1107 Human Ovarian Surface Epithelial Cells Can Give Rise to Papillary Serous Carcinoma under the Influence of Specific Oncogenes and the Microenvironment

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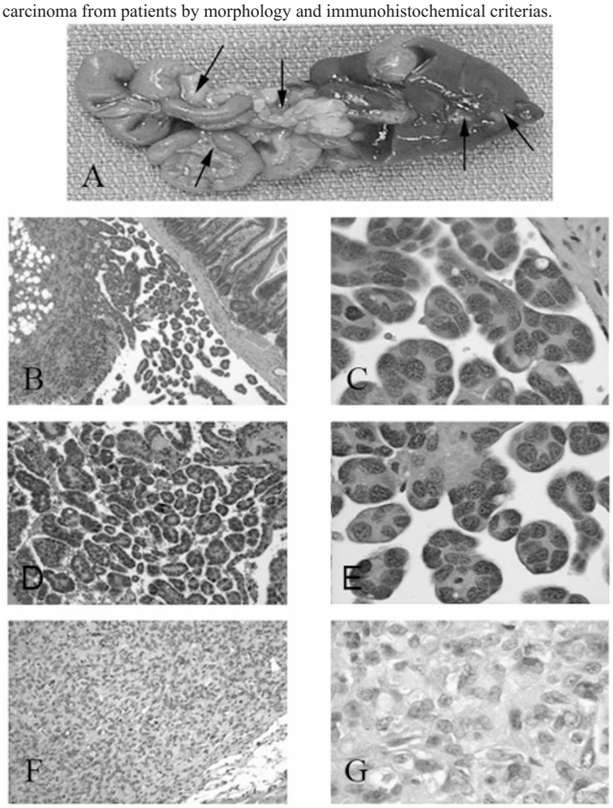
Background: Several possible cell origins have been proposed for human ovarian cancer including ovarian surface epithelial cells, fallopian tube epithelia, and rete ovarii. However, data supporting such conclusions are largely from morphologic and genetic correlative evidence, a causal relationship between the cell origin and ovarian cancer has not been established.

Design: In this study, we determined whether human ovarian surface epithelial cells could be the origin of ovarian cancer by introducing genetic elements into normal human ovarian epithelial cells and test whether such genetic modifications can induce the development of ovarian cancer.

Results: Enforced expression of HER-2/neu into two nontumorigenic human ovarian surface epithelial cells previously immortalized with SV40 T/t antigen and the catalytic subunit of telomerase resulted in anchorage independent growth of these cells on soft agar. HER-2/neu transfected cells injected sub-cutaneously developed carcinoma with morphology resembling undifferentiated carcinomas (Fig 1F-G). However, when these tumor cells was injected into the peritoneal cavity of nude mice, one of two lines developed papillary carcinoma similar to high grade papillary serous carcinoma (Fig. 1A-E). The tumor cells are strongly positive for cytokeratin, WT-1, p53, and CA125 staining. Thus, this genetically created papillary carcinoma resembled high grade papillary serous carcinoma from patients by morphology and immunohistochemical criterias.



Conclusions: These results provide first casual genetic evidence that ovarian surface epithelial cells can serve as the origin of high grade serous carcinoma and that the development of papillary serous carcinoma is also dependent on the tumor microenvironment. This newly created ovarian cancer model should greatly facilitate the study of pathogenesis of human ovarian cancer.

1108 HPV Genotypes and Cervical Carcinoma: Analysis of 163 Cases in a US Population

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Background: This study examines the distribution of human papillomavirus (HPV) genotypes and the relative association with different histologic cell types in 163 invasive cervical cancers in a US population.

Design: Cervical cytologic samples were genotyped using the LINEAR ARRAY® HPV Genotyping Test (Roche Diagnostics, Branchburg, NJ) that target the *L1* gene using PGMY09/PGMY11 consensus primers. High risk (HR) HPV genotypes included HPV16, 18, 31 and 45. Other carcinogenic HPV genotypes were classed as intermediate risk (IR). HR-negative cases using this system were secondarily tested using PCR with HPV type-specific primers for *E6* and *LCR* corresponding to HPV16, 18, 31, 33, 35, 39, 45, 52, 58 and 68.

Results: 153 histologically confirmed cancer cases were positive for one or more HR or IR HPV types using the PGMY09/PGMY11 primers. Of the 10 HPV-negative cases, 5 were subsequently shown to have HPV DNA using type-specific primers, including HPV16 (n=3), HPV18 (n=1) and HPV45 (n=1). After all analyses, 3.1 % remained HPV-negative.

HPV Genotypes in 163 Cervical Cancers

CELL TYPE	SQUAMOUS N (%)	ADENO CA N (%)	ADENO SQ N (%)	SMALL CELL N (%)	TOTAL N (%)
HPV16	84 (67.7)	7 (33.1)	3 (30.8)	0	94 (57.7)
HPV18	14 (11.2)	9 (42.9)	7 (61.5)	4 (100)	34 (20.9)
HPV16+18	5 (4.0)	1 (4.8)	1 (7.7)	0	7 (4.3)
HR, NOT 16,18	6 (4.8)	2 (9.5)	1 (7.7)	0	7 (4.3)
IR	13 (10.4)	0	1 (7.7)	0	14 (8.6)
NO HPV	3 (2.4)	2 (9.5)	0	0	5 (3.1)
TOTAL	125	21	13	4	163

Columns total 100%

The most frequent HPV genotypes associated with cancers other than HPV16 or 18 were HPV45 (4.3%) and HPV33 (2.4%). The association between HPV genotype and histologic cell type was highly significant ($p < .001$). HPV16 was closely associated with keratinizing squamous cancers while non-keratinizing cancers were more heterogeneous. IR genotypes were the highest risk genotypes in 8.6% of cancers.

Conclusions: This study documents the pattern of HPV genotypes in cervical cancers in a US population in the pre-vaccination era. HPV genotyping using PCR for *L1* can fail to identify HPV DNA in a small percentage of cancers, likely due to loss/modification of *L1* during integration. A small percentage of cancers remained HPV-negative after extensive HPV testing. Sampling variation may explain many of these cases.

1109 Prepartum Cervical Cytologic Changes Correlated with Abnormal Placental Changes and Preterm Delivery

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Background: The understanding of clinical implications of abnormal cytology screening results in the pregnant population is incomplete. The objective of this study was to determine clinical implications of abnormal cervical cytologic screening on the outcome of pregnancy.

Design: A 12-year review of cases from our institution yielded 2480 cases with data for comparison of the impact of abnormal cervical cytology on placental changes and pregnancy outcome in terms of prematurity.

Results: Analysis showed a statistically significant correlation between reactive, inflammatory, infectious, atypical, and dysplastic prepartum cytologic changes with abnormal placental diagnoses including but not limited to accelerated/delayed placental maturation, various placental infections and vasculopathies (Table 1). All but dysplastic cytologic changes showed a statistically significant association with preterm labor (Table 1). Statistically significant associations were also present between a positive HPV DNA assay and preterm labor and abnormal placental diagnoses.

Table 1. Associations between Pap findings, placental findings, and pregnancy outcome

Pap Dx	N=2480	Abn P	*p*	OR(95% CI)	Preterm	*p*	OR(95% CI)
NORMAL	715	175	-	-	116	-	-
AGUS	29	16	<0.001	3.80(1.79-8.05)	11	<0.01	3.16(1.45-6.86)
ASCUS	290	133	<0.001	2.61(1.96-3.48)	66	<0.01	1.52(1.08-2.14)
HSIL	103	44	<0.001	2.30(1.50-3.52)	24	0.051	1.57(0.95-2.58)
LSIL	161	69	<0.001	2.31(1.62-3.30)	32	0.208	1.28(0.83-1.98)
REACTIVE	1182	482	<0.001	2.12(1.73-2.61)	278	<0.001	1.59(1.25-2.02)
Trichomonas	187	96	<0.001	3.26(2.33-4.54)	63	<0.001	2.62(1.83-3.77)
Candida	476	193	<0.001	2.10(1.64-2.70)	100	<0.01	1.37(1.02-1.85)

Notes: Pap Dx, Pap diagnosis during pregnancy. Abn P, cases of placenta with pathological findings. Differences between each Pap Dx to NORMAL analyzed using Chi square test, two-tailed *p* values are shown. Statistical significance defined as $p<0.05$. OR, odd ratio. 95% CI, 95% confidence interval.

Conclusions: These findings indicate that prepartum dysplastic cervical changes do not affect the duration of gestation or correlate with abnormal placental changes, but that the presence of infectious agents and inflammatory atypia on prepartum cervical screening may serve as a risk marker for preterm labor and abnormal placental changes.

Head & Neck

1110 Utilization and Value of Frozen Section in the Diagnosis of Thyroid Cancer

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Background: In the era of FNA evaluation of thyroid nodules, the use of frozen section (FS) in the management of patients who require surgery is controversial. FS, including its complementary use of imprints, may be unnecessarily redundant. Since treatment for thyroid cancer is a total or near total thyroidectomy an intraoperative diagnosis of cancer will avoid a second procedure. FNA has been a valuable form of preoperative evaluation primarily because the diagnosis of papillary carcinoma relies on features of nuclear morphology. FNA is less valuable in separating true follicular neoplasms from colloid nodules and of no use in the diagnosis of follicular carcinoma. The value of FS in this context is debatable since the diagnosis of follicular carcinoma requires clear-cut vascular invasion, a rare event which is sampling dependent. This study examines the utilization and value of FS in establishing an intraoperative diagnosis of thyroid cancer.

Design: We reviewed 271 thyroid FNA reports and subsequent thyroidectomies from December, 2004 to July, 2007 comparing preoperative FNA, intraoperative FS and final pathologic diagnosis. Only patients with lobectomies were included.

Results: Of the total, 97 patients underwent surgery with FNA diagnoses suggestive of follicular neoplasm or positive of papillary carcinoma. Of these, 76 also had FS examinations with the following results: papillary carcinoma (14), follicular tumor (38), and colloid nodules (12). There were no FS diagnoses of follicular carcinoma. On permanent examination, there was a total of 24 confirmed cases of papillary carcinoma: 4 with an indeterminate FNA diagnosis but confirmed as papillary carcinoma by FS; 2 deferred FS, 6 not examined intraoperatively and 2 false negatives. Three eventual

cases of follicular carcinoma were diagnosed after extensive conventional examination. In addition, 128 of the 271 FNAs were diagnosed as colloid nodules and required no further surgical intervention.

Conclusions: This data suggests that even though FNA provides accurate preoperative information, a definitive total thyroidectomy will continue to be based on tissue examination which begins with an intraoperative assessment. FS is most reliable in the diagnosis of papillary carcinoma and colloid nodular disease. The rarity of follicular carcinoma is emphasized by its FS absence in this series. Follicular carcinoma remains a diagnosis dependent on thorough capsular sampling. As long as surgeons understand that follicular carcinoma is a rare disease, follicular lesions are at an increased risk for second surgical procedure.

