1357 p27, Cks1, Skp2 and PTEN Expression in Hepatocellular Carcinoma

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Background: $p27^{Kip1}$ is a cell-cycle inhibitory protein and its downregulation is mediated by its specific ubiqitin subunits Cks1 and Skp2. PTEN is a tumor suppressor gene which upregulates p27. This study investigates p27, Cks1, Skp2 and PTEN expression in hepatocellular carcinoma (HCC).

Design: Formalin-fixed, paraffin-embedded 4μm sections, obtained from 67 HCC hepatectomy specimens with matched non-neoplastic liver, were subjected to immunohistochemistry using monoclonal and polyclonal antibodies for p27, Cks1, Skp2 and PTEN. Nuclear staining was considered as positive. Results were correlated with pathologic data and patients' survival. Mean follow up time was 30.12 months (range 1-84 months).

Results: Expression of p27, Cks1, Skp2 and PTEN was recorded in: 51/67(76%) 16/67(24%), 23/67(34%) and 63/67 (94%) cases, respectively. PTEN was also expressed in cirrhotic and non-cirrhotic non-neoplastic livers; however its expression was significantly lower compared to that of tumors (HCC: 70.91±36.46, cirrhotic livers: 31.62±12.04, non-cirrhotic livers: 10.11±9.94-p<0.001). Mean values for Cks1, Skp2 and p27 expression in HCC were: 8.1±15.5, 4.72±9.7, 17±18.4 respectively. Cks1, Skp2 and p27 expression in cirrhotic and non-cirrhotic livers was observed in rare instances. Statistical analysis revealed a loss of PTEN and p27 expression in HCC grade 3: [PTEN: grade 1: 97.3±1.9, grade 2: 71.6±27.4, grade 3: 7.4±2.5-p<0.0001. p27: grade 1+2: 19.2±11.3, grade 3: 5.3±3.1-p=0.029]. Loss of PTEN and p27 expression was also related to presence of vascular invasion (VI): [PTEN: VI(-): 92.1±5.6, VI(+): 12.4±1.2-p=0.0012. p27: VI(-): 23.1±4.5, VI(+): 5.2±2.7-p=0.013]. No association was recorded between Cks1 and Skp2 expression and tumor grade or stage. PTEN and p27 expression were reversibly correlated with disease free survival (r=- 0.61, p=0.0043 and r=- 0.47, p=0.018). Cox regression analysis revealed that vascular invasion (CI:1.231-5.604, p=0.019), tumor stage (CI:0.051-0.690, p=0.012) and PTEN expression (CI: 1.065-41.082, p=0.032), were independent prognostic factors.

Conclusions: This study demonstrates that loss of PTEN and p27 expression is associated with adverse pathological parameters and increased risk for tumor recurrence. These results support the importance of PTEN and p27 loss for the progression of HCC in humans. PTEN increased expression in cirrhotic non-neoplastic livers may reflect an effort for control of hepatocyte regeneration associating liver cirrhosis.

Neuropathology

1358 Diagnostic Utility of Immunohistochemistry in Differentiating Hemangioblastoma from Metastatic Renal Cell Carcinoma

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Background: Differentiating hemangioblastoma (HB) from metastatic clear renal cell carcinoma (CRCC) to the brain is crucial since they have different management and both can occur in patients with Von Hippel Lindau (VHL) disease. Moreover, CRCC can metastasize to hemangioblastoma as a tumor to tumor metastases. In addition, HB can demonstrate vacuolated tumor cells mimicking CRCC. We investigated the diagnostic utility of D2-40, a novel monoclonal antibody, CD10, low-weight cytokeratin (CAM 5.2), epithelial membrane antigen (EMA), RCC, estrogen and progesterone receptors (ER and PR) in HB and CRCC patients.

Design: A computer search of our hospital identified 27 cases of HB between 1997 and 2005, consisting of 10 spinal, 9 cerebellar and 8 cerebral HB. We also included 30 cases of metastatic CRCC, 8 of which were metastases to the central nervous system. Immunostaining was performed on formalin-fixed, paraffin embedded sections using HIER technique. Intensity was graded from 0-3 with a score 0 for no staining and 3 for maximal intensity. The pattern/distribution of reactivity was recorded as focal (5-10%) or diffuse (>10%). Cases which showed weak or <5% staining were considered negative.

Results: Table (I) shows our results. All cases of HB are negative for epithelial markers (Cam 5.2, CD10, EMA, RCC). None of the CRCC was negative for both CD10 and Cam5.2. Cases which were negative or focally positive for CD10, demonstrated strong positivity for Cam5.2 (5 cases) and vice versa (3 cases).

	D2-40	ER	PR	CD10	Cam5.2	EMA	RCC
HB	10/27	0/27	7/27	0/27	0/27	0/27	0/27
CRCC	10/30	0/30	9/30	25/30	24/30	27/30	22/30

HB= hemangioblastoma CRCC= clear cell renal cell carcinoma

Conclusions: In our experience, the monoclonal antibody D2-40 is not a useful marker to distinguish HB from CRCC. Epithelial markers, especially CD10, Cam 5.2 and RCC are superior markers for distinguishing between a HB and a metastatic CRCC. PR immunoreactivity was unable to distinguish between HB and CRCC metastatic to the CNS, and the staining for PR was weak and focal, suggesting that hormonal treatment is not an option for these patients.

1359 Prion Disease in Washington State: A Thirty Month Surveillance Study

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Background: Variant Creutzfeldt-Jakob disease (vCJD), thought to be acquired from eating beef affected by bovine spongiform encephalopathy (BSE), was described following a epidemic in the United Kingdom. In 2003 the first instance of BSE was reported in the USA in a cow slaughtered in Washington. The meat was distributed for human consumption. In 2004, prompted by public concern, we initiated a Center for Disease Control-sponsored collaborative program to enhance autopsy surveillance for clinically suspected prion disease.

Design: The WA Department of Health (DOH) disseminated information about the program to healthcare providers throughout the state. Enrollment was prompted by healthcare providers contacting local or state DOH, the National Prion Disease Pathology Surveillance Center (NPDPSC), or the Univ of WA (UW) to report a case of suspected prion disease. No case was declined and all costs, including transportation of the deceased, were covered. Autopsies were performed by UW Neuropathology and brains were evaluated at this site and the NPDPSC.

Results: During the first 30 months of surveillance, 30 cases of suspected prion disease were referred. Eighteen had prion disease classified as CJD. One case was familial while the remainder had sporadic (s) CJD of the following subtypes: eight M/M isoform 1, two M/M isoforms 1-2, two M/V isoforms 1-2, two M/V isoform 2, one V/V isoforms 1-2, and two V/V isoform 2. There was no case of vCJD. This represents a prevalence of 1.1 sCJD cases per million people per year in WA (population = 6.375 million), a value in close agreement with prevalence estimates in other populations. Eleven of the remaining twelve patients had a variety of structural brain changes that meet criteria for diseases that cause degeneration in cognitive and motor function. One case had no demonstrable pathologic lesions in the tissue examined.

Conclusions: This is the first epidemiologic investigation within a US state based entirely on autopsy-confirmed cases. Our results do not support the hypothesis that vCJD is an emerging illness in WA or that sCJD is more common in this state than in other regions of the world. The findings and lack of evidence for epidemic BSE in the USA is encouraging but continued surveillance for prion diseases is needed because our knowledge about emergence and transmission is inadequate. Our program may serve as an example to other states that wish to enhance surveillance of prion diseases.

1360 Evaluation of Chromosome 7 Alterations Including Epidermal Growth Factor Amplification Status in Pediatric Meningiomas

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Background: Childhood meningiomas are rare tumors corresponding to less than 3% of all primary CNS tumors. Distinct features include male predominance, infratentorial and intraventricular occurrence, and frequent clear cell and papillary subtypes. Monosomy of chromosome 22 is their best known molecular alteration ; loss of 7p has also been reported. Overexpression of EGFR correlates with enhanced malignant potential of many tumor types, including glioblastomas and astrocytomas. In adult meningiomas, high EGFR expression has been demonstrated by immunohistochemistry (IHC), but is not associated with prognosis. We evaluated 13 pediatric meningiomas utilizing chromogenic in situ hybridization (CISH), fluorescent in situ hybridization (FISH), and IHC to assess chomosome 7 alterations including amplification/expression status of the epidermal growth factor receptor (EGFR).

Design: Thirteen pediatric meningiomas were classified according to the 2000 WHO criteria (6 sporadic, 2 NF2-associated, and 6 radiation-induced). IHC, CISH and FISH were performed for EFGR status and CISH with the chromosome 7 centromere probe was used to assess ploidy status. High EGFR expression by IHC was characterized by complete membranous staining in more than 10% of the tumor cells. EGFR amplification by CISH was detected when 10 or more copies or clusters were observed in more than 50% of the cells; by FISH, when the average ratio of EGFR gene/CEP 7 signal per cell was greater than 2. Tumors with low amplification showed 6-10 copies in the nuclei. Chromosome 7 diploid meningiomas showed 2 copies in more than 50% of the tumor cells (CISH).

Results: High EGFR expression by IHC was observed in 9 (61%) meningiomas. The remaining 4 (39%) cases (1 sporadic, 2 radiation-induced, 1 NF2 associated) were negative. Of the positive cases, two showed low amplification for EGFR by CISH but not by FISH (performed in 3 cases). All cases were diploid for chromosome 7 (two cases showed triploidy in 35% of the cells).

