1453 Increased Frequency of Central Venulitis after Liver Transplantation for Primary Biliary Cirrhosis

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Background: Central venulitis (CV) can occur in the allograft liver, but the significance of this finding for graft function and outcome is not well understood. The aim of this study is to establish the frequency of CV occurrence after liver transplantation (LT) for primary biliary cirrhosis (PBC) and its association with other clinical variables.

Design: All patients who underwent LT for PBC at our center from 2000-2006 and a control group of NASH patients matched by age, gender, and date of LT were identified. All post-LT biopsies done in subjects and controls were reviewed and characterized using the Banff schema. ACR was sub-classified as portal-based ACR features without CV (P-ACR) or portal-based ACR with CV (P-ACR+CV). Isolated CV (ICV) and centrilobular inflammation (CI) were also noted. Clinical, biochemical, surgical and vascular data were abstracted and analyzed. Comparisons between ACR, P-ACR, P-ACR+CV, ICV, and CI in PBC patients versus controls were made while controlling for other clinical variables.

Results: 25 patients who underwent LT for PBC and 23 matched controls were identified. 2 PBC patients died within 3 months of LT and were excluded. No significant differences between PBC patients and controls were demonstrated with respect to length of follow-up, ischemia time, anastomosis type, immunosuppression, and cytomegalovirus viremia. ACR was diagnosed in 52% of PBC subjects and 35% of controls (p=0.23). Differences in CV and CI frequency were noted between groups.

Comparison of histological findings in 23 patients after LT for PBC and 23 controls

	Post-OLT for PBC (n=23)	Post-OLT for NASH (n=23)	p value
P-ACR	4/23 (17%)	6/23 (26%)	0.47
P-ACR + CV	8/23 (35%)	2/23 (9%)	0.03
CV with or without P-ACR	11/23 (48%)	3/23 (13%)	0.008
ICV	3/21 (14%)	1/19 (5%)	0.3
CI	16/21 (76%)	6/19 (32%)	0.004

CV and CI were more common in PBC patients than controls. By multivariate analysis, the association between pre-LT PBC diagnosis and CV overall or with concurrent portal-based ACR was independent of other variables including hepatic artery flow, piggyback anastomosis, and immunosuppressive regimen.

Conclusions: Post-LT CV is more common in PBC patients than in NASH controls. In most cases CV was associated with the presence of portal features of ACR. These findings suggest PBC patients may have more severe rejection or unique biologic mechanisms of rejection such that the central veins are more affected than in NASH patients.

1454 Superior Mesenteric Vein/Portal Vein Invasion Is Associated with Poor Survival in Patients with Stage II Pancreatic Ductal Carcinoma Who Received Pre-Operative Chemoradiation

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Background: With recent advances in pancreatic imaging and surgical technique, patients who have pancreatic ductal carcinoma (PDC) with short-segment occlusion of the superior mesenteric vein/portal vein (SMV/PV) confluence and a suitable option for vascular reconstruction but no involvement of superior mesenteric artery or celiac axis, are considered borderline candidates for resection. However, the significance of SMV/PV involvement by PDC in patients who underwent pancreaticoduodenectomy is unclear. The purpose of this study was to evaluate the prognostic value of SMV/PV involvement in patients with stage II PDC who received chemoradiation.

Design: A total of 155 consecutive patients with stage II PDC who received preopertative chemoradiation and underwent pancreaticoduodenectomy at our institution between January 1, 1999 and December 31, 2004 were retrospectively analyzed. The H&E stained slides from all cases were reviewed. The SMV/PV involvement was defined microscopically as direct tumor invasion into the lumen of the vein, vein wall or adventitia (perivascular soft tissue ≤1.0 mm from media of the vein with fibrosis extending to the media). Statistical analyses were performed using SPSS software (version 12.0; SPSS, Chicago, IL) and survival was evaluated by Cox regression analysis.

Results: Among the 155 patients (54 stage IIA and 101 stage IIB), 45 patients had SMV/PV resection and 32 of them showed tumor involvement of SMV/PV (71%). The median disease-free survival (DFS) for patients with SMV/PV involvement by tumor was 8.9 ± 1.7 months compared to 14.4 ± 3.7 months for those patients without SMV/PV involvement or without SMV/PV resection (P=0.005), and overall survival (OS) was 21.2 ± 2.3 months compared to 28.5 ± 3.6 months, respectively (P=0.004). There were no differences in DFS or OS between the group of patients who underwent SMV/PV resection but with no microscopic involvement of SMV/PV and patients who did not have SMV/PV resection. In multivariate analyses, SMV/PV involvement (p=0.05 and p=0.011) and positive lymph node status (p=0.014 and p=0.031) correlated with DFS and OS respectively independent of T stage and differentiation.

Conclusions: The involvement of SMV/PV by pancreatic carcinoma is an independent prognostic factor and is associated with worse prognosis in patients with stage II PDC who received preoperative chemoradiation.

Neuropathology

1455 Primary Peripheral T-Cell Lymphomas of the Central Nervous System: Immunological Results and Molecular Approach to Diagnosis

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Background: Primary central nervous system lymphomas (PCNSL) are an uncommon extranodal lymphoma that involves the brain, leptomeninges, eyes or spinal cord in the absence of systemic disease. The majority of the PCNSL are diffuse large B-cell lymphomas, however, peripheral T-cell lymphomas (PTCLs) have been rarely reported. It is difficult to differentiate PTCL from a reactive T-cell process, especially when small T-cells predominate. Since there are no immunohistochemical markers for T-cell monoclonality, gene rarrangement analysis can be useful to make the diagnosis.

Design: Patient demographics (age, sex) and disease characteristics (location, histological type and treatment) were collected and hematoxylin and eosin stained sections were reviewed on 5 patients with T-PCNSL. Immunohistochemical stains for CD20, CD3, CD5, CD4, CD7, CD8, CD2, TIA-1 and Granzyme B; and in situ hybridization (ISH) for Epstein Barr virus (EBER) were performed on paraffin sections. T-cell receptor (TCR) gamma gene rearrangement analysis was performed by PCR with capillary electrophoresis.

Results: The majority of the PTCLs were located in the frontal lobe. The morphology showed a striking angiocentric pattern. Four of five tumors consisted of small to intermediate sized cells with round to irregular nuclei and scant cytoplasm. All cases were CD3 positive and three of four cases with sufficient tissue showed an abnormal T-cell antigen pattern (double negative CD4/CD8, double positive CD4/CD8, or loss of CD5; see Table).

Case details

	Case 1	Case 2	Case 3	Case 4	Case 5
Age (yrs)/ Sex	46/F	71/M	44/F	36/M	53/M
Location of Tumor	Frontal lobe	Frontal lobe	Frontal lobe	Cerebellum	Meninges, Retina
Symptoms	Headache	Memory loss	Headache	Multiple sclerosis	Neurological deficit
Histology	SI	SI	SI	SI	LC
CD20	-	-	-	-	-
CD3	+	+	+	+	+
CD5	+	-	-	+	+
CD4	-	+	+	+	ND
CD7	-	+	+	+	ND
CD8	-	+	+	-	ND
CD2	+	+	+	+	ND
EBER	-	-	-	-	ND
TCR rearrangement	Clonal	Clonal	Clonal	Clonal	ND
TIA-1	-	+	+	-	ND
Granzyme B	-	+	+	-	ND

M: Male; F: Female; SI: Small/Intermediate; LC: Large cell; ND: not done

Four of four cases studied showed a monoclonal T-cell receptor gamma gene rearrangement.

Conclusions: Since primary CNS-PTCL may mimick inflammatory, infectious and vascular diseases, morphology and immunohistochemical studies may be insufficient for diagnosis. TCR gamma gene rearrangement analysis assists in confirming the diagnosis of lymphoma.

1456 Use of CD10, CA9, and RCC To Distinguish between Clear Cell Meningioma and Metastatic Clear Cell Renal Cell Carcinoma

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Background: Clear cell meningioma can be difficult to distinguish from metastatic clear cell renal cell carcinoma by standard light microscopy. Differentiation between these two entities is essential in determining patient treatment and prognosis. The purpose of this study is to evaluate the utility of immunomarkers CA9, CD10, and RCC in differentiating between clear cell meningioma and metastatic clear cell renal cell carcinoma.