1111 Methods of HPV Detection in Tonsillar Malignancies

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Background: Squamous cell carcinoma of the tonsil is strongly linked to HPV 16 and detection of this virus is associated with a specific tumor type (basaloid) and more favorable outcome. Because of the latter, HPV testing is frequently requested when these tumors are discovered, or to pinpoint the tumor source when metastatic carcinoma is detected in a cervical lymph node. The aim of this study was to determine the sensitivity of PCR and in situ hybridization techniques for detection of HPV in these tumors.

Design: The standard for comparison was a PCR-based assay by Access Genetics (AGPCR), using generic primers targeting a 450bp amplimer from the L1 region of most HPV types and resolving the HPV type by RFLP. Comparisons were made with the Ventana platform for in situ hybridization (VISH) using probes targeting high risk HPVs. In addition, an HPV-16 specific primer that targeted the HPV 16 E7 region (E7PCR), resulting in a 210 bp amplimer, was used. In the latter comparison, p16 immunostaining was also performed.

Results: Seventeen cases were concurrently tested by both AGPCR and VISH. 14 of 17 were concordant. Two weakly-positive VISH results were negative by PCR; one negative VISH was positive for an unknown HPV type. All but one VISH positive had diffuse nuclear staining consistent with the presence of episomal HPV DNA. 27 cases were analyzed by both AGPCR and E7PCR. twenty-two were concordant. Four were E7PCR only, three of which were strongly p16 positive. One was AGPCR positive, confirmed by p16 staining.

Conclusions: VISH and AGPCR have comparable performance for the detection of HPV in tonsillar carcinomas, much of which appears to be episomal. E7PCR is slightly more sensitive than AGPCR and correlates with p16 immunostaining. Increased detection with the E7 primers may reflect smaller target amplimer size, which will function over a greater range of target DNA quality. However, all three assays exhibit a sensitivity relative to each other that is 85% or higher.

1112 Squamous Cell Carcinoma of the Head and Neck in HIV-Positive Patients. A Neoplasm Infrequently Associated with Human Papillomavirus Infection

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Background: Malignant neoplasms occur at increased frequency in patients with HIV/AIDS, especially those related to human papillomavirus (HPV) infection. We have studied the clinicopathological features of head and neck squamous cell carcinomas (HNSCC) developing in HIV-positive patients, and we have analyzed the possible relationship with HPV infection.

Design: All HIV-infected patients with HNSCC diagnosed at a single institution from 1998 to 2007 were retrospectively evaluated. The clinicopathological features were analyzed and tissues were tested for the presence of HPV genome by highly sensitive PCR. Moreover, immunohistochemical studies for HIV p24, p16 INK4a, p53, Epstein-Barr virus latent membrane protein 1 (LMP-1) and human-herpes virus 8 (HHV-8), and in situ hybridization for mRNA of EBER1/2 gene of the Epstein-Barr virus were performed.

Results: Ten of 4,987 HIV-infected patients seen in this period in the Infectious Disease Department developed HNSCC. The patients median age was 45 (range 36-54), the male to female ratio was 2:1. All patients were heavy smokers. Median duration of HIV infection previous to the diagnosis of the laryngeal tumor was 10.5 years (range 7-18 years) and all patients had been on antiretroviral therapy since the identification of HIV infection. Six neoplasms developed in larynx, two in oropharynx and two in oral cavity. All tumors were keratinizing squamous cell carcinomas poorly differentiated (8 cases) or moderately differentiated (2 cases). The tumors usually presented at a high clinical stage and a half of the patients died of the laryngeal neoplasm after a mean time of 16 months. HPV 16 was detected in only one oropharyngeal carcinoma, whereas the rest of the tumors were negative for HPV, even in two patients with laryngeal carcinoma and coexisting HPV-associated gynecological neoplasm. Stainings for HIV p24, p16INK4a, LMP-1, HHV-8 and EBER were negative in all tumors, and p53 was positive in 6 of 10 cases.

Conclusions: HNSCC developing in HIV-positive patients is infrequently associated with HPV infection. This neoplasm develops in young, heavy smokers and presents at an advanced clinical stage.

1113 Clinicopathologic Features and Long-Term Survival of Follicular Variant of Papillary Thyroid Carcinoma: A Comparison to Classical Papillary Thyroid Carcinoma

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Background: Conflicting data exist in the literature regarding the clinical and pathologic differences between classical papillary thyroid carcinoma (CPTC) and the follicular variant of papillary thyroid carcinoma (FV). During a period of 25 years, 1,142 patients were diagnosed with papillary thyroid carcinoma at Barnes-Jewish Hospital, St. Louis, Missouri. Follow-up of these patients showed that patients who had FV had better progression-free and higher survival rates than those with CPTC. In this study, we compared the clinical and histopathologic features of the histologic variants in an attempt to correlate these features to the differences in patients' outcome.

Design: Of the original 1,142 papillary thyroid carcinoma cases, 294 were diagnosed as FV and 848 as CPTC. 142 FV cases had available microscopic sections, satisfied strict histopathologic criteria, and had complete medical records. We randomly selected 200 CPTC cases and used similar criteria to obtain a final sample of 118 cases. 109/142 (76.7%) of the FV and 88/118 (74.5%) CPTC patients were female. 45.0% of the FV and 40.6% CPTC patients were 45 years of age or older. All 260 patients in both groups had been treated with total thyroidectomy and post operative I-131.

Results: Using the Chi Square, Kaplan-Meier survival with long-rank (Wilcoxon) test of equivalance, we found that patients with FV have better progression-free ($p=0.0037$) and higher survival rates ($p=0.035$) than patients with CPTC. Patients with CPTC presented with a higher T stage ($p<0.0001$), more common lymph node metastasis ($p<0.0001$), and more advanced overall TNM stage ($p<0.005$). Microscopically, CPTCs were more likely to show extrathyroidal extension than FV ($p<0.0001$). There were no statistical differences between CPTC and FV with regards to patients' age, gender, the tumor's vascular invasion, multifocality or the presence of lymphocytic thyroiditis.

Conclusions: Follicular variant of papillary thyroid carcinoma has a more favorable long-term prognosis than classic papillary thyroid carcinoma. This difference is associated with earlier T stage less extrathyroidal extension and lymph node metastasis, as well as a lower overall TNM stage, at presentation. While FV may be a distinct phenotypic and molecular disease entity, our findings suggest that FV may also be an earlier form or a precursor of CPTC.

1114 Role of ProEx C and MiB-1 in Evaluation of Salivary Gland Neoplasms

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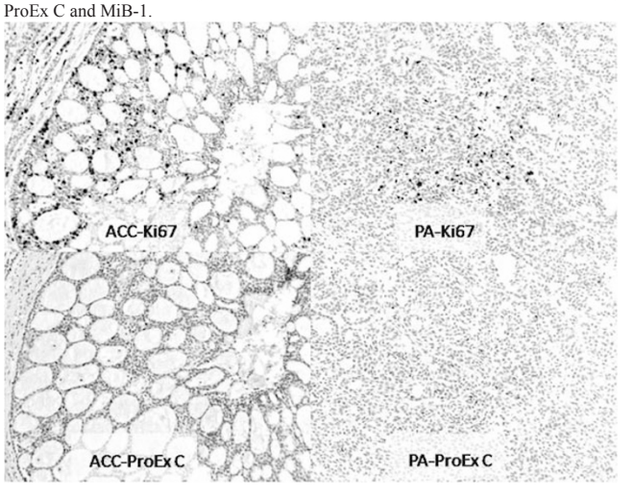
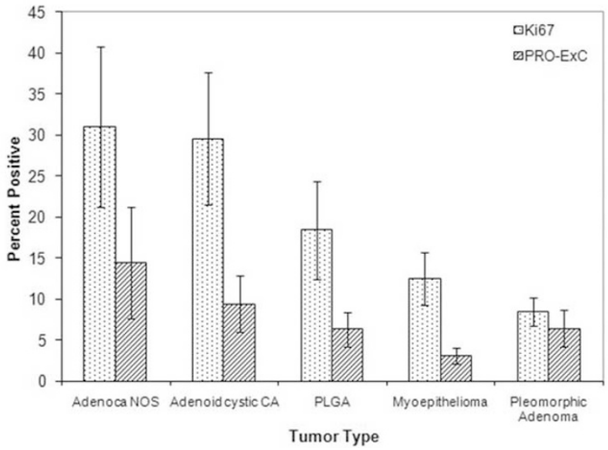
Background: Salivary gland neoplasms often present diagnostic challenges with overlapping histopathologic features. The aim of this study is to differentiate various salivary gland neoplasms using cell proliferation and aberrant S-phase cell cycle induction immunohistochemical markers.

Design: Five types of salivary gland neoplasms were selected, namely Pleomorphic Adenoma (PA), Myoeipthelioma (ME), Polymorphous Low Grade Adenocarcinoma (PLGA), Adenoid Cystic Carcinoma (ACC) and Adenocarcinoma not otherwise specified (AdCa NOS). Each tumor group included biopsy tissue samples from five patients. Each patient sample was stained with two immunohistochemical markers, ProEx C and MiB-1.



ProEx C is a cocktail of two monoclonal antibodies that target MCM2 - Mini Chromosome Maintenance Proteins and TOP2A - Topoisomerase II-alpha, which are proteins over expressed in aberrant S-phase induction. Number of positively stained tumor nuclei and total number of tumor cells were determined by computer assisted morphometric analysis.



Proportions of positively stained tumor nuclei were compared amongst various tumor groups, using Poisson regression models.

Results: There was statistically significant higher proportion of positively stained tumor nuclei for MiB-1 marker, in ACC as compared to PA, 95% confindence interval (CI) 1.34 to 8.15, p value 0.009, and in AdCa NOS as compared to PA, 95% CI 1.33 to 8.13, p value 0.009. No statistically significant difference in ProEx C staining pattern was identified among the various tumor groups, p value = 0.09.

Conclusions: MiB-1 proved useful in distinguishing ACC from PA and AdCa NOS from PA, but it did not aid in the differential diagnosis of malignant salivary gland neoplasms. ProEx C did not contribute in the evaluation of salivary gland neoplasms.

1115 Anaplastic and Squamous Thyroid Carcinoma Masquerading as Primary Mucosal Squamous Cell Carcinoma of the Trachea: Morphologic and Immunohistochemical Findings

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Background: Involvement of the larynx or trachea by thyroid carcinoma is not rare but, in well differentiated tumors, the origin is usually apparent, based on the tumor morphology. We report a series of 6 patients with anaplastic or squamous thyroid carcinoma in whom the presenting features related to a tracheal mass, and in which the biopsy showed what appeared to be primary mucosal squamous cell carcinoma (SCC).

Design: We examined the morphology of the tumours and adjacent epithelium, and expression of cytokeratins 7, 5&6, 19, thyroid transcription factor 1 (TTF1) and thyroglobulin (TG) by immunohistochemistry (all antibodies: DAKO).

Results: Five cases were anaplastic carcinoma with both spindle and squamous differentiation, 2 with coexistent well differentiated carcinoma (2 papillary,1 follicular). The sixth case was a squamous cell carcinoma of thyroid. In 5 of the 6 cases, the SCC appeared to arise within dysplastic squamous epithelium, mimicking a primary mucosal carcinoma. The tracheal component showed cytokeratin 7 positivity in 4 of 4 (focal), TTF1 (positivity in 1 of 4 (focal), and TG positivity (focal) in 1 of 4. Cytokeratin 19 was positive in 4 of 4 (focal). Cytokeratin 5&6 was positive to a varying extent in all cases.

