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Abstracts

BASIC SCIENCE

BS 1. THE ROLE OF EGF RECEPTOR HETEROGENEITY IN DRIVING GLIOMA DEVELOPMENT

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An issue that likely contributes to a lack of clinical response in GBM patients is the heterogeneous composition of the tumor, consisting of diverse cytological subtypes carrying a variety of gene alterations. An example of heterogeneity that is present in >50% of cases is amplification of the epidermal growth factor receptor (EGFR) gene. This amplification is often accompanied by structural alterations leading to expression of a variant form of the gene, deltaEGFR (also referred to as EGFRvIII, EGFR-de2-7, and EGFR*), which conveys enhanced tumor aggressiveness. This potent tumor-promoting function of deltaEGFR would suggest that it should be the predominant amplified receptor in clinical samples; however, paradoxically, deltaEGFR is usually present as minor and focal populations within tumors having global homogeneous wtEGFR amplification. This disconnect between tumorigenic potential and the frequencies and proportions of the amplified mutant EGFR and wtEGFR in GBM might arise if mutant EGFR occurs later in tumor progression, when it not only enhances the tumorigenicity of cells that express it, but also potentiates the proliferation of neighboring cells expressing amplified wtEGFR. If this were so, the potentiation loop would provide an attractive and novel therapeutic target. To understand the basis of these observations, we took two approaches. First, deltaEGFR-mediated changes in gene expression in intracranial tumors were assessed by DNA microarrays. We identified genes specifically upregulated by deltaEGFR signaling such as STAT pathway responsive ones, such as IL-6, IL-8, and interferon regulatory factor 1. Second, we assessed the ability of secreted factors from deltaEGFR-expressing cells to activate amplified levels of wtEGFR. Treatment of cells with media conditioned by cells expressing deltaEGFR resulted in activation of STAT3, Akt, Erk1/2, and, importantly, wtEGFR. Therefore, the media conditioned by deltaEGFR cells transactivates the wtEGFR and other associated downstream pathways, and it contains abundant IL-6, which contributes to STAT3 activation.

BS 2. ABERRANT EGFR SIGNALING IN GLIOMA

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Aberrant EGFR signaling is a major contributing force to glioma progression and treatment resistance. The most prevalent mutation, EGFRvIII, is an in-frame deletion of the extracellular domain that occurs in about 40% of glioblastomas and promotes growth and survival of cancer cells more effectively than overexpressed wild-type EGFR. We are investigating the mechanistic basis for this enhanced oncogenic potential. The signaling of EGFRvIII is ligand independent and does not involve receptor dimerization, and so is of low intensity. One hypothesis we are testing is that this low intensity, which allows the receptor to evade the normal mechanisms of internalization and degradation by the endocytic machinery and so makes its signal persistent, underlies its oncogenic nature. To determine whether EGFRvIII's oncogenic signal is dependent on its low intensity, we have created chimeric receptors that can be dimerized experimentally. By fusing the EGFRvIII receptor with a variant of the FKBP12 protein, we have created a chimera that can be experimentally forced to dimerize with a rapamycin derivative. We show that in the presence of the dimerization agent, EGFRvIII signaling increases severalfold in intensity, and will present data analyzing the impact of this on signal transduction and oncogenic potential. In addition to analyzing known signaling pathways downstream of EGFR, we are using shotgun phosphoproteomics based on TiO_2 recovery of phosphopeptides and mass spectrometry. Glioma cell lines expressing EGFRvIII and wild-type EGFR as well as mutants, and with different PTEN backgrounds, are being studied by this approach. Another possible mechanism behind EGFRvIII's impact on glioma biology is differential cellular localization, and we are investigating whether it partitions to the nucleus with different kinetics than EGFR. Lastly, new EGFR mutants, identified by resequencing efforts, are being investigated for their role in glioma biology.

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BS 4. EGF RECEPTOR TYROSINE KINASE MEDIATES A NOVEL PATHWAY OF DRUG RESISTANCE IN MALIGNANT GLIOMAS VIA TYROSINE PHOSPHORYLATION AND FUNCTIONAL ACTIVATION OF GLUTATHIONE S-TRANSFERASE P1

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Aberrant activation, amplification, and mutation of the EGF receptor (EGFR) tyrosine kinase are frequent alterations in human glioblastoma (GBM) and are associated with poor therapeutic response and aggressive clinical course. Similarly, elevation of glutathione *S*-transferase P1 (GSTP1), a major drug/carcinogen metabolizing and stress response signaling protein, has been associated with drug resistance and poor patient survival in GBM. Recently, we provided the first evidence of a linkage of EGFR and GSTP1, namely, that the human GSTP1 is a direct downstream target of EGFR. Here, we report that GSTP1 undergoes tyrosine phosphorylation by EGFR and that this mediates a novel pathway of drug resistance in human malignant gliomas. We show that in the EGFR-overexpressing U87MG.wtEGFR cells, EGF stimulation increased both the level of tyrosine phosphorylation and the catalytic activity of GSTP1 and is associated with increased resistance to cisplatin, which was completely reversed by pretreatment with AG1478, a specific EGFR inhibitor. Enzyme kinetic analysis showed phosphorylation by EGFR to significantly enhance the ability of the GSTP1 protein to metabolize cisplatin and other GSTP1 substrates. Using a combination of LC-MS/MS, a GSTP1 peptide microarray, and mutant GSTP1 peptides, we identified the EGFR-specific phospho-acceptor residues in GSTP1 to be primarily Tyr3, Tyr7, and Tyr198. Computer-based modeling of the GSTP1 crystal structure showed a significant increase in electronegativity of the GSTP1 active site following phosphorylation of the active site residue, Tyr7. In in vivo growing xenografts of the EGFR-overexpressing, cisplatin-resistant U87MG.wtEGFR cells, tyrosine-phosphorylated GSTP1 levels were significantly elevated, while undetectable in the parental U87MG tumors. These findings are strong evidence that the tyrosine phosphorylation and functional activation of GSTP1 by EGFR are critical mediators of resistance to chemotherapy in human GBM. The results support exploration of strategies involving double targeting of both EGFR and GSTP1 as a novel therapeutic approach in GBM.

BS 5. MULTIGENE PREDICTOR OF OUTCOME IN NEWLY DIAGNOSED GLIOBLASTOMA

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Patients with newly diagnosed glioblastoma exhibit variation in response to initial therapy as well as overall survival. Since this variation is only partially accounted for by clinical factors, it is likely that molecular genetic alterations in the tumor account for a major portion of differential response to therapy. We identified a multigene predictor of outcome in glioblastoma using independent microarray data sets. Using real-time quantitative PCR on 68 retrospective paraffin samples, a subset of these genes (n = 10) remained predictive of outcome. To refine and validate this set, we tested these 10 genes on an additional set of 109 tumors from patients uniformly treated with concurrent/adjuvant temozolomide. Nine out of 10 genes remained highly associated with both overall and progression-free survival and were combined into a single metagene score that could be dichotomized

into favorable/unfavorable outcome prediction. MGMT methylation status was also assessed, and multivariate analyses showed MGMT methylation and the nine-gene set to be independent predictors of outcome, suggesting that the multigene set could complement MGMT. Ongoing work will further refine our multigene predictor, but results to date indicate the feasibility of developing a molecular genetic test to prospectively predict response to therapy in newly diagnosed GBM.

BS 6. USING GENE EXPRESSION PROFILING TO IDENTIFY A PROGNOSTIC MOLECULAR SPECTRUM IN GLIOMAS

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Because histopathological classification of gliomas is often clinically inadequate due to the diversity of tumors that fall within the same class, convincing molecular prognostic factors are necessary for risk grouping of high-grade gliomas. The goal of this study was to identify prognostic molecular features in diffusely infiltrating gliomas using gene expression profiling. We selected 3,456 genes expressed in gliomas, including 3,012 genes found in a glioma-expressed sequence tag collection. The expression levels of these genes in 152 gliomas (100 glioblastomas, 21 anaplastic astrocytomas, 19 diffuse astrocytomas, and 12 anaplastic oligodendrogliomas) were measured using adapter-tagged competitive PCR, a high-throughput RT-PCR technique. The samples were mainly recruited from a phase II clinical trial of nimustine, carboplatin, vincristine, and interferon-β with radiotherapy. We applied unsupervised and supervised principal component analyses to elucidate the prognostic molecular features of gliomas. The gene expression data matrix was significantly correlated with the histological grades, oligoastro histology, and prognosis. Using 110 gliomas, we constructed an outcome prediction model based on the expression profile of 58 genes, resulting in a scheme that reliably classified the glioblastomas into two distinct prognostic subgroups. The model was then tested with another 42 tissues. Multivariate Cox analysis of the glioblastoma patients using other clinical prognostic factors, including age and the extent of surgical resection, indicated that the gene expression profile was a strong and independent prognostic parameter. The methylation status of the MGMT promoter was not obviously correlated with our 58 gene-based subgroups of glioblastoma. In addition, the possibility that was useful for a prediction of the early progression in grade 3 cases was suggested. Our predictor demonstrated a stable performance in two publicly available data sets. In conclusion, we identified gene expression-based prognostic score in astrocytic and oligodendroglial tumors, which showed an independent prognostic value in glioblastoma cases.

BS 7. SPHINGOSINE-1-PHOSPHATE$_1$ RECEPTOR REGULATES GLIOMA CELL PROLIFERATION AND CORRELATES WITH SURVIVAL OF PATIENTS WITH GLIOBLASTOMA

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Sphingosine-1-phosphate (S1P) is a bioactive lipid that signals through a family of five G-protein–coupled receptors, termed S1P$_1$–S1P$_5$, and regulates cellular proliferation, migration, survival, cytoskeletal rearrangement, and angiogenesis. We investigated the expression and role of S1P receptors in human astrocytomas. Astrocytomas of various histologic grades expressed four types of S1P receptors, S1P$_1$, S1P$_2$, S1P$_3$, and S1P$_5$, by qRT-PCR analysis. Expression of S1P$_1$ receptor was significantly lower in glioblastomas than those in normal brains and low-grade astrocytomas. The expression levels of S1P$_1$ receptor decreased significantly as tumor grade increased. Immunoblot using specific antibody against S1P$_1$ receptor showed that normal brain, low-grade astrocytomas and anaplastic astrocytomas expressed more S1P$_1$ receptor protein than did glioblastomas. Immunohistochemistry showed that S1P$_1$ receptor was immunolocalized predominantly to the majority of astrocytes, neurons, and endothelial cells in the normal brain, but no staining was observed in neoplastic astrocytes in glioblastoma specimens. Downregulation of S1P$_1$ receptor expression correlated with poor survival of patients with glioblastomas. Patients with glioblastomas whose tumors showed high levels of S1P$_1$ receptor expression had 76% survival rate at 1 year, whereas those with low levels of S1P$_1$ receptors expression had 37% survival rate at 1 year. Furthermore, we examined the mechanism of S1P$_1$ receptor pertaining to glioma cell proliferation by either overexpressing or knocking down, by RNA interference, in glioma cell lines. Forced expression of S1P$_1$ receptor in low-expressor cell lines (U87, U251) resulted in decreased cell growth concomitant with the activation of S1P$_1$ receptor. Cells transfected with S1P$_1$ receptor small interfering RNA in high expressor cell lines (T98G, G112) promoted cell proliferation. This is the first demonstration that S1P$_1$ receptor signaling negatively controls cell proliferation in astrocytomas. Dysregulation of S1P$_1$ receptor expression or function may underlie glioma proliferation.

BS 8. 17-ALLYLAMINO-17-DEMETHOXYGELDANAMYCIN DOWNREGULATES HYALURONIC ACID–INDUCED GLIOMA INVASION BY BLOCKING MMP-9 SECRETION

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Hyaluronic acid (HA), a principal glycosaminoglycan found in the extracellular matrix of human brain, facilitates cell adhesion, cell migration, cellular proliferation, and tumor progression through interactions with receptors such as CD44 and RHAMM. We found that PTEN suppresses HA-induced secretion of MMP-9 and invasion in U87MG cells via dephosphorylation of focal adhesion kinase (FAK), and that HA stimulates secretion of matrix MMP-9 through NF-κB in glioma cells. However, the molecular mechanism and therapeutic strategy to block HA-induced MMP-9 secretion were not well studied. Here, we elucidated the Hsp90 inhibitor 17-allylamino-17-demethoxygeldanamycin (17-AAG) as a blocker of MMP-9 secretion and further elucidated signaling mechanisms involved in HA-induced NF-κB activation that increase MMP-9 secretion and invasion by using this drug. HA-induced activation of NF-κB is mediated by IκB kinase (IKK), which phosphorylates the NF-κB inhibitor IκBα and promotes its degradation. In addition, on the basis of RNA silencing experiments, we show that FAK plays a critical role in mediating the HA-induced activation of NF-κB that resulted in increased MMP-9 expression and secretion, cell migration, and invasion. Importantly, we show that the well-known anticancer agent 17-AAG acts by inhibiting signals that lead to FAK activation, thereby blocking IKK-dependent IκBα phosphorylation/degradation, NF-κB activation, and MMP-9 expression and suppressing HA-induced cell migration and invasion. Therefore, we propose 17-AAG as a potential therapeutic candidate for the treatment of highly invasive gliomas that resulted from HA-induced NF-κB–mediated MMP-9 secretion.

BS 10. THE ROLE OF CHEMOKINE SDF-1 AND CXCR4 IN GLIOMA ANGIOGENESIS AND INVASIVENESS: INTERACTION BETWEEN GLIOMA AND GLIOMA-DERIVED ENDOTHELIAL CELLS

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Glioma cells and tumor endothelial cells (ECs) in hypoxic areas obtain prominent angiogenesis and invasiveness, resulting in resistance to radiation and chemotherapy. We investigate the role of SDF-1α/CXCR4 in glioma angiogenesis and invasiveness both for tumor cells and tumor-derived ECs under normoxic and hypoxic conditions in order to suggest new treatment strategy for gliomas. Methods were as follows: (1) Isolation and culture of glioma derived ECs: two ECs from two glioblastoma patients were isolated and cultured with endothelial growth medium (GBMEC-1, -2). (2) SDF-1α/CXCR4 mRNA expression: total RNA of glioma cell lines (U87, U251, T98G) and ECs (GBMEC-1, GBMEC-2, HUVEC) cultured under normoxic (21%) and hypoxic (1%) conditions were harvested, and SDF-1α/CXCR4 expression was measured by RT-PCR. (3) Inhibition of glioma cell invasion was measured by Matrigel Boyden chamber assay. (4) Inhibition of vascular endothelial growth factor (VEGF)-induced EC proliferation measured by WST8 assay. Results were as follows: (1) SDF-1α/CXCR4 expression varies between glioma cells: SDF-1α/CXCR4 expression is upregulated with hypoxia in U87, but not in U251 and T98G. AMD (a CXCR4 antagonist) and tannic acid (a SDF-1α antagonist) significantly inhibited U87 invasiveness under normoxic and hypoxic conditions. (2) SDF-1α/CXCR4 expression is different between normal ECs (HUVECs) and glioma-derived ECs (GBMECs). GBMECs had strong expression of VEGF, upregulation of SDF-1α expression with hypoxia, and no expression of VEGFR2 and CXCR4. VEGF-induced GBMEC proliferation is significantly slow compared to HUVEC proliferation. In conclusion, SDF-1α/CXCR4 are key target molecules in glioma invasiveness in hypoxic condition. The character-

istics of glioma-derived ECs are completely different from those of normal ECs. In order to inhibit glioma angiogenesis, glioma-derived ECs should be targeted, for example, by antagonizing SDF-1α expression.

BS 13. A NOVEL SWITCH THAT LINKS PTEN TO THE CONTROL OF UBIQUITINATION, TRAIL SENSITIVITY, AND IMMUNORESISTANCE IN HUMAN GBM XENOGRAFT CELLS

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TRAIL is a proapoptotic peptide that, when provided exogenously or by activated NK cells, induces a tumor-selective cell death. TRAIL resistance, however, is common in GBM and contributes to immune escape and the failure of TRAIL-based therapies. TRAIL resistance in GBM was previously associated with PTEN loss, Akt activation, and increased production of the antiapoptotic protein FLIPs. We found, however, that PTEN loss and Akt activation also contribute to TRAIL resistance by decreasing FLIPs ubiquitination and degradation. Because regulation of ubiquitination is a novel and previously unreported function of PTEN, we pursued the mechanism by which PTEN regulates FLIPs ubiquitination and TRAIL sensitivity. To assess the role of PTEN in regulating FLIPs ubiquitination and stability, we identified two candidate regulators of ubiquitination: USP8, a deubiquitinase; and AIP4, an E3 ubiquitin ligase. Levels of these proteins were then manipulated in PTEN wild-type or knockout-transformed mouse astrocytes and in PTEN wild-type or mutant human GBM xenograft cells, after which the effects on FLIPs ubiquitination, stability, and sensitivity to killing by TRAIL or activated NK cells were assessed. Akt activation suppressed levels of the deubiquitinating enzyme USP8, leading to ubiquitination and inactivation of the E3 ligase AIP4. Inactive AIP4 was in turn unable to ubiquitinate and target FLIPs for proteasomal degradation, leading resistance to TRAIL-mediated and NK cell–mediated killing. Conversely, overexpression of USP8 or AIP4 reversed resistance to both TRAIL-mediated and NK cell–mediated killing. Furthermore, in human GBM xenografts, pAkt levels correlated with USP8 and FLIP levels, TRAIL sensitivity, and killing by activated NK cells. These data define USP8 and AIP4 as components of a PTEN-regulated switch that controls FLIPs protein ubiquitination and stability, TRAIL sensitivity, and immunoresistance, and define control of ubiquitination as a novel means by which PTEN mediates its tumor suppressive functions.

