of the center. One section from each zone of each axis, 2 sections from small leiomyomata (1-2 cm) and 2 sections from matched myometrium were arrayed on one block (0.6 mm tissue cores). Expression of the tumor markers, including ER, PR, MIB1, RXR, GCR, HMGA2, EGFR, F8 and CD24 was scored by semi-quantitation.

**Results:** All tissue sections were reviewed on H&E stained slides. Large leiomyomata had varied amount of hyalinization, accounting for approximately 25 % of the tumor volume. The distribution of hyalinization tended to be higher in the center of the tumors. Tissue cores for microarray were selected from the cellular area. The large leiomyomata showed minimal changes of ER and PR overall, compared with matched myometrium. However, a significant up regulation of ER and PR was noted in zone 2 with scores of 1.2 and 1.8 in comparison with the mean scores of 0.4 and 0.5 in the large leiomyomata. Up regulation of CD24, HMGA2 and RXR was constantly highest in zone 2, followed by zone 3 and 4. Scores of MIB1 were higher in zone 1, 2 and 4, and lower in zone 3, 5 and 6. There was a deep down regulation of GCR in zone 4, 5 and 6, where many fewer capillary vessels (detected by F8 staining) were counted.

**Conclusions:** Spatial distribution of the selective tumorigenic markers in the large uterine leiomyomata presents a distinct pattern. Zone 2 (next to periphery) is the most biologically active area. The expression of the markers in the small leiomyomata is similar to zone 2 of the large leiomyomata. The findings suggest that central areas of hyalinization or infarcts may not slow down the tumor growth if zone 2 is still viable.

### 975 Examination of Phosphatidylinositol 3-Kinase (PI3K) and Its Associated Signaling Proteins in Endometrial Carcinoma (EC)

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**Background:** Cell signaling mediated through PI3K plays a pivotal role in regulating cell growth, proliferation and apoptosis. PI3K has a catalytic subunit, p110, which is tightly controlled by its associated regulatory subunit p85. The biological activities of PI3K are counter-balanced by PTEN, a PIP3 phosphatase. Activation of PI3K eventually turns on several down stream target molecules including b-catenin to exert its functions. Aberrant expression of PI3K, due to mutations involving either p110, or p85, is associated with colon, cervical, ovarian and lung carcinomas. However, their role in EC has not been assessed. In the current study, the expression levels of p110 and p85 were examined along with PTEN and b-catenin in EC.

**Design:** A tissue microarray was assembled from formalin-fixed and paraffin-embedded tissue blocks of 22 cases of EC, endometrioid type, seen at NYU hospitals between 1999 and 2004. The average age of the patients at diagnosis was 62 ± 12.1 yr (43 – 85). Of the 22 cases, 18 were graded as FIGO I, 3 FIGO II, and 1 FIGO III. Two 1-mm cores of tumor tissue, the accompanying simple (SH) or complex hyperplasia (CH) as well as normal endometrium (EM), when they are present, were taken from each case. The immunoreactivity to p110, p85, PTEN and b-catenin were examined by immunohistochemical staining on the tissue array.

**Results:** PI3K p110 immunoreactivity was shown predominantly in the cytoplasm in both benign and malignant endometrial tissues. The nuclear stains tend to be seen in CH and EC. The expression levels of p110 increased along the spectrum of normal EM (0.66±0.7) to SH (1.06±0.8) to CH (1.23±0.6) to EC (1.86±0.7, $p<0.0001$ vs. normal EM, Table 1). Interestingly, we didn't observe immunoreactivity for p85 or the loss of PTEN in any of our cases. Nuclear translocation of b-catenin was shown in 8 cases of EC or CH, but not in normal EM or SH.

**Conclusions:** Our study provides the first piece of evidence suggesting a role of PI3K in EC, presumably functioning as an oncogenic protein. Since its elevation is seen in pre-malignant endometrial hyperplasia, it may suggest that PI3K participates in the early events of the tumorigenesis of EC.

Table 1: The immunoreactivity of PI3K p110 in EC

| | | 0+ | 1+ | 2+ | 3+ |
|---|---|---|---|---|---|
| | Number of Cases | Cases (%) | Cases (%) | Cases (%) | Cases (%) |
| endometrium | 9 | 4 (44.4%) | 4 (44.4%) | 1 (11.2%) | 0 |
| simple hyperplasia | 15 | 4 (26.7%) | 6 (40.0%) | 5 (33.3%) | 0 |
| complex hyperplasia | 17 | 2 (11.8%) | 9 (52.9%) | 6 (35.3%) | 0 |
| endometrial carcinoma | 22 | 0 | 8 (36.4%) | 9 (40.9%) | 5 (22.7%) |

### 976 Up Regulation of Retinoid Acid Receptor X (RXRα) in Uterine Leiomyosarcomas

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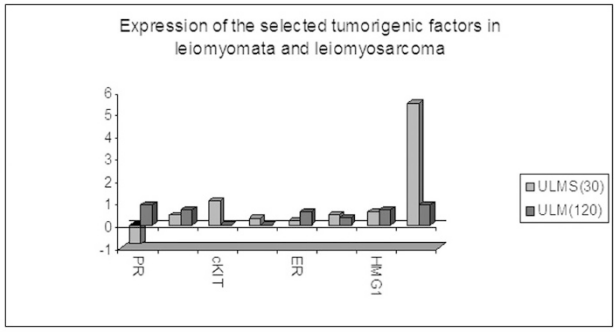
**Background:** Uterine leiomyosarcomas (ULMS) are rare type of malignant smooth tumors. The tumor pathogenesis is largely unknown. Studies suggested that ULMS may be associated with or progressed from uterine leiomyomata. However, there is no convinced evidence to establish the link between the two diseases. In this study, we intended to examine some selected tumorigenic factors by comparing the expression patterns between leiomyosarcomas and leiomyomata.

**Design:** A total of 30 uterine leiomyosarcomas, matched leiomyomata and matched myometrium were collected. 0.6mm tissue cores from ULMS (2 cores), leiomyoma (1 core), and myometrium (2 cores) were arrayed on one block. Selected immunomarkers, including SMA, ER, PR, bcl-2, Ki-67, RXRα, HAM-1 GCR, HMG1 and c-Kit were stained and analyzed by semi-quantitation. The data of the immunomarkers obtained from ULMS were compared with data of the same group of markers from 120 uterine leiomyomata (in the separate study).

**Results:** Patient's mean age was 59.2 years old (ranged from 38 to 85yrs). A total of 26 cases showed validate data from all markers. Among them, 11 cases had concurrent leiomyomas (42%). Considering the possible variation of the immunoreactivity between the cases due to no biological factors, we calculated the net values of gain or loss of immunoscores with an internal myometrial control. A significant up regulation of RXRα had been found in almost all ULMS, with an average of 5 fold increase compared with myometrium. There were slightly up regulations of bcl-2, c-kit, cyclin D1, hamartin and HMGA2. Minimal changes of ER and down regulation of PR were noted. By comparing the mean value of the selected markers from 120 leiomyomata, up regulation of RXRα and down regulation of PR were remarkable in ULMS.

**Conclusions:** There is a substantial difference of RXRα expression between leiomyomata and leiomyosarcoma. Our finding is the first time to show an up regulation of RXRα to be a factor in the pathogenesis of ULMS. A similar expression pattern of other selected tumorigenic factors between leiomyomata and ULMS indicates a possible connection in the tumorigenesis.




# Head & Neck

### 977 Leber Hereditary Optic Neuropathy Mutations in Head and Neck Cancer

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**Background:** Leber Hereditary Optic Neuropathy (LHON) is a specific mitochondrial disease, in which a primary role for mitochondrial dysfunction is confirmed by strict maternal inheritance and association with specific mutations in the mitochondrial DNA. LHON is characterized by early onset of visual loss (younger than 30 years of age), which is aggravated by the use of tobacco and alcohol. Previously, we sequenced the entire 16.6 kb mitochondrial DNA genome in 13 primary head and neck cancer and found that two secondary LHON mutations at 4216, and 13708 were present in multiple head and neck cancer. In this study, we analyze these two LHON mutations in head and neck cancer in order to identify 1) whether these two mutations are associated with increased risk of developing head and neck cancer; and 2) whether these two mutations affect tumor behavior and survival of head and neck cancer patients.

**Design:** One hundred and thirty three (133) head and neck cancer and 107 cases of normal skin of age-matched control subjects were obtained from the Department of Pathology at John L. McClellan Memorial Veterans Hospital in Little Rock. DNA samples from head and neck cancer and normal skin specimens were subjected to PCR-based restriction fragment length polymorphism (RFLP) analysis.

**Results:** T4216C and G13708A mutations were detected in 15/133 (11.3%) and 26/107 (19.5%) cases of head and neck cancer and in 12/107 (11.2%) and 20/103 (19.4%) cases of normal skin derived from control subjects. There is no statistical significant difference in the prevalence of these two mutations between head and neck cancer patients and control subjects. Using Kaplan-Miere Survival analysis. G13708A mutation was found to be significantly correlated with increased 2-year disease-free survival (decreased tumor recurrence) ($p = 0.03$) and increased overall patient survival ($p = 0.03$). T4216C mutation was not significantly correlated with tumor recurrence ($p = 0.59$) or patient survival ($p = 0.40$).

**Conclusions:** 1) G13708A Mitochondrial LHON mutation is significantly correlated with increased 2-year disease-free and 5-year overall patient survival. 2) The presence of T4216C and G13708A LHON mutations does not seems to increase an individual's risk of developing head and neck cancer.

### 978 Hierarchical Cluster Analysis of Several Myoepithelial Markers in Adenoid Cystic Carcinoma and Polymorphous Low Grade Adenocarcinoma Shows Expression of Smooth Muscle Actin, Calponin, Metallothionein and Smooth Muscle Myosin Heavy Chain To Be Most Discriminative

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**Background:** Morphologic similarity between adenoid cystic carcinoma (ACC) and polymorphous low-grade adenocarcinoma (PLGA) of the salivary glands frequently requires additional studies for their differentiation. We performed hierarchical cluster analysis to evaluate the expression of several myoepithelial / basal cell markers in ACC and PLGA in order to identify the most useful marker or combination of markers in their differential diagnosis.

**Design:** Archival tissues from 23 ACCs (10M: 13F; age range 23-82 years, median age 52) and 24 PLGAs (6M:18F; age range 32-94 years, median age 57) were studied by immunohistochemistry for the expression of smooth muscle actin (SMA), calponin, metallothionein (MT), smooth muscle myosin heavy chain (SMMS-1), maspin, and p63. The expression was interpreted as negative (no expression or expression in <10% tumor cells), focal (10-33% tumor cells) and diffuse (>33% tumor cells). The

expression was analyzed using hierarchical cluster analysis and Yates corrected chi-square test. The concordance between the expressions of different markers was analyzed using kappa statistics.

**Results:**

Expression of Markers in ACC and PLGA

| Markers | SMA (D) | Calponin (D) | MT (D) | SMMS (D) | Maspin (D) | p63 (D) |
|---|---|---|---|---|---|---|
| ACC (n 23) | 21 (20) | 21 (15) | 23 (22) | 18 (15) | 23 (22) | 22 (21) |
| PLGA (n 24) | 4 (1) | 5 (1) | 20 (8) | 11 (1) | 22 (14) | 20 (14) |
| p value* | <0.0001 | <0.0001 | 0.0001 | 0.0003 | 0.07 | 0.1 |

*Yates corrected chi-square, D diffuse

Diffuse expression of SMA, calponin, metallothionein and SMMS was significantly associated with ACC, and highly predictive of it. Hierarchical cluster analysis of the expression of these 4 markers was virtually identical with one another, which appears to suggest little advantage to their use in combination with each other.

**Conclusions:** The mere presence or absence of expression of myoepithelial / basal cell markers may not be sufficient to differentiate ACC from PLGA. However, diffuse expression of SMA, calponin, metallothionein and SMMS as defined by expression in more than one third of the tumor cells is highly predictive of ACC. SMA as a single marker may be most informative and economical.

### 979 Differential Disruption of the Rb Pathway in HPV-Related Head and Neck Carcinomas

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**Background:** Human papillomavirus (HPV), particularly the 16 subtype, has been confirmed as a causative agent in a subset of head and neck squamous cell carcinomas (HNSCCs). HPV-16 is present in most oropharyngeal cancers, but in only a small percentage of non-oropharyngeal HNSCCs. In HPV-positive carcinomas, transcription of the viral oncoprotein E7 inactivates the Rb gene product, causing a perturbation of other components of the Rb pathway. The purpose of this study was to confirm the site specificity of HPV-16 infection over a large group of anatomically diverse HNSCCs, and to establish the reliability of p16 and Rb expression as surrogate markers of HPV-16 infection.

**Design:** A tissue microarray was assembled from 177consecutive patients with HNSCCs from various sites (oral cavity, n = 61; larynx, n = 54; oropharynx, n=43; hypopharynx, n = 9; sinonasal cavity, n = 6; unknown, n = 3; nasopharynx, n = 1). Tissue array sections were evaluated by HPV-16 in-situ hybridization (ISH), p16 IHC, and Rb IHC.

**Results:** HPV-16 was detected in 38 of 177 (21%) cases by ISH. When stratified by site of origin, HPV-16 was detected in 37 of 43 (86%) tumors from the oropharynx, but in only one of 134 (0.7%) tumors from a non-oropharyngeal site ($p < 0.001$). P16 expression was associated with the presence of HPV-16: 31 of 38 HPV-16 positive tumors exhibited p16 expression, whereas only 9 of 139 HPV-16 negative tumors were p16 positive (82% versus 6%, $p < 0.001$). P16 expression also correlated with site of origin: 28 of 43 oropharyngeal tumors were p16 positive whereas only 9 of 134 non-oropharyngeal tumors were p16 positive (65% versus 7%, $p<0.001$). Rb expression inversely correlated with the presence of HPV16 ($p<.0001$): 33 of 38 HPV positive tumors demonstrated low/absent Rb expression, whereas only 16 of 139 HPV negative tumors demonstrated low/ absent Rb expression (87% versus 12%, $p<.0001$). Moreover, down regulation of Rb expression was much more likely to occur in p16 positive tumors than in p16 negative tumors (85% versus 21%, $p<.0001$). Low/absent Rb expression was much more common in oropharyngeal tumors than non-oropharyngeal tumors (70% versus 9%, $p<0.001$).

**Conclusions:** Oropharyngeal carcinoma is a biologically distinct subtype of HNSCC. In contrast to carcinomas arising in non-oropharyngeal sites, oropharyngeal carcinoma is commonly associated with HPV-16 and, in turn, with distinct perturbations of the Rb pathway including up-regulation of p16 and down-regulation of RB.