Conclusions: We conclude that (1) amplification of EGFR is not a common feature in pediatric meningiomas, (2) high EGFR expression by IHC does not correlate with EGFR amplification by FISH or CISH, (3) CISH is a better method than IHC for evaluation of true EGFR status amplification, and (4) most pediatric meningiomas show normal chromosome 7 ploidy.

1361 Chromosome 7 Polysomy Detected by Chromogenic In Situ Hybridization (CISH) Is a Common Finding in Sporadic Chordomas

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Background: Chordomas are malignant bone tumors most often located in the axial skeleton. The cytogenetic and molecular features of chordomas are largely unknown but reportedly complex. Copy number gains of chromosome 7 have been detected in some sporadic and familial chordomas by various methods including comparative genomic hybridization, G banding, and FISH. In this study, we evaluated chromosome 7 ploidy status in a group of 11 sporadic chordomas using chromogenic in situ hybridization (CISH).

Design: Eleven sporadic chordomas were analyzed by H&E and immunohistochemistry profile with vimentin, EMA, AE1/AE3, and S100 for confirmation of diagnosis. MIB1 and p53 stains were also obtained. For detection of abnormalities on chromosome 7 we used CISH. Polysomy of chromosome 7 was detected when 3 or more signals were found in the nuclei of more than 50% of the tumor cells.

Results: Chromosome 7 polysomy was detected in 9/11 (82%) sporadic chordomas. All cases showed typical physaliferous cells by H&E and stained positive for all markers. p53 and MIB1 staining was rarely detected and associated with recurrent tumors and necrosis.

Conclusions: Chromosome 7 polysomy is a common event in sporadic chordomas. This finding suggests that this region may harbor an oncogene potentially relevant in the tumorigenesis of sporadic chordomas.

1362 Primary Sarcomas of the Central Nervous System – The UCSF Experience 1985-2005

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Background: Primary sarcomas of the central nervous system (CNS) are extremely rare. Unlike their counterparts in soft tissues and extremities, there is limited information on the clinicopathological characteristics of CNS sarcomas, most in the form of case reports. It is important to recognize the unique characteristics of primary CNS sarcomas ,and identify their distinctions from similar tumors in the soft tissues and other solid organs.

Design: We searched UCSF Pathology and Cancer Center Registry databases for all CNS sarcomas diagnosed between 1985 and 2005. We excluded sarcomas at other sites, as well as Ewing's sarcoma, sarcomatoid variants of neuroepithelial or meningothelial neoplasms, and chordomas. We have studied the clinical characteristics, patient demographics, histopathological and immunohistochemical characteristics of all CNS sarcomas using a large panel of antibodies, and compared these with the soft tissue sarcomas of the same histological type.

Results: In the 21 years between 1985 and 2005, we identified 1600 soft tissue and extremity sarcomas, and 25 CNS sarcomas that fulfilled the inclusion criteria. Hemangiopericytomas consituted the majority of this group with 14 cases. In addition, 4 tumors were associated with radiation, and 4 were ultimately discovered to have extracranial origin. The remaining 3 cases occurred in male patients and were not associated with extracranial disease or radiation. These tumors included a histiocytic sarcoma, low grade parafalcine chondrosarcoma, and an undifferentiated sarcoma with a fibroblastic phenotype. Clinical and immunohistochemical features of all cases are presented.

Conclusions: Primary CNS sarcomas are extraordinarily rare and their clinicopathologic characteristics remain elusive. Tumors with the histological and immunohistochemical characteristics of Hemangiopericytoma are the most common primary CNS sarcomas. Radiation-induced sarcomas, or sarcomas metastatic from other sites are more frequent and the finding of an apparently spontaneous primary CNS sarcoma should prompt a careful search for an extracranial primary, or a prior history of radiation. Even though the cell of origin is much in debate, and the concept appears to be disappearing for soft tissue tumors, Hemangiopericytomas constitute the most common primary CNS sarcoma, and still distinguish themselves in terms of behavior from meningiomas and solitary fibrous tumors and other mesenchymal tumors of the CNS.

1363 Apoptotic Markers in Type II (Taylor Type) Malformations of Cortical Development (MCD)

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Background: Bcl-2, bcl-X_L, and bax are proteins variably expressed in brain tissue which are involved in the regulation of apoptosis. Bcl-2 and bcl-X_L act as inhibitors of apoptosis while bax promotes apoptosis. P53 is a tumor suppressor gene which, when inactivated, leads to uncontrolled neoplastic growth. The purpose of this study is to evaluate bcl-2, bcl-X_L, bax and p53 immunoexpression in MCD (cortical dysplasia) type II marked by either dysmorphic neurons (n=21) and/or balloon cells (n=18).

Design: A retrospective review of immunostaining with apoptotic markers bcl-2, bcl-X_L, bax, and p53 in 21 patients with type II MCD who had undergone surgical excision for treatment of epilepsy.

Results: Twenty-one patients (13 males, 8 females) with chronic epilepsy formed the study group. The age range of the patients at the time of surgery was 6 weeks to 57 years (mean 9.2 years). Twelve specimens were from the frontal lobe, one specimen each was from the parietal and occipital lobes, and seven were from multiple lobes. Five patients had tuberous sclerosis and three had a ganglioglioma. The dysmorphic neurons stained positively for bcl-X_L, bax and bcl-2 in 71%, 76% and 24% of cases, respectively. The balloon cells stained positively for bcl-X_L, bax and bcl-2 in 89%, 78% and 17% of cases, respectively. Eighteen of the twenty-one specimens (86%) showed some immunoexpression of p53. Of particular note was the observation that 13/18 (72%) cases with balloon cells showed positive staining of these cells, and in most of these cases, the staining was confined to the balloon cells alone.

Conclusions: There is increased expression of apoptosis-associated proteins in the balloon cells and dysmorphic neurons of MCD. The dysmorphic neurons and balloon cells had similar frequencies of expression of these markers. Most MCD type II (86%) showed some expression of p53, with the majority showing expression of p53 most prominently in balloon cells. Previous work has shown gangliogliomas and dysembryoplastic neuroepithelial tumors (DNTs), both MCD-associated neoplasms, to also demonstrate aberrant expression of apoptotic markers, suggesting a possible common mechanism of development for these two processes in patients in whom they coexist.

1364 Clinicopathologic Correlation in Progranulin Mutations

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Background: Familial frontotemporal dementia (FTD) with parkinsonism is often linked to chromosome 17q21(FTDP-17). Those with mutations in the microtubule associated protein tau gene, *MAPT,* have tau positive neuropathology. Affected individuals with ubiquitinated inclusions (FTLD-U) and linkage to 17q21 lack mutations in *MAPT*. Mutations in the *progranulin* (*PGRN)* gene were recently reported in some of these cases. Thus far, ubiquitinated neuronal intranuclear inclusions (NIIs) have been demonstrated in all cases with *PGRN* mutations.

Design: *PGRN* mutation analysis was performed in six cases, chosen because complete clinical and pathologic material was available, the pathology was FTLD-U or FTLD-MND, and DNA or frozen brain tissue for analysis was available. Informed consent was obtained from next of kin under an IRB-approved protocol. We then compared clinical and pathologic findings in those cases with and without mutations.

Results: *PGRN* mutations were found in three patients, one with clinical behavioral variant of FTD (FTD-bv) and a positive family history, and two with clinical primary progressive aphasia (PPA) – one with a family history and one reportedly without. All three had FTLD-U pathology including NIIs. FTLD-U with *PGRN* mutation is associated with greater striatal atrophy, greater frontal, temporal, and thalamic neuronal loss and gliosis, more frequent frontal ubiquitinated cytoplasmic inclusions (CIs) and dystrophic neurites, less frequent dentate gyrus CIs, more frequent frontal and striatal NIIs, and the absence of clinical and pathologic ALS.

Conclusions: *PGRN* mutations at 17q21 may occur in FTLD-U cases that lack an obvious family history of similar dementias and in cases presenting with either PPA or FTD-bv. Thus far, all cases with *PGRN* mutations have ubiquitinated NIIs, and specific pathologic differences are observed among FTLD-U cases with and without *PGRN* mutations.

1365 Characterization of the Phosphatidylinositol 3-Kinase Pathway in Neurofibromatosis-1 Associated Tumors

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Background: Neurofibromatosis I (NF-1) is a genetic disorder with loss of a single copy of the NF-1 tumor suppressor gene. Cell culture studies and mouse models demonstrate increased activation of the phosphatidylinositol 3-kinase (PI3K) pathway in NF-1 deficient cells. PI3K proteins are important regulators of proliferation and apoptosis. The purpose of this study is to evaluate the PI3K pathway using phospho-specific antibodies in NF-1 associated neoplasms and controls.

Design: Immunohistochemistry for p-AKT, p-mTOR, p-p70S6K, p-4E-BP1 and p-AMPK (Cell Signaling Technology) and PTEN (clone 6H2.1, Cascade Bioscience) was performed on a tissue array of 44 NF-1 associated malignant peripheral nerve sheath tumors (MPNSTs), 22 non-NF-1 associated MPNSTs, 24 NF-1 associated plexiform neurofibromas, 8 localized neurofibromas and 22 schwannomas. Western blots were performed with antibodies to p-AKT, p-4E-BP1 and PTEN.

Results: The western blots demonstrated high expression of p-AKT in all samples, high PTEN expression in all but one MPNST and one schwannoma and variable expression of p-4E-BP1 (high expression in 3/5 MPNSTs, 2/5 neurofibromas, 1/1 plexiform neurofibromas and 3/4 schwannomas). The immunohistochemical profiles demonstrated expression of PI3K pathway proteins at lower levels then the western blot data. Appropriate IHC controls were run concurrently.