Design: A retrospective review of immunostaining with CA9, CD10, and RCC in 18 clear cell meningiomas compared with clear cell renal cell carcinomas (n=39).

Results: Eighteen patients (9 males, 9 females) with clear cell meningiomas were studied. The age range of this group at the time of surgery was 16 to 86 years (mean 58.5 years). The most common tumor sites included the meninges overlying the frontal lobe (n=7), the clinoidal region (n=2), and the cavernous sinus (n=2). Clear cell meningioma showed some immunoexpression of CA9 and CD10 in 38% (n=7) and 28% (n=5) of tumors, respectively. Most cases with positive staining showed < 5% positivity. No immunoexpression of RCC by clear cell meningioma was observed. Thirty-nine patients (27 males, 12 females) with either primary or metastatic clear cell renal cell carcinoma made up the renal cell carcinoma component study group. The age range of the patients at the time of surgery was 39 to 87 years (mean 63.5 years). Seventeen of the tumors were metastases and 22 were primary. The clear cell renal cell carcinomas stained positively for CA9, CD10, and RCC in 100%, 96%, and 42% of cases, respectively, with the majority of cases showing diffuse expression.

Conclusions: The immunohistochemical stains CA9, CD10, and RCC are useful in differentiating clear cell meningioma from metastatic clear cell renal cell carcinoma. The immunohistochemical marker RCC is not expressed in clear cell meningioma in this study, while CA9 and CD10 have limited expression in a minority of meningiomas. The combination of these stains may prove to be a useful panel when confronted with this differential diagnosis.

1457 Evaluating Interobserver Reliability in a Classification of Malformations of Cortical Development (MCD)

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Background: Malformations of cortical development (MCD) are a well-recognized cause of intractable epilepsy. A simplified histologic classification of MCD has been proposed which divides MCD into mild MCD and focal cortical dysplasia (FCD) types I and II (Palmini A et al. Terminology and classification of the cortical dysplasias.

Neurology 2004(62)6 S3, S2-8). Analysis of reproducibility has not been performed to date. The purpose of this study is to evaluate interobserver agreement within this classification system.
Design: Twenty-six epilepsy resections were selected representing the range described in the classification system. After being provided the reference cited above, eight neuropathologists classified each case as one of the following: Normal/gliosis, mild MCD, FCD type IA, FCD type IB, FCD type IIA, or FCD type IIB. After correlation of data, kappa analysis for concordance between multiple raters was performed.
Results: Concordance between the eight neuropathologists for the 26 cases was moderate overall (k=0.4968). However, a difference was noted in concordance within the FCD type II diagnoses and all others. Of the 14/26 (54%) cases in which the majority (≥ 6/8) of participants agreed, 12/14 (86%) cases were classified as FCD type IIA or IIB. In contrast, of the 12/26 (46%) cases without significant agreement, the majority (11/12, 92%) were notable for a conspicous absence of FCD type II diagnoses.
Conclusions: Concordance between eight neuropathologists using this MCD classification system is moderate overall. There are clear differences in concordance between FCD type II diagnoses and all others. The limitations of this system are evidenced by the lack of reproducibility when making the diagnoses of mild MCD and FCD types IA and IB, as well as the difficulty in differentiating between the more subtle MCD/FCD type I lesions and normal/gliosis.

1458 SOX2: A Glioma-Specific Marker and a Potential Target for Therapy

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Background: The Central Brain Tumor Registry of the United States reports that gliomas account for 78% of malignant brain tumors. (CBTRUS, 2006). 5-year survival rates range from 90.3% for pilocytic astrocytomas to 3.3% for glioblastomas (CBRTUS, 2006). Gliomas are currently diagnosed by histopathologic features, according to World Health Organization (WHO) criteria; however the process is subjective, and this leads to considerable diagnostic and prognostic variability among and within grades. Due to extensive molecular heterogeneity within individual histologic subgroups, a prognostic molecular/genetic model has not been established. We have identified a transcription factor, SOX2, which appears to be a sensitive and specific marker of gliomas. We hypothesize that SOX2 is a glioma-specific marker associated with tumor initiation and/or progression. This transcription factor functions to maintain pluripotency in the stem cell of the developing embryo and is expressed during neurogenesis in the adult human CNS.
Design: We analyzed 125 glial tumors and 44 non-glial primary CNS tumors by immunohistochemistry for expression of SOX2 protein. We also evaluated expression array data for SOX2 in 181 separate glioblastomas.
Results: SOX2 expression was found in 119/125 gliomas, including astrocytomas (WHO grades 1-4), oligodendrogliomas (WHO grade 2, 3), ependymomas (WHO grades 1-3) and oligoastrocytomas (WHO grade 2). Of 44 non glial primary CNS tumors, 41 were nonreactive for SOX2, including 16/17 tumors with neuronal features. Our expression array data showed strong SOX2 expression in 156/181 glioblastomas.
Conclusions: Our preliminary data suggest that SOX2 is expressed in the great majority of gliomas and in nearly all tumor malignant cells, but not in significant amounts in normal brain. The fact that SOX2 is an active transcription factor believed to regulate stem cell self-renewal suggests that inhibiting its function may have a therapeutic effect.
*This work was funded by Barrow Neurological Institute.

1459 Integrins Mediate Adherence of Medulloblastoma Cells to Extracellular Matrix and Activate Pathways Associated with Survival and Proliferation

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Background: Medulloblastoma spreads by leptomeningeal dissemination rather than by the tissue infiltration that characterizes the spread of many central nervous system tumors. Although in vitro adhesion of medulloblastoma to primary leptomeningeal cells was first demonstrated more than 20 years ago, this approach has received scant research attention since then while the understanding of cellular adhesion molecules, ECM molecules, and pathway activation has advanced considerably.
Design: This study represents an in vitro investigation using a system similar system to that previously described combined with ELISA assays for adhesion molecules not described at that time to define the molecules responsible for the adhesion of medulloblastoma to the leptomeninges. Western blots for Akt and MAPK activation were also used to define the pathway signaling that allows the tumor cells to survive and proliferate in thet novel environment emcountered during leptomeningeal dissemination.
Results: The results demonstrated adhesion of a medulloblastoma cell line (D283) to a glial extracellular matrix (ECM). Of 15 integrins tested (9 alpha subunits, 3 beta subunits and 2 alpha beta heterodimers), the adhesion was significantly associated only with the alpha 9 and beta 1 subunits. Antibody blockade of alpha 9 and beta 1 integrin subunits on the surface of D283 cells eliminated attachment to ECM. Glial ECM was enriched in only one of the proteins, tenascin, which is known to serve as a binding partner for the alpha 9 beta 1 heterodimer. The results of this study also demonstrated enhanced D283 cell survival and proliferation following engagement of the ECM. Western blots performed on adherent cells showed marked activation of MAP kinase p42/44 (Erk1/2) and only modest activation of Akt.
Conclusions: Our results suggest that the expression of alpha 9 beta 1 in the D283 cells and tenascin in the ECM are necessary for adhesion, and that adhesion is necessary for cell survival and proliferation.

