

**Design:** Needle biopsies of pancreas were obtained prospectively from patients suspected of having AIP on clinical grounds. Duodenal mucosa was obtained retrospectively by sampling mucosa from Whipple resections done for pancreatic masses later confirmed as AIP. For controls, we selected a series of duodenal biopsies, including 20 diagnosed as normal, 19 with Giardiasis, 20 with celiac disease, 24 with peptic duodenitis and 10 associated with adenoma. Immunohistochemical stain for IgG4 was scored semiquantitatively as previously described: 0 = 1 to 5 positive cells/high power field; 1 = 6-10 positive cells; 2 = 11-30 positive cells; 3 = >30 positive cells.

**Results:** All needle biopsies of pancreas had increased staining for IgG4, helping to confirm the diagnosis of AIP. Duodenal mucosa from patients with known AIP did not show more IgG4-positive cells than controls.

IgG4 score	IgG4 stain in pancreatic biopsy and duodenal mucosa						
	Pancreas AIP	Duodenal mucosa Normal	Giardia	Celiac	Duodenitis	Adenoma	
0	0	7	18	17	14	20	3
1	2	1	2	2	5	4	5
2	2	1	0	0	1	0	2
3	5	0	0	0	0	0	0

**Conclusions:** Immunohistochemical staining for IgG4 is a useful adjunct to the diagnosis of AIP in needle biopsies, particularly since helpful histologic clues such as periductal lymphoplasmacytic infiltrates and obliterative phlebitis may not be sampled by needle biopsy. IgG4 staining of duodenal biopsies will not help identify patients with AIP.

## Neuropathology

### 1318 CADASIL: New Evidence of Vascular Degeneration

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**Background:** CADASIL, or Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy, is a hereditary form of vascular dementia caused by mutations in the Notch 3 gene which is located on Chromosome 19. The clinical course is characterized by early onset of migraine headaches, progressive occurrence of transient-ischemic attacks and strokes, depression, dementia, and premature death. The target structures in CADASIL include mainly medium and small vessels within the cerebral white matter, but extra-CNS sites such as skin may also be affected. Although the Notch 3 mutations are present from birth, the disease does not manifest itself until the third or fourth decade of life. To understand the possible mechanisms of disease progression, we examined gene expression related to Notch signaling in postmortem brain tissue from patients with CADASIL or normal aging.

**Design:** Formalin-fixed paraffin-embedded tissue sections were immunostained to detect Notch 1, Notch 3, smooth muscle actin, ubiquitin, and insulin-like growth factor, type 1 receptor. RNA was extracted from fresh frozen brain tissue to perform real time quantitative RT-PCR analysis of the same genes to determine if their altered levels of expression were mediated at the level of transcription.

**Results:** Immunostaining studies demonstrated that the granular degenerative changes in the media of white matter and leptomeningeal arterioles was associated with increased smooth muscle actin fragmentation and ubiquitination of proteins. In addition, the levels of insulin-like growth factor (IGF-I) receptor expression in the vessels were reduced. Real time quantitative RT-PCR studies using RNA isolated from white matter vessels confirmed the significantly reduced IGF-I receptor expression, as well as down-regulation of smooth muscle actin and both Notch 1 and Notch 3. Further in vitro experiments showed that Notch expression was regulated by IGF-I signaling.

**Conclusions:** Vascular degeneration in CADASIL is associated with down-regulation of genes encoding the IGF-I receptor, smooth muscle actin, Notch 1, and Notch 3. Since IGF-I regulates Notch expression, and Notch regulates cytoskeletal function, impaired IGF-I signaling in vessels may contribute to the progression of CADASIL vasculopathy with increasing age.

### 1319 Evaluation of NF2 Gene Deletion in Sporadic Schwannomas, Meningiomas and Ependymomas by Chromogenic In Situ Hybridization

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**Background:** Neurofibromatosis type 2 (NF2) is an autosomal dominant cancer syndrome characterized by the development of bilateral vestibular schwannomas and other central nervous system (CNS) tumors, in particular meningiomas, gliomas and ependymomas. The NF2 gene has been isolated from chromosome 22 and germ line mutations have been identified in NF2 patients. Fluorescence in situ hybridization (FISH), loss of heterozygosity (LOH)-testing, and comparative genomic hybridization (CGH) have been used to detect NF2 gene alterations in both sporadic and NF2-associated CNS tumors. In this study, we performed chromogenic in situ hybridization (CISH) to evaluate for NF2 deletion in a group of sporadic schwannomas, meningiomas and ependymomas.

**Design:** Eighteen sporadic lesions, including nine ependymomas, six meningiomas and three schwannomas were included in this study. CISH was performed utilizing the NF2 deletion probe (Zymed Laboratories). Deletion of NF2 gene was identified when the NF2 gene copy number was less than the centromeric copy number in more than 50% of tumor cells. Cases were also categorized as normal diploid when two copies were present in > 50% of tumor cells and aneuploid when 3-5 copies were seen.

**Results:** Deletion of the NF2 gene was identified in 10 tumors, including 2 out of 3 schwannomas, 5 out of 6 meningiomas, and 3 out of 9 ependymomas. The remaining eight cases were diploid.

**Conclusions:** Our results show that schwannomas, meningiomas and to a lesser degree ependymomas express a high incidence of NF2 gene deletion, and support the hypothesis that NF2 gene plays an important role in the tumorigenesis of these tumors. We have used CISH as an efficient, economic and reliable method for routinely assessing NF2 gene deletion in sporadic schwannomas, meningiomas and ependymomas.

### 1320 1p/19q Loss in Gliomas: Microsatellite Amplification in Comparison with FISH

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**Background:** 1p/19q loss is a favorable indicator of chemosensitivity and prolonged survival in anaplastic oligodendroglioma. Determination of 1p/19q status may also help to subclassify oligoastrocytomas and to distinguish glioblastoma from anaplastic oligodendroglioma. Fluorescent in situ hybridization (FISH) and microsatellite amplification by polymerase chain reaction (PCR) are the two main techniques used for 1p/19q loss determination.

**Design:** To compare the results of FISH and PCR in 1p/19q loss determination, both techniques were carried out on 48 glioma tissue samples. These included 9 grade II oligodendrogliomas (ODs), 8 grade III oligodendrogliomas (AODs), 2 grade II oligoastrocytomas (OAs), 3 grade III oligoastrocytomas (AOAs), 4 grade III astrocytomas (AAs), and 22 glioblastomas (GBs). In regard to FISH, for each chromosome we used a probe specific for the potentially deleted arm and a probe for the opposite arm as control. A total of 300 cell nuclei were evaluated per slide. As for PCR, 4 microsatellites were amplified for each chromosome (D1S199, D1S214, D1S508 and D1S2734 for 1p; and D19S112, D19S219, D19S412 and D19S596 for 19q). LOH was analyzed with GeneScan. Peripheral blood or normal tissue from the same patient was used as control.