Immunohistochemical profiles of NF associated tumors and controls

	p-AKT	p-mTOR	p-4E-BP1	p-p70S6K	PTEN	p-AMPK
MPNST (NF-1 / non NF-1)	2% / 36%	0% / 0%	42% / 33%	0% / 5%	68% / 64%	14% /14%
Plexiform neurofibroma	0%	0%	9%	0%	58%	0%
Localized neurofibroma	0%	0%	0%	0%	13%	0%
Schwannoma	14%	0%	36%	0%	95%	23%

Conclusions: The western blot data demonstrates activation of the PI3K pathway in NF-1 associated neoplasms. The concurrent expression of PTEN (a negative regulator of AKT) with p-AKT and p-4E-BP1 indicates that activation of the PI3K pathway in NF-1 associated neoplasms is independent of PTEN status. The lower detection rate of phosphorylated PI3K pathway proteins by immunohistochemistry suggests limited clinical utility for evaluation of NF-1 associated neoplasms with anti-phospho antibodies in formalin fixed paraffin embedded tissues.

1366 Aurora B Expression in Ependymomas

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Background: Aurora kinases are critical for normal chromosome segregation and cell division, and the deregulation of these kinases has been shown to cause chromosomal missegregation and polyploidization. Overexpression of Aurora B has been reported in a variety of tumors, and has been associated with shortened survival in glioblastoma multiforme. The purpose of this study was to evaluate the expression of Aurora B in ependymomas and to determine its association with prognosis.

Design: Thirty-two cases of grade II ependymoma, 10 cases of grade III ependymoma, 9 cases of subependymoma, and 16 cases of myxopapillary ependymoma were retrospectively studied. Aurora B expression was evaluated by immunohistochemical staining and nuclear staining was graded as: 0 (absent), 1+ (<5% cells), 2+ (5 – 25% cells), 3+ (>25 – 50% cells), or 4+ (>50% cells). Additionally, immunohistochemical staining for MIB-1 was performed on 27 grade II ependymomas and 8 grade III ependymomas.

Results: The patients consisted of 38 males and 29 females (mean age 37.1 years, range 1.5 – 74 years). Thirty-seven patients (55.2%) were still alive with no recurrence of disease (mean follow-up 68.9 months), 20 patients (29.9%) developed recurrence (mean follow-up 94.1 months), and 11 patients (16.4%) died because of the disease (mean follow-up 49.5 months). Aurora B expression (1+ to 4+) was identified in 20 (62.5%) grade II ependymomas (p=0.001, Fisher exact test), and 5 (50%) grade III ependymomas (p=0.4, Fisher exact test). There was no difference in the level of expression (1+ to 4+) between grade II and III ependymomas. Aurora B was expressed in only 1 myxopapillary ependymoma and none of the subependymomas. There was no association between Aurora B expression and patient age, gender, tumor recurrence, or overall survival. Among grade II and III ependymomas, the MIB-1 labeling index (LI) was higher in cases with Aurora B expression (grade II mean 1.9%, range 0 – 12%; grade III mean 11.8%, range 5.4-21.2%) versus negative cases (grade II mean 0.6%, range 0 – 2.1%; grade III mean 7.4%, range 0.3 – 16%). However, this finding was not statistically significant (grade II p=0.265 and grade III p=0.450, Student's t-test).

Conclusions: Aurora B expression is present in about half of grade II and III ependymomas, but is only rarely or not identified in myxopapillary ependymomas and subependymomas. Grade II and III ependymomas with Aurora B expression tend to have a higher MIB-1 LI. Aurora B expression does not correlate with prognosis in ependymomas.

1367 Are Germinomas of the CNS Genetically Similar to Non-CNS Gem Cell Tumors? A Fluorescent In-Situ Hybridization Study of 25 Cases

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Background: Testicular germ cell tumors are characterized by various chromosome 12 anomalies, including the presence of isochromosome 12p and 12p gain. Unlike seminoma, little is known about the genetic characteristics of central nervous system (CNS) germinoma and most of the literature consists of occasional case reports or short series.

Design: We performed dual color fluorescent in situ hybridization (FISH) analyses with a centromeric α-satellite probe for chromosome 12 and a subtelomeric probe for 12p on paraffin embedded sections from 25 intracranial germinomas.

Results: Chromosome 12p abnormalities were detected in all but one germinoma (24/25; 96%). Isochromosome 12p was found in 13/25 cases (52%) while all 24 germinomas with 12p abnormalities showed 12p gain. Subsequently, 39% had only 12p gain. In all, 52% had both isochromosome 12p and 12p gain.

Conclusions: Similar to Non-CNS germ cell tumors, chromosome 12p abnormalities are very common in germinomas of the central nervous system. The consistent gain of genetic material from chromosome 12 in CNS germinomas suggest that it has a crucial role in their development. FISH analyses for chromosome 12p abnormalities may provide a useful diagnostic adjunct for confirming the diagnosis of germinoma and for distinguishing it from nongerm cell malignancies that enter into the differential diagnosis.

1368 Germinal Center Classification of Diffuse Large B-Cell Lymphoma of the Central Nervous System

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Background: Primary central nervous system lymphomas (PCNSL) are rare neoplasms characterized by dismal prognosis relative to other extranodal lymphomas. Approximately 98% of PCNSLs are B-cell lymphomas and most belong to the diffuse large cell type. Recently, diffuse large B-cell lymphoma has been subclassified as germinal center B-cell-like (GCB) and nongerminal center B-cell (non-GCB) types using immunohistochemical expression of CD10, Bcl-6 and MUM1 (Hans et al, 2004). Studies have shown that the overall survival rate of the germinal center B-cell group is better than that of the nongerminal center B-cell group. In this study, we investigated primary CNS diffuse large B-cell lymphoma (DLBCL) utilizing this scheme.

Design: Sixteen cases of PCNSLs of the DLBCL type were retrieved from the archival files of two medical centers. All cases were reviewed and immunostained for CD10, Bcl-6, MUM1 and MIB-1. Subclassification was carried out as previously described where CD10 and/or BCL-6 positivity and negativity for MUM1 were considered diagnostic of GCB subtype and the opposite expression of non-GCB subtype. Furthermore, the proliferative activity was semiquantitatively assessed using percent positive cells of MIB-1.

Results: Of the 16 cases examined, 12 (75%) were found to belong to the non-GCB type. Specifically, 0/12 cases stained positive for CD10, 4/12 had focal BCL-6 immunostaining and 10/12 were MUM1 positive. The two cases with MUM1 negative staining were also CD10 and BCL-6 negative and therefore were categorized as non-GCB. The MIB-1 index in these 12 cases ranged from 30-95% (mean, 69%). The four GCB type PCNSLs were all characterized by CD10/BCL-6 positive and MUM1 negative immunoprofile. They had a MIB-1 index between 70-90% (mean, 81%). Surprisingly, none of our patients were known to be HIV positive. Three of the four GCB group patients died within the first month after initial diagnosis while one remains alive almost 4 years later. Six of eight non-GCB group patients died of disease after an average survival period of 12 months.

Conclusions: Most PCNSLs belong to the non-germinal center B-cell like subtype. However, both GCB and non-GCB subgroups had high proliferative rates. While diffuse large B-cell lymphomas of the germinal center type are thought to be prognostically more favorable than the non-germinal center type, our initial findings showed no significant differences in survival between the two groups.

1369 Aberrant Methylation of Promoter Is an Infrequent Mechanism of Inactivation of *PTEN* In Astrocytomas

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Background: *PTEN* is a relevant tumor suppressor gene whose protein is a phosphatase involved in the control of astrocytoma angiogenesis. *PTEN* protein is lost by different genetic mechanisms such as mutation, LOH deletion, and gene promoter methylation. It has been suggested that loss of *PTEN* expression may be associated with *PTEN* methylation. The reported results about *PTEN* gene promoter methylation are contradictory. As well as, there are no enough studies in astrocytomas searching the relationship of *PTEN* promoter methylation with both the expression of the protein and LOH.

Design: 48 surgically resected brain astrocytomas -9 grade II, including 3 gemistocytic astrocytomas; 10 grade III and 29 grade IV- from our Department bank tissue tumour were studied by a combined approach including: methylation specific PCR (MSP-PCR), LOH of *PTEN* gene locus (10q23.3) using four microsatelite markers (D10S579, D10S2491, D10S541, AFMa086wg9) and immunohistochemical evaluation of the PTEN protein using a clonal antibody (clone 6H2.1449, Cascade). Parametrical test was applied.

Results: In 3 out of 48 astrocytomas (6%) methylation of *PTEN* promoter was observed: 0 of grade II, 1 (1%) of grade III and 2 (7%) of grade IV. *PTEN* expression was noticed in 90% of grade II, 30% of grade III and 62% of grade IV. We found LOH of *PTEN* in 33% of grade II (gemistocytic astrocytomas), 50% of grade III and 57% of grade IV. All *PTEN* promoter methylated astrocytomas exhibited loss of the *PTEN* protein according to a diffuse or a focal patterns. As well as, all *PTEN* methylated astrocytomas showed LOH. Astrocytomas with both LOH of PTEN and non-methylation associated with loss of the protein (Chi-square, Pearson (p=0.01).