1460 Regional Differences in Proliferation and Redox State in Gliomas. Where To Biopsy?

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Background: Gliomas share a diffuse infiltration of brain tissue and intratumor heterogeneity which can be an issue when assessing tumor grade in small tissue samples. Mutations in the p53 gene arise early in the development of gliomas.The cellular redox state is a potential mediator of p53-dependent apoptosis.In addition, oxidative stress relates to tumor viability and growth in gliomas, and plays an important role in the mechanism of action of cancer therapeutic agents. Methylguanine-methyltransferase (MGMT) is a DNA repair enzyme with an important role in cancer cell resistance to O6-alkylating drugs.
Design: The aim of this project was to study intra-tumor heterogeneity in gliomas, by investigating the differences between central and peripheral regions of nine brain tumors (six gliomas and three metastatic tumors) from gross total resection specimens.We studied the status of p53, Ki-67 and MGMT by immunohistochemistry; and evaluated the redox status by determining Mn-SOD, Citocrome C Oxidase,oxygene consumption and ROS productionWe compared these results with the clinical outcome in these patients.Data was tested for significance by t-test.
Results: All six gliomas were positive for p53 and five showed MGMT immunoreactivity with significant differences between central and peripheral regions.Ki-67 expression was variable,with values ranging from 5 to 85%, and a significantly higher central proliferation.All tumors showed a significantly higher production of oxygen radicals, higher COX activity (mitochondrial activity) and higher oxygen consumption at the periphery; whereas the center of the tumors showed higher values for Mn-SOD (anti-oxidant mechanism).Gliomas showed a significant higher activity for superoxide dismutase in the central regions. Glioma patients received chemotherapy with Temozolamide and radiotherapy or Temozolamide alone.Of the 9 patients, 2 were alive and free of disease,5 were alive with residual/recurrent disease and 2 were dead of disease at the time of evaluation.
Conclusions: These results reveal that differences exist between central and peripheral regions in brain tumors, particularly in regards to proliferation, mitochondrial activity and redox state.We believe that this work will contribute to advance the knowledge of the physiopathology of gliomas and may have an impact in treatment protocol designs.

1461 Rosette-Forming Glioneuronal Tumors: A Clinicopathologic Study of Three Cases Not Associated with the Fourth Ventricle

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Background: The 2007 WHO classification of CNS tumors introduced several new entities, including "rosette-forming glioneuronal tumor of the fourth ventricle (RGNT-4th)." Tumors with similar rosette-forming neuropil islands at sites other than the 4th ventricle (RGNT) have been also recently reported. Unlike tumors associated with the 4th ventricle, many of the RGNTs had an infiltrative glial component, and a propensity for progression and occasional leptomeningeal spread. It was therefore proposed that outside of the fourth ventricle region, neuronal differentiation in the way of rosette formation may not impart the same favorable prognosis and thus may not deserve categorization as a separate entity.
Design: We describe three examples of RGNTs occurring outside the 4th ventricle. Analysis of the clinical, radiographical, immunohistochemical, molecular and ultrastructural studies is performed.
Results: Tumors were located in the right frontal lobe, cerebellar vermis away from the 4th ventricle and cervical spinal cord. Radiographically, the tumors appeared as well-circumscribed, solid masses with focal contrast enhancement. All three tumors were characterized by extensive well-formed rosette formation surrounding neuropil islands. The latter stained intensely with synaptophysin and negatively for GFAP. MIB-1 labeling was virtually undetectable. The glial component was characterized as low-grade astrocytic in two cases, and as infiltrative oligodendroglial-like with relatively high cellularity and proliferative activity in another. The oligodendrolial component did not show 1p/19q co-deletion on FISH analysis. Ultrastructural examination showed some of the cells to have secretory granules, while others have processes filled with glial filaments. All three patients remain recurrence free approximately two years following surgery.
Conclusions: The striking morphologic resemblance between RGNT-4th and RGNT suggests that these are the same entity; even if one chooses to designate them both as mixed glioneuronal tumors or alternatively as glial neoplasms with divergent differentiation. There is precedent in both neuropathology and surgical pathology for site related differences in tumor behavior. In view of this, whether RGNTs arising in the vicinity of the 4th ventricle prove to have a more favorable outcome than those outside may be of less importance than morphology in the diagnostic classification of these tumors.

1462 Downregulation of Numerous Genes on Chromosome 22q11-13 and 1p34-36 in Higher Grade Meningiomas

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Background: Meningiomas are the second most common type of intracranial neoplasms. Although WHO grade I meningiomas are usually associated with favorable prognosis, higher grade (WHO grade II and III) menigiomas are more aggressive with less favorable outcome. The molecular changes associated with the differential biologic behaviors have not been well established. Expression profiling using DNA microarrays may offer clues to such molecular changes and point to potential therapeutic targets. We examined the expression profiles in 10 WHO grade I and 13 WHO grade II/III meningiomas in an attempt to identify potential differentially expressed genes.

Design: Fresh meningioma tissue samples were used for extraction and purification of total RNA. The Human Genome U133 Plus 2.0 Array (Affymetrix) was used for expression profiling. Synthesis and purification of cDNA and cRNA, hybridization to the microarrays and image scanning were performed according to the manufacturer's protocols. The expression signals were calculated by using the GeneChip Operating Software (GCOS, Affymetrix). Per chip and per gene normalization were used to process the data. Differential expression was statistically analyzed by t-test. Real-time quantitative PCR (qPCR) was used to further assess the expression of selected genes.
Results: Statistical analysis of the expression data showed that over three hundred genes were differentially expressed between WHO grade I and WHO grade II/III meningiomas with a P value less than 0.01. Less than 20% of these were up-regulated, but most were down-regulated. Significantly, in addition to genes located on 22q11-13 (including NF2), genes of 1p34-36 region were also over-represented in the group of down-regulated genes. Selected genes (including HDAC1 of 1p34) were further validated by using qPCR (with beta-actin as internal control). We describe here the functional analysis of these differentially expressed genes.
Conclusions: Our gene expression profiling data showed numerous genes on chromosome 22q11-13 and 1p34-36 are down-regulated in higher grade meningiomas. These molecular changes may have contributed to the pathogenesis of the more aggressive meningiomas.

1463 Chordoid Glioma: Molecular Characterization of Four Cases

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Background: Chordoid gliomas are rare, slow-growing neoplasms of the anterior third ventricle. Although an ependymal origin has been postulated, little is known about the genetic characteristics of this neoplasm.
Design: We report a case of chordoid glioma in a 41 year-old man with obstructive hydrocephalus. Basic histological characteristics, as well as immunohistochemical characterization, was completed. A variety of molecular diagnostic assays, including fluorescence in-situ hybridization (FISH), and array comparative genomic hybridization (aCGH), was performed on this case. Array CGH was also performed on 3 additional chordoid gliomas.
Results: Histologically, the tumor consisted of polygonal epithelioid cells admixed with spindle cells in a myxoid stroma. A prominent lymphoplasmacellular infiltrate was present. The tumor cells expressed glial fibrillary acidic protein (GFAP), epithelial membrane antigen (EMA), vimentin, CD31, CD34, epidermal growth factor receptor (EGFR), and S100 but were negative for pankeratin and E-cadherin. The MIB-1 proliferation index was 1-3%. No clonal proliferation of p53 positive cells was seen. FISH showed no evidence of *EGFR* amplification or of chromosome 7 hyperploidy. Tumor microdissection with subsequent FISH and polymerase chain reaction (PCR) analysis showed no deletions of chromosomes 1p, 9p, 17p, 10q, or 19q. Array comparative genomic hybridization detected a deletion in chromosome 11q in this case, as well as in 1 of 3 additional cases analyzed. This deletion was confirmed by FISH.
Conclusions: These are only the 5th through 8th cases of chordoid gliomas with molecular characterization. Our results suggest a distinct genetic origin from other gliomas.