Conclusions: Anaplastic and squamous cell thyroid carcinoma invading the trachea may mimic a primary mucosal squamous cell carcinoma, even appearing to arise from dysplastic tracheal epithelium. Immunohistochemistry is of limited value in clarifying the diagnosis, although TTF1 and TG may show focal positivity.

1116 Larynx and Trachel Transplantation: Histopathological Features of Rejection

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Background: Larynx and tracheal transplantation is an emerging therapeutic modality performed on selected patients who have irreversible damage to the larynx and who have impaired quality of life as a result. The largest worldwide clinical experience with larynx and tracheal transplantation in humans has been developed in Colombia. However, the histopathological features of rejection of larynx and trachea in humans have not been studied in detail. We also wanted to know what changes occur in the tissues in the weeks prior to the frank clinical manifestations of rejection as a way to determine what histological findings predict rejection.

Design: Twelve patients underwent larynx and tracheal transplantation. Post-transplant follow-up biopsy tissue glass slides were analyzed for 14 histological features to determine which ones were predominant and indicative of rejection. Those findings were extrapolated from previously reported experiences on animal models as well as on the current patient's clinical manifestations. The features included: Acute inflammation of the epithelium, epithelial lymphocytosis, lymphocytic perivascular infiltrates, epithelial individual cell necrosis, presence of columnar/ciliated epithelium, submucosal fibrosis, erosion/ulceration, necrosis, granulation tissue, eosinophils, epithelial atypia, squamous metaplasia, fibrin thrombi, and infection. These findings were evaluated on the biopsy tissues obtained around the time the patients had severe clinical manifestations of rejection as well as on biopsy tissues obtained in the weeks prior to that clinical episode. They were also compared to the group of patients who did not developed clinical rejection.

Results: Severe clinical rejection occurred between 4 and 10 months after the transplant and it manifested in 5 patients as cough accompanied by necrosis and purple appearance of the mucosa. Histologically, biopsy tissues showed necrosis, epithelial lymphocytosis, lymphocytic perivascular infiltrates, epithelial atypia, squamous metaplasia and eosinophils. In the 4 weeks prior to the severe clinical manifestation, 4 of the 5 patients had in common 2 predominant findings: Epithelial lymphocytosis and perivascular lymphocytic infiltrates.

Conclusions: Predictors of clinically significant rejection can be observed histologically in the biopsies obtained in the weeks prior to the full-blown manifestation. The main predictors are epithelial lymphocytosis and perivascular lymphocytic infiltrates.

1117 Frequency of Mitochondrial DNA (mtDNA) Somatic Mutations in Oncocytic Salivary Gland Neoplasms

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Background: Oncocytic salivary lesions comprise a spectrum of hyperplastic and neoplastic lesions. Oncocytic features are also observed in Warthin's tumors and mucoepidermoid carcinoma. These lesions share mitochondrial (mt) proliferations, with characteristic oncocytic appearance. The role and significance of this feature in these lesions remain uncertain. At issue are two fundamental questions: 1) is mt accumulations in hyperplastic and neoplastic lesions a compensatory/reactive or clonal process? 2) in neoplastic lesions, do mt alterations play a primary or a secondary role in tumorigenesis? This study focused on question 1.

Design: Mutational screening in selected regions of the mitochondrial genome in SG oncocytic lesions were performed. Tumors**:** 1 oncocytic hyperplasia, 2 oncocytomas, 5 Warthin's tumors (WT) and 1 malignant WT. Genomic DNA from frozen specimens was extracted, mtDNA fragments from the D-loop and COX II regions were amplified, PCR products purified and analyzed. Genetic polymorphism was identified using MITOMAP database.

Results: In D-loop , the number of point mutations varied (2-39). All of the samples had mutations in the hypervariable segment 1 (HVS 1) (2-24). The highest frequency of mutations occurred in the HVS 2 region. Of the detected point mutations, 90-100% were homoplasmic, 0-10% of the mutations heteroplasmic. Homoplasmic mtDNA mutations were G-A, C-A or G-T transitions. HVS 1 has previously been identified as a "hotspot" for both germline and somatic mutations, but the functional significance of mutations in this region remains unknown. In the COX II region, the homoplasmic mtDNA point mutation numbers varied between none and 11, with no heteroplasmic mutations. WT had highest frequency of mutations (5-11) compared to oncocytoma (1) and oncocytic hyperplasia (4).

Conclusions: Our analysis, limited to D-loop and COX II regions suggests that: 1) most mitochondrial mutations are homoplasmic. 2) HVS 1 and HVS 2 are the most frequent sites. 3) Mt DNA alterations of the D-loop occurred in both hyperplastic and neoplastic lesions, and may not be associated with tumorigenesis. 4) Mt DNA alterations of the COX II region were more frequent in neoplastic than in hyperplastic lesions. Further mutation analysis of other respiratory chain complex regions are ongoing in SG oncocytic lesions and cell lines developed from these neoplasms. Functional analysis of cellular O2 contents will be performed on the cell lines, at different points in culture, to assess the biological evolution of these tumors.

1118 Koilocytosis Is Associated with Human PapilomaVirus Infection in Laryngeal Squamous Cell Carcinoma

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Background: Human papiloma virus (HPV) is a pathogenic agent in some carcinomas, like those of the uterine cervix . Some head and neck squamous cell carcinomas (SCC) have also revealed infections by HPV . In these carcinomas, p16INK4A (p16) expression has been correlated with HPV infection. We have identified koilocytosis in some infiltrating laryngeal SCC (LSCC), similar to the observed cytopatic effect of HPV on cervix. We have evaluated whether this effect is related to HPV infection and p16 expression.

Design: Forty-five biopsies corresponding to 30 infiltrating LSCC were analyzed during 2007 in our laboratory. All cases were evaluated for koilocytosis and for p16 expression, with immunostaining scored as positive (diffuse cytoplasmatic and nuclear or focally intense) or negative if none or few isolated cells showed positivity. All cases were also evaluated for HPV viral DNA by PCR.

Results: Twenty-nine of 30 cases were men. Mean age was 63.2 ± 12.2 years, with a five years difference between HPV-negative (65.1 ± 13,4) and HPV-positive (60.3 ± 10,2) LSCC. Koilocytosis was present in 14 (47%), while HPV was detected in 12 (40%) of the cases. All HPV subtypes were high risk, and type16 was the most frequent (60%). p16 expression was positive in 15 (50%) of the cases, out of which 7 were diffuse and 8 focal. Seventy-one per cent (10 of 14) of the LSCC with koilocytosis were HPV-positive. In contrast, 88% (14 of 16) of the LSCC not showing koilocytosis were HPV-negative. Seventy-five per cent (9 of 12) of the HPV-positive carcinomas showed p16 expression, and of those 100% (9 of 9) showed koilocytosis. Conversely, 67% (12 of 18) of the HPV-negative cancers lacked p16 immunoreactivity, and of those 75% (9 of 12) did not show koilocytosis. Here we report a sensitivity, specificity and concordance coefficient for koilocytosis and p16 expression of 83%, 78%, 0.595 and 75%, 67%, 0.400, respectively.

Conclusions: These data show not only that there is a relationship between koilocytosis and HPV status in LSCC, but also that koilocytosis correlates better than p16 expression with the presence of the HPV-DNA in LSCC, being more specific and sensitive. Koilocytosis may therefore serve as a surrogate marker that could replaced more expensive analysis, and help to define a biologically different tumor entity in LSCC.

1119 *In Situ* Hybridization Signal Patterns in Recurrent Laryngeal Squamous Papillomas Indicate That HPV Integration Occurs at an Early Stage

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Background: Laryngeal papillomas are histologically benign tumors that frequently recur. They may spread throughout the respiratory tract and can compromise airways. Children have a higher incidence than adults (4.3 vs. 1.8 per 100,000). The overall prevalence of HPV (mostly low-risk HPV genotypes 6 and 11) in these papillomas has been estimated at 76 percent; HPV11 may be associated with a more aggressive disease course. This study investigates HPV genotype, physical status, and protein expression, in relation to papilloma recurrence in children and adults.

Design: Forty-seven archival laryngeal papilloma specimens were obtained from nine children (ages 1 – 16 years) and fourteen adults (ages 16 – 82 years). In cases of recurrent respiratory papillomatosis (6 children, 10 adults), the first and last papillomas were assayed. HPV type was determined by GP5+/6+ PCR of sample DNA extracts followed by dot blot hybridization. I*n situ* hybridization (ISH) was performed on thirty-eight specimens; the data were recorded in terms of diffuse (episomal HPV) and punctate (integrated HPV) signal patterns. Immunohistochemistry for the HPV L1 capsid protein, a marker of HPV productive status, was performed on thirty-four samples.

Results: All forty-seven samples tested HPV positive: overall, HPV11 was identified in 2/9 (22.2%) children and in 7/13 (53.9%) adults with single infections [p=0.2]; 7/9 children and 6/13 adults tested HPV6 positive; a recurrent HPV6/11 double infection was noted in one adult. HPV11 was identified in 1/6 (16.7%) children and in 6/9 (66.7%) adults with recurrent papillomas [p=0.12]. ISH signals (punctate ± diffuse) were detected among 6/9 (66.7%) child and 11/13 (84.6%) adult patients. Among patients with recurrent papillomas, punctate signals (± diffuse) were found in the first as well as the most recent sample. L1 staining was detected in 1/9 (11.1%) children and in 6/10 (60%) adults [p=0.057].

Conclusions: These data support the idea that integration of low-risk HPV types into the cell genome is an early and important event in the etiology of recurrent laryngeal papillomas of both children and adults. Larger studies are required to investigate the apparent abrogation of the productive HPV life-cycle (due to integration) in children. Further studies are also warranted to examine whether HPV11 is more prevalent in adulthood than childhood laryngeal papillomas.

1120 Identification of Human Papilloma Virus (HPV) in Basaloid Squamous Cell Carcinoma of the Head and Neck and Its Clinicopathologic Significance

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Background: Basaloid squamous cell carcinoma (BSCC) of the head and neck is a rare, clinically aggressive malignancy that has a predilection for the larynx, hypopharynx and oropharynx. The histologic features are distinct from, but often confused with, those of HPV-related oropharyngeal nonkeratinizing squamous cell carcinoma (NKSCC), which has been shown to be associated with a more favorable outcome. The purpose of this study was to determine the prevalence of HPV in oropharyngeal and nonoropharyngeal tumors with true BSCC histology, and to compare their immunohistochemical reactivity to p16 and p53. In addition, the biological significance of HPV positivity in terms of patient survival was explored.

Design: Cases with a diagnosis of BSCC were selected from the Pathology Department files and those meeting Wain's criteria (Hum Pathol 1986) were included. Tumors with NKSCC morphology were excluded. In-situ hybridization for high-risk HPV subtypes and immunohistochemical staining for p16 and p53 were performed. Detailed medical records and follow up information for all patients were reviewed.