Conclusions: *PTEN* promoter methylation is a infrequent genetic event in high-grade astrocytomas. By other way, unmethylated astrocytomas with LOH of *PTEN* associated with loss of the protein. *Grant of Spanish Government: FISS G03/114.*

1370 Neuropeptide Y (NPY) Receptors in Neural and Endocrine Tumors: Marked Overexpression in Glioblastomas and Ileal Endocrine Tumors

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Background: Numerous peptide hormone receptors, like somatostatin receptors, are overexpressed in human cancer, allowing receptor-targeted scintigraphic tumor imaging and radiotherapy. Among such receptors, those for neuropeptide Y (NPY) have recently been found to be highly overexpressed in selected tumors such as breast carcinomas and adrenal gland tumors. As NPY is a wide-spread neurotransmitter in the CNS and in endocrine tissues, we evaluated brain and endocrine tumors for their NPY receptor expression.

Design: Twenty-eight glioblastomas, 9 astrocytomas, 8 supratentorial (s.t.) PNET, 19 medulloblastomas, 11 meningiomas, 39 pituitary adenomas, 10 ileal and 8 pancreatic endocrine tumors, 12 lung carcinoids, and 8 medullary thyroid carcinomas were assessed for their NPY receptor expression by *in vitro* NPY receptor autoradiography using ^{125}I-labelled peptide YY (PYY) in competition with analogs selective for the NPY receptor subtypes Y1 and Y2. Receptor density was quantitatively measured using a computer-assisted imaging program.

Results: Brain tumors expressed NPY receptors in moderate to high incidence and density; 82% glioblastomas, 89% astrocytomas, 38% s.t.PNET, 47% medulloblastomas, and 64% meningiomas were receptor positive. Highest receptor density levels were found in glioblastomas (mean density 5263 dpm/mg tissue). Y2 was the predominant receptor subtype. Conversely, endocrine tumors expressed NPY receptors in moderate incidence and moderate to low density; 36% pituitary adenomas, 70% ileal and 25% pancreatic endocrine tumors, 42% lung carcinoids, and 25% medullary thyroid carcinomas expressed predominantly the Y1 receptor subtype, with highest levels in ileal carcinoids (mean density 1315 dpm/mg tissue). Y1 receptors were also present in intratumoral blood vessels in varying incidence and density.

Conclusions: NPY receptors could represent a molecular marker for various brain and endocrine tumors. Biologically, NPY receptors may regulate tumor cell proliferation, analogous to their function in normal glial cells and pancreatic islet cells, endocrine tumor activity, in analogy to their role in rat insulinoma cells, or tumoral blood supply. With regard to clinical applications, NPY receptors, in particular Y2 in glioblastomas and Y1 in ileal carinoids, may represent *in vivo* targets for a receptor-directed imaging and therapy of these tumors.

1371 The Cadherincatenin Complex in Meningiomas

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Background: Epithelial cadherin (ECAD) is a 120 kDa type 1 transmembrane cell-adhesion molecule that represents the major constituent of *zonulae adherens*. ECAD binds another ECADs on adjacent cells and links to the cytoskeleton via a complex composed of α-, β- and γ-catenins (CAT) and p120. Reduction or loss of ECAD has been demonstrated in a variety of cancers and correlates with increased aggressiveness. Mutations of ECAD are characteristic of familial gastric carcinoma and breast lobular carcinoma. In other cancers such as colon and hepatocellular carcinoma, reduction of ECAD is mainly due to methylation of the promoter region. Mesenchymal cadherin (NCAD) shares homologies with ECAD and it is expressed in mesenchymal tissues and the central nervous system. Only five studies have investigated the CAD/CAT complex in meningiomas and they have produced conflicting results. In addition, none of them explored the expression of NCAD in meningiomas and the expression the CAD/CAT complex in arachnoid villi.

Design: We examined the CAD/CAT complex in meningiomas to determine i) whether arachnoid villi and meningiomas differ in the expression of the complex; ii) whether aggressive meningiomas show a defective expression of one or more molecules forming the complex. Using the immunoperoxidase technique with antibodies against ECAD, NCAD, α-CAT, β-CAT, we studied archive samples of 5 normal arachnoid villi, 20 WHO grade I meningothelial, transitional and fibroblastic meningiomas, 8 grade II, including one chordoid and one clear cell and 2 grade III examples (one rhabdoid type).

Results: Cap cells of arachnoid villi expressed ECAD and α and β-CAT but not NCAD. Grade I and II tumors expressed ECAD focally and the reaction was weaker than arachnoid villi. Grade I meningiomas retained of α- and β-catenin in the majority of cells; these molecules were conversely reduced in grade I cases with mitotic activity and grade II tumors. Grade III meningiomas were negative for all the proteins. A few grade I and II meningiomas expressed NCAD in regions where ECAD was negative.

Conclusions: Our results suggest that the CAD/CAT complex is defective in meningiomas and particularly in aggressive examples. The difference between arachnoid villi and conventional meningiomas also suggests that the CAD/CAT complex is involved in the pathogenesis of these tumors. Further studies on expression of γ-catenin and p120 and on methylation of the ECAD promoter need to be performed.

1372 Ikaros Transcription Factors Are Expressed in a Variety of CNS Neoplasms

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Background: The Ikaros (Ik) family of transcription factors has critical functions in lymphohematopoiesis, immune regulation and the hypothalamic-pituitary axis. Ik influences cell fate decisions through transcriptional activation of target genes and its interaction with chromatin remodeling complexes. Many CNS tumors, especially gliomas, are highly heterogeneous in their cellular phenotype and genomic methylation profile. However, the events leading to differing cell fate at the level of transcription factors and chromatin remodeling remain poorly understood. Previously identified downstream targets of Ik in other tissues include FGFR4 and the JAK/STAT signal transduction pathway. FGFR4 has been correlated with malignancy in human astrocytomas while the STAT family of proteins is an effector in meningiomas. This study was designed to evaluate the expression of Ik in different types and grades of CNS neoplasms.

Design: A tissue microarray (TMA) was assembled from archival paraffin-embedded material of 70 CNS tumors diagnosed according to the WHO 2000 classification. The TMA included 9 diffuse and 2 pilocytic astrocytomas, 3 ependymomas and 3 anaplastic ependymomas, 3 subependymomas, 3 gangliogliomas, 1 DNT, 5 medulloblastomas, 24 meningiomas, 10 esthesioneuroblastomas and 7 other tumors of variable histology. Expression of Ik, CD45 and CD68 was determined by immunohistochemistry using standard avidin-biotin techniques.

Results: Ik immunoreactivity was detected in a subset of cells for the majority of neoplasms. In all tumors, staining was nuclear, a pattern associated with Ik isoforms 1 and 2. Three types of cells were labeled: lymphocytes, intratumoral microglia, and neoplastic nuclei. In high-grade gliomas, Ik was detected in 8-10% of nuclei. Gangliogliomas showed abundant Ik reactivity in mature ganglionic cells. Variable but high Ik labeling of up to 25% was observed in some meningiomas, the majority in neoplastic nuclei.

Conclusions: Ikaros is expressed in a variety of CNS neoplasms. The presence of Ik in bone marrow-derived cells such as lymphocytes and microglia was expected in light of its functions in the lymphohematopoietic system. However, the detection in neoplastic cells is a new finding and may elucidate the role of these transcription factors in the biology of CNS tumors, e.g. in chromatin remodeling and DNA methylation.

1373 Epidermal Growth Factor Receptor Gene Amplification and Protein Expression in Glioblastomas

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Background: Glioblastoma (GBM) is the most frequent malignant glial tumor in adults. The epidermal growth-factor receptor (EGFR) gene is known to be associated with the genetic pathway of primary GBM, *de novo* and amplification of this gene being detected in approximately 40% of cases, but its relationship to prognosis is still controversial.

Design: We studied the gene and protein expression of the EGFR gene by fluorescence *in situ* hybridization (FISH) with the Vysis probe and immunohistochemistry with two different clones from Zymed (31G7, 1:50) and DAKO (PhamDx, prediluted)], as well as the immunohistochemistry of the PI3K, Akt, PTEN and p53 on tissue array blocks of the 74 cases of primary GBMs (from SNU Hospital, 1999 to 2003). Other factors possibly linked with GBM prognosis, such as gender, age of onset, extension of the surgical resection, and modalities of the adjuvant treatments, were also evaluated, and all of these results were compared with the patient´s survival.

Results: The mean age of the patients was 45 years, and the male-to-female ratio was 1: 1.4. On the EGFR FISH study carried out on the 74 cases, 18 (24.3%), 23 (31.1%) and 23 (31.1%) of cases showed low polysomy, high polysomy, and gene amplification, respectively. Immunoexpression of the EGFR gene by PhamDX and 31G7 was found in 71.2% (intensity: ≥2+) and 32.9% (intensity: ≥2+) of cases, respectively. PI3K, Akt, and p53 were upregulated in 37.8%, 26.0%, and 28.4% of cases, respectively. The immunohistochemical results for the 31G7 EGFR antibodies was well correlated to the results of the FISH ($p<0.01$). In the case of PharmDx, the more than 2+ group was only correlated with amplification by FISH. PI3K overexpression was statistically correlated with EGFR upregulation by FISH, PharmDX and 31G7 ($p<0.05$). Eighty per-cent of patients expired, their overall survival being 22.4 months. According to a Kaplan-Meier survival analysis, nothing that we analyzed, including the EGFR gene status and the above-mentioned protein expression, was significantly associated with patient's survival.