### 980 p63 and EGFR Expression in Olfactory Neuroblastoma

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**Background:** Numerous studies have searched for prognostic indicators in patients with olfactory neuroblastoma (ONB), an uncommon neuroectodermal neoplasm of the head and neck. While the Kadish stage and completeness of surgical resection provide useful prognostic information, certain histologic variables and immunohistochemical features, such as the presence of necrosis and the degree of Ki-67 and S-100 protein expression, also have been suggested to have prognostic value. We performed the current study in order to determine the expression and potential prognostic utility of p63 and EGFR in ONB, as well as to assess the possible prognostic importance of Ki-67 and S-100 expression.

**Design:** Forty-five patients with ONB were selected from the clinical files of the Dept. of Otolaryngology at the University of Virginia. We retrieved case material from nine patients for initial evaluation (all but one were Kadish stage C at presentation). Case material consisted of routinely processed, paraffin-embedded tissue from the Dept. of Surgical Pathology archives. We selected a representative block containing the largest amount of viable tumor from each case for evaluation of p63 and EGFR expression by immunohistochemistry. For each case, we also examined S-100 expression and determined the Ki-67 labeling index (LI).

**Results:** All nine cases studied thus far showed no immunoreactivity for EGFR. Four of nine cases showed occasional nuclear staining for p63. The tumors with focally positive p63 expression ranged in Hyams grade from two to four and were all from patients with stage C disease. All tumors that lacked p63 expression had Ki-67 LIs < 1.0, whereas two of the tumors showing p63 expression had the highest Ki-67 LIs (4.5 and 14.6). All cases expressed S-100, with peripheral staining of tumor nests (6 cases), staining of neurofibrillary material (2 cases), and focal staining of individual tumor cells (1 case).

**Conclusions:** All nine ONBs studied thus far lacked EGFR expression. This finding adds another neoplasm to the list of solid tumors lacking immunoreactivity for EGFR, suggesting that there is no role for selective EGFR tyrosine kinase inhibitors as adjunctive treatment of this rare neoplasm. Four cases, however, did show focal p63 expression and two of these four were the tumors with the highest Ki-67 LIs. Additional case material and clinical follow up from this patient cohort is being obtained in order to determine whether or not these markers have any prognostic value.

### 981 Papillary Thyroid Microcarcinoma: In Support of the Porto Proposal. A Review of 181 Patients

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**Background:** Papillary thyroid microcarcinoma (PMC) is defined by the World Health Organization as a tumor equal to or smaller than 10 mm in size. It is commonly found at autopsy, incidentally following thyroidectomy for another indication, or as an oncologically significant neoplasm in its own right. Controversy exists as to whether patients with incidental PMC require further treatment.

**Design:** Pathology files from MSSM were searched for all cases of PMC over a 10-year period. Slides were reviewed. Patient follow-up was achieved on 96 patients through telephone interviews (median 52 months). SAS software was used for logistic regression analysis.

**Results:** Two hundred forty-one patients were included in this study. Thiry-seven patients presented with PMC and lymph node metastastaes (oncologically significant PMC); 144 cases were incidental to benign thyroid disease and had no evidence of lymph node metastases (incidental PMC). Extrathyroid spread, multifocal PMC, PMC larger than 5 mm, and two specific histologies (solid and sclerotic) were each associated with oncologically significant PMC as opposed to incidental PMC. On logistic regression analysis, extrathyroid spread was the only determinant to remain significant ($p = 0.026$). The locoregional recurrence rate for oncologically significant PMC was 16% (6 of 37 patients) after a median of 12 months. No patient with incidental PMC developed recurrent disease, or "converted" to positive lymph node status after a median of 54 months.

**Conclusions:** The single most important consideration for PMC is the clinical lymph node status at presentation. Incidental PMC disovered after hemi- or total thyroidectomy, in all likelihood, requires no further therapy. If extrathyroid extension is present in an incidental PMC, then imaging of the ipsilateral neck, and contralateral lobe are the only recommended actions. By contrast, oncologically significant PMC are distinctly different tumors from their onset, and have a small propensity for locoregional recurrence. These patients require radioactive uptake imaging and therapy, and close follow-up for local recurrence or pulmonary metastastasis.

### 982 Tissue Distribution of HPV-16 Viral Integration in Patients with Tonsillar Carcinoma as Visualized by HPV-16 In-Situ Hybridization and p16 Immunohistochemistry

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**Background:** Human papillomavirus, particularly the HPV-16 subtype, has been strongly associated with tonsillar squamous cell carcinoma (TSCC). Although HPV-16 is detected in the majority of TSCCs, very little is known about its distribution in tonsils that harbor carcinoma or, for that matter, those that do not harbor carcinoma.

**Design:** Using a highly sensitive signal amplification method that permits recognition of viral copy numbers even at low levels, we performed HPV-16 in-situ hybridization (ISH) on the ispsilateral and contralateral tonsils from 8 patients with TSCCs and from 5 age-matched patients without TSCC. P16 immunohistochemistry was also performed because HPV-16 integration is believed to disrupt the retinoblastoma pathway and induce overexpression of p16. To establish the specificity of p16 overexpression for HPV-16 viral integration into tonsillar crypt epithelium, we also analyzed 37 non-HPV-related lymphoepithelial cysts of the head and neck.

**Results:** HPV-16 was detected in all 8 of the TSCCs by ISH. In these cases the hybridization signal was strictly confined to the carcinoma and associated dysplastic epithelium. HPV-16 was not detected in histologically unremarkable epithelium of the ipsilateral tonsil, the contralateral tonsil, or control tonsils from patients without TSCC. P16 overexpression was more widely distributed: Not only was p16 overexpression present in all 8 TSCCs, but it was consistently present in the specialized reticulated epithelium of the tonsillar crypts of patients with and without TSCC. Moreover, p16 overexpression was also noted in lymphoepithelial cysts of the lateral neck (9 of 29, 31%) and parotid gland (7 of 8, 88%).

**Conclusions:** Although the palatine tonsils are known to act as important reservoirs and replication sites for certain pathogenic viruses, we were unable to detect HPV-16 integration in non-neoplastic tonsillar tissues. Instead, visualization of HPV-16 was strictly localized to TSCC and associated dysplastic epithelium, implicating viral integration as a critical and causative step in malignant transformation of the tonsils. Strong p16 staining of the reticulated epithelium - irrespective of HPV-16 status - underscores the biologic distinctiveness of this epithelium, and calls for caution when using p16 staining as a diagnostic or biologic tool for HPV-16 detection.

### 983 Down Expression of Syndecan-1 (CD138) in Nasopharyngeal Carcinoma (NPC)

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**Background:** Nasopharyngeal carcinoma (NPC) is one of the important head and neck malignancies in Taiwan. Pathologically, most NPC tissues are poorly differentiated

squamous cell carcinoma. The differentiation of squamous cell was positively correlated with expression of syndecan–1 (CD138). The expression of CD138 in NPC has never been studied before.
**Design:** Detection of expression of CD138 in primary, recurrent, and metastatic NPC was performed in formalin–fixed paraffin–embedded sections using adequate antibody against CD138 by immunohistochemistry. The quantity of CD138 mRNA in tumor cells of primary and metastatic NPC samples was detected by real time PCR using laser capture microdissection. The results were analyzed and correlated with clinical data.
**Results:** Totally 86 primary, 37 recurrent, and 16 metastatic NPC samples were available for detecting the expression of CD138 protein in paraffin sections. Of them, 68 (79.1%) primary, 28 (75.7%) recurrent, and 9 (56.3%) metastatic cases were negative for staining. The real time PCR data revealed low amount of CD138 mRNA in both primary and metastatic NPC as compared with internal housekeeping gene. Correlated with clinical data, positive CD138 staining was more in patients survived less than 5 years.
**Conclusions:** Our findings concluded that low expression of CD138 was found in NPC and the down regulation of CD138 in NPC was at the transcription level. The expression of CD138 in NPC tumor cells was related with worse prognosis of patients ($p<0.05$).

### 984 Chemokine Receptor Expression in Metastatic Destination of Nasopharyngeal Carcinoma (NPC)

*CL Chen, DL Ou, LI Lin, AL Cheng.* Taipei Medical University; School of Medicine, National Taiwan University, Taipei, Taiwan.
**Background:** Nasopharyngeal carcinoma (NPC) is one of important head and neck malignancies in Taiwan. Clinically, early metastasis to neck lymph nodes and lately to viscera including liver and lung are common in NPC patients. Metastasis of tumor involves several steps and chemokines and their receptors may play certain role in promoting metastasis and enhancing growth of tumor cell in distant organ.
**Design:** Quantitative analysis of the expression of chemokine receptors (CCR1–CCR10, CXCR1–CXCR6, XCR and $CX_3CR1$) was performed in five human NPC cell lines to determine whether chemokine/chemokine receptor interactions are involved in NPC metastatic process. The expression of CCR7, CXCR4, and CXCR6 in primary and metastatic NPC was also done on paraffin sections by immunohistochemistry.
**Results:** All five NPC cell lines exhibited CCR7, CCR9, CXCR4, and CXCR6 mRNA of different amount. Chemotactic analysis and actin polymerization assay confirmed the biological relevance of chemokine–mediated chemotaxis of the cells bearing corresponding chemokine receptors. By immunohistochemistry, CCR7, CXCR4, and CXCR6 were randomly expressed in primary NPC (n=59) but interestingly were highly expressed in 18 metastatic NPC (n=18).
**Conclusions:** Our findings suggested that chemokines and their receptors might have a critical role in determining the metastatic destination of NPC tumor cells.

### 985 Allelic Loss of Three Different Tumor Suppressor Gene Loci in Benign and Malignant Endothelial Tumors of the Head and Neck

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**Background:** Angiosarcomas are rare malignancies that often arise in the head and neck. No definitive precursor lesion is known and only rare reports of malignancy developing in pre-existing hemangiomas exist. The pathogenesis of angiosarcomas and the DNA-based changes that accompany carcinogenesis are poorly understood. Recent evidence at the cell culture level suggested that loss of heterozygosity of 13p may be involved in malignant transformation of endothelial cells. Although p53 and WT-1 are thought to be over-expressed in angiosarcomas, little is known about the molecular changes in these genes. This study sought to compare the molecular profile of angiosarcomas with that seen in hemangiomas and granulation tissue.
**Design:** Cases of angiosarcoma, benign hemangioma, and granulation tissue were included in this study. Microdissection of unstained blank histologic sections was performed. DNA was extracted and PCR amplifications with fluorescently-labeled primers were done for eleven microsatellite markers adjacent to the tumor suppressor genes WT-1, RB, and p53. Amplification products were analyzed for LOH by capillary electrophoresis using the ABI PRISM 3100 Genetic Analyzer. Allelic loss was assessed by comparing the tumor ratio with the ratio obtained in corresponding normal tissue. Fractional allelic loss (FAL) was calculated using the number of allelic losses divided by the number of informative markers.
**Results:** Mean fractional allelic loss (FAL) was 43% for angiosarcomas and 29% for benign hemangiomas. 83% of angiosarcomas had allelic loss in loci at 17p (p53), 66% at 13q (WT-1) and 50% at 11p (RB). Allelic loss was seen in 80% of benign hemangiomas on both chromosomes 13q and 17p respectively, but only 20% at 11p. Two of the cases of benign hemangiomas did not have any LOH. The granulation tissue had no allelic losses.
**Conclusions:** These data support the cell culture evidence that transformation of endothelial cells likely involves loss of chromosomes 11p, 13q and 17p. In our study, 11p and 13q allelic losses were present in both hemangiomas and in angiosarcomas, while most allelic losses at 11p were seen in angiosarcomas. There were no major differences in FAL between angiosarcomas and hemangiomas. These results may provide interesting insight into the pathogenesis of benign and malignant vascular tumors and also suggest genotypic overlap between hemangioma and angiosarcoma.

### 986 Comparative Genetic Analysis of Intestinal and Seromucinous Sinonasal Adenocarcinomas: Distinct and Common Alterations from Primary Colorectal Carcinomas

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**Background:** Sinonasal adenocarcinomas (SNAC) are composed of two main histopathologic subtypes that include the intestinal (ITAC) and the seromucinous (SMAC) subtypes. To identify genetic events associated with their development and progression, an analysis of common genetic events seen in primary colonic adenocarcinomas was performed.
**Design:** Sixteen SNAC from an equal number of patients who underwent curative resection or biopsy at M.D. Anderson Cancer Center comprised the material of the study. Clinical information along with pathology data were retrieved from the medical records. Gene mutation analysis for K-ras, APC, ß-catenin, hMLH1 and hMSH2 genes and p53 expression using immunohistochemistry was performed.
**Results:** The study patients included 13 males and two females. The tumors were predominantly located in the ethmoid sinus. Eight cases were ITAC and seven were SMAC. One case of metastatic colonic adenocarcinoma was included. Nine patients were smokers and four were non-smokers. A smoking history was not available for four patients. Two of eight patients with ITAC had a history of wood dust exposure. Mutational analysis showed K-ras gene mutations in two of the eight ITAC at the 12A or 12B codon. Microsatellite instability at hMLH1 was identified in a single case of ITAC. No alterations of hMSH2, APC or ß-catenin were found among patients with ITAC. None of the SMAC had genetic mutations. One patient with ITAC and 2 patients with SMAC had overexpression of p53.
**Conclusions:** A subset of ITAC share the K-ras mutation with colonic adenocarcinomas. However, the ITAC lacks other alterations commonly seen in colonic primaries as well as in the seromucinous subtype. P53 alterations are associated with aggressive tumor behavior in both subtypes.