**Results:** In 41 of the 48 cases, FISH and PCR results were completely coincidental. In 4 cases (3 ODs and 1 AOD) tissue was insufficient for FISH evaluation. In 1 case (AA) PCR showed 1p deletion undetected by FISH, and in 2 GBs FISH suggested 1p and 19q losses not shown by PCR. Combined 1p/19q losses were present in 4 ODs, 3 AODs, 1 OA and 2 GBs, by either FISH or PCR, or by both. Isolated 1p loss was seen in 1 AA and 4 GBs, and isolated 19q loss in 1 AOA and 5 GBs.

**Conclusions:** Our findings indicate that FISH and PCR provide virtually coincidental results in regard to determination of 1p/19q loss. It seems thus that the experience and facilities available should be the main considerations to be taken into account by each institution when deciding which method to implement for the study of 1p/19q status.

### 1321 Diagnostic Utility of Microtubule Associated Protein-2 in Separating Schwannoma from Meningioma Including the Fibrous Subtype

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**Background:** Both schwannomas and meningiomas can occur at the cerebello-pontine angle and histologically display a spindle cell morphology. Separating schwannomas from meningiomas is usually not difficult based on clinical, histologic and immunohistochemical studies. However, fibrous meningioma can show some histological and immunophenotypic features of schwannomas such as cellular and acellular areas, thickened hyalinized blood vessels, elongated and twisted nuclei, positive immunostaining for S-100 and negative immunostaining for EMA. Microtubule-associated proteins are a major component of cytoskeleton family proteins associated with microtubule assembly and is specifically expressed in the central and peripheral nervous systems. However, the expression of MAP-2 in schwannoma, as well as diagnostic utility of MAP-2 in separating schwannoma from meningioma including the fibrous subtype, has not been studied.

**Design:** A total of 40 cases, consisting of 20 schwannomas and 20 meningiomas including 3 fibrous meningiomas, were retrieved from the hospital database. Immunostaining with antibodies to MAP-2 were performed on paraffin-embedded tissue. Immunostains were performed on an automated immunostainer with appropriate positive and negative controls.

**Results:** Diffuse and strong MAP-2 immunoreactivity was demonstrated in 19/20 (95%) of schwannomas, while focal immunoreactivity was demonstrated in 1/20 (5%) meningiomas. None of the fibrous meningiomas exhibited immunoreactivity for MAP-2.

**Conclusions:** MAP-2 is expressed in schwannomas, but not in most meningiomas, including fibrous meningiomas. The expression of MAP-2 may be useful in distinguishing schwannomas from fibrous meningiomas especially when limited material is present in a brain biopsy.

### 1322 PTEN Loss by Gliomas Induces Endothelial Tissue Factor Expression

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**Background:** Glioblastoma (GBM) is a high grade, rapidly fatal infiltrative astrocytoma distinguished by pseudopalisades, which are hypoxic, densely cellular zones surrounding necrosis. Pseudopalisades are critical to the rapid biologic progression of GBM, yet initiating mechanisms are unknown. We have proposed that intravascular thrombosis promotes pseudopalisade formation following PTEN loss. The aim of this study is to define factors secreted by gliomas following PTEN loss that lead to endothelial tissue factor (TF) expression and thereby promote thrombosis.

**Design:** We used a PTEN null human GBM cell line (U87MG) with a stably transfected muristerone-inducible wt PTEN (23.11 cells) to model glioma progression. Conditioned media from 23.11 +/- PTEN was collected after 96 hours of hypoxia (1% O<sub>2</sub>) or normoxia (21% O<sub>2</sub>) and tested for its ability to induce TF expression by human dermal microvascular endothelial cells (HDMEC). Conditioned media was added to HDMEC for 24 hours and endothelial TF was analyzed by Western Blot. VEGF and

TNF- $\alpha$  proteins were directly measured in conditioned media by ELISA. We also exposed HDMEC to increasing concentrations of VEGF (10ng/ml and 100ng/ml) and TNF- $\alpha$  (5ng/ml and 10ng/ml) and measured TF by Western Blot after 24 hours.

**Results:** We found that conditioned media from 23.11 cells lacking PTEN induced endothelial TF expression compared to 23.11 cells with PTEN. Conditioned media from glioma cells in hypoxia did not induce endothelial TF. However, exposure of HDMEC to hypoxia potentiated the effect of conditioned media on TF expression induced by PTEN (-) gliomas. Both TNF- $\alpha$  and VEGF caused HDMEC upregulation of TF when added to cells directly. We also found that PTEN loss caused a slight increase of VEGF in conditioned media ( $1.70 \pm 0.08$ ng/ml compared to  $1.40 \pm 0.17$ ng/ml), whereas TNF- $\alpha$  levels remained the same following PTEN loss (0.04ng/ml).

**Conclusions:** Gliomas secrete a factor following PTEN loss that induces endothelial TF expression and may be important to intravascular thrombosis and necrosis in GBM. This effect is potentiated by endothelial hypoxia. VEGF is secreted at increased levels following PTEN loss and may contribute to this effect.

### 1323 IGFBP2-Associated Diffuse Glioma Initiation and Progression Demonstrated in the RCAS-tva Mouse Model System

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**Background:** Insulin-like growth factor binding protein 2 (IGFBP2) has recently been shown to be overexpressed in 80% of the most advanced type of diffuse glioma, glioblastoma (GBM), and overexpressed IGFBP2 correlates with poor survival in diffuse gliomas. *In vitro* functional studies have shown that IGFBP2 increases glioma cell migration and invasion. We hypothesize that IGFBP2 is a key regulator of glioma progression.

**Design:** We tested our hypothesis using the somatic gene transfer RCAS-tva mouse model system, which permits the introduction of specific genes into specific cell lineages. In this system, avian virus receptor is expressed exclusively in glial cells via linkage to the neuroglial-specific nestin promoter. Genes of interest are cloned into an avian RCAS vector and viral particles are expanded in DF1 avian fibroblasts. When injected into the neonatal mouse brain, the virions infect only glial cells and the genes of interest borne by the virions are only expressed in glial cells. For these experiments, the study genes were IGFBP2, PDGFb, K-Ras, and Akt, which were delivered separately and in combination.

**Results:** Our results show that PDGFb signaling leads exclusively to the formation of low-grade oligodendrogliomas. PDGFb delivered in combination with IGFBP2 results in the formation of oligodendrogliomas and anaplastic oligodendrogliomas. The higher-grade tumors are characterized by increased cellular density, vascular proliferation, and poor survival. Combined K-Ras and Akt leads to the formation of astrocytomas; K-Ras alone or Akt alone do not result in tumor formation. IGFBP2 in combination with K-Ras produces astrocytomas, which are histologically similar to the gliomas resulting from K-Ras/Akt stimulation. No tumor formation resulted from the simultaneous delivery of Akt and IGFBP2, suggesting that IGFBP2 and Akt likely lie in the same pathway or in converging pathways.