BS 14. CENTROSOME AMPLIFICATION INDUCED BY SURVIVIN SUPPRESSION ENHANCES BOTH CHROMOSOME INSTABILITY AND RADIOSENSITIVITY IN GLIOMA CELLS

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Glioblastoma is characterized by invasive growth and a high degree of radioresistance. Survivin, a regulator of chromosome segregation, is highly expressed and known to induce radioresistance in human gliomas. In this study, we examined the effect of survivin suppression on radiosensitivity in malignant glioma cells, while focusing on centrosome aberration and chromosome instability (CIN). We suppressed survivin by small interfering RNA transfection and examined the radiosensitivity using a clonogenic assay and a trypan blue exclusion assay in U251MG (p53 mutant) and D54MG (p53 wild-type) cells. To assess the CIN status, we determined the number of centrosomes using an immunofluorescence analysis and the centromeric copy number by fluorescence in situ hybridization. The radiosensitization differed regarding the p53 status as U251MG cells quickly developed extreme centrosome amplification (one-quarter of CIN) and enhanced the radiosensitivity, while centrosome amplification and radiosensitivity increased more gradually in D54MG cells. TUNEL assay showed that survivin inhibition did not lead to apoptosis after irradiation. This cell death was accompanied by an increased degree of aneuploidy, suggesting mitotic cell death. We conclude that survivin inhibition may be an attractive therapeutic target to overcome the radioresistance while, in addition, proper attention to CIN (centrosome number) is considered important for improving radiosensitivity in human glioma.

BS 15. THE WARBURG EFFECT AND TUMOR CELL SURVIVAL IN HUMAN GBMS

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GBMs are resistant to apoptosis induced by the hypoxic microenvironment and standard therapies including radiation and chemotherapy. We postulate that the Warburg effect, a preferential glycolytic phenotype of tumor cells even under aerobic conditions, plays a role in these prosurvival signals. In this study, we quantitatively examined the expression of hypoxia-related glycolytic genes within pathological and MRI-defined center and periphery of GBMs. We postulate that expression of hypoxia-induced glycolytic genes, particularly Hexokinase2 (*HK2*), favors cell survival and modulates resistance to tumor cell apoptosis by inhibiting the intrinsic mitochondrial apoptotic pathway. GBM patients underwent conventional T1-weighted enhanced MRI and MR spectroscopy studies on a 3.0-T GE scanner, prior to stereotactic sampling (formalin/frozen) from regions that were T1-Gad enhancing (center) and T2-positive, T1-Gad negative (periphery). Real-time quantitative PCR (qRT-PCR) was performed to quantify regional gene expression of glycolytic genes, including *HK2*. In vitro functional studies were performed in U87 and U373 GBM cell lines grown in normoxic (21% pO_2) and hypoxic (<1% pO_2) conditions, transfected with *HK2* siRNA followed by measurement of cell proliferation (BrdU), apoptosis (activated caspase 3/7, TUNEL, cytochrome c release), and viability (MTS assay). There exists a differential expression profile of glycolytic enzymes between the hypoxic center and normoxic periphery of GBMs. Under hypoxic conditions, there is increased expression of *HK2* at the mitochondrial membrane in GBM cells. In vitro *HK2* knockdown led to decreased cell survival and increased apoptosis via the intrinsic mitochondrial pathway, as seen by increased mitochondrial release of cytochrome c. Increased expression of *HK2* in the center of GBMs promotes cell survival and confers resistance to apoptosis, as confirmed by in vitro studies. In vivo intracranial xenograft studies with injection of *HK2*-shRNA are currently being performed. *HK2* and possibly other glycolytic enzymes may provide a target for enhanced therapeutic responsiveness, thereby improving prognosis of patients with GBMs.

BS 16. GENETIC AND HYPOXIC MECHANISMS OF TISSUE FACTOR EXPRESSION AND THROMBOSIS IN GLIOBLASTOMA

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Glioblastoma (GBM) is a malignant astrocytoma distinguished by necrosis and microvascular hyperplasia. We have proposed that vasoocclusion caused by intravascular thrombosis (IVT) could initiate or propagate hypoxia and necrosis. In a histologic study of 297 CNS tumors, we found IVT in 92% of GBM resections and 0% anaplastic astrocytomas (AA), tumors that lack necrosis. Tissue factor (TF), the most potent human cellular procoagulant, is overexpressed in GBMs. We investigated mechanisms of aberrant expression of TF by neoplastic and endothelial cells in GBM. PTEN loss and EGFR amplification are specific for GBM compared to AA. Overexpression of EGFR or mutant EGFRvIII in human glioma cells caused increased basal TF expression, and stimulation of EGFR by EGF led to a dose-dependent TF upregulation that caused accelerated plasma coagulation in vitro. EGFR-induced TF depended on AP-1 transcriptional activity and was associated with c-Jun N-terminal kinase (JNK) and JunD activation. Restoration of PTEN expression in PTEN-deficient GBM cells diminished EGFR-induced TF by inhibiting JunD/AP-1 transcriptional activity. PTEN mediated this effect by antagonizing PI-3K activity, which in turn attenuated both Akt and JNK activities. We also investigated factors secreted by gliomas following PTEN loss that induce aberrant endothelial TF expression. Conditioned media (CM) was collected from PTEN-null U87MG cells with an inducible wt-PTEN ($23.11^{+/-}$ PTEN). We found that CM from PTEN-null cells induced endothelial TF expression compared to $PTEN^{+}$ cells and that hypoxia potentiated this effect. Using isotope-coded affinity tags (ICATs) and tandem mass spectrometry (MS/MS), we identified MMP-2 (1.5-fold) and macrophage migration inhibitory factor (MIF; 1.3-fold) as proteins secreted by PTEN null compared to $PTEN^{+}$ gliomas. Hypoxia induced secretion of IL-8 (3.2-fold) and insulin-like growth factor-binding protein 3 (IBP-3; 3.2-fold). Both purified IL-8 and MIF induced endothelial TF and could be secreted factors that cause thrombosis in GBM.

BS 17. GATA4 EXPRESSION IN THE NORMAL CNS AND ITS FREQUENT LOSS IN HUMAN GLIOMAS

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The GATA transcription factors recognize the consensus DNA-binding motif (A/T)-GATA-(A/G). GATA transcription factors regulate myriad normal biological functions and have also been implicated as tumor suppressor genes (TSG), including our work on GATA6 in murine and human gliomas. The expression and functional role of GATA4, a closely related GATA6 family member, in the mouse and human nervous system and gliomas are unknown. Normal brain specimens were evaluated for GATA4 expression by IHC in embryonic and adult human and murine brains. There was ubiquitous expression in a variety of CNS cells: neurons, astrocytes, oligodendrocytes, ependyma, choroid plexus, brain endothelium. Isolated normal murine and human astrocytes expressed abundant GATA4 by Western blot analysis, whereas it was lost in human GBM cells and operative samples. Tissue microarray demonstrated GATA4 loss in a large percentage of human GBMs, with variable loss in gliosarcomas, low-grade astrocytomas, and oligodendrogliomas. GATA4 siRNA gene silencing of predisposed nontransformed astrocytes (oncogenic *ras* or $p53^{-/-}$) increased their proliferation, soft agar and in vivo growth. Conversely, reintroduction of GATA4 in transformed human and murine GBM cells shifted the cells from an S/G2M to a G1 phase, with increased apoptosis. Collectively, these results suggest that GATA4 is a novel TSG in human gliomas. Our data are the first to demonstrate the expression profile of GATA4 in the CNS and implicate it as a TSG in murine and human gliomas. Current studies are focusing on mutational analysis to investigate how GATA4 expression is lost in gliomas; transformation assays to determine if loss of GATA4 in normal astrocytes can initiate transformation of normal glial cells; cross-talk among GATA family members in gliomagenesis; and transcripts regulated by GATA family members that contribute to gliomagenesis, which may serve as therapeutic targets.

BS 18. THE EXPRESSION LEVEL OF S1P1 IS RELATED TO MIB-1 LABELING INDEX THAT PREDICTS SURVIVAL IN GLIOBLASTOMA

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Astrocytomas represent the most frequent primary tumors of the CNS. There are many reports on the clinical use of MIB-1 labeling index (LI) that linked to proliferative activity in astrocytomas, and its significance varies from one study to another. There are no molecules known that directly link to the MIB-1 LI in astrocytomas. The aim of this study was to evaluate the clinical value of MIB-1 LI in our human astrocytic tumor cases of different histopathological grades (WHO) and explore the molecules that could influence the MIB-1 LI. An immunohistochemical study of the MIB-1 proteins using the avidin-biotin-peroxidase method was performed, and MIB-1 LI was determined in 70 astrocytomas (18 grade II, 10 grade III, and 42 grade IV). Clinical variables considered included gender, age, extent of surgical resection, tumor's location, and survival. In the same cases, EGFR, EGFRvIII, PTEN, PDGFR, and S1P1 (sphingosine-1-phosphate 1 receptor) were detected and quantified by qRT-PCR, Western blotting, and immunohistochemistry. Our data showed increasing values of MIB-1 LI with increasing grade of malignancy in astrocytomas (2.2% grade II, 5.4% grade III, and 26.7% grade IV). Kaplan-Meier survival curves for 40 patients with glioblastoma showed that high MIB-1 LI correlates with short survival ($p < 0.005$). On the other hand, univariate analysis did not show any correlation between survival and gender, extension of surgical resection, tumor location, or patient age at surgery. Among molecules tested, only low expression levels of S1P1 were significantly correlated with high MIB-1 LI in glioblastomas ($p < 0.05$). This study establishes MIB-1 LI as an important predictor for the grade of astrocytic tumors and prognostic factor in human glioblastomas. S1P1 may play an important role in proliferative activity in glioblastomas.

BS 20. THE RNA-BINDING PROTEIN 76 CONTROLS GENE EXPRESSION IN GBM

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We are studying mechanisms of deregulated gene expression in GBM and its contribution to tumor biology. Our studies are based on the premise that the principal gene regulatory functions in GBM act at the posttranscriptional level. Moreover, our work provides a rationale for clinical use of oncolytic viruses with GBM-specific gene expression. We discovered that double-stranded RNA-binding protein 76 (DRBP76) exhibits drastically different subcellular distribution, RNA-binding capacity, and translation regulation in the normal human brain and in GBM patients. Our empirical studies with recombinant viruses with selective replication in GBM suggest that DRBP76 represses translation of certain mRNAs in the normal brain, while permitting efficient protein synthesis in GBM. We have now performed RNA coimmunoprecipitation and microarray studies to identify the pool of endogenous mRNAs recognized by DRBP76 in the human brain. Our investigations show that DRBP76 recognizes histone mRNAs and controls their translation. We conclude that RNA-binding proteins with tumor-specific properties are responsible for regulating key classes of gene products involved in the malignant phenotype. Posttranscriptional gene regulation occurs independently of transcription rate and enables rapid adjustment of protein levels. Our studies elucidate how RNA-binding proteins exert gene regulatory functions in GBM that determine tumor properties.

BS 21. EXPRESSION AND FUNCTIONAL SIGNIFICANCE OF INHIBITORS OF APOPTOSIS PROTEINS (IAPS) IN HUMAN GLIOMAS

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Inhibitors of apoptosis proteins (IAPs) are a conserved family of proteins that inhibit the final apoptotic pathways mediated by activated caspase 3. Aberrations in apoptosis regulation are integral for transformation and therapeutic resistance in cancer, including gliomas. The regional expression profile and functional relevance of IAPs in glioma are largely unknown. We examined the expressional levels of the four major IAPs (cIAP1, cIAP2, XIAP, and survivin) in low- and high-grade human glioma specimens. Regional differences in IAPs, between the center-pseudopalisading and periphery-infiltrating areas, were investigated by real-time qRT-PCR on GBM cells isolated by laser capture microdissected (LCM) from these two regions. The qRT-PCR results were validated by IHC on corresponding paraffin sections. In vitro experiments with human GBM cell lines with siRNA knockdown were undertaken to investigate the functional relevance. cIAP1 was the only IAP member with increased expression in low-grade astrocytomas compared to normal brain, while all the IAPs examined were differentially expressed in GBMs. cIAP2 was increased in the periphery-infiltrating compared to the center-pseudopalisading GBM cells. In contrast, XIAP and survivin were increased in the pseudopalisading versus infiltrating GBM cells. To determine if increased expression of XIAP and survivin may be contributing to the apoptosis resistance phenotype of the pseudopalisading cells, siRNA knockdown of XIAP and survivin was undertaken. Knockdown of both XIAP and survivin rendered the GBM cells more susceptible to hypoxia- and chemotherapy-induced apoptosis, without any alteration in proliferation. Results from overexpression studies and in vivo experiments with shRNA-mediated knockdown are currently pending. We hypothesize that aberrant increased IAPs, especially XIAP and survivin by pseudopalisading GBM cells, plays an important role in cell survival and thereby therapeutic resistance and recurrence. Modulation of these IAPs may directly decrease glioma growth or indirectly render them more sensitive to radiation and/or chemotherapy.

BS 23. TARGETING THE TUMOR STROMA: A NOVEL THERAPEUTIC STRATEGY BASED ON SEPARATE ANALYSIS OF THE MALIGNANT AND STROMAL CELL COMPARTMENTS IN BRAIN TUMORS

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The recruitment of host vasculature and the infiltrative behavior of gliomas underscore the significance of tumor–stroma interactions in brain tumor pathogenesis. The aim of this project is to identify cancer-related changes in the stroma during brain tumor progression that can be targeted therapeutically. However, targeting tumor-activated stromal cells requires further insight into the mechanisms that regulate the tumor–stroma interplay. Since any tumor biopsy contains a mixture of cancer cells and stromal cells, we are unable to determine whether a given gene expression profile or protein signature is derived from stromal or cancer cells. For the same reason, we are also unable to specify the directions of cross-talk between compartments: whether an influence is exerted upon the tumor by the surrounding stroma or vice versa. In this project, we have generated green fluores-

cent protein (GFP) expression in the nude rat by crossing nude rat with a transgenic GFP-expressing line. We implanted human glioma biopsies in GFP-immunodeficient rats. The resulting xenograft tumors were dissociated into a cell suspension and FACS-sorted into GFP-positive stromal cells and GFP-negative tumor cells. We also obtained cell suspensions of stromal cells from normal brain. Human specific nuclei antibody staining has confirmed sufficient purity of the sorted cells. Using this tool, we intend to delineate the gene expression profiles and protein signatures unique to the tumor-activated stromal cells. This information will subsequently be used to tailor drug regimens that target tumor-activated stroma and tumor–stroma interactions.

BS 24. THE ROLE OF GLIAL CELLS IN BRAIN METASTASES OF TUMOR CELLS

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The role of glial cells in the brain metastases of tumor cells is largely unknown. As the interaction between tumor cells and organ microenvironment regulates the progression of metastases, the brain environment may play a specific role in the invasion and proliferation of metastatic tumor cells. Glial cells are greater in population than are neurons in the brain and may play important roles in brain metastases, producing many cytokines and growth factors that can modulate tumor cell proliferation, tumor angiogenesis, or tissue damage. We therefore established an animal model for brain metastasis using human lung cancer–derived cells in nude mice. Activated glial cells were noted around the metastatic lesions histologically. Then the effects of glial cells on the growth of cancer cells were investigated in vitro. Microglia inhibited the growth of cancer cells, while astrocytes promoted the proliferation of cancer cells when they were cocultured. We identified the soluble factors released from astrocytes in both mRNA and protein levels. These recombinant cytokines actually attenuated the proliferation of cancer cells in vitro. These results suggest that the inhibition of inflammatory response by activation of astrocytes may attenuate tumor cell proliferation, followed by inhibition of the progression of brain metastases.

LUNCHEON SEMINAR

LS. A JAPANESE MULTICENTER CLINICAL TRIAL OF INTERFERON-B AND TEMOZOLOMIDE COMBINATION THERAPY FOR HIGH-GRADE GLIOMAS: FROM BENCH TO BEDSIDE

Toshihiko Wakabayashi, Atsushi Natsume, Souichiro Shibui, Takamasa Kayama, Masao Matsutani, Ryo Nishikawa, Hiroshi Takahashi, Nobuo Hashimoto, Tomokazu Aoki, Toshiki Yoshimine, Kaoru Kurisu, Jun Yoshida; INTEGRA Study Group, Japan

In 2006, TMZ was certified by the National Ministry of Health and Welfare in Japan, and it is now used as firstline therapy. However, its clinical outcomes depend on O^6-methylguanine-DNA methyltransferase (MGMT) status, and MGMT modification is one of the key factors to obtain greater clinical benefits in the future. IFN-β exhibits pleiotropic biological effects and has been widely used either alone or in combination with other antitumor agents in the treatment of malignant gliomas and melanomas. In the treatment of malignant gliomas, IFN-β can act as a drug sensitizer, enhancing toxicity against various neoplasms when administered in combination with nitrosourea. In particular, IFN-β and nitrosourea combination therapy has been used for glioma therapy in Japan. Previously, we demonstrated that IFN-β markedly enhanced chemosensitivity to TMZ in an in vitro study of human glioma cells; this suggested that one of its major mechanisms is the downregulation of MGMT transcription via p53 induction. This effect was also observed in an experimental animal model. These two studies suggested that IFN-β and TMZ combination therapy might further improve the clinical outcome in malignant glioma compared to TMZ plus radiation therapy. In order to evaluate the safety, feasibility, and preliminary clinical effectiveness of combination of IFN-β and TMZ, we conducted a clinical study—the Integrated Japanese Multicenter Clinical Trial: A Phase I Study of Interferon-β and Temozolomide for Glioma in Combination with Radiotherapy (INTEGRA study). In this study, eight medical institutions covering regional populations throughout Japan are participating. The preliminary results reveal that this combination therapy causes minimal toxicity. Further clinical studies are warranted. In this presentation, we provide an overview of our fundamental research and a summary of the multicenter clinical trial.

