better understand the relationship of G-CIMP with IDH1 mutation status and patient outcome, we examined G-CIMP status in detail in a larger retrospective series of glioblastomas as well as tumor samples from the RTOG 0525 clinical trial. Samples were tested for 6 CIMP markers and were correlated with patient outcomes. In the retrospective tumor set ($n = 301$), we found 3 distinct survival groups based on the number of CIMP markers: 0-1 (CIMP-negative), 2-4 (CIMP-intermediate), and 5 or greater (CIMP-positive) with median survivals 13.8, 20.1, and 90.6 months, respectively. This finding was validated in the RTOG 0525 samples (median survivals 15.0, 20.3, and 37.0 months). Among 787 cases with both IDH and CIMP data, 617 were CIMP-negative, 136 were CIMP-intermediate, and 34 were CIMP-positive. Seven hundred forty-four were wild type for IDH1 mutation, and 43 were mutant. CIMP and IDH status were positively correlated but outliers were found. Among the 610 CIMP-negative tumors, there were 7 IDH-mutant tumors, which showed no difference in outcome. Similarly, among the 34 CIMP-positive tumors, there were 21 IDH-mutant cases, which also showed no difference in outcome. However, among the CIMP-intermediate cases, there were 15 IDH-mutant cases with significantly ($p = 0.0003$) improved outcome (medians not reached vs. 18.5 months, 2 year survival 87% vs. 32%). Multivariate analysis showed that both IDH1 mutation status and CIMP status were independent predictors of outcome. These findings suggest the clinical utility of refining the CIMP status into negative, intermediate, and positive groups and the finding that both IDH1 and CIMP status are important molecular markers in GBM.

OM-36. METABOLIC CHARACTERIZATION OF GLIOMA POPULATIONS

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Gliomas constitute a heterogeneous class of tumors that develop within the central nervous system. Their malignancy is routinely graded according to histologic criteria of the World Health Organization (WHO grades 1-4). Many grade 2 and 3 gliomas undergo progressive transformation to a higher grade of malignancy. This study used the ex vivo NMR technique of proton high-resolution magic-angle spinning(1H HR-MAS) spectroscopy to help characterize the metabolic profiles of de novo and upgraded gliomas and to classify these distinct histological grades/transitions according to 29 metabolic features. Two hundred fifty-two image-guided tissue samples from 126 patients (original WHO grade N (2,3,4) = 53, 23, 50) were analyzed using HR-MAS spectroscopy. Only tissue samples with pathologically confirmed glioma and grade were included for analysis. The Wilcoxon rank-sum test was used to compare metabolite levels between different glioma subtypes, and classification was performed with Chi square rank/logistic ridge regression. We found that elevations of the following metabolites and indices were statistically significant ($p < 0.001$) and were characteristic of certain glioma subtypes: myo-inositol/total choline(MCI) in de novo and recurrent grade 2 gliomas; choline-containing species, including free choline and total choline, in de novo grade 3 gliomas and recurrent grade 2 gliomas that had upgraded to grade 3 or 4; phospho-choline/glycerophosphocholine(PC/GPC) in de novo grade 4; and 2-hydroxyglutarate(2HG) in de novo grade 2 and 3 gliomas and recurrent grade 2 and 3 gliomas that had upgraded to grade 4 (secondary GBM). While fewer than 10 parameters could explain most of the variance between different subtypes, using all of the data allowed for an average classification accuracy of 89% (range:75-100%). Interestingly, metabolic descriptions of histologic malignancy were found to differ across glioma grades. The characteristic elevation of PC/GPC in de novo GBM suggests that the greatest contribution to the in vivo total choline peak comes from PC. Information derived from this study may be helpful in defining relative intragrade and intratumoral malignancy.

OM-37. NFKBIA GENE DELETIONS AS A CLINICAL PROGNOSTIC BIOMARKER IN NEWLY DIAGNOSED GLIOBLASTOMA MULTIFORME

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BACKGROUND: The nuclear factor of kappa-light polypeptide gene enhancer in B-cell inhibitor-alpha (*NFKBIA)* gene encodes an inhibitor of the epidermal growth factor (*EGFR)* signaling pathway. Deletions of *NFKBIA* have recently been shown to provide an alternate mechanism for *EGFR* activation in glioblastoma multiforme (GBM). *NFKBIA* gene deletions have primarily been identified in nonclassical GBM subtypes lacking high-level *EGFR* amplification and are associated with shortened survival and lack of response to temozolomide chemotherapy. A clinical test for identification of *NFKBIA* deletions would thus have clinical utility for risk stratification and treatment planning in newly diagnosed patients. DESIGN: Tumor-targeted DNA extraction was performed on formalin-fixed paraffin-embedded (FFPE) tumor samples from 27 de-identified cases of newly diagnosed WHO grade III/IV GBM. Array -based comparative genomic hybridization using a clinically validated 3300-clone bacterial artificial chromosome array was performed. Cases of *NFKBIA* gene deletions were correlated with the patients' O^6-methylguanine-DNA methyltransferase (*MGMT*) status. RESULTS: *NFKBIA* deletions were identified in 6 of 27 (22%) tumors, and all *NFKBIA* deleted cases were nonclassical subtypes lacking high-level EGFR gene amplification. In the remaining 21 cases, one demonstrated high-level platelet-derived growth factor receptor alpha polypeptide (*PDGFRA)* gene amplification, and another demonstrated high-level Mdm2 p53-binding protein homolog (mouse) (*MDM2)* gene amplification. Chromosome instability was identified in all cases. *MGMT* methylation was detected in 3 of 6 *NKBIA* deleted cases, and hypomethylation was detected in the remaining 3 cases. Using the *NFKBIA* survival prediction risk model, the 3 *NFKBIA* deleted/*MGMT* hypomethylated cases were able to be further stratified into the highest risk category (Bredel et al., *NEJM*, 2011). CONCLUSION: Microarray-based chromosome analysis provides a high-throughput clinical method for determining *NFKBIA* gene status in newly diagnosed nonclassical GBM subtypes. Correlation of *NFKBIA* gene deletions with *MGMT* methylation status can be used to further refine risk assessment and prediction of response to temozolomide chemotherapy.