1477 Tumor Infiltrating Lymphocytes in Primary CNS Lymphomas

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Ophthalmic

1481 Stem Retinoblastoma Cells in Tumorogenesis and Tumor Progression

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

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

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

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

express progenitor cell markers. Studies are undergoing to identify the cell of origin of the retinoblastoma stem cells. Our results suggest that retinoblastoma stem cells are possible effective therapeutic targets.

1482 Immune Response of Retinoblastoma after Gene Transfer Using Adnoviral-Mediated Delivery of Thymidine Kinase Followed by Ganciclovir

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Background: The purpose of this study was to evaluate potential local and systemic immune responses to the adenoviral vector used for treatment of patients with retinoblastoma vitreous tumor seeding.

Design: An IBC, IRB, RAC and FDA approved pilot study of intra-patient dose escalation was initiated to examine the intravitreal injections of an adenoviral vector containing a herpes thymidine kinase gene (AdV-TK) followed by systemic administration of ganciclovir to treat retinoblastoma. Blood samples were drawn weekly before and after the intraocular injections and the T cells were examined for evidence of adenovirus-specific reactivity. All patients eventually required enucleation and specimens were compared and immunohistochemically to retinoblastoma enucleated eyes without any previous treatment to identify the cellular inflammatory component and antigen presenting cells in these eyes using the following antibodies: CD3, CD5, CD43, TdT, CD68, L-26, CD138, CD21, CD23 and CD1a.

Results: No patient had an increase in antibody titer to adenovirus following therapy. Blood samples of 4 patients had no changes in the precursor frequency of adenovirus–specific T cells. Control (after conjunctival adenoviral infection) blood sample showed marked changes in frequency of adenovirus-specific T cells. The IHC results show that there was statistical significant difference between the ocular response in gene transferred and non-treated retinoblastoma eyes. Gene tranduced tumors showed statistically increased amount of T-cells labeling with CD3, CD43, and TdT; of B-cells labeling with CD20 and plasma cells labeling with CD138 when compared with non treated retinoblastoma eyes. The tumors show no significant differences between the two groups.

Conclusions: T-cell response and B-cells with plasma cells in intraocular structures in gene therapy treated eyes is prominent compared to the control eyes. Plasma cells were present in the intraocular structures and not in tumors predominantly in gene tx patients. The results suggest that the type and location of inflammatory cells may not only play a role in therapy related toxicity but also in anti-tumor response.

1483 Variation of Monosomy 3 within Uveal Melanoma

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Background: Uveal malignant melanoma is the most common primary intraocular malignancy in the adult population. Determining the most significant prognostic variables has been elusive but important in order to stratify patients for metastasis surveillance and possible initiation of chemotherapy or immunotherapy. Chromosomal changes of prognostic significance have been found and include monosomy 3 and alterations of 8q and 6p. While traditionally, this tumor (unlike almost all other tumors) is treated without a histopathologic diagnosis, some investigators have recently begun to acquire tissue using FNA or vitrector needles, some using a transvitreal and others a transscleral approach. Previous investigators have shown that there can be variability within a given tumor for the presence of monosomy 3. Our hypothesis was that there may be differing results for monosomy 3 detection depending on whether apex or base tumoral tissue is obtained, and this difference might impact on the proper stratification of patients for non-operative treatment.

Design: Paraffin embedded enucleated eyes from 19 patients with primary uveal melanoma were studied by FISH for monosomy 3, both in the tumoral apex and base. FISH was performed using directly labeled pericentromeric chromosome enumeration probes (CEP3 and CEP4) labeled with SpectrumOrange and SpectrumGreen respectively. Twenty nuclei in 2 representative fields in the preselected apex and base areas were counted; only nuclei with at least 2 reference CEP4 signals were counted. Monosomy 3 was determined to be present if the CEP3/CEP4 ratio was ≤ 0.7.

Results: 16 of the 19 samples (84%) revealed concordance of monosomy 3 (7 of 16, 44%) or of disomy 3 (9 of 16, 56%). In 3 samples (16%), there was discordance of monosomy 3 status between the tumor base (monosomy 3) and tumor apex (disomy 3). Lack of concordance between the base and apex did not correlate with morphologic cell type. All three discordant cases demonstrated the monosomy 3 at the base with disomy at the apex.

Conclusions: Uveal melanoma displays tumor heterogeneity, genetically as well as morphologically, as evidenced by variation of monosomy 3 status within uveal melanoma. Fine needle aspiration biopsy samples obtained from the tumor base (scleral approach) vs. apex (transvitreal approach) may yield disparate results, which could effect therapeutic decisions.

Pathobiology

1484 Dysplastic Tubules and Mitochondrial DNA Mutations Support a Precursor Lesion to Renal Cell Carcinoma

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Background: Renal cell carcinoma (RCC) is the most common malignancy of the kidney and accounts for 3% of all malignancies in adults. Unlike many other organ systems, a precursor lesion for an invasive carcinoma has not been described in the kidney. In our experience, we have occasionally observed the presence of dysplastic renal tubules (DT) adjacent or remote to RCC. These tubules display a range of features of histologic atypia, including clear cytoplasm, prominent nucleoli, irregular nuclear membranes and variation in nuclear size without architectural abnormalities. Mitochondrial (mt) DNA mutations have been described in RCC. However, the molecular mutation profile of DT has not been reported. In this study, we investigated the mtDNA mutation profile of DT and compared the findings with normal renal tissue and RCC.