**Conclusions:** The present studies show that: 1) IGFBP2 is associated with progression from low-grade O to high-grade AO in gliomas initiated by PDGFb overexpression *in vivo*, and 2) IGFBP2 can synergize with the Ras pathway to produce diffuse gliomas *in vivo*. Collectively, the data demonstrate that IGFBP2 actively contributes to diffuse glioma initiation and progression. Studies are ongoing to further elucidate the signaling pathways of IGFBP2-induced gliomagenesis.

### 1324 Identification of Novel Proteins in Brain Tissue from Patients with Creutzfeldt-Jakob Disease Using a Functional Proteomics Approach: Implications for Prion Disease Pathogenesis

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**Background:** Prion diseases are a unique group of human and animal diseases being comprised of sporadic, familial, and transmitted forms, of which sporadic Creutzfeldt-Jakob disease (sCJD) is the most common human prion disease. Although studies of protein expression of selected genes have been done, global gene expression analysis has not been performed to date. We hypothesized that while isolation of intact RNA from autopsy tissue is hampered by degradation, the global proteome should be generally intact. The tandem mass spec (LC/MS/MS) approach offers a means by which a large number of proteins can be identified simultaneously, potentially lending insights into mechanisms of pathogenesis not previously appreciated.

**Design:** Proteins extracted from cerebellar homogenates from four sCJD subjects ("classic" clinicopathological phenotype, Type 1 protein, M/M at codon 129, absence of mutation), and four age-matched controls were fractionated by high-speed ultracentrifugation into mitochondrial, membrane-associated, and cytosolic fractions. Samples were run on 1D Tris-HCl 8-16% gels and differentially expressed bands were selected for LC/MS/MS analysis. The GPM (Global Proteome Machine Organization) program was used to identify MS peaks and all proteins were annotated with appropriate gene accessions for bioinformatics and data mining. The program GenMAPP was used to visualize gene expression data on maps representing biological pathways and potentially novel functional signaling and metabolic pathways.

**Results:** 76 proteins were identified in CJD samples that were undetected in control samples. Of these, 10 proteins were common to CJD and neurodegenerative disease literature including CALM, GFAP, LAMC, PARK7, 14-3-3 zeta, SNAP25, SOD2, TUBA6, TUBA8, and UCHL1. GenMAPP analysis showed that CJD genes were found to be significantly overrepresented in several expected (oxidative stress response) and unexpected (alcohol catabolism, intracellular ligand-gated ion channel activity, insulin signaling) ontologies.

**Conclusions:** To date, the proteomics approach has not been applied to prion disease research. This study indicates that the proteomics approach is feasible, and indeed has elucidated novel proteins and ontologies. The findings also substantiate our previous assertions that prion diseases share molecular mechanisms in common with non-prion neurodegeneration.

### 1325 Increased Annexin A1 Expression in High-Grade Diffuse Gliomas: A Potential Prognostic Marker

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**Background:** Diffuse gliomas constitute the most common type of malignant primary brain tumor and can be subdivided into two principal lineages: astrocytic and oligodendrocytic. Gliomas of astrocytic lineage have been shown to have a worse prognosis compared to those of oligodendrocytic lineage of comparable grade. A promising candidate prognostic marker is annexin A1 (ANXA1), a calcium binding protein implicated in arachidonic acid metabolism and EGFR tyrosine kinase pathway. This study was designed to evaluate ANXA1 expression in the different types and grades of diffuse gliomas.

**Design:** A tissue microarray (TMA) was assembled from gliomas diagnosed according to the WHO 2000 classification. The TMA included 16 oligodendrogliomas (Grade II), 9 mixed oligoastrocytomas (Grade II), 24 anaplastic oligodendrogliomas (Grade III), 9 anaplastic mixed oligoastrocytomas (Grade III), 23 anaplastic astrocytomas (Grade III), 4 gliosarcomas (Grade IV) and 40 glioblastomas (Grade IV). ANXA1 expression was determined by immunohistochemistry.

**Results:** ANXA1 was expressed in 6% of low-grade oligodendrogliomas. Higher ANXA1 levels were present in tumors with an astrocytic component and in those of higher WHO grade: 34% of mixed oligoastrocytomas, 24% of anaplastic oligodendrogliomas, 45% of anaplastic mixed oligoastrocytomas, and 57% of anaplastic astrocytomas. The highest ANXA1 levels were present in Grade IV tumors: 75% of gliosarcomas and 88% of glioblastomas.

**Conclusions:** ANXA1 expression shows a positive correlation with glioma grade, suggesting a possible role in tumor progression

### 1326 HER-2/neu Expression in Glioblastoma Multiforme

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**Background:** The HER-2/neu oncogene encodes for a transmembrane glycoprotein with intracellular tyrosine kinase activity. The HER-2/neu receptor belongs to the family of epidermal growth factor receptors which are crucial in the activation of subcellular signal transduction pathways controlling epithelial cell growth and differentiation. Overexpression of HER-2/neu is observed in 20-40% of breast cancers as well as other solid tumors. Although information is limited, one study suggested that 15% of glioblastoma multiforme (GBM) express HER-2/neu by immunohistochemistry; gene amplification by fluorescence in situ hybridization (FISH) was not investigated. Studies in this area are potentially significant due to the role of recombinant monoclonal anti-HER-2/neu antibody trastuzumab (Herceptin) in the treatment of tumors.

**Design:** A retrospective clinicopathologic review of 44 patients with GBM with HER-2/neu immunohistochemical staining and HER-2/neu gene amplification by FISH was performed.

**Results:** The study included 44 patients (17 females, 27 males; age range 20-79 years, mean 57.9 years). Initial surgery involved tumor debulking or subtotal resection in 34 pts. Thirty-six pts received adjuvant radiation therapy and 19 pts received adjuvant chemotherapy. At follow-up (range 1.0-49.5 months, mean 10.5 months), 40 pts died with tumor (mean survival 12.7 months) and 4 patients were lost to follow-up. All tumors were negative for HER-2/neu protein by immunohistochemistry. HER-2/neu gene amplification by FISH was not observed in any of the tumors.

**Conclusions:** None of the 44 GBM's demonstrated HER-2/neu protein expression by immunohistochemistry or amplification of the HER-2/neu gene by FISH. The HER-2/neu oncogene does not appear to play a role in the pathogenesis of GBM.

### 1327 Phosphohistone H3 (PHH3) Index in Diffuse and Anaplastic Astrocytomas: Correlation with Mitotic Index and Grading

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**Background:** Histone H3 phosphorylation is a mitosis-related event, leading to the formation of PHH3. Recently, it has been shown that anti-PHH3 labeling of mitotic cells in meningiomas could facilitate more rapid and objective grading of the tumor (Ribalta et al, Am J Surg Pathol, 28:1532). In diagnosing diffusely infiltrating astrocytomas, the identification of mitoses is an important criterion in the separation of WHO grade II astrocytomas from WHO grade III (anaplastic) astrocytomas. We investigated the usefulness of PHH3 immunostaining in this respect and evaluated its correlation with mitotic figure (MF) counting.