Results: Of 27 BSCC cases, 11 (40.7%) were from the oropharynx and 16 (59.3%) from the larynx or hypopharynx. There were no statistically significant differences in stage or gender by site. The average age of the oropharyngeal BSCC patients was younger but the difference was not statistically significant. Nine of the 11 oropharyngeal BSCCs (81.8 %) were HPV positive and 8 (72.7%) were reactive for p16. In contrast, none of the 16 nonoropharyngeal BSCCs were HPV positive and only one (6.3%) stained positively for p16 ($p < 0.01$). Two oropharyngeal BSCCs were both HPV and p16 negative and stained strongly for p53. All of the 11 HPV positive oropharyngeal tumors were negative or stained weakly for p53. Thirteen of sixteen (81.3%) non-oropharyngeal BSCCs stained strongly for p53 ($p < 0.01$). The overall survival was better for HPV-positive BSCCs by Kaplan-Meier analysis ($p < 0.05$).

Conclusions: HPV-positive BSCCs are associated with better survival compared to HPV-negative BSCCs. BSCC of the oropharynx is more likely to be HPV-positive than BSCC of the larynx/hypopharynx. HPV positive oropharyngeal BSCC show strong reactivity to p16 and are negative or weakly reactive to p53. These finding suggest that BSCC of the head and neck may be a mixed clinicopathologic variant.

1121 Inflammatory Myofibroblastic Tumor of the Head and Neck (IMT-HN): A Light Microscopic, Immunohistochemical (IHC) and Cytogenetic Study of 6 Cases

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Background: IMT uncommonly occurs in the HN. IMT is believed to be a true neoplasm due to the detection of clonal cytogenetic aberrations involving the anaplastic lymphoma kinase (ALK) gene located on 2p23. The rearrangement and translocation of the ALK gene to form several fusion partners with, among others, tropomyosin 3 (1q22-23) and tropomyosin 4 (19p13.1) appear to be important in the oncogenesis of IMTs.

Design: 6 cases of IMT-HN were found from our collective files. The clinical and pathologic features were reviewed. IHC was performed on formalin fixed, paraffin sections using standard avidin-biotin complex method with microwave antigen retrieval for ALK-1, vimentin, desmin, actins smooth muscle and muscle specific, cytokeratins (AE1/AE3, CAM5.2, CK907), p63, S-100, HMB-45, myoglobin and CD68. Fluorescence in situ hybridization (FISH) was performed in 3 cases with available material on formalin fixed, paraffin sections using dual color, directly labeled, break apart format probes flanking the centromeric and telomeric regions of the ALK 2p23 locus (Abbott Molecular; Des Plaines, IL).

Results: All patients were males ranging in age from 15-64 years old (median, 40.5 years). Sites of occurrence include the larynx (5) and sinonasal tract (1). Histologic findings included a submucosal cellular proliferation with storiform to fascicular growth. The cells were spindled-shaped, stellate and/or epithelioid with enlarged round nuclei, eosinophilic nucleoli and abundant basophilic fibrillary appearing cytoplasm. Distinct eosinophilic intranuclear inclusions were seen in 4 cases. Scattered mitoses were identified; necrosis was absent. A mixed chronic inflammatory cell infiltrate was present. Lesional cells were immunoreactive for ALK-1 (3/6), vimentin (5/5), actins (3/4), desmin (1/4); all of the other markers were negative. ALK gene translocation was present in 3/3 cases.

Conclusions: IMT-HN may be diagnostically challenging, especially in its differentiation from other lesions, including spindle cell squamous carcinoma. The presence of ALK-1 immunoreactivity and/or gene rearrangements greatly assists in the diagnosis. However, detection of the ALK protein by IHC varies from 36-60% and the presence of atypical translocations may result in an absence of detection by FISH.

1122 Epigenetic Regulation of Chromatin Structure in Tumorigenesis: Polycomb Group Protein EZH2 and Heterochromatin Protein 1 Expression in Malignant Thyroid Lesions

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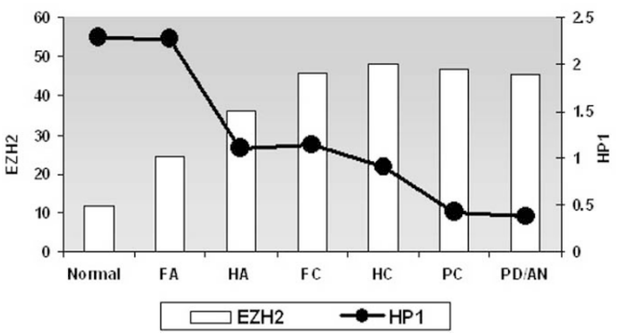
Background: Epigenetic regulation of chromatin structure plays an important role in tumorigenesis and tumor progression. The polycomb group protein EZH2, which regulates chromatin structure by methylating histones H3 and H1, is upregulated in a variety of tumors, including breast, prostate, and bladder. In addition, heterochromatin protein1 (HP1), which induces heterochromatin formation by binding to histones and thereby downregulates transcription, is lost in various tumors. In order to further understand the role that chromatin structure plays in thyroid cancer, we analyzed the expression of EZH2 and HP1β in thyroid cancer progression.

Design: Thyroid tissue microarrays (TMA) were built to include follicular adenomas (FA, N=19), Hurthle cell adenomas (HA, N=11), follicular carcinomas (FC, N=22), Hurthle cell carcinomas (HC, N=9), papillary carcinomas (PC, N=31), poorly differentiated and anaplastic tumors (PD/AN, N=10), colloid nodules (CN, N=5) and normal adjacent thyroid tissue (NT, N=80). Immunostaining was performed with antibodies for EZH2 (rabbit monoclonal, ZMD.309) and HP1β (rat monoclonal, MAC 353). Nuclear expression of EZH2 was scored as % positive nuclei using Automated Cellular Imaging System (ACIS, Clarient). Intensity of anti-HP1β staining was graded as 0, 1+, 2+ or 3+.

Results: In all thyroid lesions examined, there was a statistically significant increase in EZH2 expression when compared to NT ($p<0.01$). Furthermore, except in the case of FA, levels of HP1 beta were significantly decreased in all thyroid lesions when compared to NT ($p<0.01$). See graph below for the combined data.



Conclusions: Thyroid carcinomas exhibit significantly increased EZH2 expression, consistent with observations in other tumor types. EZH2 has been proposed to have a nonproliferative function in tumor progression. Interestingly, non-carcinomatous thyroid lesions also have increased EZH2. Thus, while EZH2 may not be sufficient for tumor initiation, increased EZH2 may play a role in tumor promotion, most likely by controlling gene expression. Similarly, the loss of HP1 in thyroid carcinomas is hypothesized to result in altered gene expression that promotes tumorigenesis.

1123 Prevalence of Salivary Ductal Inclusions in Parotid Lymph Nodes of Patients with Warthin Tumor

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Background: The histogenesis of Warthin tumor (WT) has long been debated. A predominant theory is that WT arises from heterotopic salivary ductal inclusions in intra/periparotid lymph nodes (LN); however, it is still unclear if WT is neoplastic. The prevalence of these inclusions in LN of patients with parotid WT has not previously been studied. We sought to determine this in comparison with parotids excised for pleomorphic adenoma (PA).

Design: Cases were chosen from the files of the QEII HSC, Halifax. 74 WT and 77 PA were initally selected (consecutively). H&E sections were reviewed, and cases with parotid LN were included. Aggregates of lymphoid tissue with a capsule and subcapsular sinus were defined as LN. An inclusion could be oncocytic or non-oncocytic, and was considered LN-included if completely surrounded by lymphocytes and not merging with hilar fat. Inclusions with acinar cells were excluded. Distinction between small WT and LN with oncocytic inclusions was made at 1 cm. We recorded the presence/absence of inclusions as well as the proportion of LN sections with inclusions.

Results: 46 WT and 52 PA met the above criteria (p=0.5). Among WT, the average age was 62.5 yrs (37.8-85.2); the M:F ratio was 1:1.3. Among PA, the average age was 50.2 yrs (16.4-82.3); the M:F ratio was 1:1.6. 33 WT (71.7%) and 17 PA (32.7%) had inclusions in any LN ($p<0.01$). A total of 383 and 270 LN sections were examined from WT and PA, respectively. Of these, 85 (22%) from WT and 30 (11%) from PA had inclusions (p=0.00024). Smoking status is available from 40 WT and 49 PA patients. 92.5% (37/40) of WT patients are smokers/ex-smokers, compared with 55.1% (27/49) PA patients($p<0.01$). Among PA patients, 12 of 27 smokers (44%) had inclusions compared with 5 of 22 (22.7%) non-smokers (NS).

Conclusions: Salivary ductal inclusions are more frequent in parotid LN from patients with WT than from those with PA. The high proportion of smokers among WT patients is consistent with prior studies. The M:F ratio among WT patients is lower. The results support the hypothesis that WT arises from salivary ductal inclusions found in intra or periparotid LN. The lack of a statistically significant difference in the prevalence of inclusions in smokers and non-smokers with PA suggests that smoking does not cause inclusions. We hypothesize that smoking acts on pre-existing inclusions to create WT. The study was limited by an inability to examine parotid glands that are free of neoplasia.

1124 Activation of the Mammalian Target of Rapamycin (mTOR) Pathway in Thyroid Carcinomas

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Background: Emerging data suggests that alterations of the PI3K/AKT/mTOR pathway play an important, previously unrecognized role in thyroid carcinogenesis. mTOR represents a key mitogenic output of the PI3K activation cascade and is a potential therapeutic target. Several mTOR inhibitors are in clinical trials for various solid organ tumors. Thus, we hypothesized that demonstration of this pathway using immunohistochemistry might be predictive of potential benefit from mTOR inhibitors.

Design: A tissue microarray (TMA) was built from 86 thyroid carcinomas including: papillary thyroid carcinoma (PTC, n=36), follicular carcinoma (FC, n=13), medullary thyroid carcinoma (MTC, n=21), poorly differentiated thyroid carcinoma (PDC, n=5) and anaplastic carcinoma (AC, n=11). Sections were stained with antibodies to PI3K, p-AKT, p-mTOR, p70S6K and pS6 ribosomal protein (p-S6rp). Controls stained appropriately, and slides were reviewed by two pathologists. Staining of ≥5% of tumor cells was interpreted as positive.

Results: There is a high level of expression of PI3k, p-mTOR, p70S6k and p-S6rp in all histologic types of thyroid carcinoma (see Table 1). Of note, the p-AKT expression was very low across the different types of thyroid carcinoma, probably representing an AKT-independent mTOR pathway activation.

	PTC (% Pos)	FC (% Pos)	MTC (% Pos)	PDC (% Pos)	AC (% Pos)
PI3K	89	62	67	100	100
p-AKT	3	15	0	40	0
p-mTOR	100	77	81	100	55
p70S6K	100	100	95	100	91
pS6rp	81	69	86	80	91

Table 1.

Conclusions: Our results indicate activation of the PI3K/mTOR/p70S6K/S6p pathway in all histologic types of thyroid carcinoma. This suggests a potential benefit from mTOR inhibitor in patients with thyroid carcinoma, especially in those with advanced disease. Prospective clinical trials are warranted to ascertain clinical utility.

