1342 Expression of MUC1, MUC2, MUC5AC and CDX2 in Carcinomas of the Gallbladder and Extrahepatic Bile Duct

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Background: Alteration or aberrant expression of MUC and CDX2 genes has been found in a variety of malignant neoplasms and is believed to play important roles in the tumorigenesis in these organs. The expression profile of these MUC genes in carcinomas of the gallbladder (GB) and extrahepatic bile ducts (EHB) is rarely studied, and no data has been published on CDX2 expression in these neoplasms.

Design: The expression profile of MUC1, MUC2, MUC5AC and CDX2 were investigated immunohistochemically in a total of 33 cases of carcinomas arising from GB (20 cases) or EHB (13 cases). Labeling of more than 10% of the cells was regarded as expression, as previously done in other studies.

Results: MUC1 was commonly expressed in both invasive (88%) and in-situ (60%) carcinoma of the GB and EHB. MUC5AC was expressed in approximately half of the cases (50% invasive and 67% in-situ carcinoma). In contrast, expression of MUC2 and CDX2 was rare in invasive carcinoma (2/26 for each), and slightly higher in the in-situ carcinoma (30 and 22%, respectively). These MUC2/CDX2 expressing in-situ lesions displayed intestinal morphology; however, the invasive carcinomas appeared to be ordinary carcinomas (without obvious intestinal features). No MUC1, MUC2, MUC5AC or CDX2 expression was detected in the 2 cases of small cell carcinoma.

Conclusions: MUC and CDX2 expression profiles in GB and EHB carcinomas are similar to that of pancreatic ductal adenocarcinoma, supporting the close kinship between these foregut carcinomas. MUC1, the so-called mammary type mucin, which has been implicated in tumor aggressiveness, is expressed in most cases, in keeping with the rapidly fatal outcome of these tumors. In contrast, MUC2 and CDX2, "key molecules in intestinal programming" with well-established tumor suppressor activity, are present in less than 10% of the cases. The unusual cases that express these two markers do not appear to be morphologically different. On the other hand, MUC2/CDX2 expression is slightly more common in the in-situ lesions, which typically exhibit intestinal morphology, in keeping with the intestinal metaplasia sometimes observed to precede biliary carcinomas.

1343 Histologic Evaluation of Steatohepatitis and Fibrosis (Stage) in Hepatitis C

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Background: Hepatitis C virus is a major cause of chronic liver disease in the world. Although viral loads, genotypes and coinfections with hepatitis B or HIV play a critical role in chronicity, the relationship of steatohepatitis with increased liver fibrosis is not well understood. Recent studies demonstrated that superimposed non-alcoholic steatohepatitis (NASH) might influence fibrosis in hepatitis C(hep C). The present study evaluates degree of steatohepatitis in hep C patients with/without risk factors of NASH and correlates the results with the degree of liver fibrosis (stage).

Design: Liver biopsies from 75 patients (average age of 46.3) with hep C were included in the study. Twenty-one of 75 (28%) patients had single or multiple risk factors (diabetes mellitus, hyperlipidemia, obesity, metabolic syndrome or medications) for NASH. Three of the 21 (14.3%) had an additional history of alcoholism. Five histologic parameters were evaluated on a semi-quantitative scale and expressed in numerical scores (0-10): % of fatty change (0=0, 1-33=1, 34-67=2 and >67=3), lipogranulomas (absent=0, present=1), glycogenated nuclei (GN) (absent=0, occasional=1, several=2, many=3), ballooning degeneration (BD) of hepatocytes (absent=0, present=1) & Mallory bodies (absent=0, occasional=1, several=2). Fibrosis was scored (0-4) based on standard grading criteria.

Results: Of the 21 patients with risk factors for NASH, 16 (14+2, 76.2%) had higher steatohepatitis scores (>3) compared to 25 (21+4) patients with only hep C (25 of 54, 46.3%). Twelve of these 21 (59%) had higher stage of fibrosis (scores 3-4) versus only 19 of 54 (33%) without NASH (Table1). Overall, in both groups of patients, of the 5 histologic parameters only GN & BD are correlated directly with higher stage of fibrosis (scores 3-4) (P<0.05) (Table 2).

Conclusions: 1). Hep C patients with risk factors for NASH are more prone to develop cirrhosis. 2) GN and BD of hepatocytes in hep C are related to fibrogenesis independent of risk factors for NASH

Scoring of Steatohepatitis & Staging of Fibrosis in Hep C Patients +/- Risks for NASH

Steatohepatitis scores	Hep C (n=54)	Hep C + Risks for NASH(n=21)
0-2	29/54(53.7%)	5/21(23.8%)
3-5	21/54(38.9%)	14/21(66.7%)
6-10	4/54(7.4%)	2/21(9.5%)
Fibrosis 0-2	35/54(64.8%)	9/21(42.5%)
Fibrosis 3-4	19/54(33.2%)	12/21(58.5%)

Direct Correlation between GN & BD with Fibrosis

Scores	Stage 0-2	Stage 3-4
GN = 0 (n=35)	23/35(65.7%)	12/35(34.3%)
GN = 1(n=29)	17/29(58.6%)	12/29(41.4%)
GN = 2-3(n=11)	4/11(36.4%)	7/11(63.6%)
BD = 0 (n=61)	40/61(65.6%)	21/61(34.4%)
BD = 1(n=14)	4/14(28.5%)	10/14(71.4%)

Neuropathology

1344 Hemispheric Extraventricular Glioneurocytoma: A Clinicopathological Review with Immunohistochemical Profile

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Background: Tumors expressing concomitant neuronal and glial differentiation in the same tumor cells are classified under the umbrella of glioneurocytic tumors (GNC). Tumors in this category are not recognized in the WHO classification 2000. There are no definite morphologic criteria to assess grade and prognosis of these tumors.

Design: We evaluated 10 cases of GNC diagnosed in our department from 2003-2004 with emphasis on clinico-pathological features, immunohistochemical profile and prognosis.

Results: The 10 cases of GNC showed a male to female ratio of 2:3 with age range of 23-59 years. The most common complaint was seizure. 9 cases had a tumor in the frontal lobe and one in the temporal lobe. 8 cases had hypodense, non-enhancing lesions, one of which showed a cystic component and one showed calcification. Two cases showed enhancing hyperintense lesions on T2 imaging. All cases were morphologically low grade tumors. Endovascular proliferation was present in 5 cases which was not interpreted as high grade and may be analogous to what is seen in pilocytic astrocytoma. 3 cases also showed ganglionic differentiation and 5 cases had minigemistocytic morphology. Immunohistochemically, all cases showed varying degrees of positivity for glial and neuronal markers in the same tumor cells. Synaptophysin and NSE were most sensitive in identifying neurocytic differentiation. Calretinin was a good marker for mature ganglion cells and cells showing early neuronal differentiation but not undifferentiated neurocytes. CD56 was a sensitive but non-specific marker for glial differentiation similar to S-100 and GFAP. Vimentin, CD57, neurofilament, alpha-synuclein and beta-tubulin were non-contributory.

Conclusions: GNC are a distinct entity and not rare tumors. From our study it shows that these tumors are composed of single cells with divergent glial and neurocytic differentiation. Immunohistochemistry is an essential tool to show co-expression of glial and neurocytic differentiation. Our findings show that synaptophysin and calretinin are the best markers for neurocytic differentiation and GFAP, S-100 and CD56 for glial differentiation. Some of the GNC may show atypical features such as increased mitosis and endovascular proliferation but prognostic and grading implications are still unclear. Specific morphologic criteria for grading and prognosis needs long term follow up of larger series of such tumors.