Conclusions: From our study, we could validate the positive correlation between the FISH and immunohistochemical studies of EGFR, either by 31G7 or PI3K/Akt survival signaling pathways through the EGFR gene. Amplification of the EGFR gene was found in about one-third of the studied GBMs, suggesting its implication in tumorigenesis of GBM; however, it has no prognostic significance.

1374 Array-Based Comparative Genomic Hybridization and Immunohistochemical Studies in Gliomatosis Cerebri

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Background: Gliomatosis cerebri (GC) is defined as an infiltrating glial tumor involving more than two cerebral lobes and often extending to infratentorial structures. GC histopathology is very heterogenous; it can be any grade of astroglial, oligodendroglial, mixed oligoastrocytic or uncommitted (nonspecific) glial tumor. Here we carried out array-based comparative genomic hybridization (CGH) and immunohistochemical studies to investigate the tumorigenesis of GC.

Design: A clinicopathologic review of 28 cases of </DEL> surgically proven GC having sufficient paraffin-embedded tissues was carried out from 1995 to 2005. Array-based CGH was performed in 10 cases by high-resolution GenomArray (Macrogen Inc, Korea) composed of 3801 BAC clones. In all 28 cases, an immunohistochemical study of following (result) biomarkers.

Results: The mean age of patients was 44 years (range: 0-70 years, M:F = 4:1) and the mortality rate was 48% (13/27). Histopathologically, 15 cases were low- and 13 cases were high-grade tumors. Most of the cases (78%) were immnoreactive for GFAP and nestin, and 11 cases (39%) showed p53 and CD34 positivity. Half of the cases revealed EGFR positivity; however, upregulation of PI3K, Akt and C-erbB2 was found in only 7% 11%, and 5% of cases, respectively. MGMT silencing was found in 21% of cases. In the array-based CGH, an average of 122 clones (3.23%) was gained (26 clones) or lost (96 clones) in all 10 cases. Twenty-six clones demonstrated homozygous deletions in more than half cases and only 4 clones had amplifications in more than half cases. Among the well known tumor-related genomic foci, more than 50% of cases demonstrated a gain on 17p13.1 (TP53), 22q13.1 (PDGFB), 18q21.2 (DCC), 10q23.31 (PTEN), 17q11.2 (NF1), and 2q14.2 (GLI2), whereas 40% of cases demonstrated a loss on 13q14.2 (RB1). Homozygous deletion was detected on 10p15.2-10p15.1 (KLF6) and 11p15.5 (HRAS and RASSF7) in 20% and 30% of cases, respectively. However, 10q26.3 (MGMT) was amplified (not deleted) in 2 cases. In high- grade GC, frequent loss of 10q23.1 (86%) and 14q22.1 (86%) were found, and 5q14.3 (100%) and 11p15.5 (86%) showed a high-frequency of gains, which was not detected in low-grade GC ($p<0.05$).

Conclusions: From our study, we suggest that the tumor cells composing GC might be primitive glial cells, to some degree, and they showed dominant differentiation into the astrocytic lineage. Novel genomic aberrations were found in GC, and high-grade GC had unique genomic aberrations that were not found in low-grade GC.

1375 A Unique Pattern of Antigen Expression Defining Tanycytoma

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Background: Tanycytoma is a neoplasm of specialized ependymal cells occurring in and near circumventricular (periventricular) organs. The tumor has an angiocentric pattern with myxoid stroma. Individual tumor cells have some features suggesting ependymal differentiation while others suggest piloid astrocytoma. The tumor may occur at any age. Tanycytoma continues to elicit variable classification as a tumor of astrocytic, ependymal, or neuronal origin. We undertook this study to evaluate the patterns of antigen expression in tanycytoma.

Design: Six tanycytomas were retrieved and formalin-fixed, paraffin-embedded tissue was immunostained for synaptophysin, GFAP, S-100, nestin, TTF-1, and Ki-67. Ultrastructural analysis of the tumor cells was done by electron microscopy.

Results: There were 2 females and 4 males ranging in age from 8 months to 41 years (median 6.5 years). The tumor was either suprasellar or ventricular in location, except one in the spinal cord. Two patients died within two years of diagnosis; four have recurrent tumor after 2, 3, 5, and 7 years of follow up, respectively. Microscopically, fine cellular processes originate from tumor cells containing small round lymphocyte-like nuclei. Many vessels showed cell processes radiating from their surfaces. There is microcystic degeneration with most neoplastic cells suspended in a myxoid background. Immunohistochemistry showed positivity for S100, synaptophysin, GFAP and nestin in all six cases. There was a low labeling index with Ki-67. None of the cases was positive for TTF-1. Electron microscopy of the four cases examined confirmed perivascular orientation of cell processes and showed myxoid material within the interstitium adjacent to neoplastic cells. Synaptoid endings, characteristic of tanycytic differentiation and unprecedented in astrocytoma, were also demonstrated.

Conclusions: This unique neoplasm has evoked debate with arguments correctly describing features which simultaneously imply neuronal, astrocytic, and ependymal histogenesis (synaptophysin expression – neuronal; GFAP positivity - astrocytic; S-100 positivity – ependymal). The issue of precise taxonomy remains unresolved partly due to incomplete characterization (some reports lack ultrastructural findings). Where ultrastructural observations have been made, tanycytic differentiation has been present. Matters of histogenesis have also utilized patterns antigen expression: tanycytomas have exhibited a unique and characteristic immunophenotypic signature.

1376 Expression of the Akt Signaling Pathway Molecules p-Akt, p-mTOR and p-p70S6K in Gemistocytic Astrocytomas in Comparision with Glioblastomas

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Background: Although gemistocytes typically lack mitotic activity or high proliferation indices, gemistocytic astrocytomas (GA) exhibit aggressive behavior, and are prone to progress to glioblastoma (GBM). The Akt signaling pathway is involved in cell cycle and apoptosis regulation and has been implicated in a variety of human cancers including GBM. Detailed study of these molecules in gemistocytic astrocytomas has not been documented in English literature.

Design: Formalin-fixed, paraffin embedded sections of archival tissues from 8 GA and 24 GBM, 12 of which with conspicuous gemistocytes, were stained with antibodies against the phosphorylated forms of Akt, mTOR and p70S6 kinase (Cell Signaling, Danvers, MA). Immunopositivity was scored semiquantitatively with regard to both intensity and distribution of the staining, and over-expression defined as strong at regional or diffuse distribution, or moderate at diffuse distribution.

Results: Immunostaining was primarily cytoplasmic for p-Akt and p-mTOR, and both nuclear and cytoplasmic for p-p70S6K (nuclear dominant). 6/8 (75%) GA and 23/24 (96%) GBM over-expressed p-mTOR ($p=0.135$). 10/24 (42%) GBM over-expressed p-Akt, and 0/8 (0%) GA were immuno-positive for p-Akt ($p<0.03$). Similarly, 22/24 (92%) GBM over-expressed p-p70S6K while 1/8 (13%) GA was immuno-positive for p-p70S6K ($p<0.001$). Interestingly, the gemistocytes of only 4/12 (33%) of GBM with gemistocytic features over-expressed p-p70S6K.

Conclusions: This study demonstrated an activation of mTOR in both GA and GBM with apparent differences in the activation of upstream and downstream signaling members. This data suggests that GA may undergo progression toward GBM by acquiring additional abnormalities leading to upregulated p-Akt and/or p-p70S6K, and that a subset of GBM may use an alternate signaling pathway to induce mTOR since not all GBM in this study featured increased p-Akt. Further study of the p-Akt pathway in both GA and GBM thus appears warranted.

1377 Analysis of Trends in Brain Metastases from Non-Small Cell Lung Cancers: A Clinicopathological Study

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Background: Lung carcinomas constitute the most common metastases to the brain. The exact nature and factors that lead to brain metastases are unknown, and there is limited data in the literature on the unique nature of these metastatic lung cancers. Recent evidence suggests that a subset of cancer cells within some tumors, may influence metastasis of these tumors. It is critical to identify such features that may have a greater tendency for brain metastases.

Design: In an attempt to identify trends and metastatic patterns, we reviewed the UCSF Cancer Center Registry as well as the UCSF Pathology Database for all Stage IV non-small cell lung cancers between 1995 and 2004. Demographic characteristics, metastatic patterns, frequency of surgical intervention, histological, and immunohistochemical features were recorded.

Results: There were 756 stage IV non-small cell lung carcinomas (283 female, 473 male) between 1995-2004. These included 318 adenocarcinomas, 92 squamous, 21 large-cell, and 5 adenosquamous carcinomas, 320 were not otherwise characterized. 268 patients (103 female, 165 male) had brain metastases, and the remaining 488 had metastases elsewhere. The mean age for patients with brain metastases were slightly lower that the mean age for all patients (63+11.9 vs 62.1+11.6). Only 57 (20 female, 37 male) patients with brain metastases underwent surgery (45 at UCSF), and the mean age of this group was even smaller (58.4+10.0; $p<0.01$). 18 of the surgical patients had multiple metastases, and 39 had single metastases. UCSF. Immunohistochemical stains performed in some cases demonstrated staining for cytokeratin cocktail, EMA and CK7 in 100%, TTF1 in 83.3%, S-100 in 20%, CK20 in 20%, and GFAP in 0%. Rare cases were positive for chromogranin and synaptophysin. Tissues from the primary and metastatic tumors showed discordant staining with the markers in a small number of cases.