NEW THERAPEUTIC APPROACHES

NEW 1. ENHANCING GLIOBLASTOMA CYTOTOXIC THERAPY THROUGH SMALL-MOLECULE INHIBITION OF P53

C. David James, Eduard B. Dinca, Scott R. VandenBerg, Michael D. Prados, Mitchel S. Berger; Department of Neurological Surgery, University of California–San Francisco, San Francisco, CA, USA

In this study, we have conducted bioluminescence and survival benefit analysis, examining four distinct glioblastomas in an intracranial xenograft therapy response model, for investigating the effects of p53 small-molecule inhibition when used in combination with temozolomide. Survival benefit analysis indicated that the cytotoxic effects of temozolomide were significantly enhanced by the p53 inhibitor, relative to treatment with temozolomide alone, in tumors having wild-type p53 ($p < 0.001$ for each of three tumors tested), but not when treating mice having a tumor that lacks endogenous p53. Longitudinal bioluminescence imaging results were consistent with the results of the survival analysis by showing extended suppression of intracranial tumor luminescence by temozolomide with p53 inhibitor, relative to temozolomide alone, but only in instances involving tumors with wild-type p53. Significantly, p53 inhibitor alone had no effect on survival or intracranial tumor bioluminescence compared with untreated control group animals. Analysis of tumor cells treated in vitro showed that temozolomide induces p21 expression in cells with wild-type p53 and that this induction can be blocked by cotreating cells with p53 inhibitor. The inhibition of p21 expression by p53 inhibitor suggests a scenario in which tumor cells experiencing temozolomide-associated DNA damage fail to cell cycle arrest and consequently undergo more extensive apoptosis. This study represents the first investigation of cytotoxic therapy enhancement associated with the use of a p53 small molecule inhibitor in a xenograft model, and our results suggest that wild-type p53 is a novel therapeutic target for enhancing the antitumor effects of cytotoxic therapy.

NEW 2. CYCLIN-DEPENDENT KINASE INHIBITOR FLAVOPIRIDOL ENHANCES CYTOTOXIC ACTION OF DNA-DAMAGING AGENTS ON HUMAN GLIOMA CELLS

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Previous studies have suggested that G2 checkpoint system might protect human glioma cells from the chemotherapeutic agent-induced cytotoxicity. In the present study, we therefore tested the effect of the cdk inhibitor flavopiridol (FP), which has been known not only to exhibit antitumor effects itself but also to inhibit the action of cdc2, a key protein in G2 checkpoint pathway, on TMZ-treated U87MG human glioma cells. FP potentiated the cytotoxicity of TMZ in a p53-independent manner at a low concentration of 10 nM when the cells were exposed to FP for 3 days. This FP-induced potentiation of TMZ was clearly associated with depression of antiapoptotic protein survivin and polo-like kinase 1 (PLK1) as well as increased release of cytochrome c in the cytoplasm. Furthermore, we tested the effect of FP (10 nM) on U87MG-derived TMZ-resistant clones that were established after serial treatment with TMZ over several months by dose escalation method and found that FP resensitized those TMZ-resistant cloned to TMZ when the clones showed TMZ-induced G2 checkpoint activation and PLK1 induction. We also found that posttreatment of TMZ-treated cells for 24 h with FP at higher concentration (100 nM or higher) showed enhancement of TMZ cytotoxicity, although this short exposure with FP itself did not show cytotoxicity. FACS analysis suggested these FP-induced effects with different concentrations appeared by distinct mechanisms. On the other hand, we found that FP at low concentrations did not show potentiation of cisplatin-induced cytotoxicity in human glioma cells, whereas FP at higher concentrations did in the same manner as in TMZ-treated cells. Although the mechanism of the FP-induced enhancement of the cytotoxicity of DNA-damaging chemotherapeutic agents is still unclear, our data suggest that FP could be useful not only as an antiglioma agent but also as a chemosensitization agent for gliomas.

NEW 7. MR PARAMETERS AS NONINVASIVE BIOMARKERS FOR PREDICTING SURVIVAL IN NEWLY DIAGNOSED PATIENTS WITH GLIOBLASTOMA MULTIFORME

Susan M. Chang, Sarah J. Nelson, Suja Saraswathy, Forrest Crawford, Inas Khayal; Division of Neuro-Oncology, University of California–San Francisco, San Francisco, CA, USA

Our aim was to determine whether noninvasive imaging biomarkers measured either before or after surgery and prior to radiation are predictive of outcome in newly diagnosed glioblastoma multiforme (GBM). We studied 100 patients (56 presurgery and 68 after surgery). Enhancing (CEL), nonenhancing (NEL), and necrotic (NEC) regions were delineated and applied to maps of relative cerebral blood volume (rCBV), apparent diffusion coefficient (ADC), and fractional anisotropy (FA). Choline (Cho), creatine (Cr), *N*-acetylasparate (NAA), lactate (Lac), and lipid (Lip) were assessed. Parameter values were adjusted for age and subjected to proportional hazards survival analysis. Median volumes for presurgery patients were CEL, 15.1 ± 14.1 cc; NEL, 43.4 ± 33.5 cc; and NEC, 3.46 ± 8.5 cc. Lesions with a large percentage of the overall T2 hyperintensity being in the CEL and NEC region ($p = 0.026$, $n = 56$, censored = 15) were correlated with worse survival. Poor survival was also associated with number of voxels with Cho to NAA index (CNI) > 2 and the volume within the T2 hyperintensity with nADC < 1.5. Low ADC in the CEL region or high lipid and high lactate within the CNI2 volume also predicted a worse survival. For the postsurgery patients, all of the absolute measures of residual tumor burden—volumes of the CEL, overall T2 hyperintensity, volume of CNI2, volume within the T2 lesion with nCBV > 3, and volume within the T2 lesion having nADC < 1.5—had a higher risk for poor outcome. In conclusion, the presurgery markers associated with more malignant behavior are thought to correspond to high cell density and regions of hypoxia or necrosis. The postsurgery measures of tumor volume are more closely associated with survival than are initial tumor volumes. This information is likely to be important in defining noninvasive biomarkers for stratifying patients to specific treatment protocols and for planning focal therapy.

NEW 8. TREATMENT OF CNS RADIATION NECROSIS WITH BEVACIZUMAB, AN ANTI-VEGF ANTIBODY

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Bevacizumab, a humanized monoclonal antibody against VEGF, is under investigation to determine its role in the treatment of a variety of solid tumors. This agent, alone and in combination with CPT-11, has also been evaluated in patients with recurrent malignant gliomas and, with a response rate of 63%, shown to be at least on par with other forms of chemotherapy. We hypothesized that since radiation necrosis is associated with cytokine release and what appears to be increased leakiness of small blood vessels, local VEGF release must be taking place. This led to our hypothesis that an anti-VEGF agent such as bevacizumab will reduce postradiation damage (necrosis) to the CNS and, hopefully, help limit neurological signs and symptoms due to radiation necrosis. This has been partially borne out by our recent retrospective study that showed that eight of eight patients with radiation necrosis improved following i.v. bevacizumab with respect to decreased capillary permeability (Gd-contrast enhancement), regional edema (T2 FLAIR), and their daily dexamethasone requirement. To further support this contention and to determine the length of clinical and radiographic improvement following bevacizumab, we initiated a randomized placebo-controlled study. While we cannot present the results of a complete study at this time, we will present a spectrum of patients with radiation necrosis and their diverse MRI and clinical responses to bevacizumab, and the implications of this therapy for future irradiated patients. Specifically, we will present MRI data that include axial T2*, diffusion tensor imaging with ADC mapping, coronal 3D T1 post-Gd, and DCE. In addition, we obtained data from quality of life assessment (MDASI), neurological assessment, steroid dependency, and formal neurocognitive testing. We conclude that bevacizumab is one of the most powerful modern treatments for radiation necrosis in the CNS, and its use deserves serious consideration.

NEW 9. EXPERIMENTAL BRAIN TUMOR THERAPY USING ONCOLYTIC HSV-1 ARMED WITH INTERLEUKIN 12

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Conditionally replicating herpes simplex virus type 1 (HSV-1) vectors are promising therapeutic agents for brain tumors. G47delta is a triple-mutated third-generation oncolytic HSV-1 that exhibits enhanced tumor cell killing and MHC class I expression compared with its parental second-generation G207. We have previously shown that second-generation HSV-1 vectors expressing various immunostimulatory molecules exhibited significantly better antitumor efficacy compared with the control HSV-1 in immunocompetent mouse tumor models. IL-12 was the most efficacious among the immunostimulatory molecules investigated. We further created an "armed" third-generation oncolytic HSV-1 vector (T-mfIL12) that has the single-chain mouse IL-12 gene inserted into the G47delta backbone. The virus was tested against the control virus T-01 in HSV-1–susceptible A/J mice bearing poorly immunogenic Neuro2a (murine neuroblastoma) tumors. When the left tumor alone of bilateral subcutaneous tumors received intratumoral inoculations, T-mfIL12 showed a significantly better antitumor activity than did T-01 not only in the inoculated tumors but also in the noninoculated remote tumors. When A/J mice bearing intracerebral tumors were treated by a single intratumoral inoculation, both T-01– and T-mfIL12–treated animals survived significantly longer than did mock-treated animals, although no significant difference was observed between T-01 and T-mfIL12. T-mfIL12 showed significantly higher antitumor effect than T-01 on subcutaneous tumors even when administered intravenously. Also, in A/J mice with systemic Neuro2a metastases, i.v. T-mfIL12 significantly prolonged the survival compared with T-01. Studies using athymic mice revealed that the enhancement of antitumor effect of T-mfIL12 over T-01 in remote tumors required T-cells. None of the animals exhibited toxicity at the doses tested. A clinical research using G47delta in recurrent glioblastoma patients is currently under way at the University of Tokyo. G47delta armed with IL-12 may be a good candidate for the next-generation oncolytic HSV-1 to be applied clinically.

NEW 11. DUAL-SPECIFIC IMMUNOTOXIN, D2C7 (SCDSFV)-PE38KDEL, FOR BRAIN TUMOR TREATMENT

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Brain tumors are a heterogeneous group of cancers with the poorest outcome among human cancers. National Cancer Institute statistics indicate that 20,500 new cases of malignant brain tumors were diagnosed in 2007, and 12,740 deaths from this disease were predicted to occur. The epidermal growth factor receptor (EGFR), expressed by most epithelial cells, is overexpressed in gliomas: by 27%–57% in astrocytomas, 71%–94% in anaplastic astrocytomas, and 60%–90% in glioblastoma multiforme (GBMs). Further, 58%–61% of all GBMs also express the EGFRvIII mutant, which is not found in normal tissues. Monoclonal antibodies targeting either the wild-type EGFR (EGFRwt) or EGFRvIII have been developed, one of which, D2C7, a murine IgG1κ, recognizes both EGFRwt and EGFRvIII. We have cloned a novel, recombinant single-chain variable-region antibody fragment from the D2C7 hybridoma and have engineered a disulfide-stabilized linkage between variable heavy and light domains to generate D2C7 (scdsFv). The D2C7 (scdsFv) is connected to a truncated variant of *Pseudomonas* exotoxin A, carrying a C-terminal KDEL peptide for improved intracellular transport (PE38KDEL). The binding affinity of this immunotoxin for the EGFRwt and the EGFRvIII extracellular domain was 6.3×10^8 $(mol/L)^{-1}$ and 7.8×10^8 $(mol/L)^{-1}$, respectively, as measured by surface plasmon resonance. Flow cytometry of EGFRwt-transfected (NR6W), EGFRvIII-transfected (NR6M), and parental NR6 cells, a mouse 3T3 fibroblast line that lacks EGFR, further confirmed the dual specificity of D2C7 (scdsFv)-PE38KDEL for both forms of transfected cells. The immunotoxin was highly cytotoxic, with an IC_{50} of 1 ng/ml and 3 ng/ml, respectively, in cells expressing EGFRwt (NR6W) and EGFRvIII (NR6M), but not cytotoxic at 1,000 ng/ml in the parental NR6 cells. The IC_{50} of D2C7 (scdsFv)-PE38KDEL on the pediatric GBM cell line D2159MG, expressing both EGFRwt and EGFRvIII, was 3 ng/ml. Because of its good affinity and cytotoxicity, this immunotoxin merits further evaluation for treatment of brain tumors.

NEW 12. RECOMBINANT ANTIBODY-BASED MOLECULAR THERAPEUTICS FOR BRAIN TUMOR IMMUNOTHERAPY

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The limited efficacy of surgery, radiotherapy, and chemotherapy in the treatment of malignant glioma calls for innovative approaches targeting specific biological features of these tumors. Monoclonal antibodies (MAbs), with high specificity and affinity for their target antigens, can be utilized in targeted therapy for delivery of agents such as radionuclides, enzymes, drugs, or toxins in vivo. Success in MAb-based therapy for brain tumor requires the identification of glioma-associated antigens and the proper selection of target-specific MAbs for each patient. We have validated a panel of promising molecular malignant glioma targets, such as epidermal growth factor receptor type III variant (EGFRvIII) and human transmembrane glycoprotein nmb (GPNMB), and we have performed genetic and immunohistochemical (IHC) evaluation of gliomas to determine incidence, distribution, and pattern of localization of specific antigens in brain tumors, as well as survival analysis. Univariate and multivariate analyses correlated expression of GPNMB with survival of 39 GBM patients using RNA expression and IHC data, establishing that these patients, whose tumors have relatively high mRNA GPNMB transcript levels, >3-fold over normal brain, as well as positive immunohistochemistry, have a significantly higher risk of death. We have developed both murine and human high-affinity single-chain fragment antibodies from phage and yeast display libraries and immunotoxins with *Pseudomonas* exotoxin PE38 that are reactive with either EGFRvIII or GPNMB in vitro and in vivo. Antibody engineering also provides a powerful approach for redesigning Abs for use in oncological applications: in preclinical tests for malignant glioma treatment, affinity-matured recombinant Ab fragments in the form of scFv-CH3 fusion and two immunotoxins, MR1-1(dsFv)PE38 for EGFRvIII and F6V(scFv)PE38 for GPNMB, all demonstrate efficient tumor targeting and offer the promise of improved tumor control without substantial toxicity. The IND of MR1-1 immunotoxin was issued in 2007, and phase I clinical trials are ongoing at Duke.

NEW 13. INDUCTION OF IMMUNOLOGIC AND CLINICAL RESPONSES WITH EGFRVIII-TARGETED VACCINE (CDX-110) WITH CYCLES OF TEMOZOLOMIDE IN PATIENTS WITH NEWLY DIAGNOSED EGFRVIII-POSITIVE GBM

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For the treatment of GBM, the efficacy of conventional therapy is limited by nonspecific toxicity. The immunologic targeting of tumor-specific gene mutations may allow more precise elimination of neoplastic cells. The epidermal growth factor receptor variant III (EGFRvIII) is a consistent and immunogenic mutation that encodes a tumorigenic, constitutively active tyrosine kinase. EGFRvIII is not expressed in any normal tissues but is widely expressed in GBMs and other neoplasms, making it an attractive target for immunotherapy. A phase II multicenter, prospective clinical trial was undertaken to assess the immunogenicity and efficacy of an EGFRvIII-specific peptide vaccine in patients with EGFRvIII$^+$ GBM in combination with standard or continuous temozolomide (TMZ). After resection and radiation with concurrent TMZ (75 mg/m^2/day), consecutive cohorts received subsequent monthly cycles of 200 mg/m^2 ($n = 13$) or continuous 75 mg/m^2 ($n = 7$) TMZ simultaneously with intradermal vaccinations with an EGFRvIII-specific peptide (PEPvIII) conjugated to keyhole limpet hemocyanin (KLH) (CDX-110) along with granulocyte-macrophage colony-stimulating factor. Subjects received vaccinations until tumor progression or death. There was one allergic reaction, but no other SAEs. We observed no differences in vaccine immunogenicity ($p < 0.999$; binomial proportions), PFS ($p = 0.7979$; log-rank), or OS ($p = 0.7728$; log-rank) between TMZ regimens. Although TMZ induced grade II lymphopenia in 53.8% of patients, the coadministration of TMZ with CDX-110 results in strong sustained immune responses to EGFRvIII in 100% (95% CI: 0.72, 1.00) of evaluated patients. Median PFS was 16.6 months (95% CI, 9.1–22.7 months). Median survival has not been reached. Study survival is better than a matched historical control group (14.3 months; 95% CI, 13.0–16.2 months; $p < 0.0001$; log-rank) and a subgroup treated with TMZ (15.2 months; 95% CI, 13.9–20.5 months; $p = 0.0078$). CDX-110 peptide vaccination with standard-of-care temozolomide in patients with GBM appears very promising and is under investigation in a phase III, randomized clinical trial.