1345 Changes in the Internal Granular Cell Layer of the Cerebellum in Progressive Multifocal Leukoencephalopathy in 3 Patients with AIDS

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Background: Progressive multifocal leukoencephalopathy (PML) is a disease of the central nervous system caused by infection with JC polyomavirus (JCV) in immunosupressed hosts. Demyelination, viral inclusions and astrocyte pleomorphism are characteristically seen in PML, the former resulting from lytic JCV infection of oligodendrocytes and the latter reflecting restrictive JCV infection of astrocytes. Cellular alterations in the internal granular cell layer (IGCL) of the cerebellum have also been described, and productive infection of granule cell neurons by JCV was recently demonstrated in a HIV-positive patient with PML. Here we study the histopathologic changes in the cerebellar IGCL of 3 PML cases and assess the utility of in situ hybridization (ISH) and immunohistochemistry (IHC) in identifying JCV-infected cells at this level.

Design: Formalin-fixed tissue samples from 3 HIV-positive patients with neurologic dysfunction and imaging studies demonstrating demyelination in the cerebellum were assessed, 1 at autopsy and 2 by open biopsy. PML was confirmed histologically in all 3 cases. JCV ISH was performed using a hybridized biotinylated DNA probe. Single and double-label IHC were performed using antibodies specific for the VP-1 capsid of the JC virus and MAP-2.

Results: Morphologic, ISH and IHC changes of PML were present in the cerebellar white matter of all 3 cases. Marked neuronal loss in the IGCL of the cerebellum was featured in 2 cases. In 1 case, the cellularity of the IGCL was unaltered but enlarged ovoid nuclei with glassy chromatin were focally noted. There was no significant difference between JCV ISH and IHC staining of the IGCL. In 2 cases, JCV ISH and single-label IHC were positive in a moderate to high number of cells that appeared to represent granule cell neurons, and double-label IHC revealed VP-1 immunoreactivity in MAP-2 positive cells. In 1 case with pronounced neuronal loss, JCV ISH and IHC were negative in the IGCL.

Conclusions: Our results support that JCV can infect IGCL neurons and suggest that this may lead to neuronal loss. Enlarged ovoid nuclei with glassy chromatin in the IGCL are the result of JCV infection. ISH and double-label IHC are similarly useful in demonstrating infection of cerebellar granule cell neurons, including morphologicaly normal ones. In cases with severe neuronal depletion, enlarged nuclei with glassy chromatin are not found in the IGCL and ISH or IHC for JCV may be negative.

1346 Immunoreactivity for Claudin-1 Can Help Distinguish Meningiomas from Potential Histologic Mimics

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Background: The diversity of histologic variants of meningioma can occasionally make the distinction between meningiomas and other tumors difficult. Ultrastructural studies have shown that a distinguishing feature of meningothelial cells and meningiomas is the presence of cell adhesion complexes similar to epithelial desmosomes. Claudin-1 is a recently identified component of tight junctions, whose expression is believed to be important in maintaining the blood-brain barrier. The

purpose of this study was to determine whether immunoreactivity for claudin-1 could help distinguish meningiomas from potential histologic mimics, including solitary fibrous tumors of the meninges (SFT), meningeal hemangiopericytomas (HPC), and acoustic schwannomas (AS), in comparison to immunostains commonly used in this differential diagnosis.

Design: 36 tumors were studied: 7 meningothelial meningiomas (MM), 10 fibrous meningiomas (FM), 7 SFT, 5 HPC, and 7 AS. Immunohistochemical studies for claudin-1, EMA, S-100 protein, CD34, and GFAP were performed on paraffin-embedded sections. Cases were considered positive if >5% of tumor cells were immunoreactive. Slides were evaluated in a blinded fashion, and the results were compared between the different tumor types.

Results:

Immunohistochemical Results

	MM	FM	SFT	HPC	AS
Claudin-1	5/7 (71%)	4/10 (40%)	0/7 (0%)	0/5 (0%)	0/7 (0%)
EMA	6/7 (86%)	10/10 (100%)	2/7 (29%)	1/5 (20%)	1/7 (14%)
S-100	0/7 (0%)	10/10 (100%)	2/7 (29%)	0/5 (0%)	7/7 (100%)
CD34	0/7 (0%)	5/10 (50%)	5/7 (71%)	3/5 (60%)	0/7 (0%)
GFAP	0/7 (0%)	0/10 (0%)	0/7 (0%)	0/5 (0%)	4/7 (57%)

MM, meningothelial meningioma; FM, fibrous meningioma; AS, acoustic schwannoma.

In total, 9/17 (53%) meningiomas were immunoreactive for claudin-1, often with a granular staining pattern, whereas none of the other tumors were positive. In contrast, 16/17 (94%) meningiomas were positive for EMA. Of note, the meningioma that did not express EMA was positive for claudin-1. A subset of the other tumors were focally positive for EMA. Claudin-1 was also detected in the (perineurial) capsule of a schwannoma.

Conclusions: Claudin-1 is a highly specific marker for meningiomas in comparison to other tumors arising in the dura. Although the sensitivity of claudin-1 for meningiomas is relatively low, it may be helpful when used in a panel of immunostains to distinguish meningiomas from potential histologic mimics.

1347 Primary Intracranial Germinoma: A Retrospective Clinicopathologic Analysis of 27 Cases

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Background: Primary germinomas of the central nervous system (CNS) are rare tumors of children and young adolescents. They are largely midline lesions in which imaging studies play an important role in their diagnosis. Fortunately, these tumors are exquisitely radio- and chemosensitive allowing for successful treatment without surgical debulking. Historically, most patients were treated with craniospinal irradiation (CSI) with reported event-free and overall survival rates between 85% and 95%. However, because of the potentially devastating effects of radiation on the developing brain, efforts have been made to limit the dose and volume of radiotherapy (RT).

Design: The archival pathology files and tumor registry of the Indiana University Medical Center were searched, over a 20 year-period, for primary intracranial germinoma. The search yielded 27 cases. The pathology material was available on all cases, detailed imaging studies were present on 21, and clinical follow-up was up-to-date on 20.

Results: The median presenting age was 11 years with a range of 5 to 32 years. The male to female ratio was 3:1 in the pineal region but roughly 1:1 in the neurohypophyseal region. All germinomas were situated in the midline; exclusively in the pineal and neurohypophyseal regions, the latter being twice as common. Synchronous lesions in the pineal and neurohypophyseal regions were present in two patients. Radiographically, germinomas were well-circumscribed, lobulated lesions demonstrating mostly isointense signals on T1, T2, Flair, PD, and DWI sequences. They enhanced heterogeneously. Calcifications were found solely in the pineal region. The average follow-up period among the 20 patients with available clinical data was 7.5 years. 12 of 20 patients received chemotherapy (CT) and focal RT and of that cohort, 4 (30%) relapsed. In addition, 2 other patients who had received only CT also relapsed. Four relapsed patients were subsequently salvaged with either CSI or combined CSI and CT while two died of disease progression. No relapses were recorded in those patients who received CSI as a component of their initial therapy.

Conclusions: Primary CNS germinomas typically present in the pineal or suprasellar region, sometimes synchronously. Germinomas are well-circumscribed, lobulated, enhancing masses. In our series, patients had poorer disease free survival outcome than expected for pure germinoma. Conforming to historical data highlighting the effectiveness of CSI, no patient treated with CSI relapsed.

1348 Correlation between Real Time RT-PCR Expression of PTEN and Tumoral Grade in Astrocytomas

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Background: PTEN gene is a relevant tumor suppressor gene whose protein is a phosphatase involved in the control of astrocytoma angiogenesis. This gene is frequently mutated in high-grade astrocytomas. PTEN protein is intensively immunoexpressed in a relevant subgroup of high-grade astrocytomas. For an adequate interpretation of this interesting phenomenon, quantification of the PTEN by RT-PCR has been carried out.

Design: 25 surgically resected brain astrocytomas, 5 grade II, 8 grade III and 12 grade IV from bank tissue tumor were studied both immunohistochemically and by RT-PCR. As a reference, gliotic brain tissue from temporal lobe and one glioblastoma cell line with mutated PTEN gene (TG98) were used by the same way. Real time RT-PCR from the samples was performed on an ABI-7700R® by using TaqMan probes. Immunohistochemical evaluation of the pPTEN using a clonal antibody (clone 6H2.1449, Cascade®) was obtained. Non parametric comparison tests were used.