1464 Expression of Alternative Splicing Variants of Survivin in Diffusely Infiltrating Astrocytomas

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Background: Diffuse astrocytoma (WHO grade I), anaplastic astrocytoma (WHO grade III), and glioblastoma multiforme (WHO grade IV) comprise the bulk of diffusely infiltrating astrocytomas. Recent studies showed that expression (particularly nuclear expression) of one of the inhibitor of apoptosis proteins (IAPs), survivin, is closely associated with astrocytoma grade. It has been postulated that differential expression levels of alternative splicing variants of survivin might contribute to the differential subcellular localization of survivin protein. We examined the expression status of the six alternative splicing variants of survivin in diffusely infiltrating astrocytomas to see whether some variants were preferentially expressed in astrocytomas.
Design: Total RNA was isolated from fifty-eight fresh astrocytoma tissue samples, including 17 diffuse astrocytomas, 15 anaplastic astrocytomas, and 26 glioblastomas. RT-PCR was used to assess the mRNA expression of survivin isoforms, using b-actin as internal control. Primers were designed to amplify specifically the six splicing variants of survivin (survivin, survivin delta-EX3, 2B, 2a, 3B, and 3a), respectively, according to the cDNA sequences in GenBank.
Results: RT-PCR analysis showed that the following four of the six alternative splicing variants were expressed in diffusely infiltrating astrocytomas: survivin, delta-EX3, 2B, and 2a. The overall positive expression rates of these variants in the 58 astrocytoma samples were 56.9%, 67.2%, 58.6% and 18.9%, respectively. The 3B and 3a isoforms were not detected in these samples. The positive expression rates of survivin, delta-EX3, and 2B all increased with tumor grade (from 29%-40% in diffuse astrocytomas, to around 80% in glioblastomas). However, the 2a isoform was virtually absent in diffuse and anaplastic astrocytomas, and was only detected in 38% of glioblastomas.
Conclusions: Four of the six alternative splicing variants of survivin (survivin, delta-EX3, 2B, and 2a) are expressed in diffusely infiltrating astrocytomas. The expression of the first three isoforms increass with tumor grade, but the 2a isoform appears to be preferentially expressed only in a subset of glioblastomas.

1465 The Survival Impact of Deletions of Chromosomes 1p and 19q in Glioblastoma Multiforme

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Background: Deletions on chromosomes 1p and 19q have been shown to correlate with prognosis and chemosensitivity in anaplastic oligodendrogliomas. Glioblastoma multiforme (GBM) 1p/19q chromosomal co-deletions have been reported to occur in up to 21% of tumors, 1p deletions in up to 19%, and 19q deletions in up to 30%; however, the impact on prognosis of these alterations in GBM is unclear.
Design: 337 GBMs resected between 2001 and 2006 were evaluated using florescence in-situ hybridization (FISH) for evidence of deletions of 1p and 19q. 1p deletion (with intact 19q) was observed in 21 (6.2%) tumors. 19q deletion (with intact 1p) was found in 18 (5.3%) tumors. A search of the electronic medical record was used to determine age at diagnosis, treatment modalities (including chemotherapy and radiation therapy) and survival. Cox regression was used to compare survival between these two groups and a control group of 1p and 19q intact tumors.
Results: 17 patients (pts) (9 males, mean age at diagnosis = 61 years (yrs), range (R) = 35-84 yrs) were found to have 1p deletions; 8 (47.1%) had chemotherapy and 13 were known to have had radiation therapy. The mean survival for this group was 10.8 months (mos) (R = 1-50 mos). 18 pts (7 males, mean age = 56 yrs, R= 25-76 yrs) had 19q deletions; 9 (50%) had chemotherapy and 8 were known to have had radiation therapy. The mean survival of this group was 8.4 mos (R = 1-17 mos). A control group of 20 pts (13 males, mean age = 60 yrs, R = 40-80 yrs) was selected, of whom 8 (40%) had chemotherapy and 12 were known to have had radiation therapy. The mean survival in this group was found to be 16.4 mos (1-59 mos). 9 (3.7%) of the tumors had codeletions of 1p and 19q; further testing is needed to evaluate the size of these deletions. Isolated 1p and 19q deletions did not significantly correlate with survival. Adjusting for sex, age, and chemotherapy, the 19q deleted group had a significantly lower survival (HR = 2.8, p = 0.025) than the other groups.
Conclusions: The incidence of isolated 1p or 19q deletions was 6.2% and 5.3%, respectively. In contrast to anaplastic oligodendrogliomas, 1p and 19q deletions alone were not found to improve survival of patients with GBM; however, when adjusted for age, sex and chemotherapy 19q deletions appear to negatively impact survival .

1466 An Immunohistochemical Study of MUM-1/IRF-4 and BCL-6 Indicating Activated GCB Phenotype in Primary Central Nervous System Lymphomas

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Background: Systemic diffuse large B-cell lymphomas (sDLBCL) have been divided into 3 subgroups: germinal center B-cell-like (GCB), activated B-cell like (ABC) and unclassified cases. GCB subgroup has better survival that the ABC subgroup. Primary central nervous system lymphomas (PCNSL) are thought to be of germinal center (GC) origin, yet have a poor prognosis even in immunocompetent patients. Studies of BCL-6 in PCNSL have shown conflicting prognostic results. MUM-1 is a transcription factor that regulates gene expression in lymphoid cells. MUM-1 has been identified as a marker of the ABC subgroup in sDLBCL, but with scant data in PCNSL. We studied the expression of BCL-6 and MUM-1 in PCNSL by immunohistochemistry to evaluate the phenotype of cases in our practice.
Design: A search for PCNSL in our pathology archives identified 9 cases of DLBCL in immunocompetent patients. The HE slides were reviewed independently by two pathologists and the diagnosis confirmed. Immunohistochemical staining was performed for BCL-6 and MUM-1, on formalin-fixed paraffin-embedded sections using Envision Plus System. Staining for MUM-1 was stratified into 3 groups based on percentage of positive tumor cells as strong (>75%), moderate (26-75%) weak (11-25%) and negative (<10%). Staining in >5% of tumor cells for BCL-6 was considered positive.
Results: Of the 9 cases of PCNSL 7 were BCL-6+ (78%) and 7 were MUM-1+ (78%). Six cases were MUM-1+/ BCL-6+ (67%), 1 MUM-1+/BCL-6- (11%), 1 MUM-1-/ BCL-6- (11%) and 1 MUM-1-/BCL-6+ (11%). The 1 MUM-1+/BCL6- patient died within 3 months of diagnosis. Of the MUM-1+/BCL-6+ patients, 1 died within 1 year, 1 had persistent tumor despite treatment 5 months after diagnosis, 1 was disease-free 1 year after diagnosis. No follow up was available in the other 3 cases. Of the 2 MUM-1- cases, one patient with plasmablastic lymphoma died within 5 days, and the other was alive 5 months after diagnosis.
Conclusions: In our study the majority of PCNSL showed MUM-1+/BCL-6+ expression indicating activated GCB phenotype. Since MUM-1 expression may be associated with a poor prognosis, it will be important to establish whether MUM-1 rather than BCL-6 is the dominant predictive biomarker in PCNSL.

1467 Expression of Chemotherapy-Response Marker MGMT in Neuro-Ectodermal Tumors

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Background: Chemo- and radiation-therapy remain the backbone of treatment of infiltrating gliomas and other neuro-ectodermal tumors (NET) of CNS. The most widely used alkylating agent is temozolamide (TM). Intranuclear enzyme: O-6-methylguanine-DNA methyltransferase (MGMT) removes TM-induced cross links between DNA and its nuclear expression predicts a resistance to alkylating agents. We tested MGMT expression in 150 NETs and found significant differences among various groups of tumors. The objective was to evaluate validity of immunohistochemical (IHC) detection of MGMT in prognostic studies of a response of NETs to treatment with TM.
Design: Semi-quantitative analysis of immunoperoxidase staining for MGMT was performed on paraffin embedded tissue. A previously established cut-off of 20% of tumor nuclei being positive for MGMT was used as a predictor of tumor resistance to TM.
Results: We have found that only 10% of WHO grade II-III oligodendroglial tumors were MGMT positive (mean % of positive nuclei 15±30%). In contrast, among WHO

grade II-IV astrocytomas 34% (25±21%) were positive. Staining of limited number of cases of primitive NET/medulloblastomas showed a high prevalence (60%) of immunopositive cases (46±47%). Similarly, all pilocytic astrocytomas, which are relatively resistant to chemotherapy, were found to be MGMT(+) (60±30%).
Conclusions: Our data confirm a validity of IHC staining for MGMT in NET. We demonstrate a higher prevalence of MGMT expression among astrocytomas, PNETs and pilocytic astrocytomas, which is consistent with a higher resistance of a larger fraction of these types of tumors to alkylating agents. Additionally, the presence of MGMT-immunoreactive oligodendrogliomas predicts that a sizable fraction of these tumors can be relatively resistant to TM. Thus immunohistochemical staining for MGMT has a high value in clinical practice in selection of the most effective modality of treatment.