1125 Characterization of Epigenetic Alterations of Tumor Suppressor Genes in Chemoresistant Head and Neck Squamous Cell Carcinoma

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Background: Despite multimodality treatment regimens, the overall 5-year survival rate for HNSCC has not exceeded 50% for the past three decades. One major factor for this dismal survival is the development of chemoresistance, resulting in frequent recurrences. Molecular mechanisms of chemoresistance in HNSCC have been extensively studies but remain elusive. Here, we attempt to characterize the patterns of epigenetic alterations of tumor-associated genes in a laryngeal cancer cell line that develop resistant to most chemotherapeutic agents.

Design: A HNSCC cell line, HEp2, which is highly resistant to Docetaxel and Cisplatin (DR-HEp2) and its parental sensitive cell line (HEp2) were used for RNA extraction, followed by AffyMetrix microarray analysis using human U133A and U133B gene chips, which contain 33,000 unique genes. Genes that displayed reduced expression in DR-HEp2 as compared to parental HEp2 cells, and subsequently showed increased expression following treatment with 5-aza cytidine, a DNA demethylating agent were selected. Those with promoter CpG island were then subjected to PCR-based restriction fragment length polymorphism (RFLP) analysis to determine the status of promoter methylation.

Results: Among 33,000 genes, there were 129 genes in DR-HEp2 cancer cells that showed decreased expression by 2-fold or greater as compared to its parental HEp2 cells, and increased in expression by 2-fold or greater following the treatment of 5-aza-cytidine. The promoter sequences of these 129 genes were then analyzed and 23 genes were identified, which contained a CpG island in the promoter. Five of these 23 genes, CLGN, BCAT1, HDGFRP3, MAP1B and CaRF were further analyzed by PCR-based RFLP and 2 of them, namely HDGFRP3 and MAP1B demonstrated promoter hypermethylation in DR-HEp2 but not in HEp2 cancer cells.

Conclusions: Using microarray technology combined with genetic analysis for promoter CpG island in chemoresistant head and neck cancer, we have tentatively identify a subset of tumor suppressor genes that may undergo gene silencing through promoter methylation as compared to its parental sensitive cells. These data should be invaluable in designing epigenetic therapies aiming at overcoming chemoresistance commonly seen in human cancer.

1126 Long Term Follow-Up of Patients with Follicular Variant of Papillary Thyroid Carcinoma (FVPTC)

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Background: FVPTC is a distinct morphologic variant of papillary thyroid carcinoma. Its behavior is not well-characterized and guidelines for management are lacking. Our goals were to characterize cases with a histologic diagnosis of FVPTC, assess their long-term outcome, and examine utility of completion thyroidectomy (CT) in patients with FVPTC diagnosed in partial thyroidectomy (PT).

Design: 41 patients with FVPTC on surgical resection in 1997-8 were retrieved from the pathology files. Patients with concurrent malignant thyroid neoplasm other than FVPTC were excluded. Clinico-pathologic data and follow-up (thyroglobulin and/or tissue sampling) were extracted from medical records. To assess long term outcome, first diagnosis (FD) of FVPTC included all histologic data from initial diagnosis (total thyroidectomy or combined PT and CT if within 1 year). To examine utility of CT, histologic data for PT and CT within 1 year were considered separately.

Results: **Patient/ tumor characteristics:** Mean patient age at FD was 47.9 yrs (21.9 – 76.7), with male to female ratio of 9:32. FVPTC presented as a single nodule (27 patients) or multiple (12: 2 in 5, 3 in 4, 4 in 1, "multiple" in 2). Mean size of the dominant nodule was 1.9 cm (0.2 – 4.5). Tumor capsular invasion (CI) was present in 6 (2 focal), extrathyroidal extension (ETE) in 1, lymphovascular invasion (LVI) in 1, and lymph node (LN) involvement in 2 cases. **Long term outcome:** 25/41 cases had follow-up (thyroglobulin in 20, tissue in 1, both in 4), with mean follow-up time of 7.7 yrs (2.2 – 16.7). 2/25 had recurrences of FVPTC: 1) FVPTC metastasized to a rib 7.3 yrs later, and 2) a 3mm FVPTC within a follicular adenoma was found in the contralateral lobe to that with the FD 16.7 yrs later. In both, FD FVPTC was an encapsulated single nodule (2.5 cm and 2.0 cm) with no CI, ETE, LVI, or LN involvement). **Utility of CT in patients with FVPTC diagnosed in PT:** 17 patients with FVPTC in PT had CT within 1 year. CT showed additional FVPTC in 5 patients. No others had significant findings. None of the 17 patients had recurrences of FVPTC.

Conclusions: Clinical follow-up was available in 61% of our FVPTC patients. In our experience, FVPTC is an indolent disease, with definitive metastasis in 1/25 (4%) of these patients. The microscopic FVPTC in the contralateral thyroid lobe 16 years after FD most likely represents an incidental finding rather than metastasis. None of the CT performed soon after PT had significant additional pathology, suggesting CT may not be necessary in these cases.

1127 Fluorescent In-Situ Hybridization for Detection of *MAML2* Rearrangements in Oncocytic Mucoepidermoid Carcinomas: Utility as a Diagnostic Test

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Background: Oncocytic mucoepidermoid carcinoma (OMEC) poses diagnostic challenge because of its overlap with other oncocytic salivary gland lesions, including Warthin's tumor (WT) when there is a prominent lymphoid stroma. While the prognostic value of the t(11;19) *MECT1-MAML2* fusion gene has been established in MEC, its potential use to discriminate OMEC from other mimics has not been established.

Design: Eleven cases of OMEC were selected and categorized based on predominant growth pattern: solid, cystic, or Warthin-like (defined by prominent lymphoid stroma). Available clinical and pathologic parameters, including grade were obtained. Cases were evaluated for *MAML2* rearrangements by fluorescent in-situ hybridization (FISH) using a *MAML2* - 11q21 break apart probe (SpectrumGreen-labeled BAC probe RP11-676L3 and SpectrumOrange-labeled BAC probe RP11-16K5; Children's Hospital Oakland Research Institute, Oakland, CA, USA) spanning the entire chromosome region of the *MAML2* gene. At least 60 interphase tumor cell nuclei were evaluated per case. A minimum of 20% of cells with split signal was considered positive.

Results: All OMEC were parotid tumors with a median age of 55.3 years (range: 9-83) and a female to male ratio of 2.7:1. Grade distribution was as follows: low-grade - 5, intermediate-grade - 3, and high-grade - 3. The histologic patterns observed were: solid - 5, cystic - 3, Warthin-like - 2, and mixed solid/Warthin-like - 1. Seven of the 11

cases (64%) showed a *MAML2* rearrangement by FISH. No direct correlation was seen between rearrangement status and histologic grade or growth pattern.
Conclusions: *MAML2* rearrangement is frequently detected by FISH in OMEC and is a potentially useful diagnostic tool in discriminating OMEC from other oncocytic lesions.

1128 *BRD4-NUT* Fusion Oncogene Analysis by RT-PCR in Sinonasal Undifferentiated Carcinoma (SNUC) and Other Aggressive Primary Sinonasal Malignancies: A Study of 15 Cases

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Background: *NUT*-rearranged carcinomas (NRCs), also termed *NUT* midline carcinomas (NMCs), have been characterized as poorly differentiated carcinomas of midline anatomic location that affect young patients and invariably follow a lethal clinical course. Although the most common balanced chromosomal translocation is represented by a t(15;19) causing a *BRD4-NUT* fusion, other fusion variants involving *NUT* and other partners have been observed—the so-called *NUT*-variant carcinomas. Recently, *NUT* rearrangements have been identified using fluorescent in-situ hybridization (FISH) in a subset of undifferentiated carcinomas of the sinonasal tract in older patients. This study employed reverse-transcription polymerase chain reaction (RT-PCR) to evaluate the incidence of the *BRD4-NUT* oncogene in sinonasal undifferentiated carcinomas (SNUCs) and other primary sinonasal malignancies.
Design: We carried out RT-PCR for the detection of the *BRD4-NUT* translocation in 15 cases of primary sinonasal malignancy. Eight of the 15 cases were classified as SNUCs by morphology and immunophenotype. The remaining cases studied were diagnosed as poorly differentiated carcinoma (3), squamous cell carcinoma (3), and lymphoepithelial carcinoma (1). The patients were predominantly older adults (median age, 61.3 years) and there were 8 men and 7 women. Each sample was evaluated twice with different primer sets (BR2276F/NUT1194R and BR2334F/NUT1132R). Two previously reported cell lines from t(15;19) carcinomas served as positive controls.
Results: RT-PCR studies revealed no *BRD4-NUT* fusion products with either primer set in any of the cases evaluated (0/15). *BRD4-NUT* fusion products were reproducibly obtained with both primer sets in the positive controls whereas no such products were obtained in the negative controls.
Conclusions: Our evaluation by RT-PCR of eight SNUCs showed none to have a *BRD4-NUT* fusion oncogene. Our results suggest that *BRD4-NUT* fusions are uncommon in SNUCs but our RT-PCR-based approach cannot exclude the possibility of variant *NUT* rearrangements in some cases of SNUC. Previously reported sinonasal tumors with *NUT* rearrangement are likely *NUT*-variant carcinomas that are more amenable to detection by FISH.

1129 Clinical HPV Testing in Oral Cavity/Oropharyngeal Squamous Carcinomas of the Head and Neck: Analysis of Site Distribution, Morphology, and Staining Patterns

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Background: HPV is an important pathogen in head and neck squamous carcinomas (SCC). The relative distribution varies by subsite, with the tonsil/tongue base being more frequently positive. Some studies have suggested basaloid (BSCC) and nonkeratinizing (NKSCC) have higher rates of HPV positivity than keratinizing (KSCC). We perform HPV testing on all oral SCCs. It is not well understood whether HPV is uniformly present in tumors, and whether biopsies are adequate samples for assessing viral presence. Furthermore, the copy number within unique tumor cells has not been studied, particularly with reference to the morphologic subtype of carcinoma.
Design: The surgical pathology files were searched for all reported clinical HPV results for an 18 month period. The site of tumor was recorded (oropharynx vs. oral cavity). The slides were reviewed to assess the relative percentage of KSCC, NKSCC, and BSCC components in the tumors. The HPV ISH was examined for signal (positive vs. negative), distribution of staining (focal vs. diffuse), and staining density (copy number estimation as compared to copy specific positive controls, low vs. high copy number).
Results: 56 cases were reviewed (47 male; 9 female). 46 were from the oropharynx (tonsil/tongue base) and 10 were from oral cavity (oral tongue/floor of mouth). 30/56 cases had mixed morphologies; KSCC predominated in 10, NKSCC in 17, and BSCC in 3 cases. 26 had pure morphology: 12 KSCC, 10 NKSCC, 3 BSCC and 1 LEC. HPV was positive in 29/56 cases (28/46 oropharynx, 1/10 oral cavity). 10/26 histologically pure tumors were HPV positive (1/12 KSCC, 5/10 NKSCC, 3/3 BSCC). 19/30 morphologically mixed tumors were HPV positive (3/11 KSCC predominant, 13/17 NKSCC predominant, and 2/3 BSCC predominant). 19/29 HPV positive cases had diffuse staining while 7 showed focal staining. 5/26 had low HPV copy number in the tumor cells (0/4 KSCC predominant tumors, 2/19 NKSCC predominant, and 3/5 BSCC predominant).
Conclusions: Our clinical experience with HPV assessment in oral SCCs supports prior literature suggesting that HPV is predominantly found in oropharyngeal locations and in BSCC and NKSCC subtypes, whether pure or predominant in mixed tumors. However, we also found HPV in at least 4 tumors that were predominantly keratininizing. Most tumors had high copy number HPV. But, 3/5 of the BSCC predominant tumors had low copy number.