### 987 Novel Mitochondrial DNA Mutations and Polymorphisms in Head and Neck Cancer

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**Background:** Mitochondrial genome is highly susceptible to mutations due to the high concentration of reactive oxygen species produced during respiration, and a relative lack of DNA repairs capacity. Mitochondrial DNA mutations are implicated in the development of cancer because mitochondrial dysfunction can affect apoptosis and other key cellular processes in tumor development. In this study, we sequenced the entire mitochondrial genome in 13 cases of head and neck cancer to identify a spectrum of mutations and/or genetic polymorphisms that is specific for this tumor type.
**Design:** Thirteen cases (13) of fresh head and neck cancer and matched stromal soft tissues were obtained from the Department of Pathology at John L. McClellan Veterans Hospital in Little Rock. DNA samples from these specimen were subjected to high-fidelity PCR amplification, for the entire 16.6 kb mitochondrial genome, followed by direct DNA sequencing.
**Results:** A total of 163 alterations were identified. Thirty-eight (38) alterations have never before reported and these include 4 tumor-specific mutations (mutations that are present in tumor but not in adjacent stroma) and 34 novel genetic polymorphisms. Among 125 previously reported alterations, 6 are linked to specific mitochondrial diseases and these are A3796G, T4216C, A4917G, G5460A, A12308G and G13708A, affecting six patients. The prevalence of mitochondrial disease-associated polymorphisms in this study is thus estimated at 46% (6 of 13). T4216C, A4917G and G13708A polymorphisms are correlated with Leber Hereditary Optic Neuropathy (LHON). A3796G is associated with Adult-Onset Dystonia (AOD) and G5460A is linked to Alzheimer's Disease (AD). A12308G is associated with Chronic Progressive External Ophthalmoplegia (CPEO). All disease specific polymorphisms resulted in amino acid changes except for A12308G, which affects the tRNA for Leucine.
**Conclusions:** 1) majority of the mitochondrial DNA alterations are polymorphisms that are maternally inherited and present in both tumor and the matched stromal soft tissue. These genetic alterations may contribute to an individual's susceptibility to cancer. 2) Tumor-specific mutations constitute only a small fraction of mitochondrial DNA alterations and may play important roles in the development of cancer.

### 988 Salivary Gland Pleomorphic Adenoma: ß-Catenin Immunoexpression Does Not Correlate with *PLAG1* Activation Related to t(3;8)(p21;q12)

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**Background:** Salivary gland pleomorphic adenoma (PA) is characterized, among others, by t(3;8) (p12;q21) that activates, through promoter swapping, the *PLAG1* gene and leads to reduced levels of *CTNNB1*/ß-catenin gene. ß-catenin is a protein involved in mechanisms of epithelial dedifferentiation and maintenance of a mesenchymal phenotype, which are postulated to occur in PA. In order to evaluate at tissue level whether the alteration of ß-catenin gene expression is related to altered topography of protein localization, and in which cells it occurs, we studied two groups of PA, with and without t(3;8). E-cadherin, as a potential modifier of ß-catenin localization, was also evaluated.
**Design:** Twenty one cases of PA were selected, based on the presence of 8q lesions. FISH analysis of PLAG1 rearragements and immunohistochemical analysis of ß-catenin and E-cadherin were performed. For comparative purposes of ß-catenin staining intensity, SW480 colorectal carcinoma cell line, with known levels of protein,

was used. The membranous, cytoplasmic and nuclear staining was scored 1-3+ in epithelial, myoepithelial and metaplastic mesenchymal areas. E-cadherin was scored 1-3+, the latter being the normal continuous decoration of cell membranes and 2+ being a discontinuous staining pattern.
**Results:** Fourteen cases had t(3;8) and the remaining had alternative 8q lesions. *PLAG1* gene rearrangements were present in 12 cases. All but one case showed modification of ß-catenin localization, there being no difference (p=0.08) between cases with and without t(3;8). Nuclear expression of ß-catenin was found in 10 and 6 cases with and without t(3;8), respectively. A 3+ intensity was only found in cells with myoepithelial and mesenchymal differentiation, the epithelial cells being always negative, either duct-type or metaplastic squamous. E-cadherin was shown in all cell types with scores 2 to 3+.
**Conclusions:** Modification of ß-catenin localization does not correlate with the downregulation of *CTNNB1* related to activation of *PLAG1* in tumors with t(3;8) or with E-cadherin alterations. PA cells exhibiting a mesenchymal phenotype probably represent, as previously suggested transit cells involved in epithelial-mesenchymal transition.
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### 989 Expression of Fascin, Epstein-Barr Virus(LMP-1) and Ki-67 in Nasopharyngeal Carcinoma

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**Background:** Nasopharyngeal carcinoma (NPC) is a distinctive, highly invasive head and neck carcinoma with a close association with Epstein-Barr virus (EBV). Up-regulation of motility is essential for enhancement of a carcinoma's invasive potential. Fascin, an actin-bundling protein, has been shown to have an important role in cell-matrix adhesion, cell interaction and cell migration in some tumors. The expression of fascin is greatly increased in EBV infected B-lymphocytes and virus-transformed fibroblasts. EBV-encoding latent membrane protein-1 (LMP-1) is a primary oncogene since it shows transforming activity in various cell types in vitro and is suggested to enhance the invasive properties of NPC. The relationship between expression of fascin and LMP-1 in NPC is unknown. In this study, we evaluated the correlation of fascin expression with LMP-1 and Ki-67 in NPC.
**Design:** 24 nasopharyngeal carcinoma biopsy specimens and a lymph node with NPC metastasis were selected from the pathology archival files of Bellevue Hospital. The tumors were classified using the World Health Organization (WHO) classification. Fascin, LMP-1 and Ki-67 expression was investigated by immunohistochemistry using a semiautomated immunostainer (NexES).
**Results:** 22 of 25 tissue samples came from Chinese patients. Fascin was expressed in 72 % of NPC (18 of 25) and 33.3 % of them (6 of 18) showed positive staining in more than 50% of the tumor. The undifferentiated carcinoma (WHO type III) accounted for 68 % (17 of 25) of all tumors but could be segregated into two distinct groups of staining; 8 of 25 had both high fascin and Ki-67 expression and 6 of 25 had low fascin but mixed Ki-67 expression. WHO type II NPCs uniformly had intermediate expression of both fascin and Ki-67. High EBV latent membrane protein-1 expression was noted in all cases. In the lymph node metastases, fascin was low with a moderate Ki-67 expression.
**Conclusions:** An important property of invasive tumors is the ability to become mobile. In this study, we found a positive association between Ki-67 and fascin in NPC. There was a uniform but moderate expression of fascin and Ki-67 for WHO type II NPC but there are two divergent groups for WHO type III NPCs. EBV gene status was not correlated because of its ubiquitous nature in all cases. The WHO type III may have unpredictable behavior depending on the expression of fascin and Ki-67; however, WHO type II NPC is should behave in a relatively more predictable manner.

### 990 FHIT (Fragile Histidine Triad Gene): Microsatellite Instability, Loss of Heterozygosity and Decreased Protein Expression in Oral Squamous Carcinoma

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**Background:** The FHIT gene encompasses the FRA3B locus and is thought to be altered primarily by deletions and alterations. FHIT likely functions as a tumor suppressor and comes in to play early in the carcinogenesis pathway. Loss of FHIT protein expression is seen in tumors from various sites and some investigators believe that FHIT gene therapy may be possible ultimately. We wished to study this gene and its product in oral cavity squamous cell carcinoma (OCSCC).
**Design:** 53 patients with initially treated OCSCC were followed a minimum of 5 years or until death. FHIT protein expression was studied in all patients with immunohistochemistry and was considered reduced when less than 10% of the tumor was stained. A subset of patients (20) were studied for allelic loss of heterozygosity (LOH) and microsatellite instability (MSI) using microsatellite primers that lie adjacent to the FHIT locus (D3S1766, D3S1289, D3S2408 and D3S1300). These primers were fluorescently labeled and used in a polymerase chain reaction employing microdissected paraffin embedded tissue for normal or tumor DNA. This reaction product was assayed by automated capillary gel electrophoresis. LOH was determined by calculation of the ratio of the peak heights of normal and tumor alleles. LOH was defined as a normal:tumor peak height ratio of less than 0.5 or more than 2.0. MSI was defined by the presence of novel peaks in the tumor DNA compared to normal. A tumor exhibited high MSI (H-MSI) if more than one novel peak out of the four loci was seen and low MSI (L-MSI) if there was only one novel peak. Clinical findings and followup were correlated with the molecular abnormalities and protein expression.
**Results:** 56% (10/18) of patients with reduced FHIT expression were dead of disease while 37% (13/35) of patients with preserved FHIT expresssion were dead at 5 year followup. FHIT expression did not correlate to nodal disease, stage or size of tumor. LOH was seen in 10, L-MSI in 4, and H-MSI in 1 of the 20 patients tested. We found no correlation between LOH at loci near the FHIT gene and protein expression. Neither LOH or MSI were found to correlate to clinicopathologic observations of stage, nodal status or survival.
**Conclusions:** FHIT appears to be associated with a worse survival outcome when its expression is reduced in OCSCC. LOH or MSI at or near the FHIT locus does not appear to be associated with loss of protein expression or clinicopathologic findings. This suggests that alterations of FHIT protein expression may lie at levels other than the gene.

### 991 p16INK: A New Marker To Identify Papillary Squamous Cell Carcinomas of the Head and Neck

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**Background:** Exophytic and papillary squamous cell carcinomas of the head and neck can be diagnostically challenging, especially in small biopsy samples. Furthermore, there are very few special stains that can help to differentiate these tumors from their benign counterparts, such as keratoses or papillomas. Prior evidence suggests that p16INK, the protein product of the p16 tumor suppressor gene, is expressed in mucosal dysplasias of the head and neck. This study examined p16INK expression in a group of papillary and exophytic squamous cell carcinomas of the head and neck.
**Design:** Cases with the diagnosis of papillary or exophytic squamous cell carcinoma were selected from the archives of the files of the University of Pittsburgh Medical Center. The tumors were examined for evidence of invasion and for morphologic findings. Immunohistochemical stains were performed using a standard streptavidin-biotin approach. The stains were assessed semi-quantitatively for the percentage of cell staining and the stain intensity using a scale of 0-3. Controls included benign squamous mucosa and other benign papillary and keratotic lesions.
**Results:** 21 papillary and exophytic squamous cell carcinomas of the head and neck were included in this study. The tumors occurred predominantly in larynx (11 case) and oral cavity (6), with various other sites being in the minority (4 cases). The mean age of the patients was 69 years. 20 of 21 tumors had at least some p16INK staining, with the mean staining intensity being 64%. Fourteen tumors showed significant staining of greater than 40% of the tumor component. No correlation was found between the subtype (exophytic vs. papillary) or the presence of invasion and the stain intensity. Benign control lesions were essentially negative for p16INK staining as was non-dysplastic adjacent mucosa in the cases.
**Conclusions:** Papillary and exophytic squamous cell carcinomas of the head and neck can present a diagnostic challenge to the surgical pathologist, particularly in biopsies. Morphologically, benign and malignant papillary lesions can have a similar appearance. In this study, benign squamous and keratotic lesions were essentially negative for staining with p16INK. In contrast, papillary and exophytic squamous cell carcinomas showed strong staining in the majority of cases. The high prevalence of strong positive staining with p16INK in these difficult lesions may provide a useful tool to aid in diagnosis of small or superficial biopsies.

### 992 Microsatellite Instability in Lymphoepithelial Carcinomas of the Head and Neck

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**Background:** Microsatellite instability is reportedly present in high levels in undifferentiated nasopharyngeal carcinomas (NPC) that are EBV positive in endemic areas. In conventional squamous cell carcinoma, the reported rates of microsatellite instability are low, as these tumors develop through pathways that involve the biallelic inactivation of tumor suppressor genes. Lymphoepithelial carcinomas (LEC) arising in other locations have a similar histologic appearance to NPC, but are usually negative for EBV and may not have the same etiology. This study examined a group of LECs in the United States for the presence of microsatellite instability (MSI).
**Design:** Unstained slides were microdissected and DNA from tumor and normal was extracted. PCR was performed using fluorescent-labeled primers for the 5 NCI-recommended microsatellites (D2s123, D5s346, D17s.250, BAT 25, and BAT 26). The PCR products were analyzed using capillary electrophoresis. The normal tissue was examined first, and then any novel sized PCR products in the tumor tissue were scored as MSI. Two or more loci with MSI indicates MSI-high level, one locus is MSI-low level, and no loci with MSI means the tumor is microsatellite stable.
**Results:** 19 cases of LEC of the head and neck from the United States were included in this study. They arose in nasopharynx (8 cases), tonsil (4 cases), and other sites (7 cases). The mean age of the patients was 61 years. The patients did not have any history of other tumors, nor any family history of carcinomas. Only 4/14 cases were positive for EBV using in situ hybridization. Six cases had microsatellite instability, but only three of these had high level MSI (2/5 loci in two cases and 5/5 loci in 1 case). 8 cases showed loss of heterozygosity at the markers tested for MSI and only one of these cases had concomitant MSI.
**Conclusions:** Microsatellite instability was present in only 6/19 cases in this series of head and neck LECs from the United States. This is in contrast to prior reports of LECs in Asia that showed frequent MSI. These data support the theory that Western LECs that are not usually EBV-related may not have an association with DNA mismatch repair defects. Rather, it is more likely that inactivation of tumor suppressor genes that is involved in carcinogenesis in conventional squamous cell carcinoma is also involved in these Western LECs.

### 993 Post-Therapy Histologic Patterns of Tumor Regression in Cervical Lymph Nodes Correlate with RT-PCR-Detected Minimal Residual Disease and Locoregional Recurrences in Metastatic Head and Neck Squamous Cell Carcinoma

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**Background:** Nodal metastasis is a significant adverse prognostic factor for patients with squamous cell carcinoma (SqCC) of the head and neck and is an indication for radiation therapy (RT), or concurrent chemoradiation therapy (CRT) followed by neck dissection (ND). The purpose of this study is to evaluate the significance of histologic patterns of tumor regression in neck dissection specimens following preoperative RT or CRT.

**Design:** We reviewed the records and slides of patients with primary mucosal head and neck SqCC treated with RT or CRT and subsequent planned ND between 1995 and 2002. Lymph nodes from each ND (22 post-RT; 39 post-CRT) were histologically subclassified into five groups; those with residual tumor, granulomatous reaction (keratin granulomas or abundant foamy histiocytes with or without giant cells), fibrosis, and normal-appearing lymph node. Reverse transcriptase PCR (RT-PCR) for keratin 5 (CK5) and squamous cell carcinoma antigen (SCCA) was performed in paraffin-embedded tissue in all groups. Positivity for either CK5 and/or SCCA was considered evidence of minimal residual disease in H&E negative cases.