1468 Cell Signaling and Proliferative Factors in Astrocytic Tumors: Association of the "Funnel" Factor p4E-BP1 and Cyclin D1 with Ki67 Marker in High Grade Gliomas but Not in Pylocytic Astrocytomas

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Background: In most gliomas there is activation of cell signaling pathways fired by different oncogenic alterations. Four grades of malignancy are admitted by the WHO classification. Pylocytic astrocytoma (PA) is considered grade I and benign tumor. Glioblastoma multiforme (GBM) can derive from low grade (AGII) and anaplastic astrocytomas (AGIII), or arise de novo.In GBM, overexpression of PDGFR, EGFR amplification, lack of PTEN expression and other genetic alterations have been described. In previous studies we identified that p4E-BP1, a factor that plays a central role in protein synthesis of cyclin D1, myc, VEGF, FGF and survivin, acts as a "funnel" factor in carcinomas, reflecting the oncogenic role of the cell signaling pathway. The aim of this study is to analyse the expression of p4E-BP1 in gliomas and its association with malignancy and proliferation.
Design: Tissue microarrays of 70 astrocytic tumours were performed, including 43 GB (61%), 9 AGIII(13%), 9 AGII (13%) and 9 (13%) pilocytic astrocytomas (PA). Immunohistochemistry for EGFR, p4E-BP1, pMAPK, pAKT, cyclin D1, Ki67 and p53 was done. Levels of expression were semiquantitatively evaluated as percentage and intensity of stained cells (histo-score).
Results: In grade II, III and IV, p4E-BP1 expression increased significantly regarding malignancy grade (p=0.007). EGFR expression was higher related to malignancy grade, from grade I to IV. There is a significant correlation between Ki67 and p4E-BP1 expression (p=0.003). Ki67 and cyclin D1 associated with progression of astrocytomas with values ranging from less than10% in AGII to over25% in high grade gliomas. PA showed moderate levels of cyclin D1 and p4E-BP1 and<1%Ki67 positive cells. No differences of pMAPK expression were seen between low and high grade tumors.
Conclusions: In gliomas p4E-BP1 is highly overexpressed in AGIII and GBM while AGII showed a mild nuclear expression. Moreover, p4E-BP1 in gliomas II-IV associated with cyclin D1 and Ki67 proliferative markers. These results support the role of p4E-BP1 as a funnel factor in gliomas grade II-IV where may reflect accumulative oncogenic alterations in the cell signaling pathway. Conversely PA showed moderate p4E-BP1 which correlated with cyclin D1, but not with Ki67 and EGFR. The lack of association between p4E-BP1 and cyclin D1 with Ki67 in PA could reflect a novel biochemical pathway activated in PA.

1469 Neuronal DNA Replication and Cell Cycle Reactivation in Vascular Cognitive Impairment and Cortical Lewy Body Disease

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Background: Normal neocortical neurons are post mitotic and do not enter the cell cycle. They retain elements of the cell cycle that may be reactivated by neuronal injury leading to initiation of cell death programs. Interruption of this partial reactivation process prevents neuronal death. Increased expression of neuronal cell cycle proteins suggesting cell cycle reactivation occurs in neurodegenerative disorders and stroke.This increase may reflect functions of these proteins unrelated to the cell cycle. Demonstration of DNA replication is needed to confirm partial cycle reactivation. It has been documented only in Alzheimer's disease with FISH to detect extra nuclear DNA signals (Perry A, et al. Brain Path 4:584, 1994; Yang Y, et al. J Neurosci 21:2661-68, 2001).
Design: FISH was performed on five micrometer paraffin-embedded autopsy sections of temporal neocortex from 5 cases of vascular cognitive impairment (VCI), 3 cases of cortical Lewy body disease (CLBD), 5 cases of Alzheimer's disease (AD), and 3 cases of non-demented elderly persons using a commercial fluorochrome-labeled centromeric DNA probe for chromosome X (Vysis Inc., Downers Grove, IL). Nuclei were counterstained with DAPI (Insitus, Albuquerque, NM) and the sections examined by fluorescence microscopy with appropriate filters. In each case sections with 60-80% of neuronal nuclei showing fluorescent signals were identified. In each of these sections, the number of fluorescent signals in 100-200 nonoverlapping, intact neuronal nuclei with signals were counted. The percentage of neuronal nuclei with extra signals was calculated for each case. Adjacent sections in each case were stained with H&E and gallocyanin and examined for mitotic figures and apoptosis.
Results: Neuronal nuclei with extra DNA signals occurred in 5/5 VCI cases (range 3-18%, median 6%), 2/3 CLDB cases (3% and 38%), 5/5 AD cases (range 4-18%, median 7%), and 0/3 nondemented elderly cases. Mitotic figures and apoptosis were not observed in any of the H&E or gallocyanin stained sections.
Conclusions: Neuronal DNA replication and partial cell cycle reactivation occur in VCI and CLBD in addition to AD. The reactivation pathways in these disorders are potential therapeutic targets for prevention of neuron death. Partial reactivation of the cell cycle leading to neuronal death may be a feature of other neurodegenerative and cerebrovascular disturbances.

1470 MicroRNA-124 Functions as a Potential Tumor Suppressor in Medulloblastoma

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Background: Based on the observation that miR-124 is predominantly expressed in differentiating neurons and the current hypothesis that external granule progenitors of cerebellum are the cells-of-origin for medulloblastoma, we investigated if miR-124 plays a role in the oncogenesis of this pediatric neoplasm.
Design: Quantitative RT-PCR analysis revealed that miR-124 transcript was significantly down-regulated in 21/29 (72%) of medulloblastomas and 7/7 of cell lines examined. Forced expression of miR-124 in miR-124-deficient cells inhibited cell proliferation and induced apoptosis, suggesting that miR-124 may function as a tumor suppressor. Using computational and expression analyses SLC16A1 was identified as a candidate target of miR-124.
Results: Transfection of miR-124 resulted in down-regulation of SLC16A1 at transcript and protein levels. Reporter assay with 3'untranslated region of SLC16A1 cloned downstream of the luciferase gene showed reduced luciferase activity in the presence of miR-124, providing evidence that miR-124 is a direct regulator of SLC16A1. Expression analysis further showed that SLC16A1 transcripts were elevated in 26/29 (90%) of tumors and all cell lines examined. Importantly, RNA interference of SLC16A1 induced cell death in medulloblastoma, a biological sequel mimicking that observed with miR-124 overexpression.
Conclusions: We speculate that knockdown of SLC16A1, which functions to export lactic acid accumulated in tumor cells undergoing glycolysis, results in a decline of intracellular pH to a lethal level. In conclusion, our findings provide novel insights into the molecular pathogenesis of medulloblastoma and suggest a potential tumor suppressor role for miR-124.

1471 Neoplastic Schwann Cells Express Transcription Factors Involved in Schwann Cell Development

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Background: A number of transcription factors have emerged as important in guiding normal neural crest and Schwann cell development in animal studies. The role of these transcription factors in neoplastic human Schwann cells in poorly defined.
Design: A set of tissue array blocks was assembled with representative samples from 34 malignant peripheral nerve sheath tumors (MPNSTs), 76 schwannomas and 105 neurofibromas. The schwannomas include sporadic and NF2 associated ones. The neurofibromas include localized, diffuse and plexiform variants; some of the non-plexiform neurofibromas were from sporadic cases. The MPNSTs included sporadic as well as neurofibromatosis (NF) 1 associated cases. Sections were stained with antibodies to SOX5, SOX9, SOX10, FoxD3, AP2alpha and PAX7. The nuclear staining was graded as 0 to 3+. Chi squared testing was performed.
Results: SOX9 is widely expressed in Schwannomas (100% positive, i.e.1+ to 3+staining), neurofibromas (93% positive) and MPNSTs (88% positive). SOX10 is also found in a large proportion of Schwannomas (99% positive), Neurofibromas (99% positive) and MPNSTs (94% positive). There is some variation in the staining intensity for SOX9 and SOX10 between the three tumor groups. Fox D3 reactivity is stronger and found in more Schwannomas (94% positive) and MPNSTs (94% positive) than Neurofibromas (64% positive). AP2alpha is positive in 30% to 40% of cases in all subgroups but stronger 2+ and 3+ reactivity is only found in MPNSTs and Schwannomas. Expression of PAX7 and SOX5 is restricted to smaller subsets of MPNSTs (about 20% for each). All markers showed statistically significant differences in the three-way comparison between the three different tumor groups ($p<0.05$). The two-way comparison between neurofibromas and MPNSTs showed statistically significant ($p<0.05$) differences for all markers except SOX9 and SOX10. No clear differences were found between different tumor subsets (e.g. familal vs. sporadic, neurofibroma types or tumor site for schwannomas).
Conclusions: The same transcription factors that guide normal Schwann cell development are also expressed in Schwann cell neoplasms. SOX9 and SOX10 are identified as general markers of Schwann cell neoplasm that are expressed in almost all Schwannomas, neurofibromas and MPNSTs. FoxD3, AP2alpha, PAX7 and SOX5 are upregulated in MPNSTs compared to neurofibromas and may be markers of malignant transformation.