**Design:** Formalin-fixed, paraffin-embedded tissues from fourteen diffuse astrocytomas (WHO grade II) and thirteen anaplastic astrocytomas (WHO grade III) were examined for PHH3 labeling using a polyclonal anti-PHH3 antibody (Upstate Biotechnology, Lake Placid, NY) and standard labeled streptavidin-biotin immunostaining with AP red as chromogen. PHH3 labeling index was assessed according to Ribalta et al and expressed as PHH3(+) cells/10 high power field (HPF).

**Results:** PHH3 labeling index showed an average of 0.13/10 HPF in the diffuse astrocytoma (WHO grade II) group, and 4.1/10 HPF ( $p < 0.01$ ) in the anaplastic astrocytoma (WHO grade III) group, which correlated well with mitotic figure count obtained from standard HE-stained slides, being less than 0.1/10HPF and 3.0/10HPF, respectively, in the two groups. The non-zero value of MF in WHO grade II astrocytomas came from an occasional MF in an otherwise typical grade II tumor that did not allow

upgrading according to the WHO criteria. PHH3 staining in anaplastic astrocytomas highlighted mitotic cells and made it much easier to quickly estimate the mitotic index and hence to grade the tumor.

**Conclusions:** PHH3 labeling correlated well with MF counting in separating WHO grade II diffuse astrocytomas and WHO grade III anaplastic astrocytomas. Use of PHH3 staining might save time by sparing a pathologist from looking for MFs as well as avoid counting in equivocal MFs.

### 1328 Activation of C-Jun NH<sub>2</sub>-Terminal Kinases (JNKs) Correlates with the Histologic Grades and EGF Receptor Expression in Diffuse Gliomas

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**Background:** The c-Jun NH<sub>2</sub>-terminal kinases (JNKs) play an important role in both apoptosis and tumorigenesis. They regulate gene expression through the phosphorylation of various transcription factors. As one of the downstream pathways of EGFR, the role of the JNK pathway in glioma formation and/or progression is unclear.

**Design:** The present study was designed to evaluate the activation of JNKs in human diffuse gliomas using high-throughput tissue microarrays. A glioma tissue microarray was constructed using formalin-fixed, paraffin-embedded archival tissue blocks. The tissue microarray included 142 diffuse gliomas, including 52 glioblastomas, 32 anaplastic astrocytomas, 3 low-grade astrocytomas, 29 anaplastic oligodendrogliomas, and 26 low-grade oligodendrogliomas. The expression levels of phospho-JNKs and EGFR were evaluated by immunohistochemistry on tissue sections cut from the array block. The anti-phospho-JNK antibody (Santa Cruz Biotechnology, Inc., CA) detects isoforms JNK1, JNK2, and JNK3 when phosphorylated at Thr-183 and Tyr-185.

**Results:** Phospho-JNKs was found to be overexpressed in 82.7% of glioblastomas (43/52), 46.9% of anaplastic astrocytomas (15/32), 48.3% of anaplastic oligodendroglioma (14/29), 26.9% of low-grade oligodendroglioma (7/19), and 0% of low-grade gliomas (0/3). Overexpression of phospho-JNK correlated significantly with the histologic grades of gliomas ( $p < 0.05$ ). In addition, the activation of JNKs correlated significantly with the overexpression of EGFR in glioblastomas and in the overall tumor group ( $p < 0.05$ ).

**Conclusions:** The results from our study showed that activation of JNKs correlated with the histologic grades of diffuse gliomas, suggesting a role of JNK activation in glioma progression. Our study also showed, for the first time, that the overexpression of phospho-JNKs correlated with the expression of EGFR in diffuse glioma.

### 1329 The Controversial Nomenclature of Schwannomas: Neurofilament Protein Staining Demonstrates Intra-Tumoral Axons in Many Schwannomas

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**Background:** Schwannomas are benign peripheral nerve sheath tumors believed to be composed purely of cells with ultrastructural features of Schwann cells; these tumors are believed to develop eccentrically from the periphery of nerves and not to contain axons, other than immediately beneath the capsule. This concept has recently been disputed in cases associated with NF-2. These tumors are generally said to be distinguishable from neurofibromas in which intra-tumoral axons are usually present.

**Design:** Eighty typical schwannomas (20 conventional, 20 cellular, 20 ancient, 10 gastric and 10 plexiform) were retrieved from the authors' files. H&E stained slides were reviewed, diagnoses were confirmed and all tumors were stained for S-100 protein and neurofilament protein (NFP). Appropriate positive and negative controls were used. The amount (rare, focal, multifocal and diffuse) and distribution (central and/or peripheral) of axons within the tumors were analyzed.

**Results:** All tumors were strongly and diffusely positive for S-100 protein (nuclear and cytoplasmic staining). NFP-positive axons were present in 11 of 20 (55%) conventional schwannomas (2 rare, 4 focal, 3 multifocal and 2 diffuse; 5 central, 4 peripheral and 2 both central and peripheral) and in 15 of 20 (75%) cellular schwannomas (3 rare, 6 focal and 6 multifocal; 12 central, 1 peripheral and 2 both central and peripheral). Of the 20 ancient schwannomas, 7 cases (35%) showed intratumoral axons, highlighted by NFP immunostaining (4 focal, 1 multifocal, 1 diffuse and 1 rare; 4 peripheral, 2 central, 1 both central and peripheral). Most cases of gastric schwannoma showed no evidence of intratumoral axons; 9 cases (90%) were negative for NFP and only 1 case (10%) was positive (focal and central). Seven of 10 (70%) plexiform schwannomas were negative for NFP, while 3 cases (30%) showed positive axons (2 multifocal and 1 focal; all central).

**Conclusions:** The unexpected but quite frequent presence of intra-tumoral axons in schwannomas argues against conventional views of these lesions' pathogenesis as an eccentric encapsulated lesion. Although NFP-positive axons were most often present in conventional and cellular schwannomas, their presence was also observed in a minority of ancient, gastric and plexiform schwannomas. Differentiation between neurofibroma and schwannoma in cases with overlapping cytomorphologic features should not be based on the presence or absence of NFP-positive axons within the tumor.