**Results:** There were no significant differences in mean interval from end of treatment to ND or from diagnosis to recurrence between the groups. Excluding cases with unsuccessful RT-PCR, a patient lost to follow-up, and one who developed a lung metastasis, the results show that both the RT-PCR positivity rate and the rate of locoregional recurrence were highest in patients with residual tumor, intermediate in patients with normal or fibrotic H&E negative nodes and lowest in patients with negative nodes with granulomatous reaction.

| Histologic Pattern | RT-PCR+ | Recurrences |
|---|---|---|
| Residual tumor (n=16) | 8/9 (88.9%) | 8/15 (53.3%) |
| Fibrosis (n=6) or normal (n=6) | 5/10 (50%) | 3/12 (25%) |
| Keratin granulomas (n=8) or histiocytic response (n=10) | 7/16 (37.5%) | 2/18 (11.1%) |

**Conclusions:** Our study confirms that the persistence of metastatic tumor in cervical lymph nodes following radiation or chemoradiation is a strong predictor of locoregional failure. A somewhat surprising result is that a post-therapy granulomatous response predicts a favorable outcome, confirming the findigs of another study (Westra WH et al Head Neck 1998;20:515-21). Further studies are needed to fully understand the biologic significance of this finding.

### 994 P16 and pRb Expression in Oral Suqamous Cell Carcinoma and Cervical Cancers-Different Carcinogenetic Pathways?

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**Background:** High-risk human papillomaviruses(HPV) are known as the causative agents of cervical squamous lesions and cancers and are also believed to be etiologically involved in a subset of squamous cell carcinomas of the head and neck region. It is generally assumed the same pathways are involved in human papillomavirus-induced carcinogenesis—that the malignancies arise through upregulation of the protein product of gene $p16^{INK4}$ (p16) by the human papillomavirus oncoproteins E6 and E7, which will disrupt the pathways of p53 and the product of the retinoblastoma (Rb) gene. We test the hypothesis that all HPV-related malignancies lead to the same carcinogenetic pathway.

**Design:** 38 HPV-positive oral squamous cell carcinomas (OSCC), 47 HPV-negative OSCC, eight HPV 16-positive cervical squamous cell carcinomas, and nine HPV 11 and 6 –positive low grade squamous intraepithelial lesions (LSIL) were examined by immunohistochemistry for p16 and pRb.

**Results:** There are no differences of expression of pRb and p16 between HPV- and non-HPV- related OSCC groups. Comparisons of the OSCC and combined cervical data showed that cervical cancers and squamous intrapeithelial lesions were significantly more likely to have overexpression of p16 and downregulation of pRb than HPV-related OSCC.

**Conclusions:** These findings represent new evidence that HPV-induced mucosal carcinogenesis in different anatomic locations may lead to dissimilar molecular pathways.

### 995 Decreased Immunohistochemical Expression of E-Cadherin but Not ß-Catenin Is Associated with Increased Risk of Vascular Invasion and Decreased Survival in Head and Neck Squamous Cell Carcinomas

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**Background:** One step in the development of tumor metastasis is detachment of malignant cells from their site of origin. E-cadherin and ß-catenin are cell membrane-associated proteins involved in cell-cell adhesion, and loss of expression of the cadherin/catenin complex has been described in various human malignancies. The aim of our study was to correlate the immunohistochemical expression of E-cadherin and ß-catenin to other clinicopathologic features and survival in a series of head and neck squamous cell carcinomas.

**Design:** The study included 45 patients who underwent resection of head and neck squamous cell carcinoma along with evaluation for cervical lymph node metastases. Thirty-two tumors arose in the oral cavity (5 stage I, 3 stage II, 4 stage III, and 20 stage IV), 9 tumors originated in the larynx (1 stage I, 4 stage III, and 4 stage IV), and 4 tumors began in the hypopharynx (1 stage II and 3 stage IV). All patients were followed for a minimum of 3 years or until death, and the 3-year survival rate was 64%. Formalin-fixed, paraffin-embedded tumor sections from each resection were stained immunohistochemically for E-cadherin and ß-catenin expression. Only membranous staining (of any intensity) was evaluated. Tumors were considered to exhibit "high expression" if greater than 50% of the tumor cells were positive and "low expression" if less than 50% of the tumor cells were positive for membranous staining.

**Results:** Low expression of E-cadherin was observed in 38% of cases and was significantly associated with the presence of vascular invasion (59% vs. 25%; p=0.031) and decreased 3-year survival (35% vs. 82%; p=0.003). There was no relationship between the degree of E-cadherin expression and tumor site, T status, tumor stage, cervical lymph node status, tumor grade, or perineural invasion. No statistically significant association between the degree of ß-catenin expression and the investigated clinicopathologic variables was detected.

**Conclusions:** In this series of 45 patients with head and neck squamous cell carcinomas, decreased immunohistochemical expression of E-cadherin but not ß-catenin was significantly associated with increased risk of vascular invasion and decreased survival. These findings raise the possibility that loss of cell-cell adhesion due to reduced expression of E-cadherin could contribute to vascular invasion and poor outcome in head and neck squamous cell carcinomas.

### 996 Does HPV Infect Glands in Biphasic Papillomatous Lesions of the *Bronchus* (Bronchial Adenoma Papilliferum, BAP), *Salivary Gland* (Sialadenoma Papilliferum, SAP), and *Skin* (Syringocystadenoma Papilliferum, SCP)?

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**Background:** Recently described biphasic lesions with glandular proliferation in association with surface squamous papillomatosis such as BAP and SAP are suspected to be HPV related. A closely related biphasic lesion of the skin, SCP has recently been shown to be HPV positive. To our knowledge, there is no study that address infection of glandular/ductal epithelium in the above locations. In this study we wish to evaluate whether HPV infection is present in these biphasic lesions and whether it simultaneously infects glands/ducts.

**Design:** We evaluated 23 archival papillomatous lesions of the upper and lower airway and skin: 1 BAP, 1 SAP, 7 SCP, 11 laryngeal and oral papillomatosis and 1 recurrent lung papillomatosis. All cases had *adjacent glands* for evaluation. In situ hybridization using a *low risk, LR* (6,11, 42, 43, 44) and a *high risk probe, HR* (16, 18, 31, 33, 35, 45, 51,52, 56, 58, 59, 68, 70) and Ventana automated BenchMark System, was performed. Negative control with out probe was used for each specimen.

**Results:** Of the 23 specimens, 7 were positive for **LR (43%)** in a diffuse nuclear pattern (*episomal*) on the surface of the **squamous/papillomatous** areas. All 23 specimens were positive for **HR(100%)** in a sparse punctate nuclear pattern (*integrated*). The staining for HR was scattered randomly and included the basal layer. In the **glandular/ductal areas** 1/23 (**4%**) was positive for LR in similar pattern as above and 15/23 (**65%**) for HR in a punctate pattern as above. See table for the breakdown of cases. The pattern was reproducible in two cases where recurrences were examined (see * in table). The negative controls were uniformly negative.

| LESION | #OF SPECIMENS | SQUAMOUS COMPONENT | | DUCTAL,GLANDULAR, NON SQUAMOUS PAPILLARY COMPONENT | |
|---|---|---|---|---|---|
| | | LOW RISK | HIGH RISK | LOW RISK | HIGH RISK |
| SAP | 1 | 1 | 1 | 0 | 0 |
| BAP | 1 | 1 | 1 | 0 | 0 |
| SCP | 7 | 1 | 7 | 0 | 7 |
| Lung Papillomatosis | 3* | 3 | 3 | 0 | 1 |
| Oral and Laryngeal Papillomatosis | 11 | 4 | 11 | 1 | 7 |
| Total # | 23 | 10 (43%) | 23 (100%) | 1(4%) | 15 (65%) |

**Conclusions:** BAP, SAP and SCP are positive for LR and HR HPV in the squamous component. Surprisingly, the ductal/glandular components are predominanly positive for HR in a high proportion of cases, in a sparse punctate nuclear pattern implying integration. The latter may serve as a viral reservoir that may predispose the epithelium to recurrent lesions or malignancy.

### 997 Alternative Epithelial Markers p63 and MOC-31 in Spindle Cell Carcinomas of the Head and Neck

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**Background:** Spindle cell (sarcomatoid) carcinomas of the head and neck region are aggressive, often grossly ulcerated and polypoid, variants of squamous cell carcinoma. These tumors often stain only focally or not at all by immunohistochemistry for traditional epithelial markers such as pancytokeratin (CK) and epithelial membrane antigen (EMA). These features make distinction from true sarcoma or other nonsarcomatous spindle cell lesions difficult for the pathologist, particularly on small biopsy specimens where growth patterns can't be evaluated and where surface epithelium or a traditional squamous cell carcinoma component are absent. We sought to evaluate the alternative epithelial markers p63 and MOC-31 for their ability to stain spindle cell carcinomas by immunohistochemistry and to also test their specificity for epithelial differentiation on a series of sarcomas, melanomas, and granulation tissue polyps.

**Design:** Formalin-fixed, paraffin-embedded archival tissue was obtained from 19 primary spindle cell carcinomas of the head and neck, 41 various primary and metastatic sarcomas, 8 primary and metastatic melanomas, and 8 granulation tissue polyps from the head and neck. All were stained with monoclonal antibodies to CK, EMA, MOC-31, and p63 with results recorded by a single observer (JSL).

**Results:** Among the 19 spindle cell carcinomas, the spindle cell component was positive in 12/19 (63%) for p63, 3/19 (16%) for MOC-31, 5/17 (29%) for CK, and 9/19 (47%) for EMA. In the sarcomas, 3/41 (7%) were positive for p63, 5/41 (12%) for MOC-31, 6/41 (15%) for CK, and 19/41 (46%) for EMA. Of the 8 melanomas, only one was focally positive for MOC-31. All markers were negative in the 8 granulation tissue polyps.
**Conclusions:** p63 is a sensitive and specific marker of epithelial differentiation in spindle cell carcinomas of the head and neck and should be useful diagnostically in conjunction with immunohistochemistry for CK and EMA, particularly in challenging cases.

### 998 High Incidence of Extracapsular Tumor Spreading in Persistent or Recurrent Neck Mass from Nasopharyngeal Carcinoma (NPC) Patients after Radiotherapy

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**Background:** For nasopharyngeal carcinoma, radiotherapy with or without adjuvant chemotherapy is the treatment of choice. In Taiwan, more than 60% of patients presented neck metastasis when initial diagnosis was made. After radiotherapy, however, the neck mass may decrease in size but still clinically detectable. Also, a few patients may come up with new neck mass after radiotherapy. In order to elucidate a better treatment protocol, we retrospectively performed analysis on the clinical and pathological features of NPC patients who have received surgery because of persistent or newly developed neck mass after radiotherapy.
**Design:** From the archives of Dept. of Radiooncology and Dept. of Otolaryngology between 1990 and 2003, a total of thirty-five NPC patients received surgical treatment because of persistent or newly appeared neck mass after radiotherapy. Radical neck dissection or neck mass excision was both used as surgical approaches for these patients. Clinico-pathological data was retrospectively reviewed and analyzed.
**Results:** Among 35 patients, 17 patients showed residual neck mass (group A) and 18 patients developed new neck mass after radiotherapy (group B). Eleven patients in group A and 12 in group B received radical neck dissection while the remained patients received neck mass excision only. Tumor cells were identified histopathologically in 70.6% (12/17) of group A patients and in 88.9% (16/18) of group B patients. EBER-1 in situ hybridization (ISH) or anti-keratin immunohistochemistry (IHC) can increase the rate of tumor detection, especially in cases with severe fibrosis. Extracapsular spreading of tumor cells was found in 50% (6/12) and 62.5% (10/16) of patients in group A and group B, respectively. Regional distribution of neck metastasis was diverse since all neck lymph node groups (level I to level V) were involved. Local recurrence and /or distant metastasis were also noted in 70.6% (12/17) and 61.1% (11/18) of patients in group A and group B.
**Conclusions:** Combined IHC with EBER-1 ISH, NPC tumor cells can be identified up to 80% of the patient's neck mass after radiotherapy. And more than half of them show extracapsular tumor spreading microscopically. Therefore, we would recommend aggressive surgical procedure (radical neck dissection) in time when neck mass exist in NPC patients after radiotherapy.

### 999 Expression of Caspase-3, Survivin, and Bcl-2 in Adenoid Cystic Carcinoma Using Tissue Microarray Technology

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**Background:** The expression and possible role played by caspase-3 and survivin in the pathogenesis of adenoid cystic carcinoma (ACC) of salivary glands have not been investigated. Caspase-3 is a pro-apoptotic protein with a central function in the distal effector pathway of apoptosis. Caspase-3 regulates a series of molecular events which ultimately culminate into apoptotic cell death. Coversely, survivin and bcl-2 are anti-apoptotic proteins. Survivin is a member of the Inhibitor of Apoptosis (IAP) family of proteins which regulates activation of a group of caspases, including caspase-3.
**Design:** 45 untreated ACCs of major and minor salivary glands were reviewed and classified as grade 1, grade 2, and grade 3 based on the presence or abscence of a solid component. Tissue microarrays (TMAs) were constructed by taking four representive tissue cores extracted from each tumor and arrayed into a recipient block using a tissue microarray instrument (Beecher Instruments). Sections from the recipent block were stained with antibodies to caspase-3 (Santa Cruz Biotechnology), suvivin (Novus Biologicals), and bcl-2 (Dako).
**Results:** The tumors were classified as: 27 (60%) grade 1, 11 (24%) grade 2, and 7 (16%) grade 3. Caspase-3 expression was negative in 44/45 (98%) tumors. Survivin and bcl-2 overexpression was present in 44/45 (98%) tumors. One grade 1 tumor was negative for survivin and bcl-2, whereas one grade 3 tumor was positive for caspase-3.
**Conclusions:** Upregulation and overexpression of survivin and bcl-2 is seen in the majority of ACCs and is independent of tumor pattern and tumor grade. As in other malignancies, upregulation of survivin and bcl-2 with inhibition of tumor cell death appears to be a significant event in the tumorigenesis of ACC. The mechanisms responsible for overexpression of caspase-3 and bcl-2 in ACC remain largely unknown. TMA is a powerful tool in the investigation of protein expression in large numbers of ACC despite the morphologic diversity of these tumors.

### 1000 Non-Random Promoter Methylation of Cancer-Related Genes (*RASSF-1, RARbeta-2, DAPK* and *MGMT)* in Salivary Gland Malignancies

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**Background:** CpG island methylation is an important mechanism of tumor suppressor genes' inactivation in carcinogenesis. The role of this epigenetic event in the development and progression of salivary gland carcinomas is largely unknown.
**Design:** To determine the concurrent methylation status of selected cancer related genes in salivary gland malignancies, methylation-specific PCR analysis of *RASSF-1, RARbeta*2, DAPK and MGMT genes was performed on five normal salivary glands and 41 different salivary gland carcinomas. The results were correlated with phenotypic categories and the histopathologic features.
**Results:** No methylation at any gene was noted in histologically normal salivary glands. Methylation in at least one gene was found in 16 (39.0%) of the 41 carcinomas. Ten of the 15 salivary duct carcinomas (66.6%), two each of the eight-acinic cell and mucoepidermoid carcinomas (27%), and two (20%) of the 10 adenoid cystic carcinomas manifested aberrant methylation. The most frequently affected gene in all tumor types was the *RASSF*-1 with 29.3% methylation rate. *RASSF*-1 gene hypermethylation was noted in 60% of salivary duct carcinomas and was significantly different from other histologic subtypes (p=0.008). RARbeta2 methylation was also noted in a subset of salivary duct carcinomas (27%) and was concomitantly associated with *RASSF-1A* alterations in three of four cases. Methylation of DAPK and MGMT genes was absent or infrequent in all types of tumors (0-13%). Significant correlation between methylation and lymph node status was noted (p=0.01).
**Conclusions:** We conclude that: 1) methylation at specific genes is restricted to certain tumor phenotypes, 2) salivary duct carcinomas manifest the highest incidence of methylation at the *RASSF1A* and *RARbeta2* genes, 3) methylation status correlates with aggressive tumor features and 4) DAPK and MGMT are rarely methylated in salivary gland carcinomas.

### 1001 Overexpression of *ICAM-5 (Telencephalin)* Gene in Head and Neck Squamous Carcinoma: Role in Tumorigenesis and Perineural Invasion!

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**Background:** ICAM-5 (telencephalin) is an intercellular adhesion molecule reported to be expressed only in the somatodendritic membrane of telencephalic neurons. We recently identified high ICAM-5 expression in a cDNA array study of head and neck neoplasms with a propensity for perineural invasion.
**Design:** To determine the association of this gene in tumorigenesis and perineural invasion, we analyzed the expression and functional status of *ICAM-5* mRNA transcripts in 30 different human cancer cell lines and 25 head and neck squamous carcinoma specimens by reverse-transcriptase polymerase chain reaction (cell lines and specimens) and *in vitro* functional assays (cell lines) including transfection, colony forming and si-RNA experiments.
**Results:** ICAM-5 transcripts were detected in 28 (93%) of 30 cell lines of head and neck, colon, thyroid, cervical, pancreatic, skin, and adenoid cystic carcinoma origins. In cell lines, small interfering RNA blocked ICAM-5 expression and inhibited cell proliferation. Treatment with the phosphatidylinositol 3'-kinase (PBK) inhibitor LY294002 resulted in ICAM-5 down-regulation. In tissue specimens, none of the 25 histologically normal oral mucosal specimens had detectable ICAM-5 level, whereas 16 (64%) of the 25 matched primary squamous carcinomas showed expression. Carcinoma specimens high ICAM-5 expression had a high incidence of perineural invasion.
**Conclusions:** Our study indicates that ICAM-5 may play a role in tumorigenesis and perineural invasion, most likely through the P13K/Akt-signaling pathway.

### 1002 Characterization of Lymphoproliferative Lesions of the Conjunctiva Using Immunohistochemical and Molecular Genetic Studies

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**Background:** Marked advances in immunohistochemistry and molecular genetics have led to a more intimate understanding of the biologic nature of lymphoproliferative lesions. However these studies have not been widely applied to conjunctival lymphoproliferative lesions.
**Design:** Retrospective case series examining biopsies from 16 consecutive patients with conjunctival lymphoproliferative lesions. Lesions were classified according to the WHO classification. The histopathologic, immunohistochemical and molecular genetic features as well as the frequency of tumour type, prognostic implications, clinical features and treatments offered were characterized.
**Results:** Sixteen cases, 7 males and 9 females with an age range of 35-88 years, were reviewed. Patient follow-up ranged from 7 months to 10 years. The diagnosis was lymphoma in twelve cases (10 Stage 1E and 2 Stage 2E), atypical lymphoid hyperplasia (ALH) in 1 case and reactive lymphoid hyperplasia (RLH) in 3 cases. The primary lymphomas consisted of 5 MALT, 1 FL, 2 DLBCL, 1 LPL and 1 T-cell lymphoma (TCL). Primary lymphomas were treated with radiation (n=7), surgery (n=1) and combined radiation/local chemotherapy (n=1). Two cases of recurrence to the contralateral conjunctiva were treated with radiation and both remain disease free. Complete remission was achieved in 9 of 10 (90%) primary lymphomas. Only one case of primary DLBCL progressed to systemic dissemination. Secondary lymphomas included 2 DLBCL, one treated with radiation the other with chemotherapy. Complete remission was seen following radiation in one, while the patient treated with chemotherapy was lost to follow-up. The one case of ALH presented bilaterally and achieved complete remission following primary treatment. The three RLH cases were surgically managed and remain disease free. Immunophenotyping disclosed 11/

12 lymphomas to be of B-cell lineage. Molecular genetic studies, IgH rearrangement by PCR showed clonal bands in 6/12 lymphomas, 1/3 RLH (polyclonal by immunophenotyping) and in the 1 case of ALH. The BCL2-IgH [t(14;18)] rearrangement was seen in 8/12 cases (4 MALT, 1 FL, 3 DLBCL) by real-time Q-PCR.

**Conclusions:** Conjunctival lymphomas are B cell tumours with a high prevalence of MALT lymphomas. They have a favourable prognosis and respond well to local radiation therapy. Unreported BCL2-IgH rearrangements were seen in 4/5 MALT cases in our series.

### 1003 p16 Expression Is Associated with Basaloid Morphology in Oral Cavity Squamous Cell Carcinomas

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**Background:** The p16 and Rb gene products are part of the Rb pathway which controls the G1-S transition of the cell cycle. HPV has also been linked to the Rb pathway in some carcinomas. There have been few studies of p16 and Rb expression in oral cavity squamous cell carcinomas (OCSCC). We wished to study the expression of p16 and Rb proteins in patients with OCSCC as well as determine if HPV plays a role in these carcinomas.

**Design:** 45 patients with resected (primary therapy) OCSCC were followed for 5 years or until death. Immunohistochemical stains using antibodies to the Rb and p16 gene products and PCR for HPV types 6,11 and 16 were carried out on paraffin embedded tissue. Both Rb and p16 expression were considered negative if no tumor cell nuclei were positive but controls were positive.Clinicopathologic features of stage, node status, metastasis, tumor differentiation and survival outcome were collected.

**Results:** Rb expression was seen in 39/45 patients (87%) and p16 expression in 6/45 patients (13%). There was a significant inverse correlation between Rb and p16 expression ($p < .001$) in the tumors as Rb negative cases were p16 positive and vice versa. Only one tumor that also expressed p16 was positive for HPV (type 16). When examined for clinicopathologic correlates, it was found that in the 6 tumors that expressed p16, 5 of the 6 (83%) had marked basaloid differentiation (greater than 50% basaloid cells). Four of the p16-expressing tumors were Stage 4 at time of presentation but only 2 of these patients are dead at 5 year followup. Minimal basaloid differentiation (10% of the cells or less) was seen in the other 39 tumors that did not stain for p16. We found no clinicopathologic correlates or survival differences with the presence of Rb expression.

**Conclusions:** A statistically signficant inverse relationship was seen between expression of Rb and p16 in this series of oral cavity squamous carcinomas. In addition, a striking association between p16 expression and loss of Rb expression in tumors with marked basaloid morphology was seen. Marked basaloid differentiation was not seen in the tumors that did not express p16. No significant correlation of expression of either p16 or Rb was found with survival or stage. HPV involvement by common viral subtypes does not appear to be a factor in these tumors' genesis. The findings of basaloid differentiation with p16 expression/loss of Rb expression suggest a possible link with morphology and cell cycle regulatory protein expression in OCSCC.

### 1004 Incidence of Oral and Oropharyngeal Cancer in Young Americans, 1971-2000

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**Background:** Many published studies in the the US and Europe suggest that the incidence of head and neck cancer in young adults is increasing. We previously evaluated the incidence of oral and oropharyngeal squamous cell cancer (OOSCC) in patients <40 years of age in a large metropolitan biopsy service in the Southeastern US from 1981-2000, and did not show any increase in the incidence in this cohort comparing the 80's and 90's. Because this data did contrast sharply with other studies we evaluated 1971-1980 as well to determine if any increase in OOSCC in young adults could be seen.

**Design:** A retrospective review of all cases of OOSCC diagnosed from 1971-2000 from the Emory University Oral Pathology biopsy service was performed. A comparison of demographic information, incidence data and histologic grade was made between these cases as a whole and those occurring in a subset of patients <40 years old. Chi-square analysis was performed.

**Results:** Oral and oropharyngeal squamous cell carcinoma (OOSCC) was a rare event in patients <40 in the Emory Oral Pathology biopsy service, with the first case reported in 1974. One case was reported in 1977 and 1978 each, 2 cases in 1979 and 3 cases in 1980. The incidence of OOSCC in this cohort compared to all cases of OOSCC was 2.3%. This was statistically significant when compared to 1981-2000 ($p<.002$). A 1.7:1 male:female ratio was not different from the 1981-2000 data. Five of the 8 cases seen in the 70's occurred on the tongue (62.5%). The tongue was the most common location in young patients in the 80's (55.5%) and 90's (51.6%) as well. When all ages were analyzed, only 25.7% OOSCC were tongue cancers. This incidence in tongue cancer in younger patients was statistically significant ($p<.001$).

**Conclusions:** We demonstrated a sharp increase in the incidence of OOSCC in young patients <40, beginning in 1974 and peaking in the late 80's and remaining stable. The tongue is the most common location in this age group.

### 1005 Absence of p16 Gene (CDKN2A) Deletions in HNSCC Occurring in Young Adults

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**Background:** Head and neck cancer (HNSCC) is the 6th most common cancer worldwide and is strongly associated with tobacco and alcohol use. According to the molecular progression model, loss of 9p21-22 is the most frequent event. Homozygous deletions and methylation of the 5'-CpG promoter region of p16 have frequently been identified in HNSCC, suggesting that functional inactivation of p16 is a common event. The inactivation of this tumour supressor gene has been linked to tobacco use. Recently, an increase in the number of young people (<40 years old) developing HNSCC has been reported, many of them with no history of tobacco use.

As loss of p16 function is common in HNSCC, we sought to determine whether deletion of the gene for p16 (CDKN2A), or p16 expression varied in the HNSCC in relation to age of the patient and smoking status. Because of the involvement of p16 in the tumour biology of HPV, presence of high-risk HPV DNA was also ascertained.

**Design:** Fresh tissue from 26 oropharyngeal carcinomas was obtained, including twelve samples from patients < 40 year olds. Smoking status was recorded. Laser capture microdissection and CGH microarray analysis were performed to detect deletions in the p16 gene. PCR analysis of all 26 cases to detect high risk types HPV 16 and HPV 18. Finally analysis of p16 expression was performed using immunohistochemistry.

**Results:** Loss of the CDKN2A(p16) gene occurred significantly more often in the older cohort ($p< 0.01$). In fact it was not deleted in **any** of the young cohort. The p16 protein was overexpressed in 42% of the young cohort, but in only 22% of the older cohort Interestingly, smokers did not show significantly more p16 expression than the nonsmokers, and all female samples lacked p16 overexpression.

**Conclusions:** Our study shows that CDKN2A(p16) deletions are absent in our young HNSCC samples, raising the possibility that loss of this gene might be an age-related event rather than merely a smoking related event.

In relation to the p16 protein, overexpression was found more frequently in the younger group, and in only 40% of these cases was HPV 16 detected. HPV16 was detected in **all** the over 40 year old p16 positive cases.

The results from this study suggest that molecular events thought to be of paramount importance in HNSCC tumorigenesis may not be involved the development of HNSCC in young patients.

### 1006 Sebaceous Cell Carcinoma of the Eyelid: Histopathological Analysis of 44 Cases

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**Background:** Sebaceous carcinoma (SC) is a rare tumor of the skin adnexae and its diagnosis can be a challenge for the clinician and the pathologist. Clinically, SC of the eyelid can mimic recurrent chalazion or inflammation. Histopathologically, SC can mimic squamous cell carcinoma (SqCC) and basal cell carcinoma (BCC). We evaluated 44 cases of SC.

**Design:** 31 cases of SC of the eyelids were retrieved from the Henry C. Witelson Ocular Pathology Laboratory, McGill University - Canada, and 13 cases were retrieved from the Department of Ophthalmology, Hospital Dr. L. S. Bulnes - Mexico. All hematoxylin-eosin stained slides were rewied by a pathologist without knowledge of the clinical information.

**Results:** 37 cases (84%) were classified as poorly differentiated lesions. Cytoplasmatic vacuoles were a highlight feature in 48% of the cases. 75% of the SC have features similar to SqCC and dyskeratosis was seen in 30% of them. Atypical mitosis were seen in 80% and cellular anaplasia in 84% of the cases. 3 cases (7%) resemble BCC and 4 cases (9%) could be misdiagnosed as basaloid SqCC. 1 case has features of Merckel cell carcinoma. 86% of the cases have focal well differentiated sebaceous areas within the tumor. A solid and a lobular pattern of growth was seen in 11 lesions (26%) each. Interestingly, an adenoid pattern was seen in 1 lesion and an acidophilic extracellular matrix was present in 1 case. Superficial spread as carcinoma *in situ* was seen in 13 cases (33%), as pagetoid in 4 cases (10%) and a combination of both was seen in the other 13 cases (33%). Comedocarcinoma was observed in 14 lesions (32%). Non-invasive SC (*in situ)* correspondes to 25.8% of the cases from Canada and 0% from Mexico.The mean size of the tumors from Canada was 19.73 and from Mexico was 35.15.

**Conclusions:** Most of the SC are poorly differentiated lesions that resemble SqCC with several patterns of growth. The pathologist should look for cytoplasmatic vacuoles, cellular anaplasia and well differentiated sebaceous areas in all eyelid lesions to rule out SC. We speculate that due to the chalazion protocol at McGill University, where all the chalazions are sent to the Ocular Pathology Laboratory, there are differences in the prognostic factors, particularly in the size of the tumor, between the two patient's populations.

### 1007 Molecular and Immunophenotypic Comparison of Respiratory Epithelial Adenomatoid Hamartoma, Sinonasal Adenocarcinoma, and Chronic Sinusitis

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**Background:** Respiratory epithelial adenomatoid hamartoma (REAH) is a benign lesion in the sinonasal tract that is composed of a proliferation of glands lined by ciliated respiratory mucosa surrounded by dense hyalinized basement membranes. Little is known regarding the pathogenesis of these proliferative lesions and their possible relationship to sinonasal adenocarcinomas (SNAC). To date, no studies have examined and compared the location and presence of basal cells or myoepithelial cells or the molecular profile in normal sinus mucosa, REAH, and SNAC.