NEW 14. TYPE 1 DENDRITIC CELL (DC) VACCINES IN COMBINATION WITH POLY-ICLC IN PARTICIPANTS WITH RECURRENT MALIGNANT GLIOMA

Hideho Okada[1], Frank S. Lieberman[1], Pawel Kalinski[1], L. Dade Lunsford[1], Amin Kassam[1], Arlan H. Mintz[1], David L. Bartlett[1], Charles K. Brown[1], Herbert Zeh[1], Theresa L. Whiteside[1], Lisa H. Butterfield[1], Ronald L. Hamilton[1], Andres M. Salazar[2], Ian F. Pollack[1]; [1]Department of Neurological Surgery, University of Pittsburgh, Pittsburgh, PA, USA; [2]Oncovir, Inc., Washington, DC, USA

Our previous preclinical studies have demonstrated that intramuscular (i.m.) administration of a Toll-like receptor 3 ligand poly-ICLC remarkably enhances induction of type 1 cytotoxic T-lymphocytes (CTLs) when combined with vaccinations against glioma-associated antigen (GAA)-derived CD8$^+$ T-cell epitopes. Based on these studies, we have developed a phase I/II trial of poly-ICLC–assisted type 1 dendritic cell (DC)-based vaccines. Human leukocyte antigen (HLA)-A2$^+$ participants with recurrent malignant glioma received intra-lymph nodal injections of 1×10^7 type 1 DCs loaded with HLA-A2–binding peptides derived from EphA2, IL-13Rα2, YKL-40, and GP100 for four times with 2-week intervals. Participants also received i.m. injections of 20 μg/kg poly-ICLC twice weekly. Primary end points are assessments of safety and immunological responses. Clinical and radiological responses were also evaluated. To date, scheduled four vaccinations spanning 7 weeks were completed in five participants with no major adverse events, despite the historical median time for re-recurrence of recurrent glioblastoma multiforme of 6–8 weeks. Two participants withdrew from the study due to rapid tumor progression before or following the first vaccination. Immunological analyses were completed in three of five participants who completed at least four vaccinations. Increased CD8$^+$ cells reactive to HLA-A2.1-EphA2 (883–891) and HLA-A2.1-IL-13Rα2 (345–353) tetramers were detected in postvaccine peripheral blood mononuclear cells (PBMCs) in two participants. Upregulation of a chemokine receptor CXCR3 on CD8$^+$ PBMCs was also noted following vaccinations, suggesting induction of type 1 CTL responses. One of these participants with recurrent GBM exhibited partial radiological response, which persisted for 7 months with booster vaccines. Biopsy of the postvaccine tumor in this participant revealed intensive infiltration of CD8$^+$ T-cells and macrophages. These data demonstrate preliminary safety and immunological activity of poly-ICLC–assisted type 1 DC-based vaccines. The present study especially demonstrates for the first time induction of specific reactivity against novel IL-13Rα2–derived and EphA2-derived epitopes in vaccine recipients.

NEW 16. REAL-TIME MONITORING FOR CONVECTION-ENHANCED DELIVERY OF INTRATUMORAL CHEMOTHERAPY IS PREDICTIVE OF RESPONSE IN CANINE SPONTANEOUS GLIOMAS

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Convection enhanced delivery (CED) of therapeutic agents into brain is a promising treatment strategy for the treatment of intracranial gliomas. However, current inability to accurately define the extent and location of individual infusions in real time considerably limits therapeutic efficacy at the individual level as well as the ability to objectively assess therapeutic efficacy in prospective clinical trials. We hypothesized that newly developed nanoparticle agents can be used to treat brain tumors, via systemic administration or CED with MRI guidance. For systemic treatment, nanoliposomal CPT-11 is a novel liposome-based nanoparticle featuring highly stable drug encapsulation in a long-circulating carrier. In the rat intracranial U87 tumor xenograft model, i.v. treatment with nanoliposomal CPT-11 produced 13-fold higher drug exposure in tumors based on tissue AUC than did free CPT-11. Systemic treatment with nanoliposomal CPT-11 resulted in significantly improved survival, including apparent cures in some animals, compared with free CPT-11. Using CED of liposomal nanoparticles containing the topoisomerase I inhibitor CPT-11 and the surrogate marker gadoteridol, we show that direct infusion into spontaneously occurring gliomas in dogs is feasible and efficacious. Moreover, clinical efficacy as defined by decreased tumor volume, apparent increase in survival, and modulation of tumor phenotype at necropsy was objectively shown to be correlated with infusion volume of distribution, location, and leakage as determined by real-time MRI. Delivery of both the therapeutic agent and gadolinium-based marker in a liposomal formulation resulted in minimal clinical or histopathological evidence of toxicity in normal brain. These data strongly suggest that real-time imaging should be an essential component of therapeutic trials in intracranial tumors when convection-enhanced delivery strategies are used.

NEW 17. A CLINICAL TRIAL OF CATIONIC LIPOSOMES CONTAINING INTERFERON-B GENE FOR PATIENTS WITH MALIGNANT GLIOMA

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Cationic liposome-mediated interferon-β (IFN-β) gene transfer has been found to induce regression of experimental glioma. We performed a pilot clinical trial to evaluate safety and effectiveness of this IFN-β gene therapy in five patients with malignant glioma (glioblastoma multiforme or anaplastic astrocytoma). Two patients showed >50% reduction and others had stable disease 10 weeks after the treatment initiation. Autopsy revealed that tumor tissues showed dramatic changes after therapy in all patients. Many tumor cells showed necrotic changes, and immunohistochemistry identified many $CD8^+$ lymphocytes and macrophages infiltrating in tumor and surrounding tissues, probably resulting from therapeutic effect. Simultaneously, numbers of MIB-1–positive cells were notably decreased. No adverse findings associated with the clinical trial were observed pathologically. In order to identify alterations in gene expression in brain tumors 2 weeks after gene therapy trial, we used microarray technology, which allows us to examine the expression of a large number of genes simultaneously. Interestingly, using hierarchical clustering and principal component analysis, five series of gene therapy trials were classified according to response to IFN gene therapy. There were changes in gene expression that could be predicted on the basis of previous studies. It confirms the validity of the methods. Moreover, novel patterns of altered gene expression were identified, suggesting the involvement of pathways not previously described as being involved. In sum, this study suggests the feasibility and safety of IFN-β gene therapy, which may become an important treatment option for patients with malignant glioma.

NEW 18. ANTI-EGFR ANTIBODIES IN A HIGHLY INVASIVE XENOTRANSPLANT MODEL OF HUMAN GLIOMA

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To evaluate the possible efficacy of therapeutic antibodies against EGFR, an experimental situation is desirable that recapitulates the invasive nature of human glioma cells. Direct implantation of short-term cultured human glioblastoma spheroids resulted in diffuse tumor growth. Treatment of these xenografts with intracranially administered C225 (anti-EGFR) or with intraperitoneal DC101 (anti-VEGFR) was assessed by histology after sacrifice of the animals, comparing landmark points on serial brain sections. Three of seven cases treated with C225 responded with significant tumor inhibition. Four series did not show any effect of C225. The responsive tumors showed EGFR amplification as well as expression of the truncated EGFRvIII variant. Interestingly, amplification and vIII expression were maintained in the xenografts. In the nonresponsive tumors, EGFR was not amplified and vIII was not expressed. The responsive tumors showed an increased rate of apoptosis and reduced invasion as well as proliferation. None of the four cases treated with DC101 showed any effect on any of the parameters, especially as their growth appears to be angiogenesis independent. It is apparent that inhibition of glioblastoma growth can be achieved with an antibody to EGFR that can be administered intracranially by direct infusion. The response to C225 and likely other antibodies to the EGFR will depend on its amplification and the expression of the vIII variant. Anti-VEGFR treatment appears to have no effect in a nonangiogenic situation.

NEW 19. DICER-REGULATED MICRORNAS 222 AND 339 PROMOTE IMMUNE ESCAPE OF CANCER CELLS THROUGH DOWNREGULATION OF ICAM-1

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The RNase III endonuclease Dicer plays a key role in generation of microRNAs (miRNAs) in cells. Recent studies on the roles of Dicer by others led us to hypothesize that expression of Dicer in cancer cells might regulate their susceptibility against immune-surveillance through processing of miRNAs. Using three human colorectal cancer cell lines (HCT116, DLD-1, and RKO) in which exon 5 of the human Dicer gene is disrupted ($ex5^{-/-}$), we have found Dicer ($ex5^{-/-}$) disruption upregulates intercellular cell adhesion molecule-1 (ICAM-1) and renders these cells more susceptible to antigen-specific lysis by cytotoxic T-lymphocytes (CTLs) compared to parental cells. Antibody-mediated blockade of ICAM-1 inhibited the specific lysis of the CTLs against ($ex5^{-/-}$) cells, indicating a pivotal role of ICAM-1. Other immune-response mediators examined, including HLA class I, Fas, CD40, and death receptor 5 (DR5), were not differentially expressed between ($ex5^{-/-}$) cell lines and corresponding wild-type cells. We next sought to identify specific miRNAs that can modulate ICAM-1 expression. Based on the miRBase algorithm, miRNAs 222 and 339 were predicted to bind to ICAM-1. Indeed, quantitative RT-PCR analysis showed the decrease of miRNAs 222 and 339 in all three ($ex5^{-/-}$) cell lines compared to wild-type lines. Moreover, three human glioma cell lines (U251, SNB19, and A172) exhibited higher expression levels of miRNAs 222 and 339 than did HCT116 colon cancer cells. Inhibition of these miRNAs in these glioma cells by transfection with anti-miRNA 222 and 339 RNA-based inhibitor constructs upregulated ICAM-1 expression and susceptibility of these cells against CTLs. Immunohistochemical analyses of primary glioma sections indicated strong expression of Dicer in a majority of GBM cases. Taken together, our results suggest that Dicer is responsible for the generation of miRNA 222 and miRNA 339, which suppress ICAM-1 expression on cancer cells, including glioma cells, thereby downregulating the susceptibility of cancer cells to CTL-mediated lysis.

NEW 20. THE DNA DEMETHYLATING AGENT 5-AZA-2-DEOXYCYTIDINE INDUCES THE EXPRESSION OF CANCER-TESTIS ANTIGENS IN HUMAN GLIOMAS: EPIGENETIC TARGET FOR TUMOR IMMUNOTHERAPY

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The aim of this study was to define the clinical potential of the DNA demethylating agent 5-aza-2-deoxycytidine (5-aza-CdR) for application in cancer-testis antigen (CTA)-based immunotherapy in human gliomas. By RT-PCR and Western blotting, the induction of CTA expression in a panel of glioma cells treated with 5-aza-CdR was analyzed. Methylation-specific PCR and Pyrosequencing technology determined the methylation status of NY-ESO-1, one of the most immunogenic CTAs, following 5-aza-CdR treatment. Microarray assessed the changes in gene expression following 5-aza-CdR, and human leukocyte antigen (HLA) class I upregulation was confirmed by flow-cytometric analysis. The functional activity of de novo expressed CTA was evaluated by using ^{51}Cr release cytotoxicity assays using NY-ESO-1–specific CTL lines and an orthotopic xenograft model following systemic administration of 5-aza-CdR and the adoptive transfer of NY-ESO-1–specific CTLs. While the expression of CTAs was hardly noted in human gliomas, it was remarkably induced by 5-aza-CdR in glioma cells but not in normal human cells. By quantitative measurement, following 5-aza-CdR, methylation levels were significantly decreased. Microarray revealed that the agent is capable of signaling the immune system. Antigenic peptides derived from de novo induced CTA were recognized by NY-ESO-1–specific CTLs. The adoptive transfer of NY-ESO-1–specific CTLs resulted in significant volume reduction of transplanted tumors and prolonged the survival of the animals after systemic administration of 5-aza-CdR. These results suggested that 5-aza-CdR induces the expression of epigenetically silenced CTAs in poorly immunogenic gliomas and thereby presents a new strategy for tumor immunotherapy targeting 5-aza-CdR–induced CTAs. As of January 2008, this study is in press for publication in the *International Journal of Cancer.*

NEW 22. WT1 (WILMS TUMOR GENE) PEPTIDE VACCINATION FOR PATIENTS WITH RECURRENT GLIOBLASTOMA

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The purpose of this study was to investigate the clinical responses of immunotherapy targeting WT1 gene product in patients with recurrent glioblastoma. Twenty-one patients with WT1/HLA-A*2402–positive recurrent glioblastoma were evaluated in a study of WT1 vaccine therapy. All patients

were resistant to standard therapy. Patients were intradermally injected with an HLA-A*2402–restricted, modified 9-mer WT1 peptide every week for 12 weeks. The responses were analyzed by RECIST criteria 12 weeks after the initial vaccination. Patients who achieved an effective response continued to be vaccinated until tumor progression occurred. Progression-free survival rate after initial WT1 treatment was estimated. WT1 peptide/HLA-A*2402 tetramer assay to assess WT1-specific $CD8^+$ CTL frequencies was performed in the patients before and after WT1 vaccination and in healthy controls. The protocol was well tolerated; only local erythema occurred at the WT1 vaccine injection site. The clinical responses included 2 patients with PR, 10 patients with SD, and 9 patients with PD. No patients had CR. The overall response rate (CR + PR) was 9.5%, and the disease control rate (CR + PR + SD) was 57.1%. The median progression-free survival period was 20.0 weeks, and progression-free survival at 6 months (26 weeks) was 33.3%. The frequencies of WT1-specific CTLs before WT1 vaccination were significantly higher in patients with glioblastoma than in healthy controls. There were no correlations between the induction of a clinical response and WT1-specific CTL frequencies on the PBMCs of the patients prior to vaccination. Although a small uncontrolled nonrandomized trial, this study showed that WT1 vaccine therapy for patients with WT1/HLA-A*2402–positive recurrent glioblastoma was safe and had a clinical response. WT1 protein in glioblastoma cells was thought to be naturally immunogenic. Based on these results, further clinical studies of WT1 vaccine therapy in patients with malignant glioma are warranted.

NEW 23. IDENTIFICATION OF AN HLA-A24–RESTRICTED T-CELL EPITOPE DERIVED FROM A GLIOMA-ASSOCIATED ANTIGEN, INTERLEUKIN 13 RECEPTOR A2 CHAIN

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The HLA-A24 allele is highly expressed in Asians; it is expressed in 60% of the Japanese population and also in a significant number of people belonging to other ethnicities. The type α2 receptor for interleukin 13 (IL-3Rα2) has been shown to be one of the glioma-specific antigens and to be abundantly expressed in a vast majority of high-grade astrocytomas. Here, we first investigated the suitability of IL-3Rα2 as a target antigen for malignant gliomas, and then identified a potential HLA-A24–restricted peptide derived from IL-3Rα2. The expression of IL-3Rα2 in glioma tissues was examined by RT-PCR analyses. To identify the desired epitope, we selected five candidate peptides from IL-3Rα2 using a computer-based algorithm that were predicted to bind to HLA-A24. The lytic activity of the CTLs induced by the peptide-pulsed dendritic cells was analyzed against various kinds of glioma cell lines and freshly isolated human glioma cells. In a series of gliomas from 29 glioma patients, we found that more than 50% high-grade gliomas express IL-3Rα2. Of five peptides, peptide P174 (WYEGLDHAL) was found to be the most useful for the induction of the HLA-A24–restricted and IL-3Rα2–specific CTLs. A CTL line induced by P174 also showed antigen-specific cytotoxicity against surgically removed glioma cells depending on their expression of IL-3Rα2 and HLA-A24. Our results showed that IL-3Rα2 is one of the attractive glioma-specific antigens, and the immunogenic peptide (WYEGLDHAL) may contribute to peptide-based immunotherapy against malignant gliomas for HLA-A24 patients.

CLINICAL TRIALS

CT 1. TREATMENT RESULT OF ANAPLASTIC OLIGODENDROGLIOMAS: PERSONAL EXPERIENCE

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The authors reviewed their surgically proven patients with anaplastic oligodendroglioma to assess prognostic significance of tumor location, extent of surgical removal, radiotherapy, and chemotherapy. Hospital records of the 34 cases of pathologically proven anaplastic oligodendroglioma, which had been operated on at the two institutions between 1988 and 2007, were reviewed. Clinical presentations, radiological and pathological features, and overall survival were evaluated and assessed. There were 16 men and 18 women, with mean age at diagnosis of 40 years (range, 8–70 years). The most common presenting symptom was seizure followed by headache. Twenty-two patients are alive with or without evidence of tumor with a mean follow-up of 68 (range, 8–162) months. Eleven patients died of tumor recurrences or progression to glioblastoma during the follow-up period. Recurrence or progression was noticed from 7 to 160 months after initial treatment. Seventeen patients underwent more than two surgeries. All patients underwent craniotomy with intention of radical tumor removal in noneloquent locations with aid of intraoperative ultrasound or frameless navigation. The extent of surgical removal was assessed by immediate postoperative CT or MRI scans. Gross total removal was achieved in 18, subtotal in 12, and partial in 2 cases. Postoperatively, conventional radiotherapy was given to 30 patients. Since 1999, PCV (procarbazine, CCNU, vincristine) chemotherapy was also recommended for those patients with favorable KPS. Two to seven cycles of PCV chemotherapy have been routinely given as long as the patient tolerated the regimen. In this study, the authors confirmed the good prognostic effect of radical removal of the tumor; however, additive beneficial effect of PCV chemotherapy in patients with anaplastic oligodendroglioma could not be verified. However, there was some tendency toward a higher long-term progression-free survival rate in patients receiving chemotherapy than in those undergoing radiotherapy alone.