### 1330 Loss of Smad4 Expression in a Subset of Primary Central Nervous System Lymphomas (PCNSL)

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**Background:** PCNSLs are aggressive tumors, and research on prognostic and therapeutic value of biological molecules is being actively pursued. Smad4 is a tumor suppressor gene on chromosome 18q21.1 first identified in pancreatic cancer and belongs to the Smad family whose gene products play important roles in the signaling pathway of transforming growth factor b (TGFb) family which possess broad spectrum of biological responses. One important activity of TGFb is its potent pro-apoptotic and anti-mitogenic effects which have been shown to be mediated by Smad proteins. Indeed,

escaping from Smad-induced growth inhibition and apoptosis by inactivating mutations and deletions of Smad genes is common in various tumors. Most notably, loss of expression of Smad4 has been demonstrated in tumors of the pancreas and colorectal origin. But the expression of Smad4 in PCNSL has not been well defined. **Design:** Thirty-three cases of intra-axial PCNSLs (of B cell phenotype) were retrieved from the pathology archives and were stained with an antibody against Smad4 (sc-7966 Santa Cruz Biotechnology, Santa Cruz, CA) using an automated immunostainer (Ventana Medical System). Cytoplasmic and nuclear staining pattern was scored semiquantitatively with regard to both intensity and distribution of the stain. Negative was defined as no/weak staining while positive as moderate/strong staining at regional/diffuse distribution.

**Results:** Of 33 PCNSL cases, 8 were negative for both cytoplasmic and nuclear Smad4 expression. Of the remaining 25 cases, an average of 67.2% of tumor cells were stained for Smad4 in each case. Thus, about one-third of tumor cells lost Smad4 expression. Among the 25 cases stained with Smad4, 23 showed positive nuclear staining, and 14 positive cytoplasmic staining, with 12 cases showing both nuclear and cytoplasmic staining (positive concordance).

**Conclusions:** When Smad4 negativity is defined as above, the loss of Smad4 expression occurs relatively infrequently (1/4) in PCNSL. However, even in cases with Smad4 expression, 1/3 of tumor cells have lost Smad4 protein. This implies that loss of Smad4 may be a means by which a subset of PCNSL cells escape inhibitory control exerted via Smad proteins. To define this subset of tumors further at molecular and genetic level would be of great clinical significance in designing new therapeutic approaches.

### 1331 The Value of Tandem MRI/CSF Evaluation for Predicting Disseminated Disease in Childhood Central Nervous System Neoplasms

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**Background:** The presence of leptomeningeal disease in pediatric patients with central nervous system neoplasms significantly affects their prognosis and treatment. Lumbar cerebrospinal fluid (CSF) cytology and magnetic resonance imaging (MRI) of the spine are usually performed during the staging evaluation. It has been suggested that either CSF cytology or spinal MRI alone would miss leptomeningeal spread at initial presentation in up to 14-18% of children with medulloblastoma. In this study, we extend this observation and investigate the concordance rates of lumbar CSF cytology and spinal MRI performed at the time of initial diagnosis as well as at various times during the follow-up of patients with medulloblastoma, ependymoma, germ cell tumors, and atypical teratoid/rhabdoid tumors (AT/RT).

**Design:** 78 patients with a median age of 7 years (range <1 to 22 years) were selected from the UCSF database from 1990-2005 using the following criteria: 1) A diagnosis of medulloblastoma (38 patients), ependymoma (16 patients), AT/RT (4 patients), or germ cell tumors (germinoma - 18 patients, mixed germ cell tumors - 2 patients). 2) Spinal MRI with concurrent lumbar CSF cytology performed within approximately one month of each other at the time of diagnosis and/or anytime during follow up. The median follow up time was 2.5 years (range <1 year to 13 years).

**Results:** In 78 patients, there was a total of 128 spinal MRIs with concurrent lumbar CSF cytology. Of the 113 concordant cases, 106 were negative and 7 were positive. Of the 15 discordant cases, 6 had a positive CSF with negative MRI and 9 had a positive MRI with negative CSF. Thus leptomeningeal disease was diagnosed by MRI only in 9 cases, CSF only in 6 cases, and both MRI and CSF in 7 cases. The discordant cases are presented in tables 1 and 2.

**Conclusions:** MRI correlates better with aggressive disease than CSF cytology. A positive CSF cytology and negative spine MRI portends a worse prognosis only if there is radiographic evidence of intracranial tumor spread. The significance of a positive CSF cytology with a negative spine and brain MRI is unclear and should be further investigated.

### 1332 Discrepancies between Frozen Section and Final Diagnoses in Central Nervous System Neoplasms

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**Background:** Frozen section (FS) for intraoperative evaluation of central nervous system (CNS) lesions serves several important functions. It provides an assessment of specimen adequacy and facilitates the appropriate triage of tissue for ancillary studies. FS also provides an accurate preliminary diagnosis; however, certain lesions are recognized to cause differential diagnostic challenges at the time of FS. This study sought to identify those difficult cases in order to heighten awareness of common diagnostic pitfalls in surgical neuropathology.

**Design:** All CNS FS cases involving a tumor diagnosis at FS or permanent section (n=2156) from September 1997 until June 2005 were retrospectively reviewed. Discrepancies between the FS and final diagnoses were identified.

**Results:** Of the 2156 cases identified, 57 (2.7%) discrepant diagnoses were found. The average age of the patients (29 males, 28 females) with discrepant diagnoses was 46.6 years. The vast majority of the FS diagnoses and all of the final diagnoses were rendered by one of three staff neuropathologists; however, 3 of the 57 discrepant cases were seen only by general pathologists at the time of FS. 12/57 (21.1%) discrepancies involved errors in classification of spindle cell lesions of the CNS, most commonly schwannoma versus meningioma. 12/57 (21.1%) cases involved errors in differentiating oligodendrogliomas from astrocytomas. 9/57 (15.8%) discrepancies involved errors in the diagnosis of CNS lymphoma. 8/57 (14.0%) cases involved errors in differentiating reactive from malignant processes, most frequently gliosis versus glioma. 4/57 (7.0%) discrepancies involved errors in the overgrading of tumors. The remaining 12/57 (21.1%) cases included an assortment of other discrepancies. The majority of the discrepancies did not significantly impact patient management.

**Conclusions:** FS of CNS neoplastic processes is highly accurate. Less than 3% of FS diagnoses were found to be discrepant with the final diagnoses. Approximately 80% of the discrepant cases could be classified into the following five categories: spindle cell lesions of the CNS, astrocytoma versus oligodendroglioma, differential diagnosis of CNS lymphoma, reactive versus malignant process, and overgrading of CNS tumors. Awareness of these common pitfalls at FS may help in further increasing diagnostic accuracy.

### 1333 CEACAM-1 Is Transiently Expressed in the Microvessels during Maturation of Neuroblastic Tumors and Correlate with Up-Regulation of VEGF in Differentiating Neuroblast/Ganglion Cells

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**Background:** Neuroblastoma is the most common solid extracranial tumor of childhood. As others pediatric tumors, neuroblastomas undergo spontaneous regression and/or differentiation into ganglioneuroma. Unfortunately, the majority of patients show systemic disease at diagnosis with rapid tumor progression and fatal outcome. Correlation between angiogenesis and poor outcome have been demonstrated. On the contrary, the role of angiogenesis in the maturation of undifferentiated neuroblasts towards mature ganglionic phenotype has never been considered. Human carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) has been recently shown to play a crucial role during the activation phase of angiogenesis and it is transiently expressed in newly formed immature blood vessels in most tissues and in the developing central nervous system (CNS). Vascular endothelial growth factor (VEGF) up-regulate CEACAM1 expression in endothelial cells.

