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

H Takei, Y Nguyen, V Mehta, M Chintagumpala, RC Dauser, AM Adesina. The Methodist Hospital, Houston, TX; Texas Children's Hospital and Baylor College of Medicine, Houston, TX; Texas Children's Hospital, Houston, TX.
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

M Tehrani, JJ Olson, DJ Brat. Emory University School of Medicine, Atlanta, GA.
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

AD Vanderheyden, BR DeYoung, LA Bruch. University of Iowa, Iowa City, IA.
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

L Cabrera-Muñoz, S Sadowinski, M Orjuela, F López-Casillas, G Ramón, MV Ponce-Castañeda. Hospital Infantil de México, Cuauhtémoc, DF, Mexico; Columbia University, New York, NY; IFC UNAM, Coyoacán, DF, Mexico; Hospital de Pediatría CMN SXXI, IMSS, Cuauhtémoc, DF, Mexico.
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

SEH Cameron, JD Cameron, JC Manivel, C Forster, SE Pambuccian. University of Minnesota, Minneapolis, MN; Mayo Clinic, Rochester, MN.
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.

Design: Medical records and all cases of SC of the eyelid and of the conjunctiva treated at two large medical centers during the last 27 years were reviewed. The histologic diagnosis was confirmed in each case. Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded tissue on automated immunostainers. Two EGFR clones were used, DAKO clone H11 (on the Dako immunostainer) and Zymed, clone 31G7 (on the Ventana NEXes). HER-2/neu (Dako Herceptest) staining was performed on the DAKO immunostainer. All cases were evaluated by two pathologists and scored according to the DAKO Herceptest Interpretation Manual and the DAKO EGFR pharmDx Interpretation Manual.

Results: 16 cases of sebaceous carcinoma of the eyelid comprised the study. The age range was 46 to 92 years at diagnosis. The gender ratio was M:F of 1:2.2. The tumor cells stained positively with EGFR antibody 93-100% of the cases studied, while 94% of the neoplastic cells did not stain or only weakly stained with HER-2/neu, although some normal sebaceous cells did stain.

HER-2/neu and EGFR immunohistochemical Profile of Sebaceous Carcinomas.

	HER-2/neu	EGFR (Dako)	EGFR (Zymed)
0	7/16	1/14	0/16
1+	8/16	4/14	3/16
2+	1/16	6/14	7/16
3+	0/16	3/14	6/16

*Missing values in the table are due to absence of tumor on deeper sectioning.

Conclusions: We found that sebaceous carcinoma of eyelids frequently overexpress EGFR but do not stain with HER-2/neu. This information may be useful for developing targeted therapy for metastatic sebaceous carcinoma and non-resectable primary tumors.

1392 Ophthalmic Von Hippel-Lindau Disease: Expression of Clusterin

P Chevez-Barrios, M Zhou, D Shen, EY Chew, WR Green, CC Chan. The Methodist Hospital, Houston, TX; National Institutes of Health, Bethesda, MD; Johns Hopkins University School of Medicine, Baltimore, MD.

Background: Clusterin is a multifunctional glycoprotein with an ubiquitously expressed mRNA, and high levels found in von Hippel-Lindau (VHL) target organs (brain, liver, kidney, and adrenal medulla). Interestingly, decreased clusterin secretion is reported in renal carcinoma associated with VHL disease.

Design: To investigate expression of clusterin in ocular VHL associated hemangioblastomas, we examined clusterine reactivity in 11 eyes: 9 eyes with retinal hemangioblastomas, 1 eye with family history of VHL and CNS hemangioblastomas but without ocular lesions, and a normal control eye using immunohistochemistry. In 4/9 eyes microdissection was performed to obtain ocular hemangioblastoma cells and normal cells to analyzed clusterin mRNA levels using the TaqMan fluorescent real-time quantitative PCR system (Applied Biosystems).

Results: All retinal hemangioblastomas were composed of typical VHL cells admixed with small vascular channels and/or glial cells. Marked decrease of clusterin reactivity was detected in all retinal hemangioblastomas. The normal retina with family history and CNS VHL lesions presented focal low expression of clusterin compared with the normal eye. Quantitative real-time PCR analysis confirmed the decrease of clusterin *mRNA* in the VHL hemangioblastoma compared with normal tissue in the same sample. The ratios of clusterine mRNA to beta-actin mRNA in these 4 cases (normal tissue:tumor tissue) were 18.55:7.84; 18.11:14.35;51.51:30.08; 78.17:14.92, respectively.

Conclusions: Similar to VHL-associated renal cell carcinomas, retinal hemangioblastomas lose expression of clusterine secretion . Clusterin may prove to be a useful marker of *VHL* gene status, and provide better understanding of ocular VHL disease.

1393 Malt Ocular Adnexal Lymphomas (MOAL) Are Not Associated with Chlamydia Psitacci (Cp) Even in 3 Cases of Bilateral Localizations. A Histopathological and Genetic Analysis of 14 Cases

A de Mascarel, M Parrens, B Vergier, O Fitoussi, M Beylot-Barry, B de Barbeyrac, MA Belaud-Rotureau, JP Merlio. CHU Bordeaux, Hopital Haut-Leveque, 33604 Pessac Cedex, France.

Background: The most frequent lymphomas of the ocular adnexa are Malt lymphomas (MOAL). MALT1 breakpoints involved in the t(14;18)(q32;q21) but not in the t(11 ;18) (q32 ;21) have been detected in a subset of MOAL. A possible association between Chlamydia psitacci (Cp) and MOAL has also been debated.

Design: 17 OAL were reviewed in our department. Formalin-fixed biopsies were studied by hematein-eosin stain and immunohistochemistry in particular CD20, CD5, CD43, cyclin D1, Mib1. PCR for the detection of IgH gene rearrangement and Cp DNA was performed on DNA extracted from histologically-selected formalin-fixed fragments. FISH was performed on sections using the MALT1 DNA split signal probes (Dako).

Results: 14 OAL were Malt L, 2 mantle cell L and 1 follicular cell L. For the 14 MOAL, there were 8 women and 6 men with a mean age of 59 years (13-82). Ocular localizations were orbit (n=7), conjonctiva (n=5), lacrymal gland (n=2). 3 patients presented bilateral tumors at the time of diagnosis: a 13-year-old Tunisian girl with bilateral MOAL of conjonctiva and two 71-year-old French women with bilateral MOAL of orbits. The phenotype of 14 MOAL was CD20+, CD5-, cyclinD1-; 6/14 were CD43+. The IgH gene rearrangement profile was monoclonal in 12, polyclonal in 1 and not informative in 1. In the 2 informative bilateral cases, the same monoclonal band was observed at both sites. FISH analysis did not find any MALT1 gene breakpoint (0/14). Using PCR, there was no evidence for the presence of Cp DNA in any MOAL case, even in the 3 bilateral tumors.

Conclusions: According to previous reports, the majority of OAL are Malt L. As observed in other extra gastric Malt L, CD43 can be expressed (43% in this series) but CD5 and cyclinD1 are constantly negative (14/14). Our 14 MOAL did not display MALT1 breakpoint. As compared with other reports, we did not find any association with Chlamydia psitacci even in 3 bilateral ocular tumors that would suggest alternative etiopathogenic pathways.

1394 BCL-2 Expression in Melanocytic Neoplasms of the Conjunctiva

E Furusato, AA Hidayat. Armed Forces Institute of Pathology, Washington, DC.

Background: Recent Studies indicate that bcl-2 protein is detected in benign and malignant melanocytic neoplasms of the skin in addition to follicular lymphomas. To our knowledge, there are no published reports of bcl-2 studies in melanocytic lesions of the conjunctiva. The objective of our study is to evaluate bcl-2 expression in conjunctival melanocytic lesions and compare the results with other immunohistochemical markers.

Design: We studied biopsy specimens of 123 patients with conjunctival melanocytic lesions, including benign nevi, atypical nevi, primary acquired melanosis (PAM), and malignant melanomas. Immunohistochemical studies with Bcl-2, S-100, HMB45 and Melan A were performed in all cases.

Results: Our study shows that Bcl-2 expression was positive in all benign and malignant melanocytic lesions (100%) with strong and diffuse reactivity in most cases. The expression was more consistent, stronger, and more diffuse than S100, HMB45, and Melan A. HMB 45 showed significant difference in staining between benign nevi and malignant melanomas. It showed weak or moderate expression in benign nevi, but it was strong and diffuse in malignant melanomas. The Bcl-2, S100 and Melan A did not show such difference as compared to HMB45.

Conclusions: Bcl-2 is a consistent and reliable immunohistochemical marker for benign and malignant melanocytic tumor of the conjunctive. Other markers such as S-100, HMB45 and MelanA are good, but less consistent. Because HMB45 was unique in showing a staining difference between benign and malignant lesions, we suggest using two immunohistochemical markers Bcl-2 and HMB45 for melanocytic lesions of the conjunctiva.

1395 Validating the Tumor Cell Origins of Vasculogenic Mimicry Patterns, a Significant Prognostic Histological Factor in Uveal Melanomas

AY Lin, L Leach, K Valyi-Nagy, M Apushkin, AJ Maniotis, J Pe'er, R Folberg. University of Illinois at Chicago, Chicago, IL; Hadassah Hebrew University Medical Center, Jerusalem, Israel.

Background: Multiple independent pathology groups have confirmed the prognostic significance of detecting looping vasculogenic mimicry (VM) patterns in histological sections of uveal melanomas. Although in vitro studies have demonstrated that highly invasive melanoma cells generate VM patterns, some pathologists contend that vasculogenic mimicry patterns are fibrovascular septa that originate from a host stromal response to the tumor. We have shown previously that there are morphological and immunohistochemical differences between fibrovascular septa and VM patterns. To validate our hypothesis that VM patterns are an attribute of the invasive tumor cell and not part of the host response, we studied the histogenesis of VM pattern formation in a mouse model.

Design: OCM1a human uveal melanoma cells were injected into the liver of 15 SCID mice (uveal melanoma disseminates preferentially to the liver). Animals were sacrificed after 6-8 weeks, and tumor nodules examined histologically. Sections were stained with mouse monoclonal anti-laminin antibody specific for human laminin or rabbit polyclonal anti-laminin antibody that cross reacts with both mouse and human laminin. Three-dimensional reconstruction of VM patterns were generated by laser scanning confocal microscopy assisted by immersion visualization techniques described by us previously.

Results: Vasculogenic mimicry patterns, identical to those demonstrated in human primary and metastatic melanoma, formed in the xenografts. The monoclonal antibody to human laminin stained VM patterns in the melanoma nodules but did not label vessels in the mouse liver or control mouse kidney. The polyclonal antibody that reacts with human and mouse laminin stained VM patterns in the melanoma nodules and labeled mouse vessels in the liver as well as basement membranes in the mouse kidney. Three dimensional reconstructions of VM patterns in the xenograft revealed an architecture identical to VM patterning in human tumors.

Conclusions: VM patterns in the xenograft consist of human laminin, rather than laminin coopted from the mouse host, providing another line of evidence confirming the tumor cell origin of this histologically significant marker of tumor progression and further distinguishing these patterns from host-derived fibrovascular septa.

1396 Endophthalmitis: A Ten Year Retrospective Series in a Tertiary Center

K Rishi, PL Cernoch, P Chevez-Barrios. The Methodist Hospital, Houston, TX.

Background: Endophthalmitis is a rare but potentially devastating infection likely to produce irreversible visual loss if not managed appropriately. This study focuses on the epidemiology, clinical manifestations and diagnosis (vitreous cytology, microbiology and molecular polymerase chain reaction (PCR)) of all cases of endophthalmitis in a tertiary center.

Design: We reviewed cytologic preparations of 256 consecutive vitrectomy specimens including vitreous taps and washings retrospectively from 1996 to 2006. In addition, the cultures were compared in all the patients diagnosed with endophthalmitis on cytology and molecular polymerase chain reaction (PCR) for viral or parasitic agents.

Results: Of the 256 vitrectomy specimens, 22 cases were diagnosed as endophthalmitis (acute, subacute and chronic). The patient's demographic data included 8 males and 14 females. The mean age of the patients was 55 years (age range 7 to 90 years). The most common predisposing factor included previous ocular surgery and endogenous route of infection. On cytology, the vitreous specimen contained numerous neutrophils with few histiocytes and lymphocytes in the background. The yield of vitreous aspirate in identifying organisms was 60% and that of cultures was 80% .Of the 22 cases, fungal endophthalmitis was the commonest cause (12/22), bacterial (4/22), viral (2/22), idiopathic (2/22) and nematode (2/22). The fungal organisms isolated included – Candida albicans in 6 patients; Fusarium species in 2 patient, Aspergillus fumigatus in 2 patient, Cryptococcus neoformans in 1 patient and Curvularia species in 1 patient. The nematode

endophthalmitis was seen in children, due to Toxocara canis. The commonest cause of bacterial endophthalmitis was Staphylococcus aureus. PCR was used for the 20% of cases but with only 2 cases showing positive results. The majority of the patients with endopthalmitis (60%) underwent enucleation or an irreversible visual loss.
Conclusions: Fungal organisms are the commonest causes of endophthalmitis in our center. The yield of finding the organisms on vitreous cytologic preparations is 60% as compared to cultures (80%). PCR has a high diagnostic yield in viral and nematode endophthalmitis.

Pathobiology

1397 Angiogenic Histogenesis of Stromal Cells in Hemangioblastoma: Expression of Novel Endothelial Markers

M Acon Laws, RS Saad, YL Liu, M Tung, JF Silverman. Allegheny General Hospital, Pittsburgh, PA.
Background: Hemangioblastomas are uncommon central nervous system tumors which can occur sporadically or in von Hippel-Lindau (VHL) disease. Controversy regarding the origin of characteristic stromal cells is responsible for categorizing hemangioblastoma as "tumors of uncertain histogenesis" in the current WHO classification of brain tumors. Failure to specify the histologic origin of the stromal cells in hemangioblastoma has limited our understanding of its basic biology with possible treatment implications. The aim of this study was to investigate the expression of two novel endothelial markers (D2-40, a lymphatic marker); and CD105 (endoglin, a marker for neovascular proliferation) in hemangioblastoma, and to determine whether the expression of these immunohistochemical (IHC) markers can contribute to the understanding of the histogenesis of hemangioblastomas.
Design: A computer search of our hospital identified 27 cases of hemangioblastomas between 1997 and 2005, consisting of 10 spinal, 9 cerebellar and 8 cerebral hemangioblastoma. Four cases were associated with VHL disease. Immunostaining was performed on formalin-fixed, paraffin embedded sections using automatic immunostainer with appropriate positive and negative controls. Intensity was graded from 0-3 with a score 0 for no staining and 3 for maximal intensity. Cases which showed weak or <5% staining were considered negative.
Results: All cases of hemangioblastoma showed strong IHC staining for endoglin (CD105) in the vascular channels, but negative D2-40 staining. However; the stromal cells were positive in 14/27 (52%) for endoglin and 10/27 (37%) for D2-40. Seven of the D2-40 positive cases were negative for endoglin and six cases were negative for both endoglin and D2-40.
Conclusions: Although stromal cells of hemangioblastoma are classified as undifferentiated mesenchymal tumor, they demonstrate positivity for the novel endothelial markers D2-40 and endoglin, suggesting an endothelial histogenesis.

1398 Angiogenic Ability of Metastatic Squamous Carcinoma in the Cervical Lymph Nodes from an Unknown Primary

B Agarwal, A Borges. Howard University Hospital, Washington, DC; Asian Institute of Oncology, Bombay, Maharashtra, India.
Background: Vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) molecules secreted by tumour cells and other surrounding benign cells are the principal inducers of neoangiogenesis in the growth of solid tumours beyond a size of 3-4mm. The isoforms of VEGF: $VEGF_{121}$, $VEGF_{165}$ and $VEGF_{189}$ induce angiogenesis by endothelial cell proliferation and increasing vascular permeability. Metastases from unknown primary tumours (MUP) are not an uncommon clinical problem, especially in the head and neck. The phenomenon of a tumour that is clinically undetectable at the primary site yet capable of metastasis is intriguing and begs a biological explanation.
Design: The aim of the study was to study the angiogenic differences between metastasis of squamous carcinoma with unknown primary (MUP) and metastasis of squamous carcinoma with known primary (MKP) We have investigated the expression of the angiogenic molecules VEGF and bFGF in cervical lymph node (LN) metastasis of squamous carcinoma: 50 with unknown primaries (MUP) and 52 with known primaries (MKP) by immunohistochemistry performed on paraffin sections. We have also performed RT-PCR analysis of the expression of VEGF isoforms ($VEGF_{121}$, $VEGF_{165}$ and $VEGF_{189}$) in 26 cases of MUP and 22 cases of MKP. RNA was extracted by guanidium-phenol-chloroform method and cDNA was prepared. Only those cases which were positive for housekeeping primers like underwent and RT-PCR analysis using the VEGF primers which gave a band size of 197bp with $VEGF_{121}$, 347bp with $VEGF_{165}$ and 419bp with $VEGF_{189}$.
Results: The immunohistochemical data showed that MKPs had significantly higher expression of VEGF (X^2 =46.2;p<0.001) and bFGF (X^2=22.5; p< 0.001) as compared to MUPs. The RT-PCR analysis showed that MKPs had increased expression of $VEGF_{121}$and $VEGF_{165}$ isoforms than MUPs.

VEGF isoform ($VEGF_{121}$, $VEGF_{165}$ and $VEGF_{189}$) expression in lymph node metastasis with MUP and MKP

VEGF isoform	MUP (ng/µl) (n=22)	MKP (ng/µl) (n=26)	p value
$VEGF_{121}$	1.562±0.498	3.341±.906	0.002 (significant)
$VEGF_{165}$	1.058±.366	2.064±0.6	0.007(significant)
$VEGF_{189}$	0.684±.252	1.745±1.768	Not significant

MUP: Metastasis of unknown primary; MKP: Metastasis of known primary
Conclusions: Presuming, that tumour cells in the metastatic LNs reflect the primary tumour characteristics, we suggest that MUPs represent metastasis from tumours that have not established at the primary site due to their poor angiogenic phenotype.

1399 Differential Topographic Expression and Telomerase/Telomere Profiles Determine the Kinetic Advantage in Follicular Thyroid Carcinomas

R Ashrafi, A Blanes, SJ Diaz-Cano. King's College Hospital, London, England, United Kingdom; University of Malaga School of Medicine, Malaga, Spain.
Background: The kinetic differences and telomerase/telomere profile by topographic compartments in follicular thyroid lesions have not been studied to date.
Design: We selected adenomatous hyperplastic nodules (FTHN, 18), adenomas (FTA, 19), carcinomas (FTC, 15 minimally-invasive and 15 widely-invasive), and anaplastic carcinomas (ATC, 10) (WHO criteria) to analyze by topographic compartments (internal/peripheral): Ki-67 and telomerase immunostaining, in situ end labeling (ISEL) of DNA fragments, telomere PNA-FISH and low-density selective cDNA array (LD-SelGEA; telomerase, p53, mdm2, p21, cdk2, cyclin E, pRB, Egr2, JunB, and FosB). Total RNA was extracted, cleaned from normal and neoplastic tissues (RNeasy columns), first-strand cDNA synthesized using T7-(dT24)-oligomer and used as template for cRNA synthesis. The cRNA was fragmented, Cy3-/Cy5-labeled, and hybridized to LD-SelGEA noncompetitively, cross-validating the results (expression factor>2, significance<0.01). Variables were studied regarding the histological diagnosis and molecular profile: RAS mutation (8 FTA, 7 minimally-invasive FTC, 5 widely-invasive FTC, 4 ATC), PAX8/PPARγ fusion gene (2 FTA, 1 minimally-invasive FTC, 7 widely-invasive FTC), and TP53 LOH/mutation (4 ATC) and combinations (3 widely-invasive FTC, 2 ATC).
Results: Internal compartments of benign lesions and peripheral compartments of malignant lesions revealed the most advantageous kinetic (increased Ki-67/ISEL index, due to significantly decreased ISEL index). Telomerase expression was significantly higher in internal compartments (p<0.001) and in malignant lesions (p<0.001), which only correlated with telomere PNA-FISH positive cells in internal compartments. Peripheral telomere PNA-FISH>20% was observed in high-grade lesions (widely-invasive FTC and ATC) only. Telomerase/telomere indices directly correlated with the kinetic index, being significantly higher in high-grade malignancies with multiple genetic alterations (PAX8/PPARγ-RAS in widely-invasive FTC and RAS-TP53 in ATC) and cases with upregulation of p53, cyclin E, Egr2, JunB and FosB at LD-SelGEA.
Conclusions: The kinetic advantage predominates in internal compartments of benign lesions and in peripheral ones of malignant lesions, due to inverse and opposite proliferation/apoptosis correlations. This kinetic profile directly correlates with the telomere-telomerase index, especially in FTC with multiple genetic abnormalities.

1400 Up Regulation of P-Cadherin in Uterine Fibroids Shows a Race Specific Difference

N Bailey, DA Castrodale, F Khan, JE Coad. West Virginia University, Morgantown, WV.
Background: Multiple larger uterine fibroids are a cause of pelvic discomfort in women. While their pathogenetic basis is not well defined, African American women (AAW) are more likely to have this type of fibroid than Caucasian women (CW). P-cadherin (P-cad), N-cadherin (N-cad) and beta-catenin (B-cat) expression in fibroids have not been well studied, especially for race specific differences.
Design: 28 sequential AAW fibroid uteri were identified (Age: 41 ± 5 years; Myoma size: 0.2 – 11 cm; Endometrium: 13 proliferative, 9 secretory and 6 inactive). An age, menstrual cycle and fibroid size-matched CW group was selected for comparison. These specimens provided 51 AAW and 36 CW fibroids for analysis. Routine DAB immunostaining for P-cad, N-cad and B-cat was performed on formalin-fixed paraffin-embedded tissue, according to manufacturers' recommendations. Each marker's expression was blindly evaluated in the myometrium and fibroids, using the following intensity scoring system: undetectable (0+), weak (1+), moderate (2+) and strong (3+). Differential expression between the fibroids and matched myometrium was calculated. Statistical analysis was performed.
Results: All specimens together: P-cad expression was higher in fibroids (2.0+ ± 0.8+) than myometrium (1.2+ ± 0.7+; p<0.001). N-cad expression was higher in fibroids (1.8+ ± 0.7+) than myometrium (1.0+ ± 0.6+; p<0.001). B-cat expression was higher in fibroids (0.5+ ± 0.5+) than myometrium (0.2+ ± 0.4+; p=0.005). Expression by race and tissue type is shown in the Table.

Mean Expression Scores By Race, Tissue Type And Differential Expression

	African American Women		Caucasian Women		Matched Expression Differences	
	Fibroid	Myometrium	Fibroid	Myometrium	AAW	CW
P-cad	1.9+ ± 0.8+	1.3+ ± 0.6+	2.1+ ± 0.8+	1.1+ ± 0.7+	0.5+ ± 0.9+	1.1+ ± 1.1+
N-cad	1.7+ ± 0.7+	1.0+ ± 0.7+	1.9+ ± 0.7+	1.1+ ± 0.6+	0.7+ ± 0.8+	0.8+ ± 0.8+
B-cat	0.5+ ± 0.5+	0.1+ ± 0.3+	0.6+ ± 0.5+	0.2+ ± 0.4+	0.4+ ± 0.6+	0.4+ ± 0.5+

Conclusions: Uterine fibroids have significantly increased P-cad, N-cad and B-cat expression when compared to the myometrium. While no race difference was identified for N-cad (p=0.358) or B-cat (p=0.954), P-cad showed significantly greater expression in CW than AAW fibroids when compared to the matching myometrium (p=0.007). Thus, it could be hypothesized that differential up-regulation of P-cad in fibroids may contribute to the biologic differences between African American and Caucasian women.

1401 Automation of TMA Interpretation through a Combination of Epithelial-Recognition and Specific-Recognition Algorithms

SH Barsky, LM Tatke, SH Patil, RE Jimenez, S Mahooti, AS Basu, AS Gholap. The Ohio State University College of Medicine, Columbus, OH; BioImagene, Inc, Cupertino, CA.
Background: Tissue microarrays (TMAs) are a form of high throughput screening akin to cDNA microarray and proteomic analyses, yet, unlike the latter types, are based on manual construction and subjective interpretation. Because of the increasing demand for TMAs predicted to occur over the next decade, we felt it necessary to investigate whether their interpretation could be completely automated.