CT 2. BRAIN ATROPHY AND COGNITIVE FUNCTION FOLLOWING CHEMOTHERAPY TO OLIGODENDROGLIOMA

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The notion that oligodendrogliomas show good response to chemotherapy allowed its use as an initial treatment, deferring radiotherapy. We have treated 16 oligodendroglial tumors (5 OG, 6 AO, and 5 AOA) with one to eight courses of PAV (procarbazine, ACNU, vincristine) therapy without radiotherapy. Of those, six patients developed noticeable brain atrophy on MRI 14–34 months after the initiation of the treatment. However, none of the six patients showed any significant decline in cognitive function. Therefore, brain atrophy can occur when chemotherapy was given as a sole treatment to oligodendrogliomas, although the much-feared cognitive function damage has not been observed in our series.

CT 3. AN ULTRASTRUCTURAL, IMMUNOHISTOCHEMICAL, AND MORPHOMETRIC STUDY OF OLIGODENDROGLIOMAS WITH RESPECT TO CHROMOSOME 1P DELETION

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Deletion of chromosome 1p is a common genetic alteration in oligodendrogliomas and is prognostically significant. We studied 1p deletion by fluorescence in situ hybridization as well as ultrastructural (EM), immunohistochemical (IHC), and morphometric features on 13 cases of deleted and 9 cases of nondeleted oligodendrogliomas. They included 12 grade (Gr) II oligodendrogliomas (Os), 1 Gr II oligoastrocytoma (OA), 1 Gr III O, and 4 Gr III OAs. All studies were performed in oligodendroglial components. On EM, all tumors contained oligodendroglia-like cells (OLC) with lucent to organelle-rich cytoplasm and few or no intermediate filaments. Three features, myelin-like figures, intermediate filaments (IF), and synapses, suggested oligodendroglial, astrocytic, and neuronal status, respectively. Although morphologic characteristics of these three features were identical between deleted and nondeleted tumors, nondeleted tumors had significantly higher incidence of IF. On IHC, immunoreactivity for glial fibrillary acidic protein was present in all tumors. In addition, focal NeuN or synaptophysin immunoreactivities were seen in 10 of deleted and 7 of nondeleted tumors. Statistical analysis on semiquantification for IHC revealed no significant difference between the deleted and nondeleted tumors. On morphometry for nuclei of OLC, no studied parameters except for direction differed significantly between deleted and nondeleted Gr II tumors. Conversely, all parameters except for direction differed significantly between deleted and nondeleted in Gr III tumors. In summary, astrocytic features were more frequent in nondeleted tumors; deleted and nondeleted Gr II oligodendrogliomas were morphologically indistinguishable. These results suggest that oligodendrogliomas are tumors capable of astrocytic and focal neuronal differentiation, which are the characteristics of precursor or stem cells. The 1p deletion appears to represent an epiphenomenon superimposed upon the basic phenotype of oligodendrogliomas.

CT 4. GENETIC PROFILE IN 1P- AND 19Q-DELETED OLIGODENDROGLIOMA WITH SHORT RELAPSE-FREE SURVIVAL

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Oligodendrogliomas (OLs) with combined allelic loss of 1p and 19q have been associated with longer relapse-free and overall survival. However, a subset of 1p- and 19q-deleted OLs recur early despite postoperative chemoradiotherapy. In this study, we investigated the specific genetic or chromosomal abnormalities found in 1p- and 19q-deleted OLs with short relapse-free interval. Since 2000, we have analyzed six and eight patients with histologically verified OL and anaplastic oligodendroglioma (AO), respectively. Combined loss of both 1p and 19q were confirmed in all cases using fluorescence in situ hybridization (FISH). Postoperatively, all patients received chemotherapy (eight cycles of procarbazine + ACNU + vincristine) and radiation therapy. The follow-up period was the time from diagnosis until death or final contact with the patient. Genetic and chromosomal abnormalities were determined by FISH and/or array-comparative genomic hybridization (array-CGH). Two of six OLs (33%) and two of eight AOs (25%) recurred within 24 months after initial diagnosis. The remaining 10 cases showed no tumor recurrence during the follow-up period. EGFR amplification was identified in all of recurrent OLs and AOs by FISH and array-CGH. However, only two AO cases demonstrated EGFR overexpression by immunohistochemical assay. Allelic loss of 10q23 was observed in two recurrent AO cases. In contrast, these alterations were seen in none of the patients without tumor recurrence. These results suggest that 1p- and 19q-deleted oligodendroglioma with EGFR amplification or 10q loss, which genetically mimics glioblastoma, tends to recur early. FISH is a simple and useful technique for detecting EGFR amplification in tumor cells.

CT 5. DIFFERENTIAL ASSOCIATION OF CHROMOSOME 19 SNP VARIANTS WITH OLIGODENDROGLIOMA: A CASE–CASE COMPARISON

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A t(1;19)(q10;p10) has been associated with oligodendrogliomas (OLIGO), as have combined deletions of 1p and 19q in these tumors. In small pilot studies, SNPs mapped to 19q have been associated with the development of gliomas and with glioma prognosis. The objectives of this study were to verify previous 19q SNP associations and to identify new markers on chromosome 19 that may be associated with OLIGO risk. To efficiently assess the effect of the candidate SNPs and morphology on glioma risk, we applied a case–case design comparing OLIGO and GBM (glioblastoma) and stratified by 19q deletion status. Ninety-five OLIGO, 85 mixed oligoastrocytoma (MOA), and 125 GBM cases were included in this study. The numbers of 19q-deleted and nondeleted OLIGO/MOA cases were 67 and 62, respectively. A total of 3,704 Affymetrix STY-1 250K GeneChip chromosome 19 SNPs were evaluated. Subjects with an overall call rate >90% were excluded. SNPs with genotype call rate >90% and minor allele frequency >1% were included. An ordinal logistic regression model tested the association of SNPs across groups. Comparing OLIGO/MOA with GBM cases yielded three associated chromosome 19 SNPs with p-values < 0.001: rs299170, rs2279060, and rs11670672. Comparing 19q-deleted and nondeleted OLIGO/MOA cases yielded one chromosome 19 SNP with a p-value < 0.001: rs12978873. Using an SNP-scanning method, we evaluated haplotypes across chromosome 19. A three-SNP haplotype including rs299170 and a four-SNP haplotype including rs7253105 exhibited simulated p-values of 0.00005 and 0.012, respectively, when OLIGO/MOA and GBM cases were compared. A five-SNP haplotype including marker rs12978873 exhibited a simulated p-value of 0.016 when 19q-deleted and nondeleted OLIGO/MOA cases were compared. Our results support previous observations that SNPs mapped to 19q may be associated with the development of oligodendrogliomas, especially oligodendrogliomas with 19q deletion. These associations warrant replication. (This research was supported by grant CA108961.)

CT 6. AGE, PREOPERATIVE KPS, AND MGMT PROMOTER HYPERMETHYLATION DEFINE SURVIVAL OF THE PATIENTS WITH GLIOBLASTOMA

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Glioblastoma (GBM) is a highly lethal neoplasm with a median survival of 12–14 months. Only 2%–5% of patients originally diagnosed with GBM will survive >3 years. Whether tumors from these long-term survivors (LTSs) exhibit molecular genetic differences compared with short-term GBM survivors is not known. Tumors from 18 patients initially diagnosed with GBM and having survival >3 years (LTS) was compared with 20 GBMs from short-term survivors (STSs, <1.5 years) for MGMT aberrations (promoter hypermethylation/expression), p53, PTEN mutations, and proliferation index. The STSs were chosen from patients with GBM who received more than subtotal resection and postoperative radiotherapy. MGMT promoter hypermethylation was significantly more frequent in the LTS group. Similarly, MGMT expression was more frequent in the STS group. The p53 mutation was more frequent in the LTSs, and this group had a lower rate of the PTEN mutation. Proliferation index was slightly higher in the STS patients. However, these results were not statistically different. By exclusion of therapeutic background, we noted the differences in age, preoperative KPS, and the rate of MGMT promoter hypermethylation. These results contribute to the prediction of the survival for patients with GBM.

CT 7. MGMT PROMOTER METHYLATION STATUS IS A STRONG PREDICTIVE FACTOR FOR RESPONSE TO ACNU (NITROSOUREA)-BASED RADIATION THERAPY IN NEWLY DIAGNOSED GLIOBLASTOMAS

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MGMT (O^6-methylguanine-DNA methyltransferase) promoter methylation status is associated with a survival benefit in glioblastoma (GBM) patients treated with temozolomide (TMZ). Radiation therapy (RT) plus ACNU (nitrosourea) was widely used for the treatment with newly diagnosed GBM patients in Japan before the TMZ era. A combination therapy with carboplatin and etoposide (CE) is used for recurrent GBM patients as a second-line chemotherapy. A retrospective study was performed in order to examine the relationship between MGMT methylation status and the survival benefit treated with these regimens in GBM patients. Sixty-three newly diagnosed GBM patients were treated with in National Cancer Center Hospital in Japan from 2000 to 2005. RT plus ACNU was used for 47 patients <70 years old. Sixteen patients among them were treated with CE regimen after recurrence. Sixteen patients >70 years old were treated with only RT without chemotherapy. MGMT methylation status was examined by MGMT-specific PCR. MGMT promoter was methylated in 42 patients among a total of 65 patients (64.6%). Median survival time (MST) in 32 patients with methylated MGMT promoter and 15 patients with unmethylated MGMT promoter were 17.9 months and 11.9 months, respectively ($p = 0.02$). MIB-1 staining indexes of each group are 34% and 30%, respectively. Progression-free survival (PFS) of each patient is 5.1 months and 4.4 months, respectively ($p = 0.08$). PFS with CE regimen after recurrence in 10 patients with methylated MGMT and 6 patients with unmethylated MGMT were 3.7 months and 4.9 months, respectively ($p = 0.8$). MST in 11 patients with methylated MGMT and 5 patients with unmethylated MGMT treated with only RT were 6.5 months and 4.0 months, respectively. We conclude that MGMT promoter methylation status is a strong predictive factor in survival of GBM patients treated with RT plus ACNU but not in those treated with the CE regimen after recurrence.

CT 9. PHASE II STUDY OF LOW-DOSE ICE (IFOSFAMIDE, CARBOPLATIN, AND ETOPOSIDE) FOR RECURRENT GLIOBLASTOMA

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The purpose of this study was to evaluate the efficacy and tolerability of ifosfamide, carboplatin, and etoposide (ICE) in patients with recurrent glioblastoma. This was an open-label, single-center phase II trial. Forty-two patients with first recurrent glioblastoma after surgery, standard radio-

therapy, and a firstline temozolomide-based or ACNU-based chemotherapy were enrolled. The primary end point was progression-free survival at 6 months (PFS-6), and secondary end points were response rate, toxicity, and survival. Chemotherapy consisted of ifosfamide (700 mg/m^2 on days 1, 2, and 3), carboplatin (100 mg/m^2 on day 1), and etoposide (70 mg/m^2 on days 1, 2, and 3) every 4 weeks. PFS-6 was 37%. The median PFS was 17 weeks. Response rate was 27%. Adverse events were generally mild (grade 1 or 2) and consisted mainly of alopecia. This regimen is thus well tolerated and has activity in patients with recurrent glioblastoma.

CT 10. A CLINICAL TRIAL FOR MALIGNANT GLIOMAS BY JCOG-BRAIN TUMOR STUDY GROUP (JCOG 0305)

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Japan Clinical Oncology Group-Brain Tumor Study Group (JCOG-BTSG) conducted a multi-institutional randomized controlled trial on malignant gliomas titled A Randomized Controlled Phase II/III Study of Chemoradiotherapy using ACNU versus Procarbazine and ACNU for Astrocytoma Grade 3 and 4, with the support of the Health and Labour Sciences Research Grants of the Ministry of Health, Labour, and Welfare, in order to establish a standard therapy for malignant gliomas in Japan. The patients with newly diagnosed supratentorial astrocytoma grade 3 or 4 were enrolled and randomized into two groups. The patients in group A were treated with ACNU (80 mg/m^2 i.v.) during postoperative radiotherapy (RT, 60 Gy local), while those in group B received procarbazine (80 mg/m^2 for 10 days p.o.) preceding administration of ACNU. Each regimen was continued every 8 weeks for 2 years if it was tolerable for the patients and their disease did not progress. The primary end point was the overall survival rate, and the secondary end points were the response rate on the MRI and the frequency of adverse events. Procarbazine is expected to reduce O^6-methylguanine-DNA methyltransferase (MGMT) and enhance the anticancer activity of nitrosoureas. This study started as a randomized phase II trial for group B, and it enters the phase III study after the safety and efficacy of the group B regimen is confirmed. The protocol was activated in April 2004, and 111 patients were registered by the end of August 2006 from the collaborating neurosurgical institutes of JCOG-BTSG. The overall survival of the patients treated with ACNU + RT was 16.2 months, and that of procarbazine + ACNU + RT was 18.7 months, while PFS of both groups were 6 months in the phase II stage. CTCAE grade 3/4 adverse events were observed in 40%–60% of the patients. We conclude that ACNU-based chemoradiotherapy is an effective but toxic treatment.

CT 11. PROGNOSTIC VARIABLES IN RECURRENT HIGH-GRADE GLIOMA CLINICAL TRIALS: AN NCCTG AND NABTC JOINT ANALYSIS

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To assess the impact of prognostic variables on outcomes, we analyzed results from 1,065 patients from 27 NCCTG or NABTC recurrent high-grade glioma trials. Variables assessed include gender, age, performance score (PS), race, extent of primary resection, year of study entry, last known histology, time since initial diagnosis, corticosteroid use, prior chemotherapy, prior nitrosoureas, anticonvulsant use, prior temozolomide (TMZ), TMZ in recurrent glioma protocol, number of prior relapses, academic versus community enrollment site, and prior low-grade glioma. Outcome variables were overall survival (OS), progression-free survival (PFS), and progression-free at 6 months (PFS6). For all end points, classification and regression tree (CART) models and Cox or logistic regression models with bootstrap samples were used to identify prognostic variables and to look for evidence of interactions among these variables. In addition, the data set from each group was used as an independent sample to validate the results from the other. For all outcomes, CART analysis selected last known grade as the most important variable. Additional factors associated with better prognosis regardless of outcome measure included better PS, younger age, and shorter time since initial diagnosis. Analysis required adjustment for TMZ in recurrent glioma regimens. Neither community versus academic enrollment site nor low- versus high-grade glioma at initial diagnosis was associated with outcome. There were differences in the variables selected based on the two data sets, and the variables selected in one data set were not consistently confirmed in the other data set. The results confirm prior reports that last known histology (grade) is an important factor. Other important variables include PS, age, and time since initial diagnosis. The difference in variables selected between the two data sets and the observed impact of treatment emphasize the need to be cautious in defining prognostic factors for use in subsequent trials.

CT 13. METRONOMIC TEMOZOLOMIDE TREATMENT IN PATIENTS WITH RECURRENT TEMOZOLOMIDE-REFRACTORY GLIOBLASTOMA

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Frequent regular administration of chemotherapeutic agents at low doses, known as "metronomic chemotherapy," can increase the antiangiogenic activity of the drugs, as has been confirmed by experimental tumor models. The aim of this study was to evaluate the efficacy and safety of metronomic temozolomide (TMZ) treatment in 12 consecutive patients with recurrent TMZ-refractory glioblastoma. The patients were administered by metronomic treatment schedule (continuous low-dose chemotherapy) with TMZ at a daily dose of 40–50 mg/m^2. The median overall survival (OS) and progression-free survival (PFS) from the start of metronomic treatment were 11.0 months (95% CI, 5.2–10.5 months) and 6.0 months (95% CI, 0–12.3 months), respectively. During the follow-up period, complete response (CR) was not achieved in any patient, partial response (PR) in two, and stable disease (SD) in five patients. Estimated PFS (CR + PR + SD) was 58.3% at 3 months. Grade III/IV toxicity according to the National Cancer Institute Common Toxicity Criteria (NCI CTC) was not found. These results suggest that the change of chemotherapeutic schedule from conventional to metronomic treatment overcomes the chemoresistance in patients with recurrent TMZ-refractory glioblastoma without any major toxicity.

CT 14. AN INTERGROUP PHASE III STUDY OF RADIATION THERAPY WITH OR WITHOUT TEMOZOLOMIDE FOR SYMPTOMATIC OR PROGRESSIVE LOW-GRADE GLIOMAS

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The optimal management of low-grade gliomas (LGGs) remains uncertain. Although fractionated radiotherapy (RT) prolongs time to progression (TTP) and often shrinks tumors, it is rarely curative. Increasing evidence suggests temozolomide is active in both newly diagnosed and recurrent LGGs following RT. Consequently, the EORTC and NCIC are conducting a phase III trial randomizing patients with LGGs who are ⩾40 years old or have progressive or symptomatic tumors to RT versus temozolomide. Based on potential synergistic or additive activity of temozolomide and RT, we hypothesize that the addition of temozolomide to RT may improve TTP and overall survival (OS) in LGG. Improved tumor control may translate into improved quality of life (QoL) and neurocognition. Thus, the Eastern Cooperative Oncology Group and North Central Cancer Treatment Group are initiating a phase III trial of RT + temozolomide versus RT in LGG. Major eligibility criteria mirror the EORTC/NCIC study. Stratification factors include age, 1p/19q status, preoperative maximum tumor diameter, KPS, and presence/absence of contrast enhancement. RT dose is 5,040 cGy in 28 fractions, and temozolomide dose is 75 mg/m^2 daily during RT and then 150–200 mg/m^2 days 1–5 every 28 days for 12 cycles. Patients will undergo MR scans at 3-month intervals. Additionally, the first 250 patients will undergo yearly QoL assessments with the FACT-Br and neurocognitive assessments with a 30-min battery. E3F05's primary objectives are to determine whether the addition of temozolomide to RT improves PFS and OS in LGG. Important secondary objectives include assessment of temozolomide's impact upon QoL and neurocognition and the impact of 1p/19q status on response to combined chemoradiation. Planned accrual is 540 patients over 6.5 years. We would welcome the participation of additional cooperative groups.