1472 Factors Associated with Decreased Muscle Coenzyme Q10 in Adults with and without Previous Statin Treatment and Myopathy

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Background: Inhibition of the mevalonate pathway by HMG-CoA reductase inhibitors, or "statins", may decrease the biosynthesis of both cholesterol and coenzyme Q10 (CoQ10). Controversy exists about the possible relationship between low muscle CoQ10 and statin myopathy. In addition, other factors, including old age, female gender, metabolic diseases, and high statin dosage, may be associated with increased risk of statin myopathy. The objectives of this study are to determine whether skeletal muscle specimens from adults with previous statin exposure and myopathy are CoQ10 deficient; and if other risk factors may also be associated with muscle CoQ10 deficiency.
Design: This study was approved by the IRB of the institution. Excess tissue from routine muscle biopsies was collected (between 6/1/06 and 6/30/07), and stored at -70°C until analysis. Selection criteria were (1) history of previous statin use and (2) evidence of myopathy. Specimens for disease controls were selected based upon no history of statin use or myopathy. Specimens were evaluated for evidence of myopathy by two pathologists and a trainee. Also excess muscle was tested for CoQ10 in the Clinical Laboratory of the Cincinnati Children's Hospital using a validated HPLC method

(*Clin Chim Acta* 2004;341:173-84). Multiple logistical regression applied age, gender, diabetes mellitus, hypertension, subsarcolemmal mitochondrial aggregates (SSMA) (%), and type 1 myofiber (%) effects on muscle CoQ10 content.

Results: A total of 19 muscle specimens (mean 52 y; 9 F) were collected and evaluated for this study, including 11 with histories of statin use and myopathy plus 8 controls. Groups were similar for age, F/M, SSMA (%), and type 1 myofibers (%). Muscle CoQ10 concentration (normalized to protein) was similar in myopathy vs. control groups, but was decreased in females ($P<0.05$) of both groups. Multiple regression analysis showed that only gender was significantly correlated with muscle CoQ10 content ($P = 0.05$).

Conclusions: Although somewhat limited by sample size these results indicate that statin myopathy may *not* be significantly associated with low muscle CoQ10 unless gender differences are considered. Future studies should consider gender effects when evaluating muscle CoQ10 in statin myopathy.

1473 Epithelial and Pseudoepithelial Morphology in Glioblastoma: Comparative Pathologic and Molecular Study of Different Subtypes

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Background: Glioblastomas exhibit a remarkable tendency to morphologic diversity. Although rare, pseudoepithelial components (adenoid or epithelioid) or true epithelial differentiation may occur and poses a significant diagnostic challenge.

Design: We identified 58 cases (M=38, F=20). On review of available H&E slides in all cases, tumors were classified as adenoid (A-GBM), "true epithelial" (TE-GBM) or epithelioid (E-GBM). Criteria for TE-GBM was based on morphologic evidence of epithelial differentiation (e.g. nests of cells with more generous cytoplasm than typically seen in adenoid examples, squamoid nests or glandular structures) and immunohistochemical (IHC) expression of CAM 5.2, EMA or pCEA. E-GBMs were largely characterized by often round cells with abundant, process-poor cytoplasm and well defined cell borders. IHC stains were performed as well as dual color FISH studies either on a tissue microarray (n=29) or unstained microsections (n=4) using probes targeting *EGFR*, *P16*, *PTEN* and *RB1* with respective reference probes (CEP7, 9, 10, and LSI 13q34).

Results: Median age at diagnosis was 57 years (range, 6-85) and median overall survival 7 months. A-GBM predominated (48%): the tumors were composed of proliferative cells arranged in cohesive nests or trabeculae, frequently associated with a myxoid stroma. TE-GBM were next most frequent (35%), followed by E-GBM (17%). Overall 25 (43%) cases featured a sarcomatous component. Median MIB-1 labeling indices were higher in the adenoid/epithelial (37%) vs the sarcomatous (19%) and glial (16%) components. Molecular Abnormalities identified by FISH are summarized below (see table).

Molecular Abnormalities

n (%)	P16 del/-9	-10	PTEN del	EGFR amp	RB1 del/-13q	P53 IHC (3+)
A-GBM	6/10 (60)	4/10(40)	1/10 (10)	1/10(10)	5/10(50)	5/14(36)
TE-GBM	9/14 (64)	8/14(57)	4/14(29)	4/15(27)	2/14(14)	9/17(53)
E-GBM	5/7 (71)	5/8(63)	2/8(25)	4/8(50)	0/4(0)	2/7(29)

del=deletion, amp=amplified

Conclusions: Pseudoepithelial/epithelial morphology is a rare but recognized phenomenon in glioblastomas. These patterns are associated with a poor prognosis. A-GBMs exhibit different molecular cytogenetic abnormalities than do TE-GBM or E-GBM, including an increased frequency of RB1 deletions, thus suggesting different oncogenic mechanisms in their pathogenesis.

1474 Molecular Characteristics of Metastatic Non-Small Cell Lung Carcinoma (NSCLC) to the Brain

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Background: Despite recent advances, the molecular mechanisms of brain metastasis remains poorly understood. Characterizing brain metastases will permit optimal utilization of new therapies including angiogenesis inhibitors. We investigated the role of EGFR, MIB-1, caspase-3, E-cadherin, VEGF-A, and VEGF-C in the development of brain metastasis by comparing immunohistochemistry expression of brain metastases with their primary NSCLC.

Design: We evaluated 21 patients with NSCLC metastatic to the brain that had lung and brain surgery at the Brigham and Women's Hospital, Boston. Lung and matched brain surgical pathology specimens from each patient were immunostained with antibodies against EGFR, E-cadherin, MIB-1, caspase-3, VEGF-A, and VEFG-C. Each marker was evaluated in the primary NSCLC and brain metastasis as follows: a labeling index (number of positive tumor cells/total number of tumor cells in the field of tumor showing the highest staining) was calculated for MIB-1 and caspase-3, a percentage staining in the entire slide was calculated for E-cadherin, VEGF-A, VEGF-C. A positivity score was calculated for EGFR. The results in the brain metastasis were compared with the primary NSCLC.

Results: The patients were of 11 women (52%) and 10 men (48%). Median age was 65 years (range: 39-84 years). The results of molecular markers expression in the primary and metastatic NSCLC are summarized in table 1.

Table 1. Results of various molecular markers in the primary NSCLC vs. metastatic brain NSCLC

Molecular marker	Primary NSCLC Median (range)	Metastatic NSCLC to brain Median (range)	P value
MIB-1	35 (2-70)	55 (2-80)	0.02
Caspase-3	1 (1-6)	1.5 (1-7)	0.12
VEGF-A	100 (0-100)	90 (10-100)	0.03
VEGF-C	35 (0-100)	55 (0-90)	0.32
E-cadherin	80 (5-100)	90 (0-100)	0.43
EGFR Score	2+ (0-3)	3+ (0-3)	0.03

Conclusions: Our results showed that metastatic NSCLC to the brain have a higher expression of MIB-1 ($p=0.02$), and EGFR ($p=0.03$), and lower VEGF-A ($p=0.03$) expression in brain metastases than the matched primary NSCLC. Understanding the differences between brain metastases and other metastases will allow to clarify the mechanism of brain metastases specifically and to potentially target these mechanisms for novel drug designs.