**Design:** Our goal has been to investigate the role of CEACAM-1/VEGF mediated angiogenesis in the whole spectrum of neuroblastic tumors, from the undifferentiated to the fully differentiated mature lesions. A total of 65 tumors from 26 patients have been retrieved from our archive and immunostained with antibodies against CEACAM-1, VEGF and factor VIII.

**Results:** CEACAM-1 is transiently expressed in microvessels between differentiating neuroblast/ganglion cells whereas it is completely absent in poorly differentiated/undifferentiated tumors as well as in fully mature ganglioneuromas (t-Fisher, p=0,002). Interestingly, VEGF expression has been found in the cytoplasm of differentiating neuroblast/ganglion cells adjacent to CEACAM-1 positive microvessels with a similar distribution among different cases (t-Fisher, p=0,008). VEGF expression in endothelial cells has been observed only in poorly differentiated/undifferentiated lesions.

**Conclusions:** Data suggest an important role of CEACAM-1/VEGF mediated angiogenesis during the maturation stage of neuroblastic tumors. This is in agreement with the physiologic events leading to maturation of vasculature in normal tissues, including CNS. VEGF dependent angiogenesis in poorly differentiated/undifferentiated tumors is probably sustained by others molecular pathways.

### 1334 MGMT Promoter Methylation Status and Expression in Human Glioblastoma

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**Background:** MGMT (O6-methylguanine-DNA methyltransferase) DNA repair gene methylation silencing has been associated with longer survival in patients with glioblastoma (GBM), especially receiving chemotherapy with alkylating agents. MGMT expression may be a useful prognostic and/or predictive marker in GBM patients, receiving adjuvant therapy following surgery.

**Design:** We studied samples from 50 patients with histologically diagnosed GBM. Demographics and survival data were obtained by retrospective chart review. H&E slides were reviewed and genomic DNA extracted from scrapings of corresponding formalin fixed paraffin embedded tissue sections. Methylation specific PCR (MS-PCR) was performed after bisulfite treatment by standard methods. Using the mouse monoclonal antibody MT3.1 (NeoMarkers, Fremont, CA), MGMT expression was assessed and scored in tumor cells by 2 observers, blinded to tumor methylation status and clinical data, using a 3 tiered scale (1= negative or limited to <10% positive tumor cells, 2=10-50%, 3=>50%) in the same tumor area from which the MS-PCR samples were obtained. Statistical analyses included the chi square test and Kaplan-Meier survival curve with log rank test.

**Results:** MS-PCR was technically successful in 39 cases (78%), including 22 M and 17 F (mean age 58.3, range 34-81). MGMT promoter methylation was detected in 15 cases (38.5%) and absent in 24 (61.5%). Immunostain scoring was difficult due to MGMT expression in non-neoplastic cells (endothelial cells, glia, microglia, macrophages, lymphocytes). MGMT expression was present in tumor cells in 7 cases (18%) with a score of 2. All 15 methylated samples had a score of 1. Of the 24 unmethylated samples, 17 had a score of 1, and 7 of 2 with a 70% false negative rate for detecting MGMT expression. There was no significant correlation between MGMT methylation and expression. No significant survival difference was observed in the methylated vs unmethylated groups (mean survival 487 and 453 days respectively), or negative versus positive immunohistochemistry.

**Conclusions:** Assessment of MGMT expression by immunohistochemistry is inherently complicated by its expression in a variety of non-neoplastic cells. Correlation between MGMT promoter methylation and MGMT expression is hindered by the high rate of false negative samples among unmethylated tumors. In this limited patient cohort, MGMT methylation status does not predict longer survival in GBM patients, at difference with previous reports.

### 1335 The Value of Epidermal Growth Factor Receptor in Pediatric Ependymomas: An Immunohistochemical Study

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**Background:** Aberrant signaling through the epidermal growth factor receptor (EGFR) is associated with neoplastic cell proliferation, migration, stromal invasion, resistance to apoptosis, and angiogenesis. The high frequency of abnormalities in EGFR signaling in human tumors showing that inhibition of EGFR can impair tumor growth make EGFR an attractive target of cancer therapeutics.

**Design:** Sixty-nine patients (mean age 69.7 months; range 12-216 months) of supratentorial ependymoma on whom total gross resection was performed are included in this study. EGFR immunohistochemistry was performed using a monoclonal EGFR antibody (Dako). Results were interpreted manually as follows: 0, no membrane staining; 1+, faint, partial membrane staining; 2+, weak, complete staining in >10% of tumor cells; 3+, intense complete membrane staining in >10% of invasive tumor cells. EGFR labeling was correlated with prognosis. The patients were followed-up for a mean time of 45.1 months (range 12-132 months).

**Results:** The cases consisted of 48 cases (69.5%) of grade II and 21 cases (30.5%) of grade III ependymomas. 25 patients (36%) developed recurrence 12-132 months after surgery (mean: 16 months), 11 patients died 12-132 months after surgery (mean: 52.4 months) and 33 patients (47.8%) are still disease-free after a mean time of 43.4 months (range 12-132 months) after surgery. Among the patients who developed recurrence, 3 showed 0+, 5 showed 1+, 1 showed 2+ and 16 showed 3+ EGFR staining grade. In the patients with no recurrence, 8 showed 0+, 12 showed 1+, 7 showed 2+, 6 showed 3+ EGFR labeling. In the patients who died, no tumors showed grade 0+ nor 1+, 2 showed 2+, and 4 showed 3+ EGFR labeling. In the recurrent tumors, no tumors showed 0+ or 1+, 9 tumors showed 2+, and 16 tumors showed 3+ EGFR labeling. Statistical analysis showed that EGFR labeling correlated with recurrence (P<0.0001) and death from ependymomas (P<0.0001).

**Conclusions:** This study suggests that EGFR expression is a reliable marker in predicting the behavior of pediatric ependymomas. An EGFR staining grade of 2 and 3+ on IHC in first occurrence ependymomas in children is associated with an increased risk of recurrence and death from the tumor. In recurrent tumors that did not already express the highest degree of EGFR labeling in the original tumor, progression to more expression was the rule.

### 1336 The Role of MIB-1 and Cyclin D1 in Who Grade II Pediatric Intracranial Ependymomas

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**Background:** The prognosis of pediatric ependymomas is difficult to predict based on histology alone. This is particularly true for grade II ependymomas where the presence of necrosis, vascular proliferation and focal cytologic atypia does not upgrade the tumor. In the absence of universal agreement as to the relative importance of histologic features, finding markers that correlate with prognosis become paramount. In this study, we investigate the role of MIB-1 and cyclin D1 as predictors of grade II ependymomas.

**Design:** Sixty-six cases of grade II intracranial ependymomas were collected from the files of the Division of Pathology at Cincinnati Children's Hospital. Only cases with gross total resection were included. Immunohistochemistry with antibodies to MIB-1 (prediluted, Ventana) and cyclin D1 (prediluted, Ventana) was performed. MIB-1 and cyclin D1 labeling indexes (LI) were generated by counting the number of marker-positive tumor cells in a field of 1000 tumor cells.

**Results:** The patients consisted of 45 males and 21 females. The age range was 4-204 months with a mean of 65.7 months. Follow-up showed that 23 patients (34.8%) are still alive with no recurrence of disease, 32 patients (48.5%) developed recurrence, and 11 patients (16.7%) died because of the disease. For all patients, the mean MIB-1 LI was 18% (range of 2.3-33.8%). When the tumor recurred, the mean MIB-1 LI was 22.6% (range of 14.2-32.7%). In the patients who are still disease-free, the mean MIB-1 LI was 9.1% (range 2.3-19.6%). In patients who died because of the disease, MIB-1 mean was 23.4 (range 11.9-33.8). For all patients, cyclin D1 mean was 3.3 (range 0-9.6). When the tumor recurred, cyclin D1 mean was 3.2 (range 0-8.1). In patients who are still disease-free, the mean was 2.9 (range 0-6.3) and in patients who died because of the disease, the mean was 4.2 (range 0-8.9). There was strong correlation between MIB-1 LI and the recurrence (P<0.001) and death (P<0.001) rates. There was no correlation between cyclin D1 LI and the recurrence (P>0.05) and death (P>0.05) rates. Using Kaplan-Meier survival analysis, low (<23.4) or high (>23.4) MIB-1 predicted favorable or unfavorable prognosis.

**Conclusions:** We conclude that MIB-1 LI is a prognostic factor and accurate predictor of outcome in patients with grade II ependymomas. Thus, assessment of MIB-1 LI in intracranial ependymomas is useful for outcome prediction in the routine diagnostic setting. In contrast, cyclin D1 index does not predict the behavior of these tumors.

### 1337 Melanocytic Differentiation in Epithelioid MPNSTs: A Series of 4 Cases

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**Background:** Epithelioid malignant peripheral nerve sheath tumor (MPNST) has morphologic features that overlap with melanoma and is strongly positive for S-100. They are typically described as being negative for more specific melanocytic markers. Six case reports in the English-language literature describe melanocytic differentiation. We investigated melanocytic differentiation in a series of four epithelioid MPNSTs.

**Design:** Three epithelioid MPNSTs and a tissue array of 68 MPNSTs including 1 additional epithelioid MPNST were examined. Immunohistochemistry was performed by the avidin-biotin-peroxidase complex technique using commercially available antibodies to the following antigens: S-100, HMB-45, tyrosinase, MelanA, and microphthalmia transcription factor (MITF).

**Results:** All epithelioid MPNSTs were diffusely and strongly positive for S-100. Three of four epithelioid MPNSTs had at least focal melanocytic differentiation (MelanA (2/5), HMB-45 (1/5), tyrosinase (1/5), MITF (0/3)). Immunoreactivity was focal in two of the three positive cases while the other positive case exhibited diffuse immunoreactivity. The case with diffuse immunoreactivity arose within the abdomen of a 66 year old man. Histologically, it had an extensive epithelioid and spindle cell component and also contained areas of heterologous rhabdomyosarcomatous differentiation (desmin and myogenin positive). While the epithelioid component was diffusely and strongly positive for melanocytic markers, the spindle cell component was negative. All conventional (spindle cell) MPNSTs were negative for all melanocytic markers.

**Conclusions:** Although very rare, epithelioid MPNSTs can exhibit prominent melanocytic differentiation. This might be related to the ability of neural crest to give rise to both nerve sheath and melanocytic lineages. Epithelioid MPNSTs with melanocytic differentiation may be part of a spectrum of nerve sheath neoplasms with melanocytic differentiation including melanotic schwannomas. Distinguishing epithelioid MPNST with melanocytic differentiation from metastatic melanoma is best done by identifying origin from a peripheral nerve or helpful histologic features such as areas of heterologous differentiation.

### 1338 Expression of the Polycomb-Group Protein Bmi1 in Astrocytic Tumors. An Immunohistochemical Study on 80 Cases

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**Background:** Polycomb-group (PcG) proteins form chromatin-associated complexes involved in epigenetic silencing of some developmental and cell-cycle regulatory genes. These proteins participate in stem-cell self-renewal and thus are expressed throughout life. Bmi1 protein, one PcG member, has a role in hematopoiesis and skeletal and central nervous system development, and its deregulated expression has been implicated in developmental syndromes and experimental cancers, mainly lymphomas. Bmi1 is an inhibitor of INK4a locus, a cell-cycle controller via p16 expression, and its possible role in human tumorigenesis is now under investigation. Data are available for lymphomas and breast, lung and colon cancers; however, the function of Bmi1 in brain tumors is underreported. The aim of this study was to assess Bmi1/p16 expression in a large series of gliomas and to evaluate its role in brain tumorigenesis as well as its potential prognostic meaning.

**Design:** Eighty primary gliomas were evaluated including 16 diffuse astrocytomas grade II (13 fibrillary, 3 gemistocytic), 15 anaplastic astrocytomas grade III and 49 astrocytomas grade IV (glioblastoma, gliosarcoma). Standard immunohistochemistry was applied on paraffin sections using monoclonal antibodies against Bmi1 and p16. Additional stains were done for GFAP, p53 and Ki67.

**Results:** All tumors, regardless of grade, were diffusely Bmi1 positive. Both Bmi1 and p16 were strongly and diffusely expressed in gemistocytic astrocytomas grade II and in grade III and grade IV astrocytomas with a significant gemistocytic component or a high nuclear grade. On the other hand, p16 was expressed in only 23%, 20% and 22% of fibrillary astrocytomas and in astrocytomas grade III and grade IV, respectively. No association was observed between Bmi1/p16 expression and glial differentiation (GFAP) or p53 and Ki67/MIB1 stains.

**Conclusions:** Our study shows that the expected Bmi1+/p16- pattern was present in > 75% of tested neoplasms, supporting the current experimental views of its potential role in glioma genesis. Nevertheless, its usefulness as prognostic factor appears questionable, being expressed in all gliomas, regardless of grade. The confusing double expression observed in gemistocytes is in keeping with the intriguing biology of these cells, thought to be terminally differentiated, nonproliferative cells entailing a worse prognosis. Interestingly, the same expression pattern was present in high-grade astrocytomas with gemistocytic component or very anaplastic features.

### 1339 Immunohistochemical Staining for Peripheral Benzodiazepine Receptors in the Differential Diagnosis of Low Grade Astrocytoma and Reactive Gliosis

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**Background:** The differential diagnosis of low grade astrocytoma and reactive gliosis can be a challenging problem in surgical neuropathology in the era of stereotactic and navigation-guided biopsies, usually yielding small tissue samples. Numerous criteria for differential diagnosis have been proposed, including Ki67 labeling index, p53 staining, etc. These are not very helpful and demonstrate significant overlap between gliosis and astrocytoma. Peripheral benzodiazepine receptor (PBR) is a component of a multiprotein complex located on the contact site between inner and outer mitochondrial membranes. PBR is found in various peripheral organs and glial cells of the central nervous system. The function of PBR in the glia is not clear, but it has been established that PBR expression is significantly increased in 'activated' reactive astrocytes in neurodegenerative, inflammatory and demyelinating diseases. We proposed that immunohistochemical staining for PBR could be useful in differentiating reactive gliosis from low grade astrocytoma.

**Design:** Paraffin sections from 35 cases of astrocytoma (WHO grades I and II) and 25 cases of reactive gliosis (caused by brain metastases, craniopharyngiomas, pineocytomas, demyelinating lesions and post-radiation gliosis) were stained for Ki-67, p53 and PBR.

**Results:** Both Ki-67 labeling index and p53 expression demonstrated no significant differences in the cases of gliosis and gliomas. However, immunohistochemical staining for PBR demonstrated striking differences in gliomas and gliosis. Most astrocytomas showed negative results on PBR staining, while weak focal staining was observed in three cases only. In all the cases of reactive gliosis, strong to moderate cytoplasmic staining for PBR was seen. This difference was even more striking in cases of gliosis with pilocytes and Rosenthal fibers vs. pilocytic astrocytoma and gliosis with gemistocytes vs. gemistocytic astrocytoma.

**Conclusions:** According to our results, immunohistochemical staining for PBR provided a useful clue for differentiating between low grade astrocytomas and reactive astroglia, especially helpful in small samples of tissue.

## Pathobiology

### 1340 Refractory ITP: Therapy-Related Histopathologic Changes in Spleens

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**Background:** Immune thrombocytopenic purpura (ITP) is a common disorder characterized by antibody-mediated destruction of platelets. Refractory ITP (rITP) is a chronic variant characterized by lack of response to the common therapies (e.g. corticosteroids). However, numerous newer therapies have become more widely used before splenectomy, including anti-CD20 antibodies (e.g. rituximab). Few reports exist on the histopathologic changes associated with rITP. We evaluated the splenic histologic and immunohistochemical changes in rITP after a variety of therapies.

**Design:** 24 cases were reviewed from two institutions including histology and historical information on types of pre-splenectomy therapy. A panel of immunohistochemical (IHC) stains was performed (CD3, CD20, CD79a, PAX-5, CD8, CD68, CD21 and Ki-67). Histology was reviewed for changes in the red pulp, white pulp and vasculature. The results were scored semi-quantitatively (0-3+). Patient groups were defined as follows: Group 1 - conventional therapy (corticosteroids), Group 2 - corticosteroids + additional therapies (IVIg, Rh immune globulin, danazol, cyclophosphamide/vincristine, rituximab), Group 2A (rituximab alone or with any other therapy). Results were compared using student T-test.

**Results:** The average ages (age range) were: Group 1 - 43 years (28-63); Group 2 - 49 years (13-79); Group 2A - 69 years (62-72). No statistically significant differences were identified between the histologic or IHC findings of Group 1 (conventional therapy) and Group 2. There were significant differences between Group 2 and Group 2A (rituximab). As expected, CD20 was decreased in both red and white pulp (P < 0.01, < 0.001). In addition, CD21 staining was decreased (P < 0.001) and CD8 positivity (seen in splenic cords) was increased (P < 0.05). This latter measure was inversely related to the number of macrophages. The use of CD79a and/or PAX-5 did not improve detection of B cells in cases with rituximab therapy. Marginal zone hyperplasia was seen in 14/24 cases; extramedullary hematopoiesis was seen in 5/24 cases.

**Conclusions:** The histopathologic changes seen in post-treatment spleens of rITP are broad with only few characteristic changes seen after specific therapy combinations. The use of rituximab markedly decreases B cells in rITP spleens, consistent with its known effects. It is likely that future treatment modalities will also modify the cellular composition of post-treatment spleens.

### 1341 Adenosine A2a Receptor in Bone Marrow Derived-Hematopoietic Cells Protects Liver from Ischemia/Reperfusion Injury

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**Background:** Adenosine A2a receptor (A2aR) is critical in the regulation of inflammatory responses. It has been shown that inflammatory stimulation, which causes minimal damage in normal mice, leads to extensive tissue damage in A2aR-deficient mice (KO) or normal mice (WT) treated with A2aR antagonists. On the other hand, the activation of A2aR protects mice liver from ischemic injury during reperfusion. Since A2aRs express at high levels in hematopoietic cells, such as granulocytes, we hypothesize that A2aR in bone marrow (BM) derived-cells may serve as a major anti-inflammatory mediator in ischemic liver injury.

**Design:** WT mice, in which the A2aR in BM-derived cells were selectively removed and reconstituted, were generated by transplantation of BM cells from either A2aR-KO mice (KO→WT) or WT mice (WT→WT) following irradiation. Repopulation efficiency of BM-derived hematopoietic cells in the recipient mice was determined by sex chromosome evaluation. Warm liver ischemia/reperfusion was created by clamping the hepatic artery, portal vein and bile duct for 60 min, followed by reperfusion for 23 hours. At the end of the reperfusion, the liver tissues were collected for histological examination as well as RNA isolation. Statistical analysis was performed using student t test.

**Results:** The microscopic examination of the liver tissues (H & E section) revealed a confluent coagulative necrosis in KO→WT mice. On the other hand, only small foci of necrosis and areas of ballooning degeneration were observed in the WT→WT mice. Neutrophilic infiltration was present in the necrotic areas in both KO→WT and WT→WT mice. However, a majority of neutrophils in KO→WT mice lack A2aR by immunohistochemical study using anti-A2aRs antibody. This result is comparable to the sex chromosome analysis, which demonstrated that approximately 90% of the peripheral blood cells in recipient mice were reconstituted by BM cells from the donor mice. Quantitative real time PCR analysis showed significantly elevated mRNA levels for proinflammatory cytokines, IL-1 and IL-6, in KO→WT mice compared to WT→WT mice.

**Conclusions:** Adenosine A2a receptor in bone marrow derived-hematopoietic cells protects mouse liver from ischemia/reperfusion injury. This is likely due to inhibition of proinflammatory cytokine production in neutrophils and/or hepatic Kupffer cells through A2aR-mediated anti-inflammatory mechanism.