CT 15. THE EORTC BRAIN TUMOR GROUP: CURRENT DEVELOPMENTS

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The EORTC Brain Tumor Group has completed several phase II and phase III studies, with conclusions that have led to the development of new studies that are in part conducted through a transatlantic intergroup structure. Translational research in phase II has focused on the prediction of outcome to treatment. Like others, we were unable to identify reliable biomarkers

that predict outcome to EGFR and PFDGR inhibitors. Further research within the study on anaplastic oligodendroglial tumors and anaplastic astrocytoma questions the reliability of histological diagnosis, in particular of the anaplastic oligoastrocytoma. If endothelial proliferation and necrosis is present, this entity in fact represents a tumor type with an outcome that resembles glioblastoma, and often EGFR amplification and loss of chromosome 10 is present. The interobserver variation between pathologists questions the validity of mandatory central pathology review prior to study entry. The new studies are increasingly depending on molecular characterization of the tumors. The EORTC BTG is studying newly diagnosed glioblastoma without MGMT promoter gene methylation with investigational arms without temozolomide. In another project, the addition of cilengitide to chemoirradiation with temozolomide is investigated in glioblastoma with MGMT promoter gene methylation. The subdivision of gliomas into new molecular defined entities causes several practical problems that require close collaboration of all involved specialists.

CANCER STEM CELL

STEM 2. A HIERARCHY OF SELF-RENEWING TUMOR-INITIATING CELL TYPES IN GLIOBLASTOMA

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In glioblastoma (GBM), $CD133^+$ cells have been identified as exhibiting cancer stem cell properties, but $CD133^-$ tumor-initiating cells have also been reported. To investigate heterogeneity of self-renewing tumor-initiating cells in GBM, we examine a series of newly diagnosed tumors and their neurosphere cultures. Profiling a series of GBMs by fluorescence-activated cell sorting, we found that the recently defined mesenchymal subtype of GBM contains far fewer $CD133^+$ cells than do other subtypes. Using clonal analysis of neurosphere lines, we identified three self-renewing cell types capable of initiating orthotopic tumors in nude mice and provide evidence for a lineage hierarchy of tumor-initiating cells wherein both the most primitive (type I) and most differentiated (type III) cells are $CD133^-$. Clones derived from both $CD133^+$ and $CD133^-$ cells of the same tumor culture are capable of maintaining stable CD133 expression status over many passages in vitro (>20) and of serial passage as orthotopic grafts in nude mice. Grafts of clones derived from type I, II, and III cells differ markedly in their latency to detection and in their histological appearance. Both type I and II clones, derived from $CD133^-$ and $CD133^+$ cells, respectively, generate grafts that grow rapidly and demonstrate diffuse invasion into host brain. In contrast, grafts of type III cells, a $CD133^-$ cell population that generates only $CD133^-$ progeny, arise after prolonged latency and lack diffuse invasion. Consistent with the hypothesis that clone types represent stages in a lineage hierarchy, principal component analysis reveals a continuum of expression signatures from type I to type II to type III clones. These results indicate that multiple populations of self-renewing tumor initiating cells coexist within individual GBMs and suggest that optimal treatment of GBM may require targeting multiple stem cell-like populations that include both $CD133^+$ and $CD133^-$ cells.

STEM 3. CHARACTERIZATION AND TRANSFORMATION POTENTIAL OF SYNTHETIC ASTROCYTES DIFFERENTIATED FROM MURINE EMBRYONIC STEM CELLS

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Our objective was to determine if murine embryonic stem (ES) cells, which are readily available from repositories, could be developed as a model of gliomagenesis, recognizing the difficulty in obtaining and transforming somatic astrocytes. Using a stringently controlled sequential differentiation procedure on wild-type (wt) and $p53^{+/-}$ ES cells, we established $GFAP^+A2B5^-$ synthetic astrocytes with high efficiency (90%). The synthetic astrocytes stably express several differentiated astrocyte associated structural proteins and biochemical markers, but lacked expression of differentiated neurons and oligodendrocytes. However, in contrast to somatic differentiated astrocytes, the synthetic astrocytes expressed stem cell markers, with a transcriptome profile similar to astrocytes differentiated from neural stem cells (NSCs) and somatic astrocyte cultures established from E13.5 cortex and P4 hippocampus. In addition, the synthetic astrocytes demonstrated plasticity, with ability to dedifferentiate into neuronal and oligodendrocyte lineages. Intracranial injection of postnatal differentiated somatic astrocytes or synthetic astrocytes of either wt or $p53^{+/-}$ background did not grow tumors, unlike corresponding ES cells that develop teratomas. In contrast, retroviral transduction of either wt or $p53^{+/-}$ synthetic astrocytes and not the postnatal somatic astrocytes, with relevant oncogenes found in human malignant astrocytomas (MDM2, myr-AKT, V12H-RAS), led to intracranial high-grade undifferentiated gliomas. This study demonstrates utilization of readily available ES cells of varying genetic backgrounds to model and further our understanding of gliomagenesis. Large numbers of replenishable derivative synthetic glial lineage cells retain genetic and phenotypic characteristics of progenitor cells and thereby are more amenable to transformation by genetic aberrations involved in gliomagenesis.

STEM 5. $CD133^+$ CELLS ARE STABLY ENTRAPPED IN SHORT-TERM CELL LINES DERIVED FROM MALIGNANT ASTROCYTOMA AND CONTRIBUTE TO CHEMORESISTANCE

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Brain tumors depend on transformed stem cells for initiation and growth. Characterization of such cells is important to evaluate them as therapeutic targets. Screening of a panel of short-term cultures (passage level < 15) derived from malignant astrocytoma using a panel of CD133 antibodies showed that there was a small (usually < 5%) but variable proportion of $CD133^+$ cells in different cultures and that this positivity was most pronounced in multinucleate cells. $CD133^+$ and $CD133^-$ cells were separated using magnetic bead separation and cultured in Ham's F10 media supplemented with 10% FCS or in serum-free conditions in stem cell basal media supplemented with LIF, heparin, bFGF, and EGF, and expression of stem cell markers was assessed using confocal microscopy. In serum-supplemented media $CD133^+$ cells attached to the substratum, but they proliferated in suspension, forming neurospheres in serum-free conditions. The stem cell–associated markers BMI1 and SOX2 were expressed in the nucleus in the $CD133^+$ cells, but not in $CD133^-$ cells. Musashi1 was expressed in the nucleus and cytoplasm of $CD133^+$ but not the nucleus of $CD133^-$ cells. Cells that did not express CD133 appeared to express lower levels of GFAP than did positive cells. Using a caspase-release assay, $CD133^+$ cells were more resistant to CCNU than were cells from the same tumor cell line that did not express CD133. These studies indicate that under appropriate conditions (principally the absence of PDGF), $CD133^+$ cells undergo asymmetric division, form neurospheres, and express nuclear antigens associated with stem cells. The fact that such cells are stably entrapped within cultures for long periods of time explains why these tumors are comparatively easy to grow in vitro and are intrinsically resistant to chemotherapy.

STEM 7. HUMAN ADULT BRAIN CONTAINS NEUROGLIAL PROGENITOR CELLS THAT TARGET HUMAN GLIOMA XENOGRAFTS

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Neural stem cells have been shown to have a therapeutic potential either for cellular repair in the CNS or as vehicles for drug delivery. This study has the primary goal to determine from where and how human adult neuroglial progenitor cells can be generated and how they will target intracranial xenografts of human glioblastoma. Using removed tissue from amygdalo-hippocampectomies and arteriovenous malformations, modified protocols for the culture of neural stem cells were used to establish a total of 39 cultures. The cultures were characterized for marker expression by immunocytochemistry, RT-PCR, and FACS. The homing activity toward glioblastoma was evaluated in the U-87 and G55 human glioma models. By growing as homogeneous monolayers, the cells derived from the 39 cultures expressed so-called stem cell markers such as nestin, A2B5, SOX2, BMI 1, and Musashi. Not being selected for CD133, this marker was expressed only in subsets of cells. When exposed to differentiating conditions, the cells showed differentiation along the neuronal, astroglial, and oligodendroglial pathways. Using a microarray gene-expression analysis with an Illumina Sentrix Whole Genome Platform and a bioinformatic comparison with a database derived from fetal neural stem cell cultures, the adult resection specimen-derived cultures appeared to have a distinct profile. Upon injection into the contralateral hemisphere of tumor-bearing nude mice, our adult neuroglial progenitors had a marked tropism for U87 as well as G55 and showed enrichment within the tumors. We conclude that throughout the adult brain, cells with neuroglial progenitor characteristics are present

that can be enriched in culture and show an attraction to human glioma. It now needs to be shown that such cells can be therapeutically armed to become effectively and selectively tumoricidal.

STEM 8. TUMOR-DERIVED MESENCHYMAL STEM CELLS IN HUMAN GLIOMAS

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Evidence suggests that gliomas are maintained by cancer stem cells (CSCs). However, the potential that stem cells, distinct from CSCs, are also present in gliomas has been largely unexplored. We have shown that exogenously delivered bone-marrow–derived human mesenchymal stem cells (BM-hMSCs) localize to gliomas (Nakamizo et al. *Cancer Res* 65:330, 2005). This raises the possibility that endogenous hMSCs may be recruited into gliomas. Thus, we hypothesized that endogenous hMSC-like cells can be isolated from gliomas and that these hMSCs influence the biology of the tumor. Surgical specimens from 60 patients with gliomas (31 grade IV, 21 grade III, 8 grade II) were cultured according to the same protocols used for BM-hMSCs. In 49 cases (82%), we isolated cells (called tumor-derived hMSCs [TD-hMSCs]) that met the criteria of BM-hMSCs, including spindle cell morphology, appropriate surface markers (i.e., $CD34^-$, $CD38^-$, $CD45^-$, $CD105^+$, $CD73^+$, $CD90^+$), and tridifferentiation into adipocytes, osteocytes, and chondrocytes. TD-hMSCs were $CD133^-$ and were distinct from glioma CSCs ($CD133^+$). Implantation of 10^6 cells intracranially into SCID mice from 31 specimens resulted in tumors in only one, indicating that TD-hMSCs are not tumorigenic. However, several analyses showed that TD-hMSCs contribute to glioma biology: (1) TD-MSCs were more commonly isolated from high-grade (96% of specimens) compared with low-grade gliomas (29% of specimens), suggesting that TD-hMSCs are involved in tumor progression. (2) hMSCs increase the proliferation of U87 glioma cells in both in vitro and in vivo experiments. (3) TD-hMSCs secrete the mesenchymal proteins YKL-40 and VEGF. (4) TD-hMSCs express high levels of the immunosuppressive protein TGF-β, whereas CSCs ($CD133^+$) do not. In conclusion, cells similar to BM-hMSCs can be isolated from gliomas. These TD-hMSCs increase proliferation of glioma cells, express mesenchymal proteins, and enhance immunosuppression. TD-hMSCs may represent an important new target for therapy.

STEM 10. GLOBAL ANALYSES OF POLYCOMB-MEDIATED TRANSCRIPTIONAL REPRESSION AND DNA METHYLATION TARGETS IN BRAIN TUMOR STEM CELLS

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The existence of cancer-initiating cells has been demonstrated in human neoplasia. Based on the stem cell scenario of tumorigenesis, the early epigenetic events arising in malignant precursors remain unknown. Here we examined the global targets of polycomb-repressive complex-mediated histone H3 lysine (K) 27 trimethylation (K27-3Me) and DNA methylation, which are known to be transcriptional silencing machineries for tumor-suppressor genes, in brain tumor stem cells (BTSCs). Methods and findings were as follows: (A) We established BTSCs in serum-free condition from primary glioblastomas. Continuous cultures of these cells in serum conditions resulted in profound morphological changes (i.e., adherent and spindle type) and reduced tumorigenic potential in NOD-SCID mice. Immunohistochemistry and RT-PCR analyses revealed that the morphological changes were compatible with stem cell differentiation (differentiated tumor cells [DTCs]). (B) Analysis of K27-3Me target genes with chromatin immunoprecipitation coupled with microarray (ChIP-chip) in BTSCs and DTCs revealed that 212 genes (1.2%) and 305 genes (1.8%) were the targets of K27-3Me, respectively. Many genes (193 genes) were commonly enriched with K27-3Me in both BTSCs and DTCs, suggesting that these genes were required to perpetuate the malignant phenotype. We found that several specific K27-3Me target genes in BTSCs were closely associated with cell adhesion, proliferation, angiogenesis, and development, while the targets in DTCs were associated with apoptosis, embryogenesis, and regulation of transcription by pathway analysis. Increased DNA methylation was observed in 178 genes (2.2%) during differentiation from BTSCs to DTCs with restriction enzyme-based global DNA methylation microarray analysis. Intriguingly, overlapping of K27-3Me and DNA methylation target genes in DTCs were quite rare (<0.1%), implying distinct targets of these two epigenetic silencing machineries. These results established the multiple epigenetic alterations as mechanisms for CSCs regulation, which might be a key role in stem cell–based tumorigenesis and could be a valid therapeutic target in human neoplasia.

PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

PCNSL 1. PRIMARY CNS LYMPHOMA IN JAPAN: CHANGES IN CLINICAL FEATURES, TREATMENT, AND PROGNOSIS DURING 1985–2004

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We have conducted nationwide surveys of primary CNS lymphoma (PCNSL) treated since 1985. In the present study, we newly collected data between 2000 and 2004 and investigated changes in clinical features and outcome over time. A total of 739 patients with histologically proven PCNSL undergoing radiotherapy were analyzed. Seventeen institutions were surveyed, and data on 131 patients were collected. These data were compared with updated data of previously obtained ones on 466 patients treated in 1985–1994 and 142 patients treated in 1995–1999. Recent trends toward a decrease in male:female ratio, increase in aged patients, and increase in patients with multiple lesions were seen. Regarding treatment, decreases in attempts at surgical tumor removal and increases in use of systemic chemotherapy and methotrexate (MTX)-containing regimens were observed. The median survival time was 18, 29, and 24 months for patients seen during 1985–1994, 1995–1999, and 2000–2004, respectively, and the respective 5-year survival rates were 15%, 30%, and 30%. In groups seen during 1995–1999 and during 2000–2004, patients who received systemic chemotherapy had better prognosis than those who received radiation alone. This was supported by multivariate analysis for patients in the most recent period. In patients seen in 1995–2004 with ages <70 years and performance status of 0–2, those receiving MTX-containing chemotherapy had better prognosis than those receiving other regimens. This study revealed several notable changes in clinical features of PCNSL patients. The prognosis improved during the past 10 years. Advantage of radiation plus chemotherapy, especially MTX-containing ones, over radiation alone was suggested.

PCNSL 2. RESPONSE AND SURVIVAL PATTERN IN PRIMARY CNS LYMPHOMA TREATED BY HIGH-DOSE METHOTREXATE MONOCHEMOTHERAPY: ROLE OF STAT6 AS PROGNOSTIC DETERMINANT

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We report a single center's experience of primary CNS lymphoma (PCNSL) treated with monochemotherapy with high-dose methotrexate (MTX) at a university hospital in Korea. Fifteen immunocompetent patients (median age, 54 years) diagnosed with PCNSL were enrolled in the study between 2000 and 2005. MTX (3.5–8.0 g/m^2) was administered intravenously. There were nine complete responses (60%), one partial response (6.7%), one stable disease (6.7%), and four patients (26.7%) whose tumors progressed in spite of chemotherapy. At the final follow-up, eight of the nine complete responders were alive and well without radiotherapy with a median follow-up of 24 months (four patients alive for >5 years). This group of patients received high-dose MTX of eight to nine cycles. The median progression-free survival and overall survival times in the six noncomplete responders treated with the subsequent radiotherapy (5,040–5,400 cGy) were 2 and 36 months, respectively. The toxicity was modest and was not a reason for dropout during or after the 88 cycles of chemotherapy. The tumors of all noncomplete responders showed the expression of STAT6 in the immunohistochemical study. High-dose MTX monotherapy with deferred radiotherapy is safe and efficient in terms of both survival and tolerability. Additionally, complete response to the chemotherapy appears to be closely related to the prolonged survival, and STAT6 expression might be used as a possible prognostic determinant in the patients with PCNSL.