**Design:** Immunohistochemistry was performed on 9 cases of SNAC (7 intestinal type [ITAC] and 2 non-intestinal type [non-ITAC]), 10 cases of REAH, and 10 cases of chronic sinusitis (CS) for CK7, CK20, CDX-2, p63, Ki-67, and calponin. DNA was extracted from microdissected samples and PCR was performed for loci at 9p, 11p, and 17p. Capillary electrophoresis was used to semi-quantitatively assess for loss of heterozygosity.

**Results:** Immunohistochemical staining characteristics of SNAC, REAH, and CS are shown in the table. Four out of nine (44%) SNACs expressed both CK7 and CK20. Only one non-ITAC SNAC expressed p63 in a diffuse pattern. In contrast, p63 staining was uniformly present around glands of REAH and in the basal mucosa in CS. One-third of the ITACs expressed CDX-2, and 3/7 (43%) expressed CDX-2, CK7, and CK20 concurrently. In the adenocarcinomas, the frequency of allelic loss (FAL) was 68%, while in the REAH lesions, the FAL was 24%.

**Conclusions:** Reactivity to p63 was seen around all proliferative glands of REAH and in normal respiratory mucosa, indicating the presence of a basal/myoepithelial cell type. In contrast, the SNACs did not show p63 reactivity in this specific location. The FAL for SNAC was significantly higher than that seen in REAH. These results show that there may be several novel immunohistochemical and molecular markers that can be useful to characterize REAH and distinguish REAH from SNAC.

Immunohistochemical Results

| Stain | SNAC (%) | REAH (%) | CS (%) |
|---|---|---|---|
| CK20 | 5/9 (56) | 0/10 (0) | 0/10 (0) |
| CK7 | 8/9 (89) | 10/10 (100) | 10/10 (100) |
| CDX-2 | 3/9 (33) | 0/10 (0) | 0/10 (0) |
| Ki-67 | 3/9 (33) | 1/10 (10) | 0/10 (0) |
| p63 | 1/9 (11) (diffuse) | 10/10 (100) | 10/10 (100) |
| Calponin | 0/10 (0) | 0/10 (0) | 0/10 (0) |

### 1008 Human Papillomavirus (HPV) Related Squamous Cell Carcinoma of the Oropharynx: Characterization of a Distinct Phenotype

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**Background:** We have recently shown that more than 90% of tonsillar squamous cell carcinoma in young patients, under the age of 40, harbor high risk HPV DNA & show a characteristic morphology. The purpose of this investigation is to evaluate the prevalence of this type of carcinoma in the tonsils & base of tongue in patients of all ages & to further characterize its clinicopathologic & immunohistochemical features.

**Design:** 235 cases of squamous cell carcinoma of the tonsil & base of tongue were reviewed & classified according to their histomorphology into 2 groups: classical keratinizing squamous cell carcinoma (KSCC) & nonkeratinizing carcinoma with basal cell features (NKca). 10 cases from each group were selected & evaluated for the presence of HPV DNA by polymerase chain reaction (PCR). Tumors were immunohistochemically stained for antibodies against p16, Rb, p53 & Ki67.

**Results:** 141 cases of carcinoma of the tonsils were identified. Of these 51 (36.17%) were Nkca. Of 94 base of tongue carcinomas 30 (34.5%) were Nkca. All of the 10 Nkca & only 2 of the KSCC cases were positive for HPV DNA. HPV16 was identified in 10 cases & types c31/33 in 2. All the Nkcas showed strong & diffuse reactivity for p16, higher Ki67 & lower p53 & Rb staining scores as compared to KSCC.

**Conclusions:** The study shows that about 1/3 of tonsillar & base of tongue carcinomas in patients of all age groups are positive for high risk HPV DNA & show distinct histopathologic & immunophenotypic features.

### 1009 Non-Hodgkin's Lymphoma of the Oral Cavity: A Retrospective Study of 37 Cases

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**Background:** Non-Hodgkin's lymphoma (NHL) is a heterogeneous group of malignancies with a predilection to involve extranodal tissues. NHL presenting with oral symptoms is uncommon and lesions of the oral cavity represent about 2% of all NHL. Clinical and radiographic features of NHL in the oral cavity can be nonspecific and mimic inflammatory disorders. The objective of this study was to identify cases of NHL presenting in the oral cavity, determine the most common types of NHL, sites of involvement, and clinical presentation.

**Design:** We searched the databases of the College of Dentistry and the Nebraska Lymphoma Study Group for cases of NHL presenting in the oral cavity between 1987 and 2004. Thirty-seven cases were identified. We reviewed each case and performed immunohistochemical stains as needed to reclassify the cases using the WHO classification.

**Results:** Twenty cases were females and 17 were males, their ages ranged from 21 to 99 years (mean 69 years). Nineteen cases were diagnosed as diffuse large B-cell lymphoma (51%), seven as follicular lymphoma (19%), five as extranodal marginal zone lymphoma, MALT type (13.5%), three as Burkitt lymphoma (8%), two as mantle cell lymphoma (5.5%), and one case as a monoclonal plasmacytoma (3%). The most common site was the maxilla (73%); however, this included lesions of the hard palate, soft palate, gingiva, and alveolar ridge. Five cases presented in the mandible (13.5%); four of these were diffuse large B-cell lymphoma with bone involvement and one case a follicular lymphoma, presenting as a soft tissue mass. All patients with mandibular lesions developed pain and numbness of the affected area, which prompted the dentist to take a tissue biopsy. Three lesions occurred in the buccal mucosa (8%) and two lesions presented on the lower lip (5.5%), both diagnosed as extranodal marginal zone lymphoma, MALT type. Of the 37 cases, nine had a previous history of lymphoma and had received treatment. These patients later had a recurrence in the oral cavity, the most common site of recurrence was the palate.

**Conclusions:** In our study, the most common type of NHL presenting in the oral cavity was diffuse large B-cell lymphoma, most likely involving the maxilla and presenting as a large mass in the hard palate. The type of NHL most commonly involving bone was also diffuse large B-cell lymphoma. Extranodal marginal cell lymphoma, MALT type, was common in soft tissue, such as buccal mucosa and lips, and often associated with minor salivary glands.

### 1010 Epithelial Growth Factor Receptor (EGFR) Expression by Immunohistochemistry in Benign and Malignant Laryngeal Lesions, Review of 56 Cases

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**Background:** EGFR a cell cycle proliferative marker, is up-regulated in Squamous Cell Carcinomas of Head and Neck (SCCHN). Monoclonal antibodies to EGFR and tyrosine kinase inhibitors are now available for treatment of malignancies in other organs and determination of EGFR expression is indicated in the larynx. We are studying EGFR expression in squamous epithelia from benign laryngeal lesions, dysplasias and invasive squamous cell carcinomas (SCC) of various differentiations and tumor (T) stages.

**Design:** 56 laryngeal biopsies and resections were retrieved from surgical pathology files of Henry Ford Hospital between 1996-2004. Patient age ranged between 32-83, (37 males 14 females). Benign lesions (12), squamous dysplasia (8), invasive carcinomas: T1 (8), T2 (10), T3 (9), T4(9). There were 2/36 well differentiated (WD) SCC, 28/36 moderately differentiated (MD) SCC, 6/36 poorly differentiated (PD) SCC. The formalin fixed, paraffin embedded sections were subjected to immunohistochemical staining for EGFR using polyclonal antibody (Santa Cruz Biotechnology Inc., 1:50 dilution with Avidin-Biotin Peroxidase method). Sections were reviewed for the pattern of immunostaining, staining intensity, proportion of EGFR positive cells in the benign or malignant squamous epithelium. Staining intensity was graded between 0 (absent) to 3+ (strong). Proportion was the average rate of positively stained cells in 3-5 high power fields. No cells stained (0), 0-1/3 (1+), 1/3 to 2/3 (2+) more than 2/3 (3+). Immunostaining score (IS) was the summation of intensity and proportion of positively stained cells with a range of 0-6.

**Results:** Positive staining is noted as membranous and cytoplasmic. Mean IS of WD carcinomas is 4.0, 3.21 for MD carcinomas, 2.66 for PD carcinomas. Comparison of IS in benign lesions versus invasive carcinomas shows a significant association (Fisher exact test, p: 0.0253), with benign lesions having an IS of ≤2. In this series, IS did not distinguish between benign and dysplastic mucosa, nor between grades of invasive carcinomas.

**Conclusions:** 1. There is wide variation in staining intensity and proportion of positively stained cells between benign lesions and invasive SCC of larynx. 2. PD SCC have lower IS than WD SCC and may need to be correlated with molecular studies like polymerase chain reaction, a study in progress. 3. Treatment by EGFR antibodies may need to be individualized based on intensity of EGFR expression and proportion of positively staining cells.

### 1011 Oncocytic Epithelial-Myoepithelial Carcinoma of the Salivary Gland: An Unrecognized Morphologic Variant

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**Background:** Epithelial-myoepithelial carcinoma of the salivary gland is an uncommon neoplasm. It is characterized by a dual epithelial and myoepithelial differentiation. Typically, the myoepithelial component predominates and displays "clear" cytoplasm. Oncocytic change, which is well recognized in various salivary gland neoplasms, has not been previously reported in Epithelial-myoepithelial carcinoma. Oncocytic Epithelial-myoepithelial carcinoma can mimic other benign and malignant neoplasms and can be a source of considerable diagnostic dilemma.

**Design:** We identified 3 cases of Oncocytic Epithelial-myoepithelial carcinoma and analyzed their clinicopathologic features and immunohistochemical profile.

**Results:** Two patients were female and 1 male, aged 65 to 79 years (mean 73). All 3 tumors were in the parotid gland and the patients presented with localized swelling of 3 to 6 months duration. Tumors ranged in size from 1.2 to 2.6 cm and grossly appeared firm, tan and circumsribed. Histologically, all three tumors displayed a nodular growth pattern with tumor infiltration into adjacent tissues. The nodules contained bilayered tubules and trabeculae with intervening hyalinized stroma. Both cell types contained vesicular nuclei, prominent nucleoli and abundant eosinophilic cytoplasm; with myoepithelial cells usually larger. The lumens contained PAS positive pink secretions. Mitotic figures were 1 per 10 HPFs. One tumors showed perineural invasion and there was no necrosis or vascular invasion. In 1 patient, focal metastasis were seen in an adjacent intraparotid lymph node. Immunhistochemical stains delineated the dual differentiation: Epithelial (Cam 5.2+, CK7+); Myoepithelial (Vimentin+, S100+, Calponin+, Smooth muscle actin+, Smooth muscle myosin+, p63+, CD10+). All three tumors were also reactive for anti-mitochondrial antibody. After parotidectomies, the 3 patients are free of disease (after 4, 7 and 56 months).

**Conclusions:** We have presented 3 cases of hitherto unrecognized Oncocytic variant of Epithelial-myoepithelial carcinoma of the salivary gland. The two cell components of this neoplasm can undergo oncocytic change masking their characteristic "clear cell" appearance and make its accurate identification difficult. Due to significant prognostic and therapeutic implications, discrimination of an Oncocytic Epithelial-myoepithelial carcinoma from other "oncocytic" lesions/neoplasms is crucial.

### 1012 Papillary Thyroid Carcinoma Versus Follicular Adenoma: Molecular Diagnosis by Cumulative Analysis of Gene Expression Ratios

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**Background:** The histologic distinction of follicular variant of papillary thyroid carcinoma (PTCFV) from follicular adenoma (FA) often presents a diagnostic challenge. Recent gene microarray studies identified genes consistently overexpressed in papillary thyroid carcinoma (PTC), as well as genes that show decreased expression. This suggests that mRNA expression analysis by quantitative RT-PCR (qRT-PCR) can be useful in distinguishing PTC from FA.

**Design:** Microarray data was evaluated and 7 genes with differential mRNA expression were selected: melanocyte specific gene 1 (MSG1/CITED1), surfactant protein B (SFTPB), galectin-3 (LGALS3), keratin 19 (KRT19), thyroid peroxidase (TPO), deiodinase 1 (DIO1), and trefoil factor 3 (TFF3). RNA was extracted from fresh frozen and paraffin-embedded tissues of PTC and FA and qRT-PCR was performed.

**Results:** Using RNA from fresh-frozen tissues of 9 cases [4 PTC (2PTCFV) and 5 FA], qRT-PCR confirmed high expression of LGALS3, KRT19, CITED1, and SFTPB in most PTC, whereas TFF3, TPO, and DIO1 showed higher expression in FA than in PTC. For each individual gene, however, the ranges of RNA expression level in PTC and in FA were either overlapping or showed only limited differences. We have thus devised a strategy termed Cumulative Analysis of Gene Expression Ratios (CAGER). In CAGER, genes with the same expression trends are clustered as a group, and combined gene expression ratios are calculated based on qRT-PCR data of 2 to 7 genes. Because of the cumulative effect, the differences between two biological groups are amplified, leading to a more effective separation. Applying CAGER to PTC and FA, the ratio of combined (LGALS3+KRT19) expression to combined (TFF3+TPO+DIO1) expression was found to be much higher in PTC than in FA. A molecular criterion was established and nine cases [3 PTC (1 PTCFV), 2FA, and 4 possible PTCFV] were tested, using RNA extracted from paraffin-embedded tissues. Results accurately identified 3 PTC and 2 FA, while 3 of the 4 morphologically debatable cases of PTCFV showed expression profiles closer to FA.

**Conclusions:** Based on the selected gene panel, CAGER can separate PTC from FA and suggests that some of the cases histologically interpreted as PTCFV might be FA. This technology can be applied to paraffin embedded tissues with good results and validation with a larger number of PTC and FA cases is warranted.

### 1013 Papillary Thyroid Carcinoma Versus Follicular Adenoma: Analysis of Keratin 19, Galectin-3, and CITED1 Expression by Tissue Microarray

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**Background:** Detection of cytokeratin 19 (CK19) and galectin-3 expression by immunohistochemistry has been postulated as supporting evidence for papillary thyroid carcinoma (PTC) amongst follicular lesions, with variable sensitivity and specificity reported previously. Recent DNA microarrays showed CITED1 (MSG1) overexpression in papillary carcinoma, suggesting CITED1 as a new and potentially useful diagnostic marker.

**Design:** Tissue microarrays were constructed, consisting of 50 cases of classic papillary carcinoma (PTCC), 31 papillary carcinoma follicular variant (PTCFV), and 56 follicular adenoma (FA). Each case was represented by three tissue cores. Immunohistochemical staining was performed with monoclonal antibodies against CK19 and galectin-3, as well as a rabbit polyclonal antibody against CITED1 (T. Shioda, Harvard Medical School).