Conclusions: Non-small cell lung carcinomas metastatic to the brain that are treated surgically represent a selective population of patients among all stage IV lung cancers. Staining patterns in tissues from primary tumors and their brain metastases show some differences that may be significant in their propensity for CNS spread, and may be diagnostically challenging. Studies using tissue microarrays from stage IV lung cancers with and without brain metastases are being planned to determine the validity of these observations.

1378 Glioblastomas: Correlation between Oligodendroglial Component, Genetic Abnormalities and Prognosis

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Background: The histology of Glioblastomas (GBM) is quite variable and a small fraction of these tumors may present an oligodendroglial component. The clinical significance of an oligodendroglial component in GBMs remains uncertain, but its presence has been associated with longer survival. Molecular genetic studies of GBMs with oligodendroglial component have shown heterogeneous genetic alterations, with a variable frequency of LOH in chromosomes 1p and 19q. The presence of 1p/19q deletions has been related to prolonged survival and better response to chemotherapy in oligodendrogliomas and leaded to a growing interest in search for these genetic alterations in other gliomas. In astrocytic tumors, 1p loss is usually partial, not associated with 19q deletion and also may be associated with a worse prognosis.

Design: A series of 31 GBM was investigated for the presence of 1p and/or 19q deletions, 21 of them with oligodendroglioma-like areas. Quantitative microsatellite analysis using real time PCR and/or FISH were used to access the copy number on chromosomes 1p and 19q. Clinical data was retrieved and the variables location, age, gender, adjuvant treatment and extent of resection were correlated to 1p and 19 q status. Survival analysis with all the variables was performed. This group of tumors is part of a study involving 90 GBM, with test results currently under analysis.

Results: Tumors with an oligodendroglial component showed isolated 1p loss in 2 cases and 19q loss in other 2 cases. Only one combined 1p/19q loss was observed. Conventional GBM demonstrated isolated 1p loss in 1 case and 19q loss in other 2 cases. None of these tumors revealed combined loss of 1p/19q. Survival was longer for patients who received radiotherapy and chemotherapy. In a Cox multivariate analysis, the absence of 1p loss, total tumor resection and adjuvant treatment with radiation and chemotherapy were related to reduced risk of death, but only the last one reached statistical significance. No significant correlation was observed between 1p or 19q status and histology.

Conclusions: Combined 1p/19q is infrequent in GBM with oligodendroglial areas. Although these tumors have demonstrated a hybrid phenotype, the pattern of genetic changes on chromosomes 1p and 19q seems to be more closely related to astrocytic than oligodendroglial tumors.

1379 Discrepancies between Frozen Section and Final Diagnoses in Non-Neoplastic Lesions of the Central Nervous System

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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, facilitates the appropriate triage of tissue for ancillary studies, and guides intraoperative patient management. Non-neoplastic lesions provide a particular diagnostic challenge in this venue. This study sought to review diagnostic discrepances between FS and final diagnoses in these lesions.

Design: Retrospective review of 303 non-neoplastic CNS lesions from 1997 to 2006. Discrepancies between the FS and final diagnoses were identified and reviewed.

Results: 39 (12.9%) discrepant diagnoses were identified. The average age of the patients (22 females, 17 males) with discrepant diagnoses was 37.6 years (range 2-73 years). All but one of the discrepant FS diagnoses and all of the final diagnoses were rendered by one of three staff neuropathologists. Final diagnoses in the discrepant case group included: inflammatory lesions (n=8, 20.5%), malformations of cortical development-cortical dysplasia (n=5, 12.8%), gliosis (n=5, 12.8%), vascular malformations (n=5, 12.8%), demyelination/progressive multifocal leukoencephalopathy (n=3, 7.7%), infarct (n=3, 7.7%), hemorrhage/blood clot (n=3, 7.7%), and no pathologic changes (n=3, 7.7%). The remaining 4 (10.2%) discrepant cases involved one case each of amyloid angiopathy, nonspecific vasculopathy, vasculitis, and meningioangiomatosis.

Conclusions: Among non-neoplastic cases, about 13% of frozen section diagnoses were found to be discrepant with the final diagnoses. This is notably higher than a similar study which evaluated FS discrepancies of neoplastic CNS lesions (3% of 2156 cases). Non-neoplastic lesions can be more challenging than neoplastic lesions at FS, partly because they are less commonly sampled for FS, making them less familiar to pathologists. The most common categories of discrepances were inflammatory lesions, malformations of cortical development-cortical dysplasia, gliosis, and vascular malformations.

1380 Epidermal Growth Factor Receptor and p53 Expression In a Large Series of Gliosarcomas: A Tissue Microarray Study

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Background: Gliosarcoma is an unusual variant of glioblastoma characterized by both glial and mesenchymal components. In primary conventional glioblastoma, EGFR amplification and overexpression are common. By contrast, EFGR amplification and overexpression occurs rarely if ever in gliosarcoma and secondary glioblastoma. p53 overexpression is rare in primary glioblastoma, but seen often in gliosarcoma and secondary glioblastoma. However, most series of gliosarcomas have been marked by small sample size (only two have sample size greater than 4), and the results have been highly variable. In this study, 16 gliosarcomas and a series of primary and secondary glioblastomas were examined for differences in EGFR expression and amplification and p53 expression.

Design: The University of Washington pathology database identified 25 surgical and autopsy gliosarcoma specimens. The 16 specimens with paraffin-embedded tissue available were examined to confirm the diagnosis of gliosarcoma. For comparison, 11 primary and four secondary conventional glioblastoma cases were selected. Tissue microarrays containing triplicate samples of each tumor were created, immunocytochemically stained for EGFR and p53 overexpression, and examined by fluorescent in-situ hybridization for EGFR amplification.

Results: 45 percent of primary glioblastomas overexpressed EGFR, while 13% of gliosarcomas ($p < 0.04$) and no secondary glioblastomas ($p < 0.018$) showed overexpression. EGFR amplification was seen in four of the five primary glioblastomas that overexpressed EGFR (36%), and one of the two EGFR-expressing gliosarcomas (6%; $p < 0.15$); no amplification was seen in the secondary glioblastomas ($p < 0.04$). p53 overexpression was seen in 55% of primary glioblastomas, 50% of secondary glioblastomas, and 38% of gliosarcomas.

Conclusions: This large series of gliosarcomas reveals that, despite apparant similarity in biological origin and prognosis to primary conventional glioblastoma, gliosarcomas differ with regard to EGFR overexpression and amplification. EGFR overexpression is seen significantly less frequently than in primary glioblastoma, and EGFR gene amplification is also seen with decreased incidence, although the difference is not significant. For p53, in contrast to the findings of previous series, no significant difference in p53 overexpression was seen between glioblastoma and gliosarcoma. These results help reveal the biology of gliosarcoma and its molecular relationship to primary glioblastoma.

1381 Cytoplasmic Vacuolation of Adenohypophyseal Cells in Creutzfeldt-Jakob Disease

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Background: It is well documented that pituitary tissue from patients with Creutzfeldt-Jakob Disease(CJD) contains prion infectivity and can transmit the disease. There are no reports of any histological correlate of this status.

Design: We examined pituitary glands from 9 patients who died of CJD and compared the histologic appearance to that of 2 control pituitary glands fixed and processed identically, with hand processing and post-fixation formic acid treatment.

Results: The CJD patients ranged in age from 40 to 83. One had a history of treatment with CJD-contaminated pituitary extract for growth-hormone deficient dwarfism; two had a family history of CJD; and the remainder had sporadic CJD. In each case, the adenohypophyseal cells contained variably prominent round cytoplasmic vacuoles of varying size. These closely resemble the vacuoles in the neuropil of brain tissue with CJD. Pituitaries from the 2 control cases did not show these characteristic round vacuoles.

Conclusions: We have identified a histopathological correlate of prion content in pituitaries of patients dying of CJD. While a somewhat similar vacuolar alteration of adenohypophyseal cells has been reported in an animal model of the 139H strain of scrapie(Ye and Carp, J Comp Pathol 1996, 114:291-304), these light microscopic changes in the pituitary gland have not been described previously in human prion disease.

1382 Exploring Novel Markers in the Diagnosis of Hemangioblastoma Versus Metastatic Renal Cell Carcinoma

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Background: Hemangioblastomas (HB) account for nearly a tenth of all posterior fossa neoplasms and can be the presenting finding in patients with von Hippel Lindau (VHL) syndrome. HB must be differentiated from renal cell carcinoma (RCC), also seen in VHL, as the distinction between the lesions dictates the management of these patients. Currently inhibin α and RCC marker have been used in the diagnosis of HB and metastatic RCC, both with inconsistent results. PAX-2 has been an immunohistochemical (IHC) stain of interest in the diagnosis of RCC. D2-40 and Fli-1 are IHC stains which have recently gained interest in the diagnosis of lesions with lymphatic or vascular origin. To our knowledge, no recent studies have compared the staining patterns of PAX-2, D2-40, and Fli-1 in HB and metastatic RCCs.

Design: 12 cerebellar HB and 12 metastatic clear cell RCCs to the brain were selected. All cases were stained with RCC, inhibin α, PAX-2, D2-40, and Fli-1 immunomarkers. The staining patterns were scored based on intensity and extent of tumor staining.