PCNSL 3. IMMUNOCHEMOTHERAPY WITH RITUXIMAB IN CONJUNCTION WITH BLOOD–BRAIN BARRIER DISRUPTION AND AN ICE REGIMEN FOR RELAPSED OR REFRACTORY PRIMARY CNS LYMPHOMAS (PCNSL)

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High-dose methotrexate (HD-MTX)-based chemotherapy with whole-brain irradiation improves the prognosis of PCNSL; however, up to 30% of patients are refractory to primary therapy and 60% relapse. Fewer than 50% of patients enter a second remission. Thus, novel treatment regimens for relapsed PCNSL are needed. As the majority of PCNSL are B-cell type expressing the CD20 antigen, treatment with the monoclonal antibody (mAb) rituximab might be reasonable. However, delivery of mAb to the brain would be limited by the blood–brain barrier (BBB). Osmotic BBB disruption (BBBD) is currently being used to increase the delivery of chemotherapeutic agents for the treatment of brain tumors. The activity of rituximab plus ifosfamide, cisplatin, and etoposide (R-ICE) as salvage therapy in systemic lymphomas provides the rationale for our use of the R-ICE regimen in conjunction with BBBD. Here we report our experience with i.v. rituximab with BBBD followed by ICE-based chemotherapy in patients with relapsed or refractory PCNSL. Five patients who failed therapy with HD-MTX–based chemotherapy were treated with a regimen of rituximab with BBBD followed by ICE in a 28-day cycle. BBBD was performed with 20% mannitol infused through a catheter placed into the artery of the tumor site selectively at a rate of 3 ml/s for 30–60 s. ICE included ifosfamide 900 mg/m^2/day, CDDP 20 mg/m^2/day, and etoposide 60 mg/m^2/day on days 1–5. Four patients had prior RT and had been pretreated heavily. The mean age was 50 years. Three complete remissions and one partial remission were achieved. The major toxicity was grade 3–4 neutropenia (five of five) and grade 3–4 thrombocytopenia (two of five). Hematotoxicity was prolonged in an elderly patient. The preliminary data presented here suggest that R-ICE with BBBD is an effective second-line treatment in PCNSL, and clinical trials are necessary to evaluate both the efficacy and long-term safety of this treatment.

PCNSL 4. RACIAL/ETHNIC DIFFERENCES IN PRIMARY CNS LYMPHOMA: A SEER-BASED ESTIMATE OF INCIDENCE AND SURVIVAL RATES AMONG U.S. BLACKS, WHITES, ASIAN/PACIFIC ISLANDERS, AND AMERICAN INDIANS/ALASKAN NATIVES

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The racial and ethnic associations of PCNSL have never been determined. Our clinical impression was that PCNSL was less commonly diagnosed in blacks than in whites and rarely in Asian/Pacific Islanders and American Indians/Alaskan Natives. This study was to determine if there was a racial difference in incidence and survival when we used SEER program data. The data are suggestive of a racial influence on PCNSL incidence and mortality. Incident malignancies were classified using ICD-O, 2nd edition, and specified NHL morphologies, and were stratified to those 20–49 years of age and those >50. Subgroup analyses reflect the four SEER racial groups. Age-adjusted incidence rates were calculated to the 2000 U.S. standard million population and reported per 100,000 person-years (SEER*Stat software). We calculated 95% confidence intervals (CIs) based on properties of the Poisson distribution. Among whites, PCNSL incidence rate was 0.94 per 100,000 per year (95% CI, 0.90–0.98); among blacks, the incidence was 1.10 per 100,000 per year (95% CI, 0.98–1.22). The other subgroups had much lower PCNSL incidence rates. In patients 20–49 years of age at diagnosis, PCNSL incidence in blacks (IR = 1.43; 95% CI, 1.27–1.59) was twice that of similarly aged whites (IR = 0.72; 95% CI, 0.68–0.76). For those above the age of 50 years, the incidence ratio between the two racial groups was reversed: 1.30 (95% CI, 1.22–1.38) in whites and 0.56 (95% CI, 0.40–0.72) among blacks. Survival for all races and all ages at 12 months, 2 years, and 5 years was 33%, 25%, and 16%, respectively. Among all persons 20 years and older, survival at 12 months, 24 months, and 60 months among whites was significantly higher than among blacks. When stratified into groups of those 20–49 and those >50 years of age, only 12-month survival and 5-year survival were statistically different in the two racial groups ($p = 0.03$ and $p = 0.05$, respectively).

PCNSL 5. DEL(6)(Q22) AND BCL6 REARRANGEMENTS DETECTED BY INTERPHASE FLUORESCENCE IN SITU HYBRIDIZATION (FISH) ARE UNFAVORABLE CYTOGENETIC ABNORMALITIES IN IMMUNOCOMPETENT (IC) PATIENTS WITH PRIMARY CNS LYMPHOMA (PCNSL)

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The aim of this study is to determine the prevalence and survival impact of del(6)(q22), BCL6, immunoglobulin heavy chain (IGH), and MYC gene rearrangements in IC PCNSL. We studied 76 HIV-negative newly diagnosed IC PCNSL patients treated between 1992 and 2006. Median age was 63.5 years (range, 26–87 years). There were 51 deaths. Median follow-up for 25 living patients was 399 days (range, 0–2,520 days). FISH used two-color break-apart probes (BAP) for BCL6 and MYC, two-color dual-fusion probes for IGH-BCL6, and two-color probes for del(6)(q22) on thin sections of paraffin-embedded tumor samples. Survival was calculated from the date of tissue diagnosis to date of death or date of last contact using Kaplan-Meier method. The log-rank test was used to compare survival across groups. Thirty-one (41%) cases did not show del(6)(q22) or BCL6 rearrangement and had a median overall survival (MS) of 731. Twenty-eight (36%) cases showed an isolated de (6)(q22) and had an MS of 412 days ($p = 0.0048$). Seventeen (23%) cases showed a BCL6 rearrangement and had an MS of 442 days ($p = 0.0048$). Of the cases with BCL6 rearrangement, eight (11%) showed IGH-BCL6 fusion and the remaining nine (12%) involved translocation to an unknown gene partner. Two (3%) cases showed MYC translocation to an unknown gene partner. Six cases with BCL6 rearrangement also showed del(6)(q22). A total of 34 (45%) cases showed del(6)(q22) and had an MS of 412 days ($p = 0.0198$). In this study, del(6)(q22) and BCL6 rearrangements were present in 45% and 22% of cases, respectively, and were both associated with decreased survival, seemingly independent of patient age and treatment time trends. IGH translocations were seen in only 13% of cases, which is less frequent than seen in systemic diffuse large B-cell lymphoma, suggesting a distinct pathogenesis.

MOLECULAR TARGETED THERAPY

MTT 1. PHASE II STUDY OF BEVACIZUMAB AND ERLOTINIB IN PATIENTS WITH RECURRENT GLIOBLASTOMA MULTIFORME

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Bevacizumab, a neutralizing monoclonal antibody to vascular endothelial growth factor (VEGF), has demonstrated promising radiographic response and promising survival benefit in combination with irinotecan in patients with recurrent glioblastoma multiforme (GBM). Erlotinib, an EGFR tyrosine kinase inhibitor, has shown antitumor activity in some glioma patients. Combination of bevacizumab and erlotinib has demonstrated safety and efficacy in several solid malignancies. In this study, we evaluated the combinatorial efficacy of bevacizumab and erlotinib in patients with recurrent GBM. Twenty-five patients with recurrent GBM were enrolled. The primary outcome measure was 6-month progression-free survival. Radiographic response, pharmacokinetics, and correlative biomarkers were secondary outcome measures. Patients were stratified based on concurrent use of enzyme-inducing anticonvulsants (EIAC). Bevacizumab was dosed at 10 mg/kg intravenously every 2 weeks. Erlotinib was orally administered daily with 200 mg/day for patients not on EIAC and 650 mg/day for patients on EIAC. With a median follow-up of 32.3 weeks, the 6-month progression-free survival rate was 24%. Twelve patients (48%) achieved radiographic response. This treatment combination was well tolerated. Common side effects included those previously seen with erlotinib therapy, such as rash, diarrhea, mucositis, and fatigue. One ischemic stroke and one asymptomatic intracerebral hemorrhage were observed. We conclude that the combination of bevacizumab and erlotinib is safe and well tolerated in patients with recurrent GBM. The regimen is associated with promising radiographic response and encouraging survival benefit.

MTT 3. FEASIBILITY/TOLERABILITY RESULTS FROM A PHASE I/II STUDY OF TEMSIROLIMUS (CCI-779) AND SORAFENIB IN RECURRENT GLIOBLASTOMA: NORTH CENTRAL CANCER TREATMENT GROUP (NCCTG) N0572

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The ras/raf/MEK/ERK (MAPK) and PI3K/Akt/mTOR pathways are thought to be key overactive pathways in glioblastoma. Temsirolimus is an intravenously administered mTOR inhibitor. Sorafenib inhibits multiple kinases, including raf, PDGFR, and VEGFR 2 and 3. Sorafenib has shown modest single-agent activity in phase I glioblastoma trials; temsirolimus has shown limited single-agent activity in prior NCCTG and NABTC recurrent GBM studies. Horizontal inhibition of these critical pathways is a logical strategy. We initiated a phase I/II study of temsirolimus with sorafenib in June 2006. Eligibility was restricted to patients with progressive glioblastoma, not taking enzyme-inducing anticonvulsants, with two or fewer prior chemotherapy regimens. Sorafenib was administered orally on a continuous daily schedule, and temsirolimus was given intravenously every week of each 4-week cycle. As of December 31, 2007, 12 patients had been enrolled, of whom nine were assessable for DLT (three patients were replaced for inadequate dosing). At dose level 0 (sorafenib 200 mg b.i.d. and temsirolimus 25 mg), none of the three patients experienced DLT. At dose level 1 (sorafenib 400 mg b.i.d. and temsirolimus 25 mg), two of three patients had DLT: one patient had grade 4 gastrointestinal perforation possibly related to treatment, and another patient experienced grade 3 anorexia, vomiting, and fatigue. Of three additional patients enrolled at dose level 0, one had possibly related grade 3 fatigue and one grade 2 rash. Sorafenib 200 b.i.d. and temsirolimus 25 weekly will therefore represent the phase II dose. We will present toxicity data on the first six patients treated at this dose from the phase II study. This is one of the first studies of horizontal inhibition of multiple signal transduction pathways in glioblastoma. Our data suggest synergistic toxicity between temsirolimus and sorafenib. The phase II study has opened.

MTT 4. PHASE I/II STUDY OF ERLOTINIB AND CCI-779 (TEMSIROLIMUS) FOR PATIENTS WITH RECURRENT MALIGNANT GLIOMAS (NABTC 04–02)

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Glioblastomas (GBM) frequently have amplification/mutation of EGFR and inactivation of PTEN. Although single agent EGFR and mTOR inhibitors have only modest activity, combinations of these agents may be more effective. The North American Brain Tumor Consortium is conducting a phase I/II study of the EGFR inhibitor erlotinib in combination with the mTOR inhibitor temsirolimus in recurrent malignant glioma. Eligibility criteria were histologically proven GBM and anaplastic gliomas (AG), radiologic progression, >18 years old, KPS > 60, and adequate bone marrow reserve and organ function. There was no limit on the number of prior therapies. Patients must not be receiving enzyme-inducing antiepileptic drugs. The dose of erlotinib was fixed at 150 mg/day. Patients initially received temsirolimus 50 mg intravenously once weekly, and the dose was adjusted based on toxicities. Escalation was performed in standard groups of three. The MTD was defined as the dose at which DLTs occurred in no more than one in six patients. In the phase II component, 32 GBM and 16 AG patients will be treated at the MTD. The primary end point is 6M-PFS. In the phase I component, 22 patients were enrolled (15 GBM, 7 AG). Median age was 54 years (26–74); median KPS score, 90 (70–100); median prior chemotherapy regimens, 1 (0–3). The MTD was determined to be 150 mg of erlotinib daily combined with 15 mg of temsirolimus weekly. Dose-limiting toxicities were rash, mucositis, and liver function abnormalities. Pharmacokinetic data suggest there is no interaction between erlotinib and temsirolimus. In the phase II component, 29 GBM and 13 AG patients have been enrolled to date. In conclusion, the combination of erlotinib and CCI-779 was associated with a higher than expected incidence of rash and mucositis. Detailed pharmacokinetic data from the phase I component and response data from the phase II component will be presented.

PEDIATRIC NEURO-ONCOLOGY

PNO 1. TREATMENT OF PRIMARY CNS GERMINOMATOUS GERM CELL TUMORS (GCT) WITH CHEMOTHERAPY PRIOR TO REDUCED-DOSE VENTRICULAR FIELD IRRADIATION: THE CHILDRENS HOSPITAL LOS ANGELES EXPERIENCE 2003–2007

Jonathan Finlay, Mark Krieger, Arti Nasta, Ignacio Gonzalez, Rima Jubran, Anat Erdreich-Epstein, Araz Marachelian, Girish Dhall, Barbara Britt, Floyd Gilles, Gordon McComb, Arthur Olch, Sharon O'Neil, Richard Sposto, Robert Lavey; Neural Tumors Program, Childrens Hospital Los Angeles and University of Southern California, Los Angeles, CA, USA

Our purpose was to evaluate a uniform treatment strategy for newly diagnosed CNS germinomas. Since October 2003, 20 patients with histologically diagnosed pure germinomas (n = 19) or germinomas with mature components (n = 1) received four cycles of carboplatin and etoposide at 3-week intervals, followed by ventricular field irradiation to 22.4–24.0 Gy with simultaneous integrated boost to the primary site(s), to 30 Gy in 15 fractions. Mean age at diagnosis was 15.5 years. Fifteen patients were males. Primary tumor locations were 11 pineal, 6 suprasellar, 2 basal ganglia, and 1 cerebellar. No patient had metastases at diagnosis. Elevated HCGβ levels (>50 mIU/ml) were documented in the serum (2 of 20) or CSF (4 of 20) at diagnosis. One patient (of 18 tested) had CSF AFP elevated at 2.9 ng/ml, while none of 20 had serum AFP elevations. Minimal endoscopic biopsies were obtained in 12 patients; partial or gross total resections, in 8. All but two patients continue without evidence of residual or recurrent tumor. One patient, with elevated CSF AFP at diagnosis, developed radiographic recurrence with AFP and HCGβ elevations 18 months from diagnosis. A second patient, with serum and CSF HCGβ of 323 mIU/ml and 101 mIU/ml at diagnosis, completely responded to induction chemotherapy but promptly recurred prior to irradiation radiographically and with markedly rising HCGβ, consistent with choriocarcinoma. The EFS and OS for all 20 patients, at a median of 40 months, are 89.5% and 100%. We conclude that this treatment strategy produces outstanding EFS for patients with unequivocal diagnoses of CNS germinoma. Patients with even minimal AFP elevations require more intensive regimens for nongerminomatous GCT. For patients with modest elevations of HCGβ, the risks of radical resection to accurately confirm germinoma diagnosis must be weighed against the risks of long-term morbidity of more intensive chemotherapy and irradiation and against the risks of undertreatment, as in our CHLA experience.

PNO 7. IDENTIFICATION OF GENES AND PATHWAYS CRITICAL FOR TUMOR INITIATION, MAINTENANCE, AND PROGRESSION IN A MURINE MODEL OF LEPTOMENINGEAL METASTATIC MEDULLOBLASTOMA DRIVEN BY THE SLEEPING BEAUTY TRANSPOSON

Michael D. Taylor[1], Xiaochong Wu[1], Lara Collier[2], David Largaespada[3], Jessica Mcleod[1], Paul Northcott[1], Sid Croul[1], Jeff Macdonald[1], Junjun Zhang[1], Steve Scherer[1]; [1]Division of Neurosurgery, Hospital for Sick Children, Toronto, Canada; [2]University of Wisconsin, Madison, WI, USA; [3]University of Minnesota, Minneapolis/St. Paul, MN, USA

The sleeping beauty (SB) transposon is a powerful functional genomics tool for cancer gene discovery. In the presence of its transposase (enzyme), the SB transposon is excised from genomic DNA and is pasted into a new genomic position. Tumorigenesis is caused by activation of nearby oncogenes by the MSCV-LTR contained in the transposon (activation of oncogenes) and by gene trapping using poly-A traps (loss of tumor suppressor genes). We have modified the SB transposon system by expressing SB11 transposase selectively in the murine cerebellar EGL using the Math1 enhancer/promoter (J2Q-SB11). Whereas 10%–20% of ptch$^{+/-}$ mice develop localized medulloblastoma by 8 months of age, 100% of J2Q-SB11 ptch$^{+/-}$ mice that also carry the donor transposon concatemer develop leptomeningeal metastatic medulloblastoma by 10 weeks. SB transposition sites can be determined through a relatively simple PCR-based technique followed by cloning and sequencing. We have cloned and deep sequenced more than 100,000 insertion sites from >100 SB-induced primary medulloblastomas, as well as preneoplastic cerebellar rests and leptomeningeal metastases. CIS from rests, primary tumors, and metastases target known oncogenes, angiogenic factors, and genes important in neuronal development. Analysis of the clonal insertion patterns in primary medulloblastoma versus spinal leptomeningeal metastases shows that the metastases carry all of the insertion sites seen in the primary tumor, as well as additional clonal insertions that presumably have driven tumor progression. Our new model of medulloblastoma with leptomeningeal spread is ideal for translational research as it has full penetrance (100% incidence), short latency (10 weeks), and leptomeningeal dissemination faithful to the human disease, and it occurs on an immune

competent background and involves random, easily discernible secondary genetic events. Analysis of the SB insertion sites will allow rapid dissection of the genetic events important for the initiation, maintenance, and progression of medulloblastoma.