**Results:** Strong cytoplasmic staining of galectin-3 was observed in 48 of 50 PTCC, 24/31 PTCFV, and 6/56 FA. Similarly, moderate to strong cytoplasmic staining for CK19 was seen in 50/50 PTCC, 27/31 PTCFV, and 12/55 FA. Simultaneous expression (2+-3+) of galectin-3 and CK19 was found in 48 of 50 (96%) PTCC, 23 of 31 (74%) of PTCFV, and only 5 of 55 (9%) of FA. On the other hand, only 1 of 81 morphologically diagnosed PTC was negative for both CK19 and galectin-3.

In contrast to the cytoplasmic staining of CK19 and galectin-3, CITED1 antibody showed mainly nuclear staining, with mixed nuclear and cytoplasmic staining in some cases. Thirty-eight of 50 (76%) PTCC and 28/31 (90%) PTCFV showed >2+ staining, while staining of similar intensity was seen in only 8/56 (14%) FA, confirming overexpression of CITED1 as a marker for papillary carcinoma. However, the signal/background ratio observed with CITED1 antibody was in general lower than that seen with CK19 and galectin-3 antibodies, and the distinction between weak positive and background staining was not possible in some cases.

**Conclusions:** CK19 and galectin-3 expression, particularly when observed concomitantly, supports the diagnosis of PTC. However, strong staining can be observed in occasional FA, cautioning against reliance of this finding as the sole criterion, particularly for the purpose of pre-operative diagnosis. CITED1 is a promising marker but is of limited value at present due to the suboptimal signal/background ratio with the existing antibody and the lack of a commercial antibody source. Development of additional anti-CITED1 antibodies, e.g. a monoclonal antibody, is worthy of pursuing.

### 1014 Sinonasal Tract Mucoepidermoid Carcinoma: A Clinicopathologic and Immunophenotypic Study of 19 Cases

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**Background:** Primary sinonasal tract mucoepidermoid carcinomas (MEC) are uncommon tumors that are frequently misclassified, resulting in inappropriate clinical management.

**Design:** Retrospective review.

**Results:** Nineteen cases of MEC included 10 females and 9 males, aged 15–75 years (mean, 52.7 years). Patients presented most frequently with a mass, obstructive symptoms, and/or epistaxis present for a mean of 12.6 months. The majority of tumors involved the nasal cavity alone (n = 10), maxillary sinus alone (n = 6), or a combination of the nasal cavity and paranasal sinuses (n = 3) with a mean size of 2.4 cm. Most patients presented at a low clinical stage (n = 15, Stage I & II). Histologically, the tumors were often invasive (bone or perineural invasion), with invasion into minor mucoserous glands. Surface involvement was common. The neoplastic cells were composed of a combination of squamoid cells, intermediate cells, and mucocytes. Cysts spaces were occasionally large, but the majority were focal to small. Pleomorphism was generally mild. Necrosis (n = 5) and atypical mitotic figures (n = 6) were seen infrequently. Most of the tumors were classified as low grade (n = 10), with intermediate (n = 6) and high grade (n = 3) comprising the remainder. Mucicarmine was positive in all cases tested. Immunohistochemical studies showed positive reactions for keratin, CK5/6, CK7, EMA, and CEA in all cases tested, while bcl-2 was only positive in 3 cases. GFAP, MSA, TTF-1, S-100 protein and CD117 were non-reactive. p53 and Ki-67 were reactive to a variable degree. MEC need to be considered in the differential diagnosis of a number of sinonasal lesions, particularly adenocarcinoma and necrotizing sialometaplasia. Surgery occasionally accompanied by radiation therapy (n = 2) was generally employed. Six patients developed a recurrence, with 5 patients dying with disease (mean, 2.4 yrs), while 14 patients are either alive (n = 9) or had died (n = 5) of unrelated causes (mean, 14.6 yrs).

**Conclusions:** Mucoepidermoid carcinoma usually present in middle age patients as a mass. Most patients present with low stage disease, although invasive growth is common. Recurrences develop in about a third of patients, who experience a shorter survival (mean, 6.5 yrs). The following parameters, when present, suggest an increased incidence of recurrence or dying with disease: male gender, age >60, size >3.0 cm, mixed anatomic site, tumor grade and higher stage disease.

### 1015 Correlation of Degree of Upper Aerodigestive Tract (UAT) Squamous Dysplasia with HPV Status and Expression of Cell Cycle Associated Proteins p16, p53, Rb and Ki-67

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**Background:** Squamous carcinogenesis in the UAT results from cumulative genetic and epigenetic alterations induced by exposure to carcinogenic agents. While the link between tobacco and ethanol and carcinogenesis is strong, the role of human papilloma virus (HPV) in UAT dysplasia is controversial. The reported prevalence of HPV infection in squamous dysplasia varies from zero to 80%. The nature and sequence of the genetic and epigenetic changes in dysplasia are controversial as well: some studies show inactivation while others show upregulation of cell cycle associated proteins. A recent study found an intriguing correlation between degree of dysplasia in oral cavity lesions and height of p16, p53, and Ki-67 expressing cells in the epithelium. Our study examines UAT dysplasia with HPV status and height of p16, p53, Rb, and Ki-67.

**Design:** Paraffin embedded archival biopsies of of 53 cases of squamous dysplasia (23 mild, 12 moderate, 18 severe) of the oral cavity and hypopharynx were stained with monoclonal antibodies to p16, p53, Rb, and Ki-67. The height of nuclear staining was coded 0 for no staining or 1-3 according to extension of staining into the lower, middle, or upper third of the epithelium. The proportion of positive cells was coded 0 for no staining or 1-3 for positivity of up to 1/3, 2/3 or 3/3 of the cells. Mucosal HPV type status was evaluated by PCR using consensus primers and paraffin-extracted DNA.

**Results:** For p16 staining, the percentage of positive cases was highest among mild lesions (39%), and decreased with degree of dysplasia to 25% and 11%, respectively, of moderate and severe dysplasias. Mean height and cellular proportion of p16 staining was highest among mild lesions (middle third); moderate and severe dysplasias exhibited p16 staining in the lower third of the epithelium. The height of Ki-67 and p53 correlated with increasing dysplasia. The height and proportion of Rb did not differ among the different degrees of dysplasia. HPV DNA was detected in 1/53 cases (2%).

**Conclusions:** While our results found correlation of p53 and Ki-67 stratification with degree of dysplasia, the height of p16 staining was inversely proportional to the degree of dysplasia in these essentially HPV negative cases. Our findings may indicate that cumulative alterations in p16 lead to its downregulation with increasing degree of dysplasia. Since other work has shown the converse in regard to p16, our study suggests that genetic alterations in carcinogenesis may be heterogeneous in UAT dysplasia.

### 1016 Novel Estrogen Receptor Coactivator PELP1/MNAR Gene and ERbeta Expressed in Salivary Duct Adenocarcinoma: Potential Therapeutic Targets

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**Background:** Salivary duct carcinoma (SDC) is a high-grade neoplasm with marked morphologic resemblance to mammary duct carcinoma. Recently a novel estrogen receptor (ER) interacting protein, proline-, glutamic acid-, and leucine- rich protein 1 (*PELP1*) also called the modulator of nongenomic activity (*MNAR*) has been cloned and shown to activate steroid hormone receptors in mammary carcinomas by nongenomic and genomic mechanisms.

**Design:** To determine expression and the relationship of this gene to the ER status in SDCs. We analyzed the differential expression of the *PELP1/MNAR* and ERs alpha and beta proteins in SDCs, using Western blotting and immunohistochemistry and correlated the results with patients' outcome.

**Results:** Western blot analysis of seven paired normal and tumor specimens showed increased expression of PELP1/MNAR and ERbeta in three and four of the SDCs, respectively. No detectable expression of ERalpha in any normal or SDC specimens was noted. Immunohistochemical staining performed on 70 SDCs revealed strong expression of PELP1/MNAR in 51(73%) and ERbeta in 52 (74%) tumors. PELP1/MNAR and ERbeta were co-expressed in 35 (50%), individually in 17 (24.2%) and negative in 18 (25.7%) tumors. PELP1/MNAR staining was predominantly cytoplasmic while ERbeta staining was nuclear and occasionally cytoplasmic in tumor cells. PELP-1 expression correlated significantly with decreased disease free survival of these patients.
**Conclusions:** Our results indicate: 1) PELP1/MNAR and ERbeta are co-expressed in the majority of SDCs and may play a role in the pathobiology of these tumors, 2) PELP-1 is correlated with the biological aggression of a subset of these tumors and 3) these markers can be targeted for future therapy of these tumors.

### 1017 Differential Expression of p63 Isotypes in Salivary Gland Tumorigenesis: Histogenetic and Biologic Relevance

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**Background:** p63, a homologue of the p53 tumor suppressor gene, is located on chromosome 3q27-29 region. The gene encodes for at least six distinct proteins of variable biological functions. The main isotypes are the TAp63 and deltaNp63 that differ by the presence or the lack of the amino-terminal domain, respectively. p63 gene also play a role in the regulation of the Notch receptors pathway through binding with JAG1 and JAG2 ligands.
**Design:** To determine the role of the p63 isotypes and the Notch ligand JAG1 gene in salivary gland tumorigenesis, we evaluated the expression of the TAp63 and deltaNp63 isoforms transcript and the JAG1 gene using RT-PCR based analysis in 71 benign and malignant salivary gland neoplasms and correlated the results with the clinicopathologic features and the immunostaining using an antibody to the full-length protein.
**Results:** p63 isoforms were either negative or weakly expressed in normal salivary gland tissues. TAp63 was strongly expressed in Warthin's tumor, pleomorphic adenoma, myoepithelioma, and was negative or weakly positive in malignant tumors. Conversely, deltaNp63 was highly expressed in adenoid cystic, mucoepidermoid, and myoepithelial carcinomas and was low or negative in benign neoplasms except Warthin's tumor. Immunohistochemical staining showed ubiquitous nuclear expressions in basal and myoepithelial cells of both benign and malignant neoplasms. JAG1 was expressed in most benign and malignant tumors and did not correlate with p63 isoforms expression.
**Conclusions:** Our findings indicate that: 1) p63 isotypes play different roles in salivary gland tumorigenesis, 2) high expression of TAp63 is restricted to benign tumors and deltaNp63 to certain carcinoma subtypes and 3)there is no association between p63 isotypes and the Notch pathway ligand JAG1 expression.

### 1018 Assessment of CD43 Expression in Adenoid Cystic Carcinomas, Polymorphous Low-Grade Adenocarcinomas, and Monomorphic Adenomas

*V Woo, R Kelsch, T Bhuiya.* Long Island Jewish Medical Center, New Hyde Park, NY.
**Background:** CD43 (leukosialin, sialophorin) is a sialoglycoprotein expressed on the surface of most hematolymphoid cells, including T lymphocytes, thymocytes, monocytes, and granulocytes. Expression of CD43 in T-cell malignancies, some myeloid disorders, and a subset of B-cell lymphomas has been established. In contrast, immunoreactivity in epithelial neoplasms, such as colorectal adenomas and carcinomas, has only been recently recognized. In this article, we report differential immunostaining of CD43 in three types of salivary gland neoplasms: adenoid cystic carcinoma (ACC), polymorphous low-grade adenocarcinoma (PLGA), and monomorphic adenoma (MA).
**Design:** Archived, formalin-fixed, paraffin-embedded tissue was retrieved from the Department of Pathology at Long Island Jewish Medical Center from 1990 to 2001. A total of 40 tumors were selected, including 12 ACCs, 14 PLGAs, and 14 MAs. Immunohistochemical staining with mouse monoclonal anti-CD43 antibody was performed employing standard techniques. Immunoreactivity was assessed using a scoring system based on percentage of positive cells (weak [10-25%], mild [26-50%], moderate [51-75%], and strong [76-100%]).
**Results:** Immunoreactivity for CD43 was detected in 12/12 ACCs (6 with strong staining), 1/14 PLGAs (strong staining in a lesion diagnosed as PLGA with foci more typical of ACC), and 3/14 MAs (all weak staining). Immunostaining occurred in a membranous pattern, with uniform expression in tumor cells in some cases and stronger expression in the abluminal cells in others. When present, CD43 cross-reactivity with lymphocytes and lymphoid tissue served as additional internal controls. No correlation between staining intensity and perineural or angiolymphatic invasion was observed.
**Conclusions:** CD43 appears to be preferentially expressed in salivary gland ACCs in comparison with PLGAs and MAs. Distinguishing between ACC and PLGA can occasionally pose a diagnostic challenge as a result of their overlapping histologic appearances; however, distinction between the two is of utmost importance due to therapeutic and prognostic implications. Our results indicate that use of CD43 immunostaining as an adjunct to histological examination may be helpful in differentiating ACC from other salivary gland neoplasia. The mechanism by which CD43 is favorably expressed in ACCs remains obscure at this time. Further investigation of the role of CD43 in salivary gland and other epithelial neoplasms is warranted.

### 1019 Indeterminate Cytological Category of Thyroid Lesions: A Study of 220 Cases with Cytological-Histological Correlation

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**Background:** Indeterminate category represents a gray zone in thyroid cytology. It generally includes follicular lesions of uncertain biologic behavior, such as neoplastic vs. non-neoplastic, and benign vs. malignant. The diagnostic uncertainty of this category causes difficulty in the patient's clinical management. The present study analyzed thyroid FNA cases with indeterminate cytological diagnosis, aiming at the possibility to increase the diagnostic accuracy and, therefore, to improve the role of FNA in the clinical setting.
**Design:** The cases were retrieved from thyroid FNA specimens filed at LIJ between 1992 and 2002. The cases with indeterminate cytological diagnosis were subclassified as follicular neoplasms or suspicious for malignancy. The cytological diagnosis was compared with the histological diagnosis and the discrepancies were re-evaluated to identify the source of errors.
**Results:** Among 3,342 thyroid FNA cases, 386 (11.5%) were classified as indeterminate, including 341(88.3%) cases of follicular neoplasms, and 45 (11.7%) suspicious for malignancy. 220 of 386 (57%) cases had surgical follow-up in our institution. The surgical rate in follicular neoplasm and suspicious for malignancy cases was 54.8% and 73.3%, respectively. The histological diagnosis of 187 cases of follicular neoplasm was 28 nodular goiters, 4 Hashimoto's thyroiditis, 79 follicular adenomas, 16 follicular carcinomas, 59 papillary carcinomas, and 1 parathyroid carcinoma. The histological diagnosis of 33 cases of suspicious for malignancy was 5 nodular goiters, 27 papillary carcinomas, and 1 medullary carcinoma .The overall discrepancy between cytological and histological diagnosis was 16.8%. The major source of error consisted in interpreting follicular neoplasms on less than optimal specimens and in specimens containing dominant adenomatous nodule. Significant cytological atypia caused by Hashimoto's thyroiditis also contributed to a few misdiagnoses. The key cytological features of follicular neoplasm were the presence of syncytial groups of follicular cells, a microfollicular pattern, and the absence of watery colloid.
**Conclusions:** The usefulness of the indeterminate category as a preoperative diagnostic approach is validated by this study. The cases with cytological diagnosis of suspicious for malignancy should be separated from the indeterminate category due to significant higher risk of malignancy. The accuracy in diagnosing follicular neoplasms can be improved by adhering to more stringent adequacy criteria.

### 1020 Perineurial Separation by Tumor Cells Associated with Perineural Invasion in the Squamous Cell Carcinoma of the Head and Neck Region

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**Background:** Perineurium is mainly composed of the perineurial cells that are separated by layers of the basement membrane. Perineurial space is the space between the nerve fascicle and its surrounding perineurium. Tumor cells growing in the perineurial space, termed perineural invasion (PNI), is a common pathological feature of many malignant tumors in humans, including the squamous cell carcinoma (SCC); and this feature is associated with high likelihood of local tumor spread and recurrence in SCC of the head and neck region. Currently, it is believed that the tumor cells associated with PNI penetrate through the perineurium and spread more rapidly in a less resistant pathway of the perineurial space. However, the exact relationship between the tumor cells and the perineurium is uncertain. This study was performed to understand the relationship between the tumor cells and the perineurium at the sites of PNI in SCC of the head and neck region.
**Design:** In this retrospective study, 11 cases of SCC with evidence of PNI in the head and neck region were used. One tissue block with at least one focus of PNI from each patient was retrieved, and paraffin sections were stained with H/E to demonstrate tumor cells and PNI. The nerves were confirmed by immunoperoxidase stain with S100. The architecture of the perineurium was highlighted by Collagen IV immunoperoxidase stain, which labeled the layers of basement membrane between the perineurial cells. Epithelial membrane antigen (EMA) immunoperoxidase stain was also performed to label both the flat-shaped perineurial cells and some of the tumor cells. The sections were studied under the light microscope.
**Results:** All of the 11 cases showed evidence of PNI with tumor cells in the perineurial spaces, and the number of nerves with PNI varied from one to three in each case. In eight out of 11 cases, we found tumor cells within the perineurium in one to multiple foci. These tumor cells invaded between the thin layers of circumferentially arranged Collagen IV-positive basement membrane, replacing the perineurial cells. There was no collagen IV-positive substance between the tumor cells in the areas not associated with nerves, indicating that the collagen IV-positive material surrounding the nerves at the sites of PNI represented the basement membrane in the perineurium and was not produced by the tumor cells.
**Conclusions:** The results demonstrate that SCC tumor cells associated PNI not only invade in the perineurial space, but can also invade and spread within the layers of perineurium.

### 1021 Overexpression of Phosphorylated Nuclear Factor-kappaB (p-NF-κB) in Tonsillar Squamous Cell Carcinoma and High Grade Dysplasia Is Associated with Poor Prognosis

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**Background:** Intracellular signals along epidermal growth factor receptor (EGFR)-Akt-nuclear factor-kappaB (NF-κB) pathway have been associated with carcinogenesis in varying malignant neoplasms. This investigation was to evaluate the expression of EGFR, phosphorylated (p)-Akt (p-Akt) and phosphorylated (p)-NF-κB and correlate them with clinical outcomes in patients with squamous cell carcinoma of the tonsil (SCCT).

**Design:** Forty-five patients with SCCT were studied by immunohistochemistry to evaluate expression of EGFR, p-Akt and p-NF-κB. EGFR staining was performed using EGFR pharmDx kit on a Dako Autostainer. p-Akt and p-NF-κB were stained using a semi-automatic method (manual phospho-antibody incubation overnight, followed by automatic staining using a Dako autostainer). Results for SCCT were compared with those for associated high grade dysplasia (HGD) and adjacent normal appearing epithelium. In addition, tonsillar epithelium from non-neoplastic specimens of age-matched patients was also stained for the same markers.
**Results:** In both HGD and SCCT, all three markers were overexpressed, when compared to those in the adjacent normal epithelium and the normal control tonsillar epithelium. When markers from SCCT were correlated with survival status, only p-NF-κB was a statistically significant predictor of poor survival (p=0.047). No markers in SCCT were significantly related to rate of recurrence. When analyzing marker scores from HGD tissue, both p-Akt (p=0.043) and p-NF-κB (p=0.006) were related to poor survival. Additionally, p-NF-kB from HGD tissue was related to rate of recurrence (p=0.049).
**Conclusion:** p-NF-κB, overexpressed in HGD and SCCT, is associated with worse prognosis in terms of rate of recurrence and poor survival. This significant finding in patients with SCCT suggests that p-NF-κB represents a potential therapeutic target in head and neck squamous cell carcinoma (HNSCC).

# Hematopathology

### 1022 T-Cell Receptor Signaling and Growth Pathways in T-Cell Tumors

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**Background:** In non-neoplastic T-cells, T-cell receptor (TCR) engagement induces activation of a cascade of membrane-associated and cytoplasmic kinases leading to widespread transcriptional changes that support cell survival and proliferation. The role of this important growth pathway in promoting T-cell tumors has not been well established.
**Design:** Using phosphospecific antibodies as markers of kinase activation, we examined the expression and function of TCR-associated tyrosine kinases Lck, Syk and ZAP-70 as well as downstream Akt kinase in a wide range of T-cell neoplasms (n=158) and T-cell tumor lines (n=3). We evaluated the functional upregulation and expression of Akt and its target forkhead after TCR ligation. We examined the effect of blocking the proximal TCR signaling pathway on the expression/activation of Lck, Akt, and LAT using rosmarinic acid, a putative inhibitor of the TCR-associated kinase, Lck.
**Results:** Nearly half (48%) of primary peripheral T-cell tumor samples showed constitutive activation of the TCR-associated kinases and downstream Akt, whereas most (57%) anaplastic large cell lymphomas showed absence of activated TCR-pathway components and much lower levels of Akt activation. Activation of the TCR-associated kinases and Akt in these tumors appeared to be independent of the presence or absence of surface TCR-CD3 complex suggesting a non-canonical mechanism of activation. These results were paralleled by *in vitro* stimulation studies in which some primary T-cell tumors and cell lines showed activation of TCR-associated kinases, Akt and its targets following TCR-crosslinking whereas others were not affected. We showed that the putative Lck inhibitor, rosmarinic acid, was capable of inhibiting proliferation in T-cell tumor lines in a dose-dependent manner, in parallel with inhibition of Lck kinase activity.
**Conclusions:** We thus provide evidence that constitutive activation of the TCR-associated pathway kinases are a common feature of T-cell tumors and that blocking this pathway can inhibit proliferation *in vitro*. These results support the TCR-associated kinase pathway as an important target for treatment of T-cell malignancies.

### 1023 ZAP-70 Expression in Precursor B-Cell Acute Lymphoblastic Leukemia

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**Background:** Zeta-associated protein-70 (Zap-70), a member of the Syk family of tyrosine kinases, plays a critical role in T-cell receptor signaling and is associated with a subset of chronic lymphocytic leukemia/small lymphocytic lymphomas (CLL/SLL) cases with poor clinical prognosis. ZAP-70 is also important in early B-cell development and loss of ZAP-70 results in a block at the pro-B cell stage of development. Recently, ZAP-70 expression has been reported in precursor B-cell acute lymphoblastic leukemia (pre-B ALL). The biological and clinical significance of ZAP-70 expression in pre-B ALL has not been established.
**Design:** Using immunohistochemical methods, tissue microarrays and paraffin-embedded tissue sections, we evaluated ZAP-70 in bone marrow biopsy or clot specimens from 52 adults with pre-B ALL. We also by graded expression by comparison with T-cells as follows: 0, no staining; 1+, positive but less than T-cells; 2+, positivity equal to T-cells. We correlated ZAP-70 expression with the age, immunophenotype by flow cytometry, proliferative index by Ki-67 staining, and conventional cytogenetics.
**Results:** ZAP-70 expression in pre-B ALL demonstrated both nuclear and cytoplasmic staining. 33 of 52 (63%) pre-B ALL were positive for ZAP-70. ZAP-70 expression was 2+ in 9 (17%) cases and 1+ in 24 (46%) cases. The median age was 41.5 (range 19 to 72). ZAP-70 expression (1+ or 2+) correlated significantly with older age and low CD33 expression (Table 1). Cases with 2+ ZAP-70 expression had lower proliferation rates than those with 0 or 1+ expression (p=.048, Mann-Whitney). There was a significant association between abnormal karotype and 2+ ZAP-70 expression (p=.023, Fisher exact test), with all 17 diploid patients having 0 or 1+ ZAP-70 expression. No association between the Philadelphia chromosome (13 cases) and ZAP-70 expression was found. Evaluation of the 29 untreated patients showed that 5 of 5 patients with 2+ expression of ZAP-70 were disease free at 2 years.
**Conclusions:** Expression of ZAP-70 in pre-B ALL is significantly associated with older age, lack of CD33 expression and abnormal karyotype. The prognostic significance of ZAP-70 expression in pre-B ALL merits further investigation.

Table 1

| pre-B cell ALL | Median Age | CD20-positive | CD33-positive | 2 year disease free survival |
|---|---|---|---|---|
| ZAP-70 Positive | 45 | 65.6% | 33% | 100% |
| ZAP-70 Negative | 34.5 | 37.5% | 64% | 62% |
| p-value | 0.046 (Mann-Whitney) | 0.12 (Fisher's exact) | 0.047 (chi square) | not significant |

### 1024 The Value of a Limited 4-Antibody Panel in the Detection of Hematolymphoid Neoplasia in Bone Marrow and Peripheral Blood by Flow Cytometry

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**Background:** Flow cytometry (FC) has proven to be an important tool in the diagnosis and classification of hematolymphoid malignancies. Different laboratories use varying numbers of antibodies for FC analysis of these malignancies and the optimal number of reagents needed remains controversial. CD45 is an antigen expressed by all leukocytes but at different levels of intensity among various lineages and stages of maturation. Along with light scatter properties, this marker enables the selective gating and separation of different cell types in bone marrow (BM) and peripheral blood (PB). We assessed the value of a limited 4-antibody panel in detecting phenotypic abnormalities consistent with malignancy in BM and PB.
**Design:** We reviewed 407 consecutive cases (345 BM and 62 PB) received in our laboratory that were analyzed using a comprehensive 35-antibody panel. For the purpose of this study, cases were analyzed utilizing only a 4-antibody combination of anti-(CD45, CD19, CD38 and kappa light chain) along with light scatter properties. Interpretations were based on changes in shape, density, and location of discrete cell clusters by visual inspection of the graphical FC data. Results were compared to those obtained with the comprehensive panel.
**Results:** The interpretations based on the comprehensive antibody panel included: no abnormality detected (235 cases), precursor B-acute lymphoblastic leukemia (10), acute myeloid leukemia (27), chronic lymphocytic leukemia (25), hairy cell leukemia (6), other T and B-lymphoproliferation/lymphoma (30), plasma cell dyscrasia (PC) (35), possible myelodysplastic syndrome (MDS) (17), and other abnormalities (22). Using the limited antibody panel, abnormalities were detected in 160/172 (93%) cases considered abnormal by the comprehensive panel and more specific characterization was possible in many cases. Undetected cases with the limited panel included small cell peripheral T-cell lymphoma (2), possible MDS (4), PC (1), and minimal residual disease (5).
**Conclusions:** In our study, careful FC data analysis using a limited 4-antibody panel allowed the detection of most PB and BM neoplasias, as recognized by a much larger number of antibodies. This restricted panel may be used as a screening tool. However, additional reagents would be necessary to identify all cellular elements present, confirm cell lineage, and assess differentiation pathways.

### 1025 Immunophenotypic Comparison of Peripheral Blood Stem Cells, Normal Bone Marrow Blasts and Leukemic Myeloid Blasts

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**Background:** With the expanding therapeutic use of myeloid growth factors, cases with circulating blasts ("stem cells") are increasingly encountered in practice. These may cause diagnostic dilemmas, especially in patients with myeloid leukemia. In this study, we undertook a detailed flow cytometric characterization of normal circulating stem cells (PBSC) and compared them with non-leukemic and leukemic myeloid blasts.
**Design:** Ten PBSC apheresis specimens were prospectively studied, in addition to 18 normal bone marrows (BM) and 11 cases of CD34(+) acute myeloid leukemia (AML) identified from our database. Blasts/PBSC were identified by CD34/CD45 expression and light scatter properties. The expression of the following markers was evaluated beyond isotypic control cutoffs: CD11b, CD14, CD15, CD33, CD34, CD36, CD38, CD45, CD64, CD79a, HLA-DR and myeloperoxidase (MPO).
**Results:** Three markers were most informative in differentiating between the three groups: CD15, HLA-DR and MPO. A significantly higher proportion of AML blasts expressed CD15 (mean 60.76%), compared to PBSC (14.41%; p=0.0005) or BM (17.25%; p=0.0008). Interestingly, MPO expression in leukemic blasts (mean 73.39%) was also significantly higher than that of normal BM blasts (27.31%; p=0.0009) or PBSC (13.44%; p=0.0001). MPO expression was also significantly lower in PBSC, compared to normal BM (p=0.0024). In addition, PBSC were uniformly bright for HLA-DR, while BM blasts showed more variable expression, with a significantly higher proportion below the isotypic cutoff (p=0.02). Compared to normal BM blasts, PBSC showed significantly brighter CD34 expression (p=0.01) and more variable (p=0.04) and dimmer (p=0.002) CD38 expression, suggesting a less mature stage of differentiation. Expression of other markers did not show significant differences amongst the three groups.
**Conclusions:** Compared to normal BM blasts, non-leukemic PBSC have uniform bright HLA-DR expression and a lower proportion express MPO. In combination with brighter CD34 and variable CD38 expression, this is suggestive of a less mature stage of differentiation. Of note, substantial MPO expression in blasts is a leukemia-associated feature, and, in conjunction with CD15 expression, is highly useful in distinguishing leukemic myeloblasts from PBSC and normal BM blasts.