1475 Distinguishing Chordoid Meningiomas from Their Histologic Mimics: An Immunohistochemical Evaluation

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Background: Chordoid meningioma (CM), WHO grade II, is a rare variant of meningioma with a propensity for aggressive behavior and recurrence. Recognition of this entity is important in cases that show similar morphologic overlap with other chondroid/myxoid neoplasms which can arise within or near the central nervous system (CNS). As immunohistochemical (IHC) markers can prove useful in the differential diagnosis, a formal assessment of CM versus tumors with significant histologic overlap using a panel of both novel and traditional antibodies including D2-40, S100, cytokeratin (CK), and EMA has not been previously reported.

Design: IHC staining of 10 cases of CM were assessed and compared with 11 cases of extraskeletal myxoid chondrosarcoma (EMC), 22 low grade chondrosarcomas (LGC), 27 enchondromas (EN) and 18 chordomas (CH) using monoclonal antibodies D2-40, S100, CK, and EMA. Staining was semiquantitatively evaluated on one representative section per case as negative (0, <5% cells stained), focally positive (1+, 5-10% cells stained), positive (2+, 10-50% cells stained), or diffusely positive (3+, >50% cells stained), and a mean extent (E, range 0-3) calculated. Staining intensity was graded from 0 to 3+ and a mean intensity (I, range 0-3) calculated. Statistical analysis utilized Fisher's two-tailed exact test.

Results: IHC results are summarized in the table below.

	D2-40	S100	CK	EMA
CM	8/10 (80%)	4/10 (40%)	2/10 (20%)	9/10 (90%)
	E=2,I=1.6	E=1,I=1.5	E=2.3,I=2.1	E=2.5,I=1
EMC	0/11 (0%)	9/11 (82%)	3/11 (27%)	3/11 (27%)
		E=2.2,I=1.8	E=2.3,I=1.7	E=1.7,I=1.3
LGC	21/22 (95%)	22/22 (100%)	0/22 (0%)	0/22 (0%)
	E=2.1,I=2.4	E=2.5,I=2.2		
EN	26/27 (96%)	27/27 (100%)	0/27 (0%)	0/27 (0%)
	E=2.6,I=2	E=2.7,I=2.5		
CH	0/18 (0%)	17/18 (94%)	18/18 (100%)	17/18 (94%)
		E=2.5,I=2.1	E=3,I=3	E=2.6,I=2.2
p-value (+cases)	<0.001	<0.001	<0.001	<0.001

Conclusions: While CK staining in occasional CM can be helpful in differentiating from LGC and EN, diffuse immunoreactivity with EMA is superior in this differential. Additionally, immunostaining with the novel antibody D2-40 in the majority of CM is valuable in excluding EMC and CH. In conjunction with radiographic and clinical features, IHC studies with a panel of D2-40/S100/CK/EMA can prove useful in the assessment of a chondroid/myxoid neoplasm within or near the CNS with overlapping morphologic findings.

1476 Are Vascular Proliferation and Necrosis in Astrocytoma Smear Preparations Predictive of GBM Histology?

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Background: Glioblastoma (GBM) is a high grade astrocytoma characterized by necrosis, microvascular proliferation (MVP) or both. These features are typically identified in tissue sections for establishing diagnosis. Intraoperative diagnosis of astrocytomas uses smear preparations and frozen sections (FS). While MVP and/or necrosis can often be appreciated in smear preps, it is not clear if findings are concordant among pathologists or predictive of tissue findings in FS or permanent sections (PS). Here we investigated interobserver concordance and predictive value of necrosis and MVP in smear preps of infiltrative astrocytomas.

Design: The concordance among four pathologists using a set of 30 astrocytomas, including 10 diffuse astrocytoma (A, grade II), 10 anaplastic astrocytoma (AA, grade III) and 10 GBM (grade IV) was studied. Pathologists blinded to the diagnoses individually reviewed H&E smear preps of all 30 cases and recorded the presence of MVP or necrosis. MVP was defined as tufted or glomeruloid vessels. An agreement coefficient (AC) of the pathologists was calculated for each feature. Next, the study set was expanded to 70 cases, including 20 A, 25 AA, and 25 GBM. Two pathologists blinded to diagnoses examined the smear preps and histologic sections and reached an agreement on the presence of MVP and necrosis in each prep. MVP and necrosis on smear preps were correlated with FS and PS.

Results: The AC between the four pathologists for identifying necrosis and MVP in smear preps were 0.64 and 0.59, respectively. The performance standards of smear preps for necrosis present in the PS and MVP are shown in Table 1.

Table 1

	Necrosis (%)	MVP (%)
Sensitivity	73	90
Specificity	90	80
PPV	76	64
NPV	88	95

Among cases of GBM (n = 25), necrosis was present in 68% of smear preps and 88% of FS, while MVP was present in 88% of smear preps and 60% of FS. In 8 cases of GBM, MVP was noted in smear preps but not in FS. In one case of GBM, MVP was noted in the FS but not in the smear prep.

Conclusions: Necrosis and MVP can be identified in the intraoperative smear preparation of GBM with substantial concordance. The presence of these features in smear preps are often predictive of their presence in frozen and permanent sections. MVP was more frequently identified in smear preparations of GBM than in FS. Thus, smear preps are a helpful adjunct to histologic sections for the diagnosis of GBM at the time of FS, especially with regard to MVP.

1477 Tumor Infiltrating Lymphocytes in Primary CNS Lymphomas

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Background: Primary CNS lymphomas (PCNSL) are aggressive non-Hodgkin B-cell lymphomas (NHL) generally confined to the CNS and eye. Morphologically, most PCNSL are large B-cell lymphomas (LBCLs) indistinguishable from systemic LBCLs. As with systemic LBCLs, tumor infiltrating lymphocytes (TILs) are commonly seen in primary CNS lymphomas. TILs, especially CD4+, CD25+ FOX-P3+ regulatory T-cells, have been suggested to play a role in tumor growth in both classical Hodgkin and non-Hodgkin lymphomas. However, the role of TILs have not been formerly studied in PCNSL. We evaluated the prognostic implications of regulatory and cytotoxic subsets of TILs in PCNSL.

Design: A total of 29 cases (M:F 17:12, median age 66 yrs, range 37-87 yrs) of PCNSL diagnosed between 2002 and 2007. The median survival was 5.75 mths (range 0.75-61 mths). The TIL subsets were evaluated using CD3, FOX-P3, and TIA-1 immunohistochemical stains. Enumeration of TIL subsets was performed by AT and LT independently and blinded from survival data. Cells were counted at high power (400x) and averaged over 2-4 fields depending on the specimen size.

Results: There was an average of 164 T-cells per high powered field (hpf) ranging from 25-621 cells/hpf. FOX-P1+ cells averaged 19 cells/hpf (range 1-108 cells/hpf). TIA-1+ cells averaged 74 cells/hpf (range 0-293 cells/hpf). The interobserver correlation was moderate to strong for CD3, FOX-P1, and TIA-1 cells (R=0.82, 0.72, and 0.62, respectively). No significant association between the number of regulatory cells (FOX-P1+) or cytotoxic cells (TIA-1+), and survival at 6 months was identified (p=0.53 and 0.12, respectively). No significant association between TIA-1:FOX-P3 ratio and survival was seen.

Conclusions: TILs of PCNSL contain cytotoxic and regulatory subsets. However, unlike systemic non-Hodgkin lymphomas, there appears to be no association between the TILs and prognosis. Although TILs appear to play a role in some neoplasms, it appears that its role in PCNSL may be limited. PCNSL are often aggressive and of the activated B-cell phenotype. In addition, PCNSL have been shown to often lack HLA expression. These features may limit the effect of immune regulation of these lymphomas.