Results: RCC immunomarker was positive in 5 of 12 (42%) RCCs. No HB showed expression of RCC marker (0/12). All RCCs were negative for inhibin α (0/12). Inhibin α staining was strong to moderate in 5 of 12 (42%), weak in 6 of 12 (50%) and negative in 1 of 12 (8%) HB. 9 of 12 (75%) RCCs were positive for PAX-2. 1 of 12 (8%) HB showed expression of PAX-2. D2-40 showed non-specific nuclear staining in 2 of 12 (16%) RCCs, the remaining RCCs (10/12) were negative. 4 of 12 (33%) HB expressed weak to moderate staining for D2-40. Weak Fli-1 expression was noted in the endothelium in 1 of 12 (8%) RCCs. Fli-1 was negative in all other RCCs (11/12). 9 of 12 (75%) HB showed Fli-1 positivity with strong to moderate nuclear staining in the endothelium of the vascular component, as well as, nuclear staining of some stromal cells.

Conclusions: In the differentiation of HB and metastatic RCC, D2-40 and RCC marker proved to be poor markers with less than 50% of HB and RCCs, respectively, showing positive staining. PAX-2 was superior to RCC marker in the diagnosis of metastatic RCC. Overall, while variable and with decreased staining in previously frozen tissue, inhibin α still stained a higher percentage of HB than D2-40 or Fli-1. However, with their distinct nuclear staining Fli-1 and PAX-2, in conjunction with inhibin α, may prove to be more useful and consistent stains in the diagnosis of HB versus RCC.

1383 Claudin-1 Versus EMA Staining in Low and High Grade Meningiomas

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Background: Claudin-1 has recently gained interest as a potential immunomarker for the differentiation of meningiomas from other spindle cell lesions. Our interest was to look into the staining patterns of Claudin-1 and epithelial membrane antigen (EMA) in meningiomas with World Health Organization (WHO) 2000 Grades I, II, and III (GI-III), to determine if there is a difference in staining based on tumor grade. To our knowledge, no previous studies have specifically compared Claudin-1 and EMA staining in low and high grade meningiomas.

Design: 38 meningiomas were selected and WHO 2000 grading criteria was applied to all cases. Final grading of the 38 cases revealed 20 GI, 15 GII, and 3 GIII meningiomas. Because of the small number of GIII meningiomas, the cases were divided into low grade (GI) and high grade (GII-III) groups. All 38 cases were stained with Claudin-1 and EMA.

Results: Claudin-1 positivity was seen in 17 of 20 (85%) low grade (LG) and 10 of 18 (56%) high grade (HG) meningiomas. EMA was positive in all 38 meningiomas. EMA staining in both LG and HG tumors was smooth and membranous with some cytoplasmic staining. Only membranous staining was considered positive. Claudin-1 staining in the LG and HG meningiomas showed granular membranous staining. In the LG cases, Claudin-1 staining was equal in intensity and distribution to EMA. In the HG cases, Claudin-1 staining was less intense and diffuse than EMA. There appeared to be preferential staining of the meningotheliomatous, rather than the fibrous component in the HG meningiomas with Claudin-1.

Conclusions: There is a significant difference in the staining of LG and HG meningiomas with Claudin-1 versus EMA. EMA appears to be a more sensitive stain, however, as studies have shown EMA is not specific to meningiomas. Claudin-1, on the other hand, lacks the sensitivity of EMA. Nevertheless, studies (including one done by this group) have shown that Claudin-1 is a more specific marker than EMA when trying to differentiate fibrous or atypical meningiomas from other spindle cell lesions. Claudin-1 as a singular stain in HG meningiomas may not be of benefit, however, used in conjuction with EMA, may prove to be useful in confirming the diagnosis of meningioma and distinguishing it from histologically similar entities. With further investigation, Claudin-1 may be of use as a prognostic marker in helping to distinguish LG from HG meningiomas.

1384 The Spectrum of Malignancy in Craniopharyngioma: Clinicopathologic Study of Three Cases

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Background: Craniopharyngiomas are low grade epithelial neoplasms occurring almost exclusively in the sellar/suprasellar region. Histologic malignancy is extremely rare; the literature consists mostly of isolated case reports.

Design: Clinical histories and follow-up were abstracted from retrospective chart review. All available H&E slides were reviewed. Immunohistochemical stains (streptavidin-biotin peroxidase complex method) were performed using antibodies directed against Ki67, p53 protein, β-catenin, estrogen receptor, progesterone receptor, p16 , p27, GFAP, S-100, cytokeratin AE1/AE3, smooth muscle actin (case 3), p63/racemase (case 3). The p53 and p16 counts, as well as the MIB-1 labeling index, were quantified by examination of 20 consecutive tumor fields using the CAS200 system. Microvessel density analysis was performed in case 2 in the benign and malignant components.

Results: The patients included 2 males and 1 female, age 14, 31, and 58 years at presentation, respectively. All patients expired 3 months to 9 years after first resection and 3 to 9 months after malignant transformation. Histologic malignancy developed after multiple recurrences and radiation therapy in two cases, but seemed to arise de novo in one case resembling odontogenic ghost cell carcinoma. The malignant component of the other two cases resembled squamous cell carcinoma and low grade myoepithelial carcinoma, respectively. The latter expressed smooth muscle actin and p63. Nuclear labeling for β-catenin was seen in all cases. The MIB-1 labeling index and nuclear p53 labeling were markedly increased in the malignant component in comparison with the low grade precursor. Microvessel density was higher in the carcinoma (10.73%) than in the original craniopharyngioma (2.63%) in case 2.

Conclusions: Malignant transformation in craniopharyngiomas, although rare, does exist. It assumes varied histologic appearances, usually after multiple recurrences and radiation therapy, and has a near uniformly fatal outcome.

1385 Modified Maximal Thymectomy in Patients with Myasthenia Gravis. Biologic and Therapeutic Implications

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Background: In this study the clinicopathologic features of 46 patients with myasthenia gravis (MG) who underwent modified maximal thymectomy (MMT), for therapeutic purposes, were investigated and correlated with patients' outcome after thymectomy.

Design: The study included 46 patients (17M/29F; mean age 36.60±16.09 years) with MG who underwent MMT. Osserman classification, before thymectomy, showed: stage I-5, IIA-21, IIB-17 and III-3 patients. Microscopic examination of thymus revealed: thymic hyperplasia (n=26), atrophy (n=8), thymoma type B1/B2 (according to WHO classification) (n=9), thymoma type B3 (according to WHO classification) (n=3). Patients were followed-up for 39-166(median-86) months. At the end of the follow-up period, according to MGFA criteria (Jaretzki A, et al, Neurology 55:16, 2000) patients were classified as having complete stable remission(CSR), pharmacological remission (PR), minimal manifestations (MM), improvement(IM), deterioration (DE).

Results: Follow-up data were available on 39/43 patients; 37/39(95%) patients showed improvement and 2 deterioration. More specifically, 13 patients showed CSR, 2 PR, 17 MM, 5 IM and 2 DE. ANOVA revealed that the clinical factors correlated with more frequent presence of complete stable remission were: younger age of the patients (<40 years-p=0.036), shorter interval time between disease onset and thymectomy (<1 year-p=0.009) and earlier stage of the disease (I+IIA vs. IIB+III-p=0.013). Thymus pathology did not correlate with patients' outcome. Cox regression analysis revealed that only the shorter interval time between disease onset and thymectomy (<1 year) and the earlier stage of the disease (I+IIA vs. IIB+III), constitute independent prognostic factors (95% CI:0.043-6.50, p=0.01 and 95% CI:1.023-4.529, p=0.043, respectively).

Conclusions: This study shows that a) patients with MG who underwent MMT for treatment, developed improvement in 95% of the cases and b) independent prognostic factors for therapeutic response after thymectomy are: the shorter time interval between disease onset and thymectomy, and the earlier stage of the disease.

1386 Immunohistochemical Analysis of 250 Cases of Meningioma: Molecular Biomarkers of Grade, Location, Recurrence and Overall Survival

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Background: Meningiomas are histologically and clinically diverse CNS neoplasms with few available immunohistochemical markers of differentiation and progression.

Design: With high throughput tissue microarray immunohistochemistry (TMA-IHC) we construde a TMA that includes 250 meningiomas (211 grade 1 and 39 grade 2 of the WHO clasification). All cases were stratified according to clinical behavior and histopathological findings. Antibodies primarily utilized were progesterone receptor (PR), epithelial membrane antigen (EMA), cathepsin D, E-cadherin, platelet derived growth factor (PDGF), survivin, epithelial growth factor receptor (EGFR), cox2, MIB1, p16, p21WAF, p53, Retinoblastoma, MMP2, Cyclin A, E and D1, CD44, Her2/neu, CDK1, TGFB, and vascular endothelial growth factor (VEGF). Statistical analysis.

Results: The frequencies of tumor positivity we found were similar in most cases to those previously reported using whole section IHC. Grade 2 meningiomas showed overexpression of p21WAF, and CD44 and lower expression of PR and cathepsin D

than grade1 meningiomas ($p<0.05$). VEGF, cox2 and histhological hipercellularity were associated ($p<0.05$) with tumor recurrence and we found no statistical associations with tumor location. p21WAF, number of mitosis and tumor grade were associated with CNS infiltration.p53 expression was correlated with atupical meningioma. Kaplan Meire test demonstrated PR as the only biomarker associated with overall survival.
Conclusions: We conclude that p21WAF, CD44, PR and CathepsinD are associated with tumor grade and may be useful indicators of tumor progression.