PNO 8. IDENTIFICATION OF NT-3/TRK-C–REGULATED PROTEINS IN MEDULLOBLASTOMA

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Recent reports identified high neurotrophin receptor TrkC mRNA expression as a powerful independent predictor of a favorable survival outcome in medulloblastoma (MB) patients. However, the role of activated TrkC receptors in the biology of MB remains unclear. We report on a proteomic approach to determine downstream effector proteins of TrkC signaling. The MB cell line DAOY was stably transfected with a vector containing the full-length TrkC cDNA sequence or an empty vector control. Accounting for the complexity of ligand-induced changes in cellular pathways and effector proteins, we investigated proteomic changes at multiple time points for up to 48 h following neurotrophin-3–induced TrkC receptor activation. Nineteen proteins were differentially expressed: cathepsin D, mitofilin, heterogeneous nuclear ribonucleoprotein H and K, superoxide dismutase (Mn) lamin A/C, valosin-containing protein, ULIP protein, laminin receptor 1, moesin, and vimentin were upregulated. Cofilin, caldesmon, metastasis inhibition factor nm23, vinculin, glutathione *S*-transferase P, stathmin, annexin A1, DJ-1 protein, and fascin were downregulated. Sixteen of the differentially expressed proteins were validated by immunoblotting in independent cell culture experiments and confirmed four proteins being up- or downregulated in a very reproducible way: lamin A and C showed upregulation upon NT-3/TrkC activation, whereas cofilin and stathmin showed profound downregulation. Our conclusions are as follows: The differentially expressed proteins have been implicated in affecting cell motility, migration, invasion, proliferation, apoptosis, and drug resistance. Almost all of the proteins have been described as being essential in the pathogenesis of different solid tumors but have not been related to MB pathogenesis so far. Functional analyses are under way.

PNO 10. FACTORS AFFECTING MEDULLOBLASTOMAS IN CHILDREN

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The overall survival of medulloblastoma in children has improved to 50%–70% in recent years. In addition to the revolutionary treatment modalities directly influencing the survival of patients, reported factors associated with outcomes of patients with medulloblastomas include younger age, histopathological classification/grade, metastasis (M stage) at diagnosis, postoperative residual tumor volume, clinical risk grade, and certain molecular genetics features. In patients younger than 3 years, one of the factors influencing outcomes is the diagnosis of cerebellar AT/RTs. In Taipei Veterans General Hospital, from 1970 to 2007, we collected 148 cases of medulloblastomas in children of <18 years of age. The median age at diagnosis was 6.9 years and median follow-up period was 3.9 years (5 days to 34.7 years). Cerebellar AT/RTs or At/RT-like tumors were excluded as possible. For dissemination of disease, we detected definite metastasis in 33 cases (22.3%). A total of 103 (69.6%) patients received gross total/near total resection. The majority of cases with residue tumor of $<1.5\ cm^2$ were due to tumor infiltration. For clinical risk grading, there were 74 (50%) average-risk and 58 (39.2%) high-risk patients. Relating to treatment modalities, 123 patients received primary adjuvant treatment with radiation and/or chemotherapy (CMT), 102 patients completed the adjuvant therapeutic regimens, 98 patients completed CSRT with/without CMT, and 4 infants completed only CMT regimen. For those patients who completed the treatment regimen of CSRT with/without CMT, the overall survival at 5 years, 10 years, and 15 years were 71.1%, 58.0%, and 55.0%, respectively. Radiation-induced tumors were observed in five patients with average follow-up period of 20.5 years. We analyze the prognostic factors in this series and correlate with reported literatures. Our experience and reasons for treatment failure of this particular tumor in children are highlighted.

PNO 11. ADULTS WITH MEDULLOBLASTOMA: TIME FOR A CHANGE IN MANAGEMENT

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Medulloblastoma is predominantly a malignant tumor arising in the posterior fossa during early childhood; however, it is clear that as many as 30% of medulloblastomas do arise in young adults into the fifth decade of life. Medulloblastoma represents <1% of adult intracranial tumors. The management of such adults remains unclear, other than radical surgical resection of the primary tumor followed by full-dose (3,600 cGy) craniospinal irradiation with posterior fossa boost (to 5,400 cGy)—the standard of treatment for children and adolescents back in the 1970s and early 1980s. Over the last two decades, it has become clear, both from randomized trials and for large sequential nonrandomized trials, that the outcome for children and adolescents with medulloblastoma has been substantially improved through two efforts: (1) more radical resection of the primary tumor, affected through improvements in both neuroimaging and neurosurgical techniques, and (2) the addition of chemotherapy both during and after irradiation for a duration of about 12 months. For "standard-risk" medulloblastomas (defined as near gross total resection of primary tumor, no evidence of neuraxis dissemination, and age >3 years), the current North American data (CCG A9961) report in excess of 85% 5-year event-free survivals in centrally reviewed eligible patients. All patients received the now "standard" reduced-dose neuraxis irradiation of 2,340 cGy with posterior fossa boost, along with vincristine weekly, followed by "Packer"-style chemotherapy (vincristine, CCNU and cisplatin or vincristine, cyclophosphamide). The current North American trial seeks in a randomized fashion to compare 1,800 cGy versus 2,340 cGy (in the younger patients) as well as "standard" posterior fossa boost versus focal conformal boost to the tumor bed only—along, of course, with chemotherapy. No comparable data exist for adults; no randomized trials have been completed to demonstrate benefit of the addition of chemotherapy to irradiation over irradiation alone. However, survival for adults treated with irradiation alone has not differed over the years from that reported in children—a recently published SEER population-based survey of 454 adults with medulloblastomas treated between 1973 and 2004 reported 2-year, 5-year, and 10-year survival rates of 79.5%, 64%, and 50.4%; this hardly represents cure in a disease known (especially in adults) to have very late recurrence. The SEER registry reported 20-year survival reduced to 42.7%. Recently, Christian Carrie and colleagues in France published a retrospective analysis of outcome in 253 adults treated between 1975 and 2004 with medulloblastoma; multivariate analysis of the 124 patients with "standard-risk" medulloblastomas revealed no difference in outcome between those receiving full-dose craniospinal irradiation only (>34 Gy) and those receiving reduced-dose craniospinal irradiation (<34 Gy) with chemotherapy, with 77% and 62% 5-year and 10-year survivals. One important argument against using irradiation only is the long documented observation in children and adolescents that, after irradiation alone, the first site of recurrence will be beyond the neuraxis (in bones and bone marrow) in about 10% of relapsing patients; the introduction of adjuvant chemotherapy has virtually eliminated such occurrences. In our practice, the only time we have seen such bone and/or bone marrow metastases in the last decade or more has been in adults treated with irradiation only—and cure of such patients, despite their extraneural location of tumor, is rare indeed. The debate should focus now not on whether all adults with "standard-risk" medulloblastoma should receive chemotherapy, but whether they should receive the same chemotherapy as the children. The Toronto Sick Childrens Hospital has published their sobering experience in adolescents with the "Packer" regimen, not only documenting very poor tolerance for the regimen, but also demonstrating that this poor tolerance translated into poorer survival in the group. One would anticipate such intolerance and compromised outcome to be at least as great in adults. Vincristine is less well tolerated, both acutely and chronically, in adults and needs careful dose attenuation. Cisplatin is "deafening" to young and old alike—the major long-term toxicity of the regimen. Hematopoietic suppression is more a significant problem in adults than in children, reflecting the impact of spinal irradiation upon adult marrows (note that 40% of one's marrow resides in the spine, and 36 Gy irreversibly destroys the marrow microenvironment) already more heavily overburdened with fat than that of young children. For adults with disseminated medulloblastoma, data are even more scant. Clearly, the historical data in children indicate that cure with full-dose craniospinal irradiation alone is rarely if ever achieved in such patients. The more intensive chemotherapy regimens used follow-

ing irradiation at full dose in children with disseminated medulloblastoma are even less likely to be tolerated in adults than the milder regimens used for "standard-risk" medulloblastoma patients. In order to circumvent this problem, regimens have been piloted and reported using short but intensive cycles of chemotherapy prior to irradiation in adults with "high-risk" medulloblastoma and pineoblastomas. It is surely time for neurooncologists treating adults to come forward with a multicenter, indeed, multinational trial for adults with newly diagnosed medulloblastomas, to establish demographics, and to set a new standard of management for adults, as well as to provide tumor tissue for study that will improve our understanding of the biology of this uncommon disease in adults.

PNO 14. OTX2 IS ONCOGENIC AND PREDICTS DECREASED SURVIVAL IN MEDULLOBLASTOMA PATIENTS

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Despite the fact that medulloblastoma is accepted as the most common malignant brain tumor of childhood, the identity of molecules that are useful therapeutic targets in medulloblastoma is largely unknown due to the lack of fundamental knowledge on the molecular pathogenesis of the disease. To determine the molecular mechanism underlying tumorigenesis of medulloblastoma, we explored the genome of medulloblastoma to identify novel tumor suppressor genes and oncogenes. Digital karyotyping is a gene-dosage tool that allows whole-genome screening for cancer genes at an unprecedented "single-gene" resolution. Using this method, we have identified a homeobox gene OTX2 amplification and demonstrated its overexpression in medulloblastoma associated with tumor anaplasia. We have shown that knockdown of OTX2 by small interfering RNA reduced medulloblastoma cell growth and that the cells with high levels of OTX2 expression were sensitive to treatment with retinoic acid (RA). Exogenous retinoids, when exogenously applied, have been shown to displace or repress OTX2 expression in the embryonic nervous system and also in embryonal carcinoma cells through *cis*-acting elements of the OTX2 promoter. In our studies, we observed that RA abrogated cell proliferation in every OTX2-expressing cell line in a dose-dependent manner. These studies lay the conceptual framework for clinical trials of retinoids in the treatment of a commonly lethal pediatric brain tumor. Furthermore, we have investigated the OTX2 molecular signaling pathway and demonstrated that another medulloblastoma oncogene c-Myc was transcriptionally regulated by OTX2. We expect the studies to advance our knowledge on the pathogenesis of medulloblastoma that could make significant contributions to the therapeutic intervention in this frequently lethal cancer.

PNO 16. RESULTS FROM A SINGLE-ARM, MULTI-INSTITUTIONAL PHASE II STUDY OF MULTIAGENT INTRATHECAL AND SYSTEMIC CHEMOTHERAPY WITH AGE- AND RISK-ADAPTED RADIATION THERAPY FOR CHILDREN WITH NEWLY DIAGNOSED CNS ATYPICAL TERATOID/RHABDOID TUMOR

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Atypical teratoid/rhabdoid tumor (ATRT) of the CNS is a highly malignant neoplasm primarily affecting young children, with a median survival ranging from 6.5 to 10 months. Based on a small successful pilot series of patients with newly diagnosed and recurrent disease (*J Neurooncol* 2005;72:77–84), a multi-institutional trial was conducted. Treatment included multiagent chemotherapy and intrathecal chemotherapy. Patients with M0-stage disease received 5,400 cGy conformal radiation therapy; those >3 years with M+ disease received craniospinal irradiation with 3,600 cGy and a boost to 5,400 cGy. Between February 2004 and February 2007, 22 evaluable patients were enrolled. The median age at diagnosis was 2.5 years. Primary tumors were supratentorial in 12 and posterior fossa in 10 patients. Gross total resections of the primary tumor were achieved in approximately 50% of patients. Sixteen patients had M0, one had M2 disease, and five had M3 disease. All patients received some intrathecal therapy. Seventeen patients received radiation therapy, 12 conformal, and 5 CSI. Significant toxicities included bone marrow suppression; febrile neutropenia; infection; gastrointestinal, electrolyte, and hepatic function disturbances; neuropathies; transverse myelitis; high-frequency hearing loss; and two patients who experienced radiation recall. There was one toxic death from pneumococcal sepsis. Of the 14 patients evaluable for chemotherapeutic response (pre-RT), the objective response rate (CR + PR) was 62%. The objective response rate from radiation therapy was 38%. The 1-year PFS and OS are 68% and 77%, respectively, and the 2-year PFS and OS are 48% and 67%, respectively. Sites of relapse include local (three patients), distant metastases (two), disseminated (three), and unknown (one). Median overall survival has not yet been reached. Thus, for this rapidly fatal disease, significant progress has been made in terms of improving survival. A future study is planned to incorporate growing biologic data.

PNO 17. CPT-SIOP-2000 STUDY: A PLANETWIDE CHOROID PLEXUS TUMOR STUDY: INTERIM REPORT, OCTOBER 2007

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Since choroid plexus tumors are rare, in 2000 we started a worldwide registration of both adults and children into a combined registry for all choroid plexus tumors and an open-labeled randomized treatment approach for those who require postoperative treatment. The decision of which patient should receive treatment was based on an algorithm including staging, grading, and resection grade. Treatment includes two cycles of chemotherapy (etoposide and vincristine combined with either carboplatinum or cyclophosphamide), response evaluation, irradiation for patients at least 3 years of age, and continuation with four chemotherapy courses. Since 2000, 21 nations registered 118 patients. Of those, six patients were excluded from analysis due to a different histology. Forty-two were choroid plexus papillomas (CPPs): median age, 2.2 years; 52% were male; 71% of tumors were located in the lateral ventricles; 5% were primary metastases; and 90% were completely resected. Twenty-eight were atypical choroid plexus papillomas (APPs): median age, 0.7 years; 46% male; 82% in the lateral ventricles; 14% metastases; 68% completely resected. Forty-two were choroid plexus carcinoma (CPC): median age, 2.5 years; 55% male; 91% in the lateral ventricles; 19% metastases; 52% completely resected. The 5-year event-free survival was 95% ± 4% SD for CPP ($n = 40$), 70% ± 14% SD for APP ($n = 21$), and 45% ± 14% SD for CPC ($n = 33$). Most CPP and APP could be salvaged with delayed start of treatment resulting in a 5-year overall survival of 100% and 83% ± 15% SD, respectively. The randomized treatment arms are still blinded. The treatment algorithm is effective for ascertaining the risk patients to receive primary treatment and allowing salvage therapy for the initially observed patients with tumor progression. An updated version of the algorithm is under international discussion.

NEUROCOGNITIVE FUNCTION AND OUTCOME

OUT 1. LATE EFFECTS FOLLOW-UP OF HEAD START II SURVIVORS

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The purpose of this study was to evaluate the neuropsychological late effects of survivors treated on the Head Start II protocol from 1997 to 2003. Patients younger than 10 years diagnosed with a malignant brain tumor underwent baseline neuropsychological assessment prior to autologous stem cell transplantation (AuHCR) and 3 years later. Standard risk patients received five cycles of induction chemotherapy and one cycle of myeloablative consolidation therapy, while patients with neuraxis dissemination also received intensification using i.v. methotrexate (IVMTX). Craniospinal irradiation (CSI) was used to treat residual disease at completion of induction or relapse. Baseline assessments for 49 patients (median age = 38 months; SD = 25) indicated low average intelligence (FSIQ = 88.77, SD = 17.7) and low average visual-motor abilities (VMI = 87.69, SD = 9.4), whereas expressive language skills were within the average range (PPVT = 102.11; SD = 17.1). Parents reported social-emotional and behavioral functioning within the average range (CBCL T score = 47.59; SD = 9.4). Follow-up testing was obtained on 24 of 30 survivors (80%) at mean follow-up of 39.7 months after transplantation (SD = 16.1). Analysis of FSIQ reveals a stable level of intellectual functioning between baseline and follow-up (FSIQ = 89.27, SD = 16). Additionally, learning and memory was within the average range, while academic achievement (reading, spelling, and math), receptive language, and VMI were within the low average range at time 2. Of the 10 patients (42%) who received CSI, mean radiation delay was 14.3 months (SD = 13.2). There were no significant differences in FSIQ between those who received IVMTX and those who did not. No significant correlations were found between age at diagnosis and intellectual ability, academic achievement, expressive language, or visual-motor skills.

Conclusions: Induction and myeloablative consolidation chemotherapy, with or without IVMTX, followed by AuHCR may avoid or delay CSI while preserving neuropsychological functioning, including those who were younger at diagnosis. Continued follow-up of these survivors is warranted to determine the stability of their neuropsychological, social-emotional, and behavioral functioning over time.

OUT 2. NEUROPSYCHOLOGICAL OUTCOME OF SURVIVORS OF PEDIATRIC MALIGNANT GLIOMA

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The purpose of this study was to evaluate neuropsychological late effects of survivors treated on a pediatric high-grade glioma (HGG) study (CCG-945) from 1985 to 1991. Fifty-four of 79 (68%) survivors from 25 institutions across North America were enrolled on the companion study COG L991 between 2000 and 2005 to assess their neuropsychological and emotional-behavioral functioning and quality of life. The median age at diagnosis of the 54 survivors (29 male, 25 female) was 8.8 years (0.2–19.5 years) with median follow-up of 15.1 years (9.5–19.2 years). Survivors demonstrated mean intelligence and verbal memory within the low average range, executive functioning between the low average to borderline ranges, and visual memory within the borderline range. Visual-motor and processing speed were focal areas of impairment. Survivors reported emotional-behavioral functioning and quality of life within the average range. Midline and posterior fossa tumor patients displayed lower verbal intelligence ($p = 0.02$) and processing speed ($p = 0.05$) compared with those with hemispheric tumors. Patients < 3 years at diagnosis demonstrated lower full-scale IQ ($p = 0.03$) and performance IQ ($p = 0.03$), while their family members reported more depression ($p < 0.01$), aggression ($p = 0.04$), and somatic complaints ($p = 0.03$). Female patients achieved lower verbal IQ scores ($p = 0.02$) and reported poorer physical health ($p = 0.02$), while their family members reported more frequent problems with depression ($p = 0.01$), somatic complaints ($p = 0.03$), aggression ($p = 0.04$), and motor functioning ($p = 0.05$). This is the first pediatric HGG study to examine neuropsychological late effects. Deficits in visual-motor and processing speed are consistent with cranial irradiation sequelae; however, intelligence, verbal memory, and executive functioning are notably higher than reports of adult HGG patients. Attention should be given to young patients and to females treated for HGG. Further research is warranted in the outcomes of patients with HGG that arise in the midline or posterior fossa and to gain insight into the development of normal quality of life amidst variable neuropsychological functioning.