1478 An Approach for the Development of Neuropathological Criteria for Vascular Cognitive Impairment Utilizing Honolulu-Asia Aging Study (HAAS) Resources

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Background: The HAAS cohort was established in 1991 with the examination of 3734 Japanese-American men then aged 71-93 years. Brain autopsies were done on 642 men who died between 1991 and 2003. Microscopic data available on 439 cases revealed 151 cases with clinically significant levels of Alzheimer lesions, neocortical Lewy bodies or hippocampal sclerosis. To examine the effects of vascular pathology alone on cognitive functions we restricted these analyses to the 288 brains without these other lesions.

Design: Brain sections selected for microscopy include: Frontal, temporal, parietal and occipital cortices, putamen, caudate, globus pallidus, hippocampus, cerebellum, thalamus, midbrain, pons and medulla. All gross infarcts and lacunes was recorded and confirmed by microscopy. The sections was stained for H&E then examined for microinfarcts and infarcts. The quantity and age of the lesions for each section was recorded.

Results: We found a strong correlation between cognitive impairment and microvascular infarcts supporting the commonly held belief that ischemic small vessel disease is a major cause of dementia in late life. Based on our initial exam, 118/288 had more than 2 large vessel infarcts, and/or more than 2 lacunar infarcts, and/or more than 2 microinfarcts. Of these 118 decedents, 44 had cognitive testing within 3 years of death (8 was normal, 14 marginal, and 22 demented).

Conclusions: We plan to expand our study of relationships of the numbers, types, and regional distributions of infarcts with severity of cognitive impairment in this subset (n=44) of brains as a strategy for devising provisional neuropathological criteria for vascular cognitive impairment. Our goal is to define criteria that is efficient and economical for use by pathologists. The issues to consider for this study include determining: (1) optimal stains (H&E, GFAP, CD-68, NeuN, Luxol Fast Blue), (2) brain regions to select for microscopy, (3) age of microinfarct (recent vs remote) (4) how to record the observations to facilitate analyses and interpretations (5) the number of lesions required to estimate the likelihood that these lesions caused vascular cognitive impairment.

1479 Immunohistochemical Markers To Distinguish between Hemangioblastoma and Metastatic Clear-Cell Renal Cell Carcinoma in the Brain: Utility of Aquaporin 1 Combined with Cytokeratin AE1/AE3 Immunostaining

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Background: Distinguishing hemangioblastomas from metastatic clear-cell renal cell carcinomas in the brain is a diagnostic challenge due to similar clinical and morphologic presentations. Inhibinα and aquaporin1 were shown positive markers of hemangioblastoma, but cannot reliably distinguish hemangioblastoma from metastatic CCRCC. The purpose of this study was to show that this distinction can be achieved using a combination of markers.

Design: The study group included 87 patients with either hemangioblastomas (67 lesions) or metastatic clear-cell renal cell carcinomas in the central nervous system (34 lesions). All samples (n=101) were analyzed with a panel of antibodies including aquaporin1, inhibinα, D2-40, cytokeratin AE1/AE3, EMA and CD10. Furthermore, WesternBlot analysis was performed with D2-40 and aquaporin1 antibodies.

Results: The study confirms the usefulness of aquaporin1 (97% sensitivity, 83% specificity) and inhibinα (88% sensitivity, 79% specificity) as positive markers of hemangioblastoma and shows that aquaporin1 is a superior positive marker *versus* inhibin α for the differential. Positivity of tumor cells with cytokeratin AE1/AE3 is the signature of a metastatic CCRCC (88% sensitivity, 100% specificity), CD10 expression as well (100% specificity, 79% sensitivity). The combined use of aquaporin1 and AE1/AE3 attains a high degree of sensitivity and specificity to distinguish between hemangioblastoma and metastatic CCRCC. All but one tumors aquaporin1 positive and cytokeratin AE1/AE3 negative (65/66) correspond to hemangioblastomas (97% sensitivity, 97% specificity, 98.5% diagnostic positive predictive value). Tumors with the reverse profile, aquaporin1 negative and cytokeratin AE1/AE3 positive, (25/25), correspond to metastatic CCRCC (74% sensitivity, 100% specificity, 100% diagnostic positive predictive value). We failed to find a utility for D2-40 antibody for this diffential.

Conclusions: Aquaporin1 is the most sensitive positive marker of hemangioblastoma. Although of moderate specificity, when used in combination with epithelial marker AE1/AE3 it allowed to reliably distinguishing hemangioblastoma from metastatic CCRCC.

1480 Malignant Epithelioid Glioneuronal Tumor: Unusual Phenotype or New Entity?

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Background: Malignant glioneuronal tumors of the central nervous system are heterogenous and remain incompletely codified.

Design: We report the clinicopathologic features of two unusual malignant epithelioid glioneuronal tumors in pediatric patients. Both patients were male, aged 5 and 6 respectively.

Results: In both cases, preoperative imaging studies demonstrated large fronto-parietal lobe mass lesions with heterogeneous enhancement, mass effect and marked surrounding edema. Histologic examination of the resection specimens revealed well circumscribed high-grade tumors of predominantly epithelioid morphology but also with a small cell undifferentiated cellular component. Additional features include geographic necrosis, vascular endothelial proliferation, lymphocytic infiltration and brisk mitotic activity. Immunohistochemical stains showed positivity for GFAP, vimentin, NSE and chromogranin A in both tumors, and additional positivity for synaptophysin and neurofilament protein in one tumor, consistent with both astrocytic and neuronal differentiation. Tumors cells showed co-expression of synaptophysin, chromogranin and GFAP. The Mib-1 labeling index was high, ranging from 20% to 60%. Ultrastructural examination showed few cells with intermediate filaments and membrane-bound dense core neurosecretory granules. The first patient had rapidly progressive disease and was lost to follow up, presumed dead in less than a year. The second patient has progressive disease, with a doubling of tumor size over the three month follow up period.

Conclusions: The tumors share histopathology and clinical aggressiveness with three cases previously described in adults *(Acta Neuropathol 2006;112:727),* and they represent an aggressive, hitherto undefined, form of epithelioid glioneuronal tumor that is distinct from anaplastic ganglioglioma, malignant mixed glioneuronal tumor, and other types of previously defined glioneuronal tumor.

Ophthalmic

1481 Stem Retinoblastoma Cells in Tumorogenesis and Tumor Progression

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Background: Tumor vitreous seeds, tumor treatment resistance, late recurrence and metastasis are the most challenging aspects of treating children with retinoblastoma. Stem cancer cells have been implicated in tumor chemotherapy resistance and in late recurrence and successful survival of distance metastasis in other tumors. We study the role of retinoblastoma stem cancer cells through *in vitro*, *in vivo* and human tissue studies.

Design: Y79 cell line and primary murine and human tumor cell lines were developed in stem cell media. These cells were then grafted into vitreous cavity of Rag-2 mice (immune-deficient mouse) and tumors were developed. Primary human tumors and primary transgenic mouse retinoblastoma tumors (Pax6 driven SV40/Tantigen transgene) were also studied. Tumors were characterized by culture cell growth type, morphology and immunophenotype using CD133, Nestin, and Sox2 neural stem cell markers and neuronal differentiation markers (synaptophysin, NSE) and for glial cells (GFAP) by double-labelling.

Results: Stem cell markers labeled a select small population of cells in the primary tumors of mouse and humans with specific characteristics for vitreous seeds. Cultured cell lines and primary human and mouse in stem cell media produced neurospheres and the cells labeled with CD133, Nestin and Sox2. When transplanted to the vitreous cavity of Rag-2 mice the transgenic mouse tumor cells and Y-79 human cell lines produced tumors that were metastatic to the brain. Secondary intraocular tumors and brain metastasis expressed stem cell markers in a select population of retinoblastoma cells. Some but not all of these cells marking with stem cell antibodies also expressed neuronal markers and T-antigen (mouse).

Conclusions: Functional, morphologic, and immunophenotypic characterization of retinoblastoma tumors suggest that these develop from a progenitor cell (multipotent, slow proliferation, well adapted to hypoxia, limited expression of differentiation markers) that give rise to cells with predominant neuronal differentiation. Metastases