1130 Salivary Duct Carcinoma with or without Micropapillary Components: Clinicopathologic and Immunohistochemical Comparison

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Background: Micropapillary (MP) carcinoma has been described in tumors of several organs including breast, lung, colon, ovary and urinary bladder, and reported to be associated with a poor prognosis. We compared the clinicopathologic and immunohistochemical findings of 12 cases of salivary duct carcinoma (SDC) with MP and 14 cases of conventional SDC without MP carcinoma component.
Design: Clinicopathologic findings including age, gender, site, tumor size, and proportion of MP carcinoma component as well as immunohistochemical stain (IHS) findings in 12 SDCMP were compared with 14 cases of SDC without MP carcinoma component. Follow-up data were obtained from all patients with SDCMP and SDC. IHS for CK7, CK20, ER, PR, racemase, HER-2, p53 and ki-67 was performed on tissue microarray blocks in both SDCMP and SDC.
Results: Histologically, the MP component was characterized by small tight cell clusters without fibrovascular core and distributed in clear lacunar spaces. The MP component ranged from 5 to 50% with average 19.2%. The male and female ratio was 9:3 for SDCMP and 12:2 for SDC. The tumor was slightly larger in SDCMP (3.2cm) than SDC (2.9cm). The location of SDCMP was parotid in 7 cases, submandibular in 4 cases and sublingual in 1 case (parotid- 9, submandibular- 4 and sublingual-1 in SDC). Lymphovascular invasion (LVI) was seen in 10 of 12 cases of SDCMP (4/14 cases in SDC) ($p<0.05$). 10 of 12 patients of SDCMP showed local recurrence and/or metastasis (7/14 patients in SDC) ($p<0.05$) with only 1 patient of SDCMP is alive and well (7/14 patients in SDC) ($p<0.05$). IHS results revealed that SDCMP cases were more commonly positive for p53 (67%) and Ki-67 (50%) than in SDC (p53, 50%; Ki-67, 21.4%). Recemase (10/12 and 13/14) and Her-2/neu (8/12 and 10/14) were commonly positive in both SDCMP and SDC. All but one case of SDCMP was positive for CK7 and negative for CK20. Most SDCMP and SDC were negative for ER and PR.
Conclusions: MP component was frequently seen in SDC (12/26 cases, 46.1%). In our study, SDCMP was seen in slightly younger age (59.2 yrs) than in SDC (64.2 yrs), and the tumor size of MPSDC was slightly larger (3.2 cm) than that of SDC (2.9cm). SDCMP showed more frequent LVI, local and distant recurrences, and more frequent p53 and Ki-67 positivity than SDC. Our study indicated that MP histology in SDC is an aggressive histologic variant.

1131 The Prognostic Value of Ki-67, p53, 1p36, and 9p21 in Skull Base Chordomas

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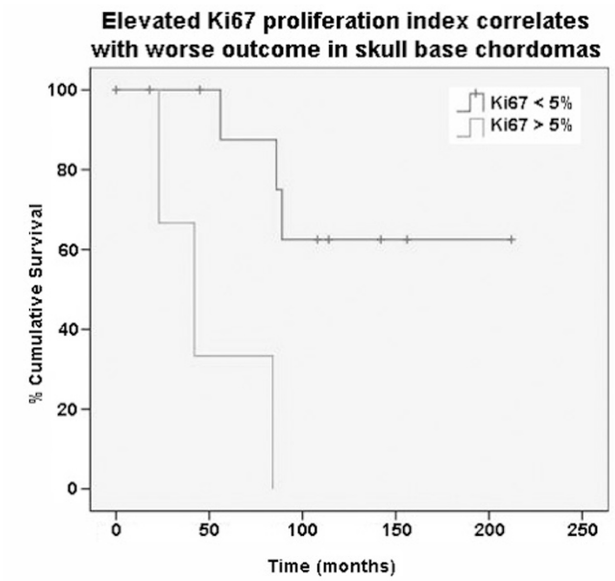
Background: Skull base chordomas (CD) are rare, locally aggressive, notochord-derived neoplasms for which prognostic biomarkers are not well established. We evaluate the relevance of Ki-67 and p53 protein expression as well as 1p36 and 9p21 loss, all of which are markers of recent interest in the literature, to the biology and behavior of CD.
Design: 15 skull base CD (collected from 1980 – 2007) were evaluated by dual-color FISH analysis for the 1p36 and 9p21 chromosomal regions. For 1p36 hybridization was performed using a spectrum orange labeled probe for 1p36 and spectrum green labeled control probe for 1q25 (Vysis dual-color probe set, LSI 1p36/LSI 1p25) Vysis, Downers Grove, IL, USA). For 9p21 hybridization was performed using a spectrum orange labeled probe for *CDKN2A* (*p16*) and a spectrum green labeled chromosome 9 centromeric probe (CEP 9) (Vysis, Downers Grove, IL, USA). Cases were considered positive for 1p36 deletion if ≥ 20% of nuclei showed deletion. For 9p21 only homozygous deletion was counted and was defined by loss of both 9p21 signals in ≥ 20% of nuclei with least one CEP 9 signal. Additionally, immunohistochemical staining for p53 (1:100, DO7, Dako, Carpinteria, CA) and Ki-67 (1:25, ki-55, Dako) were performed. Results were then correlated with clinical and pathologic parameters.
Results: Loss at 1p36 or homozygous deletion at 9p21 were in 4/15 (26.7%) with 2 cases (13.3%) showing deletion of both loci. All cases with 1p36 deletion, and all but one case with 9p21 deletion, were conventional CD. 1p36 loss was equally distributed between CD with and without prominent solid components (2 cases each), while 9p21 deletion was more frequent (3/4 cases) in tumors with a prominent solid component. Neither the deletions nor p53 expression were significant predictors of overall survival (1p36: log rank p=0.599, 9p21: p=0.363, p53: p=0.647). However, a Ki-67 index of greater than 5% predicted a worse outcome (p=0.002).



Conclusions: Ki-67 is the only marker predictive of outcome in this small series. There is a tendency for loss of 1q36 and 9p21 to occur with more 'aggressive' histologies, namely conventional CD and CD with prominent solid components. However, this study does not support these loci as major prognosticators for this tumor.

1132 Stromal Osteonectin/SPARC Expression Predicts Aggressive Behavior in a Subset of Head and Neck Squamous Cell Carcinomas

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Background: SPARC/osteonectin is a 43 kD matricellular protein involved in cell-matrix interactions, particularly in remodeling tissues such as tissues undergoing morphogenesis, mineralization, angiogenesis, or pathological responses to injury and tumorigenesis. It is normally expressed by osteoblasts and seems to have a counter-adhesive effect on cells by disrupting cell matrix interactions. Overexpression of SPARC is seen in various cancers including its recent observation in head and neck squamous cell carcinomas (SCC), with over-expression suggested to predict poorer survival.

Design: Paraffin-embedded tissue microarrays (TMAs) consisting of two tumor punches per case were constructed from 192 non-selected head and neck SCC. These were immunohistochemically stained for SPARC. TMA slides were digitally scanned and then manually graded. Composite tumor and stromal SPARC scores (0-8) were assigned based on quartile percentage and strength of staining in the tumor cells and intra/peritumoral stroma, respectively. Correlation of pathologic findings and survival time was examined by log rank tests based on histologic tumor subtype (typical keratinizing SCC or K-SCC vs. nonkeratinizing oropharyngeal SCC or NK-SCC).

Results: Of the total of 192 cases, 174 were evaluable for SPARC staining. 107 were K-SCC of numerous primary sites and 67 were NK-SCC of the oropharynx. Analysis of the entire group showed that SPARC expression did not correlate with overall or disease specific survival. In the K-SCC group, a SPARC stromal score of 7 or greater ($p=0.038$) but not tumor cell staining ($p=0.428$) correlated with differentiation (well, moderately, poorly); however, neither correlated with overall or disease specific survival. In the NK-SCC group, a SPARC stromal score of 7 or greater strongly correlated with both poorer overall ($p= 0.0106$) and disease specific survival ($p = 0.0477$). SPARC tumor cell staining did not correlate with outcome.

Conclusions: SPARC/osteonectin stromal staining is associated with poorer overall and disease specific survival in oropharyngeal non-keratinizing SCC. We do not confirm the results of a prior smaller study which suggested that SPARC expression predicts poorer survival in SCC of the head and neck from various sites combined, although our data does show a similar trend. Further investigation is necessary, but SPARC staining may nonetheless serve as a useful prognostic marker in the appropriate context.

1133 Use of Cytokeratins 10 and 19, p16, and pRb as a Diagnostic Tool for Determining the Primary Site of Cervical Lymph Nodes Metastases of Occult Squamous Cell Carcinoma

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Background: Cervical lymph node metastases from unknown primary sites account for approximately 2% to 9% of all head and neck malignant lesions. Squamous cell carcinoma is the most common type of all cervical metastatic carcinomas. Many investigators have studied the utility of immunologic expression of various protein markers in identification of the primary site of origin of a metastatic carcinoma. However, to date, there is no specific antibody for determining the site of origin of metastatic squamous cell carcinoma.

Design: We collected the 101 consecutive patients with cervical nodal metastasis who had undergone neck dissection for the treatment of known head and neck squamous cell carcinoma. The primary sites included 16 oral cavity, 38 oropharynx, 26 hypopharynx, and 21 larynx. We validated differential expression of nine protein markers, cytokeratins 5/6, 8/18, 10, 13, 14, and 19, p16, p75, and pRb in 101 cases of cervical node metastases, and evaluated the use of this immunohistochemical panel as a potential diagnostic tool for determining the primary site.

Results: Cytokeratin 10 expression was frequently observed in oral cavity primary tumor (62.5%) than in pharynx (14.1%) and larynx primary (33.3%). Cytokeratin 19 staining was less frequently observed in oral cavity primary (37.5%) than in pharynx (71.9%) and larynx (61.9%) primary. Expression of p16 and altered pRb status (0% or >50%) were exclusively observed in oropharynx primary tumors. In contrast, laryngeal primary tumors were negative for p16 in nearly all cases (20 of 21). Cytokerains 5/6, 8/18, 13 and 14, and p75 were noncontributory in differentiating these entities.

Conclusions: In the diagnosis of an unknown primary in patients with metastatic squamous cell carcinoma in cervical lymph nodes, cytokeratins 10 and 19 staining may be used in the diagnosis of oral cavity primary, and expression of p16 and altered pRb status are strongly suggestive of an oropharyngeal primary. The absence of p16 staining may also be helpful in the diagnosis of laryngeal primary.