1387 Low Level Copy Gain Versus Amplification of *myc Oncogenes* in Medulloblastoma: Utility in Predicting Prognosis and Survival

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Background: Medulloblastoma (MDB) is a malignant embryonal tumor of the cerebellum. A number of genomic alterations have been described in MDBs and are presumed to be important in determining the biology of these tumors. *c-myc* or *N-myc* amplification has been described in 10-15% of MDBs, and is frequently associated with the large cell/anaplastic (L/A) phenotype. The frequency of low level copy gain of *myc* oncogene and the relationship between low level copy number of *myc* oncogene and prognosis has not been explored.
Design: 64 MDBs were histologically reviewed and classified into 3 major subtypes: classic, nodular, L/A. Using quantitative real-time PCR (QRT-PCR), 58 cases with a pure histologic subtype were analyzed for the copy number for *c-myc* and *N-myc* oncogenes. Cases with >5-fold copy number were further analyzed using the FISH assay. Statistical analysis including Kaplan-Meier survival analysis was performed.
Results: >5-fold *myc* (*c-myc* and *N-myc*) copy number was noted in 5(20.8%), 1(5.3%), and 2(13.3%) cases of 24 L/A, 19 classic, and 15 nodular subtypes, respectively, while <2-fold copy number was observed in 5(20.8%), 5(26.3%), and 3(20%) cases, respectively. A significant number of tumors, 14(56%) of L/A, 13(68%) of classic and 10(67%) of nodular MDBs had >2<5 fold copy number. The group of patients with >5-fold *myc* amplicon copy number showed significantly shorter survival than those with <5-fold copy number (p=.045). High level amplification, defined as >10-fold copy number, was only seen in L/A subtype (5 cases). FISH readily detected most cases corresponding to tumors with >5-fold amplicon copy number by QRT-PCR, and could detect all 5 cases with >10-fold by QRT-PCR.
Conclusions: High level amplification (>10-fold copy number) of *myc* oncogenes was only seen in L/A subtype, although moderate amplification (>5<10-fold) could be detected in other histologic subtypes. There was a significant survival difference between the groups of MDB patients with and without moderate to high amplification of *myc* oncogenes. Since FISH could easily detect most cases in the moderate to high amplification group, the FISH assay has utility in detecting subsets of MDB with worse prognosis.

1388 Intravascular Thrombosis in Central Nervous System Malignancies

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Background: Intravascular thrombosis is a frequent intraoperative finding during the neurosurgical resection of glioblastoma (GBM). Microscopic studies have demonstrated intravascular thrombosis in a large percentage of GBM resection specimens and it has been suggested that vaso-occlusion due to thrombosis could promote hypoxia-induced tumor progression. The diagnostic specificity and prognostic significance of intravascular thrombosis has not been established in central nervous system (CNS) malignancies. We investigated whether intravascular thrombosis was more frequent or prominent in GBM than other CNS malignancies, including anaplastic astrocytoma (AA), metastatic carcinoma, and primary CNS lymphoma (PCNSL).
Design: We retrospectively examined all available histological sections (frozen and permanent) from the Emory University Hospital Department of Pathology and Laboratory Medicine archives (years 1999-2006) from 169 neoplasms, including 44 GBMs, 45 AAs, 31 PCNSLs and 49 metastatic carcinomas. Biopsy and resection specimens were included. Hematoxylin and eosin stained sections were evaluated for the presence of necrosis, vascular proliferation and for the degree of intravascular thrombosis (total number of vessels with complete vascular occlusion by an organized thrombus).
Results: Intravascular thrombosis was present in 75% of GBMs, 11% of AAs, 10% of metastatic carcinomas and 6% of PCNSLs. Among those tumors with intravascular thrombosis, GBMs had significantly more vessels demonstrating thrombosis (mean, 15.45 ± 2.9) than AAs (3 ± 0.85; $p<0.05$), but had a similar number of involved vessels as PCNSLs (15 ± 5.05) and metastatic carcinomas (16.4 ± 8.9). Nearly all (95%) GBMs with intravascular thrombosis also showed both necrosis and vascular proliferation; 2.5% showed necrosis alone; and 2.5% showed vascular proliferation alone. Among the 33 cases of GBM with thrombosis 75% showed thrombosis of mature vessels, 63% showed thrombosis in hyperplastic vessels and 39% showed thrombosis in both.
Conclusions: Intravascular thrombosis is much more frequent in GBM than other CNS malignancies, but is not entirely specific. The greater prominence of thrombosis in GBM than AA may indicate a role in tumor progression. The utility of intravascular thrombosis as a prognostic marker in AA has yet to be determined.

1389 The EGFR/PI3K/PTEN/AKT Pathway in Glioblastoma Multiforme: Increased PI3K Immunohistochemical Expression Correlates with Decreased Survival

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Background: Abnormalities of the EGFR/PI3K/PTEN/AKT pathway have been shown to play a role in oncogenesis in many epithelial malignancies. More recently, alterations in this pathway have been identified in astrocytic neoplasms, most notably in glioblastoma multiforme (GBM). In this pathway the epidermal growth factor receptor (EGFR) activates phosphatidylinositol-3-kinase (PI3K), which through other mediators converts AKT to its phosphorylated active form. AKT is an oncogene product that inhibits apoptosis and promotes cellular proliferation through complex downstream interactions. PTEN acts as a tumor suppressor gene by counteracting the effects of PI3K. Targeted therapies directed against EGFR have found their way into clinical use and new therapies targeting PI3K have entered clinical trials. We investigated this pathway through immunohistochemical staining to evaluate the relationship between protein expression and patient survival, and to establish methods for detecting expression of these proteins in routine neuropathology specimens.
Design: We evaluated 67 cases of primary GBM with immunohistochemical staining for EGFR, PI3K and PTEN. All tumors were obtained at presentation without prior treatment; secondary GBM were excluded. A chart review was performed to obtain data on age, survival, extent of resection and follow-up treatment. Immunohistochemical expression was graded and correlated with survival through Kaplan-Meier survival analysis.
Results: Of the 67 primary GBM, 66% showed positive EGFR expression, 41% showed decreased PTEN expression, and 77% showed increased PI3K expression. EGFR and PTEN expression did not significantly correlate with survival (p-value = 0.13 and 0.76, respectively). PI3K expression was significantly linked with survival (p-value = 0.027) with increased PI3K expression being associated with a shorter survival (average = 8.9 mo., median = 8.6 mo.) than decreased PI3K expression (average = 15.3 mo., median = 11.4 mo.).
Conclusions: Variable expression of proteins in the EGFR/PI3K/PTEN/AKT pathway has been described in GBM. We have confirmed this variable expression, and have demonstrated the feasibility of evaluating these proteins with immunohistochemical staining. Additionally, we have identified a correlation between increased PI3K expression and decreased survival. These findings establish a method to further analyze the role of PI3K expression in identifying tumors suitable for targeted therapy and/or predicting response to targeted therapy.

Ophthalmic

1390 Expression Microarrays from Short Term Cultured Primary Retinoblastomas, Allows To Discriminate between HPV Positive and HPV Negative Tumors

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Background: Previously we reported DNA from Human Papilloma Virus (HPV) detected by PCR in retinoblastoma tissues, using the Manos MY09 and MY11 consensus primers. In order to find non PCR based molecular evidence of the HPV involvement in retinoblastoma, we prospectively cultured primary tissues for short periods of time. High quality and quantity RNA from this tumor cell cultures was obtained, and used it to perform expression microarrays experiments. With this study, we present transcriptional data that correlates with the PCR based HPV status in these retinoblastomas.
Design: RNA was extracted from 14 short-term primary retinoblastomas cultures. Microarray were printed at the National University of México microarray facility, using 10 K human oligonucleotide library set A, from MWG Biotech. In order to reduce variability in the statistical analysis, a novel approach using RNA from exponentially growing Saccharomyces cerevisiae was used in every microarray experiment. To test the variability of the system, we used four unilateral cases (>36 months of age at diagnosis) and negative family history, and four bilateral cases. These four cases in each group constitute biological replicas for the two dominant clinical forms of retinoblastoma. Two cases from each group were also chosen for technical replicas in order to define the magnitude of the variation in the data obtained. Non supervised and variance analysis were used to get clusters and measure variability among biological and technical replicas. HPV status was determined for each case by PCR.
Results: Higher variability was found among technical replicas than among biological replicas. Non supervised methods for clustering, discriminated correctly HPV positive from HPV negative cases, and unilateral from bilateral cases.
Conclusions: 1 Non supervised methods for clustering, discriminate laterality and HPV status. 2 Microarray expression variance analysis, indicates that is sufficient to pool data from two biological replicas (different patients) from each clinical category or HPV status. 3 Further analysis of the differences found among HPV status and laterality, may give insights about the mechanisms and disturbed cellular pathways in both forms of retinoblastoma.

1391 Sebaceous Carcinomas of the Eyelid Are Frequently EGFR Positive and HER-2/neu Negative

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Background: Sebaceous carcinoma (SC) is a rare, aggressive eyelid malignancy that is frequently initially misdiagnosed resulting in delayed treatment. Local control can be achieved by surgical resection but there is no established protocol for treatment of metastatic disease. Treatment of other cancers has been revolutionized by the addition of monoclonal antibody therapy, specifically targeting molecular markers overexpressed by the tumor. Inhibitors of tyrosine kinase, specifically targeted against HER-1/EGFR and HER-2/neu have proven to be an effective treatment for some types of carcinoma. Sebaceous glands show HER-1/EGFR expression and cytoplasmic staining for HER-2/neu, but sebaceous carcinomas have not been rigorously studied. The aim of this study is to determine the presence of HER-1/EGFR and HER-2/neu overexpression in eyelid sebaceous carcinoma.