Design: A total of 35 cases of RCC were retrieved and reviewed. Eight of the 35 cases (23%) had DT based on histomorphologic features. The control groups include 5 normal renal tubules (NRT) in autopsy kidneys and 8 RCC. Following review of the cases, representative areas of RCC and DT were selected from each case. Three areas from the RCC and DT on each case were microdissected and DNA was extracted. We analyzed mutations of the mtDNA regulatory region or D-loop at 3 sites (D310, D512, and D4977) using PCR/electrophoresis.

Results: All cases of RCC (100%) showed mutations of mtDNA, the most common being mutation of D4977 (8/8 cases). All DT (100%) had mutations of mtDNA, with the most common being D4977 (7/8 cases). NRT showed mutation in 2 cases, but at a much lower rate than DT.

Number of cases positive for mtDNA mutation and rate of mutation

	D310		D512		D4977	
	Number of cases positive	Average rate of mutations	Number of cases positive	Average rate of mutations	Number of cases positive	Average rate of mutations
RCC	7	2	5	0.63	8	1.63
DT	6	1.1	2	0.33	7	1
NRT	2	0.63	0	0	2	0.63

RCC (renal cell carcinoma), DT (dysplastic tubules), NRT (normal renal tubules)

Conclusions: We demonstrated that mtDNA mutations are present in DT and in RCC, but not in NRT. These findings are supportive that DT may be a precursor of invasive renal cell carcinoma. Further investigation of oncogene expression in DT may also be helpful to better define the concept of "dysplastic" renal tubules as a precursor lesion in RCC.

1485 Carcinosarcomas Exhibit EMT – A Phenomenon of Tumor Progression Unrelated to Metastasis

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Background: The importance of epithelial mesenchymal transition (EMT) in tumor progression is controversial although its occurrence has been well documented in many model systems. Questions concerning its transient nature, its reversibility and most importantly its role in metastasis have been debated. Human carcinosarcomas of diverse types exhibit EMT in which there is a progression from a carcinomatous gene expression profile to a sarcomatous one and a concomitant pathological change from epithelial to mesenchymal histology.

Design: We decided to study EMT from both an observational as well as experimental perspective to address its role in metastasis. We studied 20 cases of human carcinosarcomas by E-cadherin, cytokeratin, β-catenin and vimentin IHC. We also used a transgenic murine model of breast cancer (WAP-*myc*) which exhibits EMT and pulmonary metastasis. We transfected cells from this model *ex vivo* with WAP-*cre* and lox (β-gal) reporter and with FSP-*cre* and lox reporter constructs and reinjected the cells into the cleared mammary fat pads of wild type mice. WAP is a promoter active in mammary epithelial cells whereas FSP is a promoter active in mesenchymal cells. Once the promoter becomes active in the constructs it causes an irreversible expression of the reporter.

Results: Each of the human carcinosarcomas exhibited IHC evidence of biphasic immunoreactivity yet their corresponding metastases were carcinomatous. The WAP-*myc*/WAP-*cre*/lox carcinoma cells exhibited primary tumors where both the carcinomatous as well as the sarcomatous areas expressed β-gal. The corresponding pulmonary metastases also expressed β-gal and were carcinomatous. The WAP-*myc*/FSP-*cre*/lox carcinoma cells exhibited identical primary tumors as the previous WAP-*myc*/WAP-*cre*/lox except that only the sarcomatous regions expressed β-gal. Their pulmonary metastases were exclusively carcinomatous and non-expressive of β-gal.

Conclusions: In both observational and experimental studies, there is clear evidence that EMT occurs. However the experimental studies shed further light upon the nature of EMT. EMT appears to be unidirectional (carcinoma to sarcoma and not vice versa), not derived from a stem cell switch, not transient and not metastasis promoting because if any of these latter features were true, the WAP-*myc*/WAP-*cre*/lox and the WAP-*myc*/FSP-*cre*/lox mammary carcinomas would not exhibit their pattern of β-gal expression in their primary tumors and metastases.

1486 Predilection of Pancreatic Ductal Adenocarcinoma Cells To Form Duct-Like Structures in Vascular and Perineural Spaces, Mimicking Normal Ducts and PanIN: A Peculiar Form of Tumor-Stroma Interaction

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Background: The tremendous ability of pancreatic ductal adenocarcinoma (DA) cells to rapidly disseminate, despite its relatively well differentiated morphology, is one of the most intriguing questions in tumor biology. It is our impression that the DA cells may be utilizing the connective tissue of the host as a vehicle for their spread.

Design: Infiltration patterns in 103 cases of resected DA were analyzed.

Results: 64 of 103 cases (62%) had vascular invasion, and in 25 (40%) of these there was a peculiar phenomenon in which the carcinoma cells lined the inner surfaces of the vessel walls, forming a well-defined duct-like structure. These were so well-formed and bland-appearing that they closely resembled normal ducts or PanIN. Actin and elastin stains were performed to confirm the intravascular nature of the duct like structures. The carcinoma cells appeared to replace the endothelial cells; CD34+ endothelial cells were