1134 Teratocarcinosarcoma — An Enigmatic Entity

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Background: Teratocarcinosarcoma (TCS) is an unusual and highly aggressive clinicopathologic entity. It has variegated histomorphology characterized by epithelial, mesenchymal and neuroectodermal components. The morphologic diversity, a histologic hallmark, is responsible for diagnostic errors. This study is aimed at analyzing entire morphologic spectrum in excision specimen and evaluating diagnostic criteria in biopsy.

Design: This is a single institutional retrospective study comprising 25 cases diagnosed as TCS on review of HE slides. Performed pertinent immunohistochemistry. Clinical, follow up data recorded & correlated with histology.

Results: There were 20 males & 5 females with age spanning 10-65 years. The primary sites were nasal cavity, maxillary sinus, ethmoid sinus, oropharynx & oral cavity. Presence of all 3 elements in the first biopsy enabled a confident diagnosis of TCS in **28 % cases**. In remaining cases, diagnoses of olfactory neuroblastoma, lymphoma, rhabdomyosarcoma, poorly differentiated carcinoma & inflammatory polyp were entertained depending on the predominant element in biopsy TCS exhibited intermingling of multiple tissues of varying maturity derived from 3 elements. The epithelial element comprised either of poorly differentiated carcinoma or benign glands mixed with fetal squamous epithelium. The glands showed squamous morules & were surrounded by smooth muscles imparting an organoid pattern. Fetal squamous epithelium was the characteristic diagnostic feature[10 cases] The mesenchymal elements ranged from fasciculated spindle cell sarcoma, rhabdomyosarcoma & chondrosarcoma to mature osteocartilagenous tissue, skeletal muscle tissue and myxoid matrix. The third & predominant element represented primitive neuroectodermal tissue with rosettes & neurofibrillary matrix Fifteen cases underwent excision of tumor followed by postoperative radiotherapy. Recurrence and nodal metastasis were observed in 3 patients each, bone metastasis in one. The post chemotherapy excision specimen exhibited extensive neural maturation in one case.

Conclusions: TCS exhibits heterogeneous tissues of variable maturity comprising 3 elements. Erroneous diagnoses on biopsy are common A high index of suspicion & adequate sampling are prerequisites for diagnosis. Though 3 elements are necessary for confident diagnosis on biopsy, combination of tissues derived from any 2 elements should lead to suspicion. Fetal squamous epithelium offers a diagnostic clue If diagnosed on biopsy, neoadjuvant chemotherapy can be offered to this aggressive tumor to induce maturation.

1135 p16 Over-Expression Is a Favorable Prognostic Factor in Squamous Cell Carcinoma of the Oropharynx

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Background: Alterations in p53, human papilloma virus (HPV) and overexpression of epidermal growth factor receptor (EGFR) play a significant role in the pathogenesis of squamous cell carcinoma of the head and neck (SCCHN). The presence of HPV, and p16 and EGFR overexpression have been reported to be of prognostic significance in SCCHN. We investigated the relationship between certain clinicopathological parameters and p53, EGFR, and p16 expression in oropharyngeal carcinoma (OPC).

Design: The expression of p53, EGFR and p16 in biopsy samples from 112 patients undergoing curative radiation therapy (RT), with or without concurrent chemotherapy (CT) were determined by immunohistochemistry. The expression of these molecules was correlated with clinical features and outcome.

Results: Over-expression of p53, EGFR and p16 were observed in 47% (53/112), 72% (81/112) and 59% (64/112) of patients, respectively. Twenty-two of 27 non-smokers (81%), and 43 of 55 non-drinkers (78%) were associated with p16 over-expressing tumors ($p<0.05$ and $p<0.001$, respectively). The 3-year overall survival (OS) and disease-free survival (DFS) revealed significantly better outcome for patients with p16-overexpressing tumors (92% *vs*. 61%, $p<0.0002$, and 82% *vs*. 42% $p<0.0001$, respectively). In contrast, p16 under-expression was significantly associated with the 3-year probability of relapse (47% *vs*. 12%, $p<0.0001$). P53 and EGFR expression were not correlated with clinical outcome. A multi-variate analysis was conducted to assess the independent predictive value of age, treatment and p16 expression. P16 expression status was revealed as an independent and significant prognostic factor for OS, DFS, and recurrence free rate (RFR) in this model, wherein the hazard ratio for OS was 0.24, ($p= 0.0061$), DFS of 0.25 ($p< 0.0001$), and RFR of 0.19 ($p<0.0001$).

Conclusions: In this cohort of 112 OPC patients treated with curative intent, p16 over-expression was significantly associated with non-smokers, and non-alcohol drinkers. This molecular parameter was also an independent predictor for outcome, in that OPC patients with reduced p16 expressing-tumors had reduced OS, DFS, and a higher risk of cancer recurrence.

1136 Frequent Loss of Heterozygosity of a DNA Repair Gene, hOGG1, in Arteriovenous Malformation

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Background: Arteriovenous malformation (AVM) is a vascular anomaly that frequently occurs in the head and neck region. Most AVMs are diagnosed at birth and expand slowly with growth acceleration when changes in hormonal levels occur, such as at puberty, during pregnancy, or with use of birth control pills. While AVM is considered a benign lesion from a morphological point of view, it usually behaves aggressively with frequent recurrences. On the other hand, a small subset of AVMs is diagnosed in late adulthood and these tend to grow focally. In this study, we attempt to characterize the pattern of hOGG1 gene loss in relation to the biologic behavior of AVM.

Design: Seven lesions from 2 patients with aggressive AVM and 4 lesions from 3 patients with focal AVM were included in this study. A total of 11 sections from AVM and 11 from matched squamous epithelia in the same patients were used for microdissection of tissue. DNA samples were then obtained from dissected tissue and subjected to PCR amplification using 4 fluorescent-labeled microsatellite markers (D3S1289, D3S1297, D3S1300 and D3S1274), followed by fragment analysis using ABI PRISM 3100 Genetic Analyzer.

Results: All 7 lesions of aggressive AVM are informative with at least one of 4 markers used. Among these 7 lesions, 4 (57%) showed evidence of loss of heterozygosity (LOH). Three of 4 lesions of focal AVM are informative with at least one marker and 3 (66%) showed evidence of LOH. In one patient with aggressive AVM, one original AVM, two local recurrences (left face), and one geographically separate AVM (top of scalp) were analyzed. All 4 lesions showed an almost identical LOH pattern except that the 2nd left face recurrence and the scalp AVM contained an additional LOH at marker D3S1289. In this patient, the scalp AVM is completely separate from the left face AVM, which may represent a de novo second primary AVM or a metastasis from a subclone of the original AVM on the left face that has accumulated additional gene loss.

Conclusions: Loss of heterozygosity (gene loss) of the hOGG1 gene frequently occurs in AVM and the frequency of hOGG1 LOH does not differ significantly between aggressive and focal AVM. Accumulation of LOH at hOGG1 gene locus may be related to more aggressive growth of AVM.

1137 Allergic Fungal Sinusitis Is Often Associated with a Mix of Different Fungal Pathogens: A Microbiologic Assessment of 147 Patients over a 28 Year Period

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Background: Allergic fungal sinusitis (AFS) is a form of non-invasive sinonasal fungal disease which most likely results from an allergic reaction to fungal antigens produced by fungi which have colonized the sinonasal tract. A variety of different filamentous fungi, most notably dematiaceous fungi have been associated with AFS. In this study, we present a fungal culture analysis of a series of AFS patients seen at our institution over a 28 year period.

Design: The surgical pathology archives were searched for all cases either with a diagnosis of allergic mucin or allergic fungal sinusitis from 1991-2008. The surgical pathology reports for each case as well as any previous sinus surgeries and fungal culture data were reviewed for each patient if available.

Results: A total of 233 patients (450 specimens) were included in the study. All 233 patients had at least one specimen which contained allergic mucin with or without fungal organisms. Of the 233 patients, 157 were classified as having AFS by the presence of allergic mucin with histologic evidence of fungal forms on silver stain or by the presence of allergic mucin without histologic evidence of fungus and positive fungal cultures at the time of surgery. Of the 157 documented AFS patients, 130 had fungal cultures performed. Cultures were negative in 23 patients (18%). The remaining 107 patients (82%) showed either one fungal isolate (58 patients, (54%)) or multiple fungal isolates (39 patients (46%). Of the 58 patients with one fungal isolate, *Aspergillus sp*. was the most common (40%), followed by a variety of different Dematiaceous fungi (26%), yeast including *C. albicans* (17%), molds including *Penicillium sp*. and non-sporulating molds (7%), *Scedosporium sp* (5%), and Fusarium (3%). Of the 39 patients with more than one fungal isolate, 46% grew Aspergillus and at least one other fungal isolate. One patient grew up to 10 different fungal pathogens.

Conclusions: Greater than 80% of AFS patients have at least one fungal pathogen isolated in culture. In almost half of AFS patients, multiple pathogens are isolated over the course of the disease process. In our population, *Aspergillus sp*. is the most common fungal isolate followed by the dematiaceous fungi.

1138 Application of the "Pheochromocytoma of the Adrenal Gland Scaled Score" (PASS) for the Evaluation of Clinical Behavior of Carotid Body Paragangliomas

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Background: Carotid body paragangliomas share histologic features with pheochromocytomas. Few studies have detailed the pathology associated with aggressive behavior. In 2002, Thompson (Am J Surg Pathol**.** 2002;26(5):551) developed the Pheochromocytoma of the Adrenal Gland Scaled Score (PASS) to assess which histologic parameters could predict which tumors may act in an aggressive fashion. Tumors with a PASS <4 were benign. We adapted this scoring system for evaluating carotid body tumors.

Design: 39 carotid body tumors were studied. Tumors were evaluated using PASS with scoring as follows: capsular invasion (1 point), vascular invasion (1 point); adipose tissue invasion (1 point), large nests/diffuse growth (2 points), high cellularity (2 points), cellular spindling (2 points), monotony (2 points), mitoses (>3/10 high power fields; 2 points), atypical mitoses (2 points), profound nuclear pleomorphism (1 point), and hyperchromasia (1 point). Necrosis was excluded from the PASS since almost all of the carotid body tumors had been embolized prior to resection.

Results: All 39 cases were confirmed paragangliomas. 3 were malignant because of metastases to lymph nodes (2) and lung (1). An additional 4 patients had multiple tumors either involving the opposite carotid body or the middle ear. PASS ranged from 2-12 with an average of 4.8. The most common histologic feature was profound nuclear pleomorphism (30 patients) followed by capsular invasion (14 patients), spindle cell change (13 patients), vascular invasion (12 patients) and cellular monotony (12 patients). The least common features were high mitotic activity (4 patients – 2 of which were malignant) and atypical mitoses (2 patients both malignant). 37 patients are alive with no evidence of disease and 2 patients are alive with disease. No patient has died of disease. 21 patients had PASS > or = 4. The three malignant paragangliomas had PASS >4 (5, 8, and 12). The 18 remaining patients with PASS >4 have shown no recurrence. The patients with PASS <4 are alive with no evidence of disease.

Conclusions: While most patients with "PASS" >4 behave in a non-aggressive fashion, PASS can identify patients with carotid body tumors at risk for aggressive behavior. All malignant paragangliomas had scores >5. Mitoses and atypical mitoses are rare in carotid body tumors and their presence may indicate aggressive behavior. The presence of metastases is compatible with prolonged survival in carotid body paragangliomas.