Results: Cytoplasmic expression of pPTEN in the tumor was observed in all grade II, 50% of grade III and 30% of grade IV. Immunoreactivity in high-grade astrocytomas was characterized by a very intense (+++) cytoplasmic positivity, more intense than the brain cortex in the biopsy or the gliotic temporal lobe. No immunoreactivity was observed in PTEN mutated glioblastoma cell line. Real time RT-PCR expression in grade II (1.00 +/- 0.001) was significantly higher than in grade III (0.33+/-0.16) or in grade IV (0.45+/-0.12) ($p<0.05$). RT-PCR values in high-grade immunonegative tumors were lesser than in the immunopositive ones, but non significant difference between them was observed. As reference, RT-PCR PTEN value from the gliotic tissue was higher than in the tumoral cases and no detection was found in glioblastoma cell line.

Conclusions: Real time RT-PCR expresion of PTEN correlates with tumoral grade in astrocytomas. According to a comparative results of PTEN by RT-PCR and immunohistochemistry, PTEN protein detected in high-grade astrocytomas could be an accumulated protein by an altered degradation.

1349 Patterns of Constitutive Activation of Signal Transducers and Activators of Transcription 3 and 5 (STAT3 and STAT5) in Primary CNS Lymphomas (PCNSL)

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Background: STAT proteins are transcription factors and function as downstream effectors of many cytokines, hormones and growth factors. STAT3 and STAT5 have been demonstrated to play an important role in oncogenesis by promoting cell proliferation and inhibiting apoptosis. Activation of STAT3 and STAT5 has been reported in a number of solid and hematologic malignancies, but their role in primary CNS lymphomas has not been elucidated.

Design: Thirty-three cases of intra-axial PCNSL (all of B-cell phenotype) were retrieved from paraffin-embedded archival material and immunohistochemically stained for STAT3 and STAT5 (sc8019 and sc-836 Santa Cruz Biotechnology, Santa Cruz, CA) using an automated stainer (Ventana Medical System, Tucson, AZ). Staining pattern was evaluated, and positivity was scored semiquantitatively with regard to both intensity and distribution of the stain.

Results: Positive nuclear staining for STAT5 was shown in 32 of 33 cases (97%) at moderate to strong intensity with only one case of borderline positivity. For STAT3, positive nuclear staining was present in 13 of 33 cases (39%), while positive cytoplasmic staining was observed in 18 cases (54.5%). There were 12 cases with both nuclear and cytoplasmic positivity for STAT3, 1 with nuclear staining only and 6 with cytoplasmic staining only, and 14 cases negative for both nuclear and cytoplasmic STAT3. Of 32 STAT5 positive cases, 13 co-expressed nuclear STAT3. In cases containing CNS tissue, staining for STAT3 and STAT5 was not noted in neurons and astroglia.

Conclusions: This study demonstrated constitutive activation of STAT5 in vast majority of PCNSL and co-activation of STAT5 and STAT3 in 13/33 (39%) of PCNSL. To the best of our knowledge, this represents the first study that implicates the activation of STAT5 in the pathogenesis of PCNSL and provides molecular insight into designing novel approaches for PCNSL therapy. The dysregulated nuclear translocation of STAT3 may also contribute to tumorigenesis, and the underlying molecular mechanisms warrant further investigation.

1350 Immunohistochemical Expression of OCT4 in Primary Central Nervous System Germ Cell Tumors

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Background: OCT4, a POU domain transcription factor, is expressed in embryonic stem cells and germ cells. It is involved in the regulation and maintenance of pluripotent cell population. Recent studies have shown that OCT4 has been detected in specific types of testicular germ cell tumor, including seminoma and embryonic carcinoma. The aim of this study was to evaluate OCT4 expression in the primary central nervous system germ cell tumor for their possible use in the differential diagnosis.

Design: Formalin fixed, paraffin embedded tissues of primary central nervous sytem germinoma (5), yolk sac tumor (3), mature teratoma (2), immature teratoma (2), and mixed germ cell tumors (5) were immunohistochemical studied. Five cases of mixed germ cell tumors contained components of yolk sac tumor (4), embryonal carcinoma (3), mature teratoma (2), germinoma (2), and immature teratoma (1).

Results: Diffuse and strong nuclear staining of OCT4 was detected in all cases of pure germinoma (5) and in cases of mixed germ cell tumors with component of embryonal carcinoma (3) and germinoma (2). There was no staining in pure yolk sac tumor, mature teratoma, immature teratoma, and mixed germ cell tumors with component of yolk sac tumor, mature teratoma, and immature teratoma.

Conclusions: OCT4 immunostaining is a useful marker in the identification of primary central nervous system germinoma and embryonal carcinoma and highlights pluripotent cells (embryonal carcinoma and germinoma) in primary central nervous sytem mixed germ cell tumors.

1351 Use of Tissue Microarrays in the Immunohistochemical Analysis of Olig-2 and Ki-67 in Gliomas. A Quality Control Study

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Background: Tissue microarrays (TMA) are increasingly utilized for analysis of specific markers and have the potential to rapidly assess multiple specimens while using minimal tissue and reagents. Several studies validated the concordance between whole tissue sections (WTS) and TMA in various tumors. However, few studies address this issue in infiltrating gliomas, which constitute the most common primary CNS tumors. Known to have varied geographic distribution of histologic features, the

small amount of tissue sampled presents a diagnostic challenge in gliomas. It is important to determine whether TMA of all glioma types can be used reliably to determine the presence of immunohistochemical markers. We sought to determine the usefulness of TMA as quality control assays in evaluating Ki-67 and Olig-2 staining in oligodendrogliomas and ependymomas in comparison with WTS.
Design: UCSF pathology records were searched for oligodendroglioma (1990-2003) and ependymoma (1985-1998) cases. Diagnoses were confirmed by two pathologists after review of all slides. WTS were obtained from cases with adequate tissue. Ideal regions on blocks and slides were marked for microarray sampling and a minimum of two 1.0 mm cores per case was obtained. Ki-67 and Olig-2 staining was performed on all cases in TMA as well as WTS. Ki-67 staining results were scored using a folio; Olig-2 staining was semi-quantitatively characterized.
Results: 19 oligodendroglioma and 23 ependymoma cases were included in this study. Diffuse Olig-2 staining was observed in all oligodendroglioma cases in both TMA and WTS. Oligodendrogliomas had low proliferation rates as measured by Ki-67 staining (2 to 8%) on TMA. WTS of oligodendrogliomas tended to have greater Ki-67 staining (2 to >16%). In contrast, Olig-2 staining of ependymomas in TMA was variable: 11 negative, 8 focally positive, and 4 inconclusive. TMA concordance with WTS depended on both tumor type as well as antibody used. Ki-67, expressed in cycling cells, showed less concordance. In contrast, Olig-2 seems to be expressed more uniformly throughout a tumor and was more likely to be concordant.
Conclusions: Despite the economic, practical and technical ease provided by TMA, staining variability presents a significant obstacle to its use in immunohistochemical studies. It is necessary to account for variations in both the type of tumor/tissue and the antibody to be applied before a reliable stain can be performed in TMA.

1352 Proteomic Analysis of Cerebrospinal Fluid Discriminates Malignant and Non-Malignant Disease of the Central Nervous System and Identifies Specific Protein Markers

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Background: Central nervous system (CNS) diseases are often accompanied by protein changes in cerebrospinal fluid (CSF). Analysis of CSF proteins could potentially be used to detect and monitor CNS disease. Affinity mass spectrometry (MS) offers a novel approach for identifying proteins in complex biological fluids such as CSF, and the SELDI-TOF-MS protein chip platform is practical for clinical investigation.We compared the CSF proteomes of patients with neoplastic and reactive/inflammatory CNS diseases in order to identify relevant biomarkers that discriminate them.
Design: Proteomic analysis was performed on CSF from 32 patients: 10 with known CNS malignancy (primary and metastatic), 12 with inflammatory/reactive conditions, and 10 with undefined CNS disease. SELDI-TOF-MS coupled with SAX-2 (strong anionic exchange chip surface) was used on CSF samples cleared of cellular material, concentrated, and suspended in buffer. Spectra were generated from an average of 50 laser shots at intensities L: 200, L: 230, L: 270. Protein profiles were analyzed using Ciphergen Biosystems automated protein identification software. Only protein peaks with signal/noise ratio > 3 were considered. Using molecular masses demonstrated by SELDI, we identified and isolated protein bands following gel electrophoresis and characterized them by trypsin digestion and fragment analysis by MALDI-TOF-MS. Digest patterns were compared to an NCBI database.
Results: Protein peaks identified by SELDI-TOF-MS specific to reactive/inflammatory conditions were noted at 6.7-7.1, 11.6-11.9, and 13.3-13.7 kDa. Peaks specific to malignancy were noted at 7.5-8.0, 15.1-15.9, and 30.0-32.0 kDa. Within our study set, the dagnostic panel of proteins in malignancy showed 80% sensitivity, 100% specificity, a positive predictive value of 100% and a negative predictive value of 92%. MALDI-TOF-MS identified the protein at 13.3-13.7 kDa in reactive/inflammatory conditions as cystatin C, a potent inhibitor of cysteine proteases. Carbonic anhydrase, a hypoxia inducible factor (HIF) transcriptional target, was identified as the 30.0-32.0 kDa protein in neoplastic disease.
Conclusions: We were able to distinguish neoplastic and reactive/inflammatory CNS disease based on their protein profile in CSF, independently of cytologic composition. Specific protein markers identified could be valuable for detecting and monitoring CNS disease.

1353 Expression of Herpes Simplex Virus (HSV) Entry Receptor Nectin-1 in Normal and Neoplastic Human Nervous System Tissues

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Background: HSV is an important pathogen of the human nervous system and genetically modified HSV strains are promising vector candidates for gene and tumor therapies targeting the brain. Nectin-1 is an immunoglobulin-like adhesion molecule that participates in the formation of adherens junctions and serves as an entry receptor for HSV. Tissue culture and experimental animal studies indicate that nectin-1 is widely expressed in epithelial cells and neurons. However, the expression of nectin-1 in the human nervous system is not well understood and there is no information available about nectin-1 expression in tumors of the nervous system. The aim of this study was to better understand the expression pattern of nectin-1 in the normal and neoplastic human nervous system.
Design: We have used standard immunohistochemistry to detect nectin-1 expression in normal human brain, spinal cord, trigeminal ganglia, and dorsal root ganglia (n=10), and surgical specimens of nervous system neoplasms (n=23) including oligodendroglioma (n=2), glioblastoma multiforme (n=3), anaplastic astrocytoma (n=2), diffuse astrocytoma (n=2), ganglioglioma (n=1), pilocytic astrocytoma (n=1), pleomorphic xanthoastrocytoma (n=2), ependymoma (n=1), meningioma (n=3), medulloblastoma (n=2), and schwannoma (n=2).
Results: Widespread strong nectin-1 expression was detected in the soma and processes of normal central and peripheral nervous system neurons, in choroid plexus epithelial cells, and vascular endothelial cells. Widespread weak to moderate nectin-1 expression was detected in meningothelial cells and ependymal cells. Oligodendrocytes, astrocytes, vascular smooth muscle cells, and Schwann cells showed variable immunoreactivity ranging from no to weak staining. Among tumors, schwannoma, fibrous meningioma, and medulloblastoma were nectin-1 negative. Oligodendroglioma, pilocytic astrocytoma, pleomorphic xanthoastrocytoma, diffuse astrocytoma, anaplastic astrocytoma, and glioblastoma multiforme showed focal weak nectin-1 positivity. Meningotheliomatous meningioma and ependymoma showed focal, moderate nectin-1 staining. Ganglion cells of ganglioglioma were strongly positive.
Conclusions: These studies provide novel information about the expression of nectin-1 in normal and neoplastic human nervous system tissues and may lead to a better understanding of cell targeting by HSV during HSV-induced neurological disease and in infections associated with HSV-based gene and tumor therapy.

1354 Aquaporin 1 Distinguishes Hemangioblastoma from Metastatic Renal Cell Carcinoma

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Background: The differential diagnosis between hamangioblastoma and metastatic renal cell carcinoma represents an important diagnostic challenge. In fact a strong association with von Hippel-Lindau disease is observed in both entitites. Aquaporins represents selective water channels in plasma membranes of living cells and microorganisms. Aquaporin 1 appears to be expressed along the structures lining the CFS circulation, including ependyma, leptomeninges and choroids plexus. Outside SNC Aquaporin 1 is expressed in renal tubules, capillary endothelium, lung, muscle and gut.
Design: 27 cases of hemangioblastoma, 7 primary renal cell carcinoma and 7 metastatic renal cell carcinoma were retrieved from the files of the Department of Pathology of Treviso and immunostained with Aquaporin 1 (clone 1/22, AbCAm, dilution 1:100). Material was paraffin embedded and formalin fixed.
Results: Strong membrane staining was observed in all cases of hemangioblastoma immunostained with Aquaporin 1. In all cases immunopositivity was observed in 100% of cells. All metastatic renal cell carcinomas were Aquaporin 1 negative. One case of well differentiate renal cell carcinoma showed weak Aquaporin 1 immunopositivity. Primary renal cell carcinomas featuring higher nuclear grade all proved negative.
Conclusions: 1. Aquaporin 1 appears to be a novel immunohistochemical markers enabling the differential diagnosis between hemangioblastoma and metastatic renal cell carcinoma. 2. In consideration of the pattern of expression of Aquaporin 1 in normal tissues hemangioblastoma may represent a neoplasm differentiating towards capillary endothelium or choroid plexus epithelium.

1355 Beta-Catenin Is Expressed Consistently in Meningiomas: Another Link in the Chain of "BROCN"-Family of Tumors?

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Background: There is mounting evidence in the literature suggesting that beta-catenin (BC) pathway is one of the key events in hormone-related carcinogenesis. Recently, BC has also been implicated as the common denominator in tumors that have "biotin-rich optically clear nuclei" (BROCNS), most of which are seen in women. Meningiomas are often seen in young women and suspected to be hormonally driven. Furthermore, they commonly exhibit optically clear nuclei. Although the expression of a related molecule, E-cadherin, has been tested in meningiomas, the status of BC has not been systematically investigated.
Design: Eight cases of meningioma were retrieved from our pathology department files. Standard H and E stained slides were examined for each case. Immunohistochemical staining for beta-catenin (monoclonal antibody, citrate buffer digestion, Zymed laboratories) was performed. The percentage of cells stained and intensity of staining was recorded.
Results: The eight cases included five meningotheliomatous, one mixed and two anaplastic meningiomas. On H and E slides, nuclear pseudoinclusions were seen in 5 of 8 cases. All eight cases showed strong (3+) and diffuse (100%) cytoplasmic immunostaining with prominent membranous localization. In addition, focal nuclear staining was observed in 6 of the 8 (75%) cases. Both anaplastic meningiomas showed nuclear and cytoplasmic co-localization. Two of the 3 meningiomas, which did not show nuclear pseudoinclusions, also failed to reveal nuclear immunostaining for BC, although they did have th cytoplasmic labeling.
Conclusions: Beta-catenin is strongly and diffusely expressed in meningiomas. This finding supports the role of BC pathway in the carcinogenesis of meningiomas, and as such may also serve as surrogate evidence for its hormone related nature. This study suggests that meningiomas can be included in the generic group of neoplasms characterized by "biotin-rich optically clear nuclei (BROCNs)". Further studies focusing on the BC pathway are warranted to determine its role in the pathogenesis of these tumors.

1356 BAF47/INI1/hSNF5 Expression Is Retained in Composite Rhabdoid Tumors, Including Rhabdoid Meningiomas

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Background: Rhabdoid cells are encountered in specific entities, such as malignant rhabdoid tumor (MRT) and atypical teratoid/rhabdoid tumor (AT/RT), as well as in composite rhabdoid tumors (CRTs) derived secondarily from other tumor types. Although rhabdoid neoplasms are uniformly aggressive, distinction of the entity from the phenotype remains important due to therapeutic implications. The majority of MRTs and AT/RTs affect infants, harbor chromosome 22q deletions, and inactivate the *BAF47/INI1/hSNF5* tumor suppressor gene on 22q11.2. In contrast, most CRTs affect adults, with FISH detectable 22q losses being less common. Unfortunately, this assay remains limited since 22q dosages are maintained in 20-30% of MRTs and AT/RTs. Furthermore, chromosome 22 losses are seen in several other tumor types, particularly meningiomas. The recently developed BAF47 antibody shows loss of nuclear INI1 protein expression in virtually all MRTs and AT/RTs, though its status is unknown in other rhabdoid neoplasms.

Design: We utilized immunohistochemistry and FISH to study INI1 expression and 22q dosages in 40 CRTs, including 16 meningiomas, 15 carcinomas, 3 melanomas, 2 sarcomas, 2 glioblastomas, and 1 neuroblastoma.

Results: 22q deletion was encountered in 71% of rhabdoid meningiomas, but was rare in other tumor types. With the exception of one retroperitoneal leiomyosarcoma and one renal cell carcinoma, nuclear INI1 expression was retained in all cases, including the meningiomas with 22q deletion.

Conclusions: We conclude that BAF47 immunohistochemistry is a simple, sensitive, and specific technique for distinguishing MRT and AT/RT from CRT.

1357 Does Biopsying Multiple Skeletal Muscle Sites at the Same Time Increase Diagnostic Yield?

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Background: Skeletal muscle biopsies are generally directed at muscles which are likely to demonstrate pathologic findings. Certain disorders of skeletal muscle, such as inflammatory myopathies, may show regional variability, which has prompted some to consider biopsying more than one muscle at the same time to increase the likelihood of diagnosis. There is little data in the literature to support this approach.

Design: Retrospective eight year review of 99 patients who had two (N=97) or three (N=2) muscles biopsied at the same time.

Results: The study including 99 patients (52 males, 47 females) who ranged in age from 23 to 92 years (mean 61.8 yrs) at the time of biopsy. The most common clinical symptoms prompting biopsy included weakness (N=83) and myalgia (N=15). Biopsies were performed to rule out an inflammatory myopathic condition in 32 patients. The most commonly biopsied muscles included deltoid (N=70), vastus lateralis (N=44), and quadriceps (N=27). The most common diagnoses rendered were inflammatory myopathy including inclusion body myositis (N=37), neurogenic atrophy (N=48), and type II atrophy (N=24). Diagnoses were the same in both biopsied muscles in 54 patients. In 17 patients, a diagnosis was made on only one biopsy. Both muscles were normal in five patients. Of potentially treatable inflammatory myopathies (N=29), a diagnosis could be made on only one of the two biopsies in 10 patients (34%).

Conclusions: In slightly more than half of the patients (55%), the diagnoses were identical in both biopsies. In a significant subset of patients, a potentially treatable inflammatory myopathic condition may have been missed if only one site had been biopsied, justifying biopsy of two sites in patients suspected of having an inflammatory myopathy.

1358 Fascin Expression in 90 Patients with Glioblastoma Multiforme

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Background: Fascin is a protein that serves to aggregate F actin into bundles that rearrange the cytoskeleton and promote cellular motility. Fascin has been linked to the invasive behavior of some tumors.

Design: Retrospective review of 90 patients (pts) with glioblastoma multiforme (GBM) and Fascin immunohistochemistry. Fascin positivity was graded as follows: <5%=1, 5-25%=2, 26-50%=3, 51-75%=4 and >76%=5.

Results: The study group is comprised of 53 males, (age range 11-83 yrs, mean 58.3 yrs). Initial surgery involved tumor debulking or subtotal resection in 85 pts and biopsy alone in 5 pts. Sixty-seven pts received adjuvant radiation therapy and 42 received adjuvant chemotherapy. On most recent follow-up (range: 12 days -55.5 mo, mean 11 mo), 3 pts were alive with no evidence of tumor (ANET), 5 pts were alive with residual tumor (AWT), 5 pts were lost to follow-up (LFU) and 77 died with tumor (DWT). All pts had tumors, which demonstrated positive Fascin staining. Nineteen tumors had 5+ staining, 14 tumors had 4+ staining, 23 tumors had 3+ staining, 26 tumors had 2+ staining and 8 tumors had 1+ staining. In comparison, 11 pts with low grade astrocytoma (LGA) and 10 pts with anaplastic astrocytoma (AA) were also evaluated, and all also demonstrated positive staining for Fascin. 9/11 LGA had a 3+ or lower staining. 8/10 AA had a 4+ or higher staining.

Conclusions: All GBM, AA and LGA expressed Fascin by immunohistochemistry, which may play a factor in tumor cell infiltration. Higher grade tumors generally expressed a greater degree of Fascin staining. There was no obvious correlation with the degree of staining and survival among the GBM.

1359 Cyclooxygenase-2 Expression in 100 Patients with Ependymal Tumors

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Background: Cyclooxygenase-2 (COX-2) is a cytokine-induced enzyme that metabolizes arachidonic acid into prostaglandins. Up-regulation has been described in some tumors, including astrocytomas, and COX-2 inhibitors exist which may play a role in treatment.

Design: Retrospective clinicopathologic review of 100 patients (pts) with ependymal tumors and COX-2 immunohistochemistry (IHC).

Results: The study group was comprised of 56 males, (age range 1-75 yrs, mean 30.8 yrs). Diagnoses included myxopapillary ependymoma (N=27), subependymoma (N=13), ependymoma, WHO grade II (N=48) and anaplastic ependymoma, grade III (N=12). Locations included spinal cord (n=56), 4th ventricle (n=22), lateral ventricle (n=18) and 3rd ventricle (n=4). Initial surgery involved gross total resection in 68 pts. Fifty-four pts received adjuvant radiation therapy and 16 adjuvant chemotherapy. On most recent follow-up (range: 3 days -217 months, mean 60 months), 63 pts were alive with no evidence of tumor (ANET), 20 were alive with residual tumor (AWT), and 12 died with tumor (DWT). Two pts died with no evidence of tumor (DNT) and 3 died with tumor status unknown (DTSU). Nineteen pts had a recurrence (interval 4 to 100 months) and 6 pts had 2 or more recurrences. Thirty-six (36%) pts had tumors, which demonstrated positive COX-2 staining, including 16/27 myxopapillary ependymomas, 3/13 subependymomas, 14/48 ependymomas and 3/12 anaplastic ependymomas. Tumors in 25 pts ANET were positive for COX-2 (39.7%), 7 AWT (35%), 2 DWT (16.7%) and 2 DNT or DTSU. Of the 19 pts in whom original and recurrent tumors were evaluated with COX-2, only 4 presented a discrepancy; 3 had a negative first sample and the recurrence was positive.

Conclusions: The majority of myxopapillary ependymomas and a subset of other ependymoma types expressed COX-2 by IHC. Some patients with ependymal tumors may benefit from treatment with COX-2 inhibitors.

1360 Bax/Bcl-2 Ratio as a Predictive Marker for Therapeutic Response, after Thymectomy in Patients with Myasthenia Gravis

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Background: Today thymectomy is a widely accepted therapeutic option for myasthenia gravis (MG). Because apoptosis seems to play a significant role in progress of MG, this study investigates whether there is a correlation between the ratio of bax oncoprotein (apoptosis promoter) to bcl-2 oncoprotein (apoptosis inhibitor) expression in patients with MG, and the clinical response after thymectomy.

Design: The study included 38 patients (16M/22F-median age 36yr) with MG, who underwent thymectomy for treatment. Pathology of thymus showed: hyperplasia-19, atrophy-8, thymoma-9, thymic carcinoma-2. Clinical staging (Osserman classification) included: stage I-3, IIA-19, IIB-13, III-3. Patients were followed up for 48-166 (median 100.5) months. At the end of the follow-up period, according to standard criteria (Jaretzki A, et al, Neurology 55:16, 2000) patients were classified as follows: group A: complete stable remission, group B: pharmacological remission+minimal manifestations. Paraffin sections of thymic tissue were subjected to *a)* immunohistochemistry (bax, bcl-2 protein) and *b)* in-situ hybridization (bax, bcl-2 mRNA). Bax to bcl-2 mRNA and protein ratio was determined for each sample by dividing %bax (+) cells by % bcl-2 (+) cells.

Results: Follow-up data were available on 31/38 patients. According to the aforementioned criteria 10/31 patients belonged to group A and 19/31 to group B. The Bax/Bcl-2 mRNA and protein ratios were increased towards advanced disease stages (+370% for mRNA and +391% for protein, from MG stage I to stage III). All the 10 cases of group A had a Bax/Bcl-2 ratio<1 (mean±SD: 0.58±0.04 for mRNA and 0.62±0.03 for protein), whereas all the 19 cases of group B had a ratio>1(1.47±0.07 for mRNA and 1.52±0.18 for protein). The Kaplan Meier survival curve showed higher, free of disease, survival in group A ($p<0.01$). Cox regression analysis revealed that the Bax/Bcl-2 ratio is independent prognostic factor, however the p-value is marginally significant (95% CI:1.42–16.0, p=0.049).

Conclusions: This study demonstrates that in patients with MG who underwent thymectomy, **a)** Bax/Bcl-2 ratio<1 is associated with complete stable remission, without medication, after thymectomy. **b)** Bax/Bcl-2 ratio is an independent predictive marker for therapeutic response after thymectomy. More extensive studies are needed in order to use this marker in the design and selection of the proper therapeutic modality in such cases.

1361 Anaplastic Oligodendroglioma: Distinctive Morphologic and Immunophenotypic Subsets Associated with Combined 1p/19q Allelic Loss

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Background: Anaplastic oligodendrogliomas (AOs) that exhibit combined 1p/19q loss respond more favorably to chemotherapy. There are currently no morphologic or immunophenotypic markers that are predictive of 1p/19q deletion. A subset of AOs show positivity for glial fibrillary acidic protein (GFAP). GFAP-positive tumor cells show two morphologies: gliofibrillary (GF) and minigemistocytic (MG). The relationship of these variants to molecular subtype has not been evaluated. Recently, neuronal differentiation marker expression (synaptophysin) has been described in a few cases of oligodendrogliomas that show 1p/19q loss, but this association has not been systematically studied. Results of this study show a positive association between synaptophysin expression and 1p/19q loss, as well as a negative association of the MG variant with 1p/19q loss.

Design: Three tissue microarrays (TMAs) were constructed from 86 archival AOs. Immunostaining for glial fibrillary acidic protein (GFAP; Pharmingen; clone cocktail 4A11/1B4/2E1; 1:6000) and synaptophysin (SYN; Biogenex; clone snp88; 1:50) was performed. GFAP-positive cases were subdivided into gliofibrillary (GF)-predominant and minigemistocytic (MG)-predominant subtypes. 1p and 19q deletions were determined by quantitative microsatellite analysis and/or fluorescent *in situ* hybridization.
Results: 74% (60/86) of AOs were GFAP+, 27% (23/86) were SYN+, 22% (19/86) were both GFAP+ and SYN+, and 26% (22/86) were negative for both markers. 41% (35/86) showed combined 1p/19q loss. Of the GFAP+ cases, 65% (43/66) were GF-predominant while 35% (23/66) were MG-predominant. 74% (17/23) of the SYN+ cases exhibited 1p/19q loss (p=0.0002, two-tail, Fisher Exact Test). 43% (23/60) of GFAP+ cases showed 1p/19q loss (no significant correlation). Of the GFAP+ cases, the GF-predominant subtype exhibited a trend towards a stronger association with 1p/19q loss compared to the MG-predominant subtype (p=0.065).
Conclusions: Study results show that synaptophysin expression is predictive of 1p/19q loss and, hence, suggests that the neurocytic immunophenotype characterizes a unique biologic subset of AOs with favorable molecular characteristics. Conversely, the MG phenotype shows a trend towards a negative association with 1p/19q loss and likely reflects a less favorable subset of AO.

1362 Angiogenic Patterns and Their Quantitation in High Grade Astrocytic Tumors

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Background: High grade gliomas including glioblastomas are known to be prognostically heterogeneous. Microvascular density (MVD) has been found to correlate with grading and prognosis. Recently, angiogenic patterns have been found to be independent predictors of survival in glioblastomas. Among promising markers of proliferation, DNA Topoisomerase II-alpha (T2a), a nuclear enzyme (170 KDa) with gene on chromosome 17, is expressed in S and G2/M phases, unlike MIB1 which identifies cells in nearly the entire growth fraction. The present study was undertaken to find correlation between angiogenic patterns and their quantitation versus proliferation indices as measured using T2a and MIB1 in grades 3 and 4 astrocytic tumors.
Design: Thirty six cases diagnosed in the year-2004 were stringently graded using the latest WHO schema and twenty nine cases of grade 3 (11) and grade 4 (18) were studied in detail. Immunohistochemical staining for CD34, MIB1 and T2a were performed using streptavidin biotin technique. Angiogenic patterns were studied and morphometric evaluation performed using image analysis on at least 4 hotspots on CD34 immunostained sections to determine MVD, microvascular area (MVA), aspect, mean diameter (MD) and fractal diameter (FD).
Results: Two major angiogenic patterns identified were capillary (30) and glomeruloid (6) types and were best developed in glioblastomas. Statistically significant differences were seen between grades 3 and 4 in MVD, aspect, MD and FD, but not in angiogenic patterns or MVA. T2a values strongly correlated with MIB1 labelling indices ($p<0.001$), but were lower than MIB1 LI values possibly because T2a identifies only a portion of the growth fraction (S and G2/M phases).
Conclusions: Angiogenesis is increased in transition of grade 3 to grade 4 in astrocytic tumors and may have an important pathophysiologic role, including neovascularization and vascular lengthening which may be related to their increased infiltrative property. High grade gliomas including glioblastomas can be subcategorized based on angiogenic parameters and proliferation indices. Further studies are needed to substantiate these postulates.

1363 Three Cases of Pituitary Cushing Adenomas with Concomitant GH and/or PRL Production from a Large Series of Pituitary Adenomas

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Background: Pituitary adenomas generally express their function according to the hormones defined in three cell lineages,i.e.GH-PRL-TSH, ACTH(POMC) and FSH/LH which are meticulously defined by the combination of various transcription factors and cp-factors. In our large series (total 595 cases 1996-2004) of pituitary adenomas, we have experienced three cases with concomitant production of ACTH and GH and/or PRL (designated as "trans-cell lineage" production).
Design: Three cases of ACTH producing adenomas(ACTHomas) with concomitant GH production were subjected to the immunohistochemical studies for the hormones and transcription factors(Pit-1,NeuroD1,Tpit,GATA-1,SF-1,DAX-1). Immunohisotchemical staining was done by ABC on the formalin paraffin sections. In the selected cases, in situ hybridization(ISH) was performed to analyze mRNA.
Results: In our series of surgically resected human pituitary adenomas, 48 of 595 cases(8%) were Cushing disease. From our immunoihstochemical studies for the pituitary hormones, only three cases(6.2% of Cushing's adenma and 0.5% of the entire pituitary adenomas) showed concomitant immunoreactivities for GH and/or PRL in the tumor cells. All patients were female and the ages of the patients were 53(case #1), 51(Case #2) and 65(Case #3). The tumors were "chromo phobic" and lacked Crooke's hyaline. Immunohistochemically, cases #1,2,3 showed the presence of GH-ACTH, GH-PRL-ACTH, GH-PRL-ACTH, respectively. ISH showed GHmRNA and indicated production in the tumor cells. Cases #1,3 showed macro adenomas and #2 was micro adenoma. Two cases(#1,3) showed the combination of NeuroD1, Tpit(for ACTH transcription) and Pit-1(GH,PRL transcription) immunohistochemically.
Conclusions: Three very rare tumors of concomitant "trans-cell lineage" production of ACTH and GH/PRL were demonstrated Aberrant "trans-cell lineage" expression of transcription factors was suggested as a basic molecular mechanism for this rare functional combination.

1364 Inflammatory Myofibroblastic Tumors in the CNS and Respiratory Tract: A Comparative Analysis

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Background: Inflammatory myofibroblastic tumor (IMT), often used synonymously with inflammatory pseudotumor (IP), was originally described in the lung. The term encompasses both neoplastic and non-neoplastic lesions, and the proposed distinction between the two is controversial. Some neoplastic examples have been linked with ALK gene (2p23) rearrangements. A link to human herpesvirus 8 (HHV-8) has also been proposed. IMTs in the CNS are rare and their characteristics are not well defined.
Design: Twenty-four tumors in the CNS and six in the respiratory tract (RT) were identified. The tumors were classified as either IMT or IP by histological criteria. When paraffin-embedded material was available, additional studies were evaluated. A subset of these cases was evaluated for the presence of ALK overexpression by standard immunohistochemistry (IHC). Fluorescence in-situ hybridization (FISH) was used to identify the 2p23 (ALK locus) translocation. The presence of HHV-8 was evaluated by IHC (vIL-6, monoclonal and polyclonal antibodies against latency-associated nuclear antigen) and RT-PCR for four HHV-8 related transcripts (vIL-6, v-Bcl-2, v-Cyclin D1, v-FLIP).
Results: The cohort included 13 male and 17 female patients with a mean age of 36.8 years (range 10 months-87 years). Twelve were diagnosed as IMT (6 CNS, 6 RT), eighteen as IP (all CNS). The IMT patients had a mean age of 38.1 years (range 10 months to 87 years), the IP patients 36.8 years (5 to 72 years). None of the IMT or IP cases recurred where follow-up information was available (22 cases, mean follow-up 50.5 months, range 1 month to 12 years). Seven of ten IMTs demonstrated ALK abnormalities; five IMTs were positive for ALK overexpression by IHC (3/4 in CNS, 2/6 in RT) and two had 2p23 rearrangements by FISH (1/2 in CNS, 1/6 in RT). None of the IPs was positive for ALK by IHC and/or FISH (0/4). None of the IPs (0/4) or IMTs (0/8) demonstrated HHV-8 expression by either IHC or RT-PCR.
Conclusions: Neoplastic as well as non-neoplastic lesions were identified in the CNS. The neoplastic group closely resembles the pulmonary counterpart. Our findings suggest that the neoplastic group have a relationship to ALK expression by IHC/FISH analysis, and neither IP nor IMT are associated with HHV-8 by IHC/RT-PCR. We suggest that the term IMT should be reserved for the neoplastic lesion, and should be distinguished from the non-neoplastic IP.

1365 Bcl-x: An Anti-Apoptotic Protein Showing Promise as an Alternative Marker for Glial Cells

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Background: Reactive glial proliferation (gliosis) occurs in response to various CNS injuries while neoplastic proliferation results in gliomas. The ability to confirm the identity of glial cells is useful during tissue diagnosis. GFAP has been the traditional marker for glial cells, while more recently, bcl-x, an anti-apoptotic protein, has also been shown to be expressed by some gliomas. We seek to compare the immunoexpression of bcl-x with GFAP in a variety of glial lesions, both neoplastic and non-neoplastic, and to assess its potential usefulness in the delineation of these lesions.
Design: Forty cases of brain tumors and reactive brain conditions were reviewed. The former included astrocytomas, GBMs, ependymomas, oligodendrogliomas, gangliogliomas, subependymomas and neurocytomas. The latter included cases of gliosis, cerebritis and mesial temporal sclerosis. Immunohistochemistry for bcl-x and GFAP was performed. Selected cases were also subjected to double immunofluorescent labelling using both GFAP and bcl-x.
Results: Expression of bcl-x closely follows that of GFAP with strong expression in both reactive astrocytes and astrocytomas. There is more focal expression in other gliomas. Immunostaining for bcl-x is generally more intense and distinct, compared to that for GFAP. Expression of both GFAP and bcl-x is more focal in oligodendrogliomas, with staining of mainly intervening astrocytic processes. Double immunolabelling confirms the co-expression of bcl-x and GFAP.
Conclusions: Bcl-x is co-expressed with GFAP in various gliomas and reactive brain conditions. Immunostaining for bcl-x is generally more distinct and intense. In the appropriate context, immunostaining for bcl-x may be useful in the identification of gliomas, in particular, astrocytomas.

1366 Factor XIIIa Expression in Primary CNS Lymphomas

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Background: Clotting factor XIIIa (FXIIIa) is a transglutaminase produced by histiocytic and dendritic cells. It is known to cross-link fibrin in hemostasis, but more recently has been postulated to play a wider role in inflammation and neoplasia. Proteins in the transglutaminase family form covalent bonds between protein units, and have been implicated in adhesion of cells to the extracellular matrix, cancer cell migration, and apoptosis. Decreased expression of FXIIIa and other transglutaminases has been associated with poor outcome in some carcinomas, but has not been studied in CNS lymphomas.

Design: We looked for expression of FXIIIa in 23 cases of primary CNS lymphoma from immunocompetent patients using immunohistochemistry on formalin-fixed diagnostic biopsy specimens. 19 cases were large B-cell lymphoma, 2 were high-grade Burkitt-like lymphoma, and there were one case each of lymphoblastic and marginal zone lymphoma. All cases were diagnosed at UCSF, and treated at our institution with chemotherapy that included high-dose methotrexate.
Results: Significant staining for FXIIIa was found in 13 of the 23 cases (57%), and was absent from the remaining 10 cases (43%). This staining appeared to co-localize with staining for CD68 but not S100 or GFAP, suggesting the positive cells were histiocytes. In some cases, FXIIIa staining appeared to localize to vessels in the tumor, suggesting either endothelial expression or localization of FXIII-positive histiocytes to vascular beds. No significant expression for FXIIIa was seen in normal brain tissue. Among the large cell lymphomas, only 1 of 6 patients (17%) with FXIIIa-negative tumors had a durable response at one-year, while that rate was 37% overall.
Conclusions: Lack of FXIIIa expression by tumor histiocytes is found in a significant proportion of CNS lymphoma cases, and is associated with a poor clinical response to therapy, raising the possibility that a failure of immunosurveillance by tumor histiocytes may be involved in the pathogenesis of primary CNS lymphomas in immunocompetent individuals.

1367 Colorectal Intramucosal Perikarya of Ganglion Cells

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Background: It is generally believed that perikarya of ganglion cells in the normal human colorectum are confined to plexuses that lie deep to the mucosa. Intramucosal perikarya of ganglion cells have been noted only in ganglioneuromas, neuronal intestinal dysplasia, and the appendix.
Design: We retrospectively reviewed 58 specimens from colorectal biopsies. For each specimen, the presence of intramucosal perikarya of ganglion cells, their number, location and grouping were recorded, as well as the diagnoses. Immunostains for neuron-specific enolase and cytomegalovirus were performed to attempt to confirm the presence of intramucosal perikarya.
Results: Eleven specimens (19%) contained intramucosal perikarya. Intramucosal perikarya were located throughout the large intestine and occupied the muscularis mucosa and lamina propria. Intramucosal perikarya were seen in normal mucosa, hyperplastic polyps, adenomas, carcinoma, inflammatory bowel disease, and cytomegalovirus-associated colitis. Perikarya morphologically resembled microgranulomas when they were clustered and cytomegalovirus when they occurred singly. Although some perikarya were immunoreactive with neuron-specific enolase, perikarya were often absent from additional sections used to perform immunostains.
Conclusions: We present the first study of colorectal intramucosal perikarya of ganglion cells. Our findings demonstrate that intramucosal perikarya of ganglion cells are common in normal and abnormal mucosa. Awareness of intramucosal perikarya is necessary to avoid confusion with microgranulomas or cytomegalovirus.

1368 Uveal Melanoma and Monosomy of Chromosome 3

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Background: Risk assessment for uveal melanoma currently uses attributes such as tumor size and histologic cell type. In the future, morphologic molecular pathology may complement or replace those attributes. Monosomy of chromosome 3 has been associated with an adverse outcome. Fluorescence in situ hibrydization (FISH) was used to evalute uveal melanomas for monosomy of chromosome 3.
Design: A tissue microarray was constructed using two or more cores of uveal melanomas from 15 patients. FISH using a directly labeled probe specific for the pericentrimeric region of chromosome 3 was performed. Tumor cell nucleli were evaluated for the presence of one or two chromosomes. Up to 50 nuclei were evaluated in each core. For monosomy to be present, at least 30% of the evaluated nuclei had to have no more than one chromosome.
Results: FISH was successful in 13 tumors. 8 tumors revealed monosomy 3. 5 had two chromosomes 3. Follow up varied from one year to eight years. Three patients developed metastatic disease at one, three and six years. One is dead of disease. All of the patients developing metastatic disease had chromosome 3 monosomy. All 5 patients with two chromosomes 3 had no evidence of disease at last follow up that varied from one year to five years. 5 patients with monosomy 3 were alive without evidence of disease at zero to eight years follow up.
Conclusions: FISH for chromosome 3 monosomy may complement other attributes used in risk assessment of uveal melanoma.

1369 Differential Amplification and Expression of CDK6 between Low-Grade Astrocytoma and Glioblastoma Multiforme

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Background: Astrocytomas are the most common primary brain tumors occurring in the adult central nervous system. Emerging evidence suggests that the development of glioblastoma multiforme (GBM) is a multi-step process and is result of a series of genetic alterations occurring over time. Our previous study using array-based comparative genomic hybridization (A-CGH) demonstrated marked difference in the extent of genomic gains and losses between GBM and low-grade astrocytoma (LGA). In this study, we validated the A-CGH results for cyclin-dependent kinase 6 (CDK6) copy number changes by fluorescence *in situ* hybridization (FISH) and examined the expression level of CDK6 protein between GBM and LGA by immunohistochemistry (IHC).
Design: A tissue micro-array (TMA) containing 31 GBM and 5 LGA was constructed and used for FISH and IHC. The CDK6 probe derived from a bacterial artificial clone (BAC) was labeled with SpectrumGreen dUTP. The CDK6 and CEP7 probes were hybridized with the TMA slides. Immunostaining on TMA was performed using monoclonal antibody specific for CDK6.
Results: 14 cases (45%) of GBM showed increased copy number of CDK6, whereas all five cases of LGA displayed no changes. 5 cases of GBM exhibiting high-level amplification also showed strong immunostaining for CDK6 and the other 9 cases were moderately positive. Among the cases with no copy number changes for CDK6, 15 cases of GBM and 1 case of LGA displayed weak to moderate CDK6 staining.
Conclusions: This study reveals the differential changes in CDK6 copy number between GBM and LGA. Overexpression of CDK6 protein resulting from either increasing the copy number or enhanced transcription/translation of CDK6 gene may play a role in the development of GBM and the possible progression of low-grade astrocytomas to GBM. In addition, our study demonstrates that array-based genomic profiling together with FISH validation and IHC can be effective tools for the identification of new molecular markers having potential clinical utility.

1370 Diagnostic Utility of Epithelial Markers in Separating Choroid Plexus Papillary Neoplasms from Metastatic Carcinomas with Papillary Architecture

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Background: Choroid plexus papillary neoplasms (CPPs) are epithelial tumors derived from the choroid plexus (CP). The diagnosis of choroid plexus papillary neoplasms is usually not difficult based on the histologic and clinical features. However, in patients with clinical history of a papillary carcinoma, separation of choroid plexus papillary neoplasm from metastatic carcinoma with papillary features is very important and can be occasionally challenging. The utility of immunohistochemical markers to separate choroid plexus papillary neoplasm from metastatic carcinoma with papillary features has not been investigated.
Design: A total of 38 cases including 11 normal choroid plexus, 8 choroid plexus papillary neoplasms and 20 metastatic carcinomas with papillary features metastatic to the central nervous system were retrieved. Epithelial markers of CAM5.2, CK7, CK20, CK5/6, Ber-EP4, EMA and B72.3 were evaluated. Immunostains were performed on an automated immunostainer with appropriate positive and negative controls. Statistical analysis was calculated with Chi-square method.
Results:

Expression of epithelial markers in choroid plexus papillary neoplasms (CPPs), normal choroid plexus (CP) and metastatic carcinoma with papillary architecture

Markers	CAM5.2	CK7	CK20	CK5/6	Ber-EP4	EMA	B72.3
Normal Choroid Plexus	64%(7/11)	9%(1/11)	0%(0/11)	0%(0/11)	0%(0/11)	0%(0/11)	9%(1/11)
CPPs	100%(8/8)	38%(3/8)	13%(1/8)	38%(3/8)	0%(0/8)	38%(3/8)	25%(2/8)
Metastatic carcinomas	100% (20/20)	75% (15/20)	20% (4/20)	10% (2/20)	80% (16/20)	70% (14/20)	70% (14/20)

Conclusions: 1) Ber-EP4 and B72.3 are expressed in 80% and 70% of metastatic carcinoma with papillary features but none of the choroid plexus papillary neoplasms. Therefore, Ber-EP4 and B72.3 are useful immunohistochemical markers in separating metastatic carcinoma from CPPS.
2) CK7, which detected more metastatic carcinomas than CPPs, should be included in the panel of immunostains.
3) High molecular cytokeratin CK5/6 and CK20 are not useful in separating metastatic carcinoma from CPPs.

Pathobiology

1371 Persistent Increase of Immature Reticulocyte Fraction in Sickle Cell Anemia Treated with Hydroxyurea

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Background: Sickle cell anemia is an inherited disease. The deformed red blood cells obstruct the circulation causing severe pain, hemolytic anemia, and other complications. Recent studies have shown that hydroxyurea (HU) increases the concentration of HbF that prevents the polymerization of HbS and significantly improves the microcirculation, leading to decrease in the number of vaso-occlusive episodes in patients with sickle cell anemia (Steinberg et al., 2003). Since it has been noted that the Immature Reticulocyte Fraction (IRF), a ratio of immature reticulocytes to the total number of reticulocytes, is markedly increased in sickle cell anemia, we investigated the effect of HU on IRF, which serves as an important physiologic indicator of erythropoietic bone marrow response to anemia or tissue hypoxia.
Design: Thirty-two patients with sickle cell anemia at Boston Medical Center, Boston, MA were studied, including 16 treated with HU (mean age = 24 years, M: F = 1.28) and 16 without HU (mean age = 13 years, M: F = 1.0). Laboratory observations include IRF as well as Hb, RBC, MCV, reticulocyte %, absolute reticulocyte count (ARC) by automated hematology analyzer (Beckman Coulter), and HbF by HPLC. In all cases, peripheral blood smears were also reviewed. Statistical analysis was performed using student t test.
Results: The significant differences between HU-treated vs non-treated groups were observed in HbF (12.4% vs 8.0%; $p< 0.05$), MCV (102.7 fL vs 92.2 fL; $p<0.01$), reticulocyte % (8.1 vs 12.8; $p<0.01$) and ARC (21800 vs 35000; $p<0.01$). Even more dramatic differences in HbF (18.8%), reticulocyte % (5.8) and ARC (13600) were noted in the treated group with MCV greater than 100 fL (mean 116.5; N=7). In addition, there was a marked reduction in the number of sickle cells per HPF in the