1139 Increased ALDH1 Expression in Squamous Cell Carcinoma of the Head and Neck May Be a Predictor for Lymph Node Metastases

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Background: Head and neck squamous cell carcinoma (HNSCC) affects approximately 47,000 patients a year in the U.S., and is associated with a high degree of morbidity and mortality. Over the last 30 years survival rates have gradually improved. However, mortality is often associated with both distant metastases and local recurrences that are therapy resistant. Aldehyde dehydrogenase 1 (ALDH1), is an enzyme that oxidizes aldehydes to carboxylic acid and is involved in the conversion of vitamin A to retinoic acid. High expression of ALDH1 has been reported in colorectal cancer stem cells and thought responsible for chemoresistance to cyclophosphamide (CPA). High ALDH1 expression in breast cancer correlated with a poor clinical outcome, and increased ALDH1 expression was seen in breast tissue with metastases compared to no metastases. To date, correlation of ALDH1 expression with metastasis in HNSCC has not been reported. We compared ALDH1 expression in HNSCC from patients who did not have lymph node metastases (LNM) and in patients with LNM.

Design: A total of 199 patients with HNSCC were examined; 100 cases from patients who had a primary tumor without LNM, and did not develop any LNM within a 2 year follow-up, and 99 patients who presented with LNM. In this second group, both the primary tumor and the corresponding LNM were examined. No patients received chemotherapy and/or radiation therapy prior to surgical treatment. Formalin-fixed paraffin sections were stained with goat polyclonal ALDH1A1 antibody at a dilution of 1:200 using standard procedures. The sections were analyzed by a pathologist and the sections were scored according to the following scheme: 1: <5 %; 2: 5-25%; 3: 25-50%; 4: >50% cells staining.

Results: There was a statistically significant increase in ALDH1 expression in tumors with LNM compared to tumors without LNM (2.12 vs. 1.26, p<.0003). The corresponding LNM also had higher ALDH1 expression compared to tumors without LNM (2.19 vs. 1.26, p<.0001). Although the LNM had higher ALDH1 expression than the corresponding primary tumor, the difference between these two groups was not significant (2.19 vs. 2.12).

Conclusions: Our results suggest that ALDH1 may be a potential marker for LNM in HNSCC. This finding may provide new insights into the biology of head and neck carcinogenesis. [This study is supported by Head and Neck Cancer SPORE (P50 CA128613) from National Cancer Institute.]

1140 Frozen Section Analysis of Head and Neck Tumor Resection Margins: Methods, Trends, and Error Rates at a Large Academic Institution

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Background: Frozen section (FS) analysis is an essential tool for assessing tumor resection margin status intra-operatively. Most institutions evaluate surgical defect edge tissue provided by the surgeon after the main lesion is removed. This will be an ever more utilized method, particularly as transoral laser microsurgery (TLM) is more frequently utilized, for which defect margin specimens are the only choice. There have been very few large studies evaluating error rates for this type of margin assessment or that analyze methodology.

Design: All head and neck cases with margins evaluated at FS were identified by database search. Margins from all of these cases were evaluated in the routine manner by all faculty members who cover the FS service with two H&E levels at FS and one permanent section on the remaining tissue. All cases were reviewed retrospectively for the study. Finally, a prospective one-year evaluation of obtaining an additional third, deeper H&E FS level has been undertaken.

Results: The preliminary data presented here includes all head and neck tumor cases with margins evaluated by FS during 2007 at Barnes-Jewish Hospital. 1107 total margin specimens were evaluated, 117 showing invasive tumor or dysplasia. There were 32 errors in total (2.9% of all margins). Of these, 16 were sampling errors (FS slides negative, permanent slide positive 1.5% of all margins; 14% of all margins with neoplasia; sensitivity of 86%) and 16 interpretation errors (diagnostic tissue present on frozens which was mis-called 1.5% of all margins). There were 17 bone margins

(marrow scrapings), 4 of which had neoplasia. None of these had errors. Review of the main specimens showed that all corresponding bone margins correlated with the frozen results.

Conclusions: The baseline error rate is low (2.9%) which is comparable to published data, but could be improved, particularly when considering that our current method of 2 FS levels had an 86% sensitivity for the lesion (when just considering specimens with neoplasia in them). Sampling errors constitute a significant portion of all errors (50%). This may be decreased by examination of an additional deeper level on FS. Once the prospective data is gathered, the utility of this method change can be evaluated relative to retrospective data. Bone margins evaluated by scraping have strong predictive value as well.

1141 Snail Expression Is a Rare Event in Oral Squamous Cell Carcinoma Associated with Features of Poor Prognosis

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Background: Epithelial-mesenchymal transition (EMT) is an embryological process that can be aberrantly reactivated in cancer and is thought to contribute to epithelial tumor progression. Snail is one of several transcriptional repressors of the cell adhesion molecule E-cadherin capable of EMT induction. Loss of epithelial characteristics by EMT potentially contributes to invasion and metastasis of oral squamous cell carcinoma (OSCC) and might be associated with over-expression of focal adhesion kinase (FAK), a signalling molecule involved in cell migration, and loss of p63, a marker of squamous origin.

Design: We examined the expression of Snail, E-cadherin, FAK and p63 in formalin-fixed paraffin-embedded tissue from 46 patients diagnosed with OSCC and obtained outcome data from clinical files. Primary tumors and one lymph node metastasis of each N+ case (26/46) were stained by immunohistochemistry. Two commercially available Snail antibodies were used after evaluation of their specificity. Scoring was performed on full sections to account for heterogeneity and rare/focal expression of the parameters of interest.

Results: Snail expression (nuclear, ≥5% tumor cells positive) was observed in 10 primary tumors and 5 metastases. 30 patients had at least rare individual tumor cells with Snail positivity whereas all cases had occasional to abundant Snail-positive stroma cells. E-cadherin loss was observed in 29/46 cases (63%) and was associated with presence of lymph node metastases (21/29 [72%] E-cadherin negative, 5/17 [29%] E-cadherin positive; $p<0.05$ Fisher's exact test). Cytoplasmic FAK was over-expressed in 10 (22%) cases and associated with tumor recurrence/new primary (9/10 [90%] FAKc high, 16/36 [44%] FAKc low expression; $p<0.05$). Two N+ cases showed a distinct sarcomatoid component within the primary tumor with Snail+/FAK+/E-cadherin–/p63– phenotype. The metastasis of one case displayed a Snail–, the other a Snail+ phenotype.

Conclusions: We conclude that Snail-associated EMT occurs as a rare event in OSCC and can indicate the presence of a sarcomatoid component. Absence of p63 in primary tumor or metastasis does not exclude squamous origin in the context of EMT. Additional transcriptional repressors of E-cadherin should be examined for their contribution to OSCC progression.

1142 MicroRNA Expression Profling of Salivary Duct Carcinoma and Pleomorphic Adenomas of the Parotid Gland

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Background: Salivary duct carcinoma (SDCA) is a rare but highly aggressive salivary gland malignancy that may either arise *de novo* or from pleomorphic adenomas (PA) for which pathogenesis is still largely unclear. MicroRNAs (miRNAs) are small non-coding RNA molecules that function as negative regulators of coding gene expression that have been shown to be differentially expressed between normal tissues and tumor at other organ sites. To improve the molecular understanding of these tumors, we evaluate the miRNA expression pattern parotid SDCA and PA.

Design: Human mature miRNA expresion was studied in 4 normal parotid tissues, 11 PA, and 9 SDCA (5 de novo, 4 SDCA ex PA) . Total RNA was extracted from frozen tissue using Trizol reagent (Invitrogen). RNA quality was evaluated on 2100 Bioanalyzer (Agilent). First, 4 salivary duct carcinomas, 4 pleomorphic adenomas and 4 normal parotids were analyzed for 328 human mature miRNAs using Flexmir MicroRNA Human Panel (Exiqon) on Luminex 200. Analysis of miRNA expression was performed using Luminex IS™ software v.2.3 (Luminex) relative to normal tissue. Subsequently, expression of selected miRNAs was validated and extended to include all selected samples by real-time PCR on ABI 7500 (Applied Biosystems).

Results: The miRNA expression in both SDCA and PA was different as compared to normal tissue. MiRNAs strongly upregulated (> 24 folds) in tumors vs normal tissue included miR-181d, miR-501, and miR-25, while the miRNAs most downregulated included miR-32, miR-153, miR-380-3p. While SDCA and PA showed similar levels in many miRNAs, a few miRNAs were differentially expressed between SDCA and PA: miR-154 was upregulated (>11 folds) in PA vs. SDCA, while miR-99b was upregulated (>10 folds) in SDCA vs PA and normal tissue. Interestingly, miR-424, which was upregulated in PA (77 folds vs normal), was still higher in SDCA x PA (40 folds) than in SDCA *de novo* (2 folds). A search of MiRanda and PicTar databases for miRNA target prediction demonstrated *PLAG1*, known to be commonly altered in PA, as one potential target gene for miR-424.

Conclusions: Both SDCA and PA show differential miRNA expression with respect to normal parotid tissue. Despite a similar miRNA expression profile, there are several key miRNA that are differentially expressed between SDCA and PA. MiR-424 expression is one key difference between SDCA ex PA and SDCA *de novo*. A miRNA database search raises the possibility that miR-424 may affect *PLAG1* regulation.

1143 Adenosquamous Carcinomas of the Head and Neck: An Analysis of MDM2, p53 Expression and MAML2 Rearrangements

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Background: Adenosquamous carcinoma (AsqCA), though generally considered a variant of squamous cell carcinoma (SCC), shows considerable morphologic overlap with mucoepidermoid carcinoma (MEC). To date, the question of whether the molecular alterations in AsqCA resemble those of conventional SCC or those of MEC has not been addressed. We describe our survey of AsqCA with markers that represent key molecular pathways of MEC (*MAML2* rearrangement), and conventional SCC (*MDM2* – *TP53*), respectively.

Design: Twenty cases of AsqCA were selected for immunohistochemical (IHC) analysis and fluorescent in-situ hybridization (FISH). Anti-p53 (1:100, clone DO-7, Dako, Carpinteria, CA) and anti-MDM2 (1:50, clone IF2, Invitrogen, Carlsbad, CA) staining was visualized using a brown (DAB) chromogen. Only strong staining in greater than 25% of nuclei was considered positive. FISH analysis was performed as follows: *TP53* - SpectrumGreen-labelled CEP17 and SpectrumOrange-labeled 17p13.1 (*TP53*) probes (Vysis, Downers, IL); *MDM2* - SpectrumGreen-labeled CEP12 (Abbott Molecular, DesPlaines, IL) and SpectrumOrange-labeled BAC probe (RP11-775J10 and RP11-450G15 12q15, Roswell Park Cancer Institute, Buffalo, NY*)*; *MAML2* - 11q21 break apart probe (SpectrumGreen-labeled BAC probe RP11-676L3 and SpectrumOrange-labeled BAC probe RP11-16K5 spanning *MAML2*, Childrens Hospital Oakland Research Institute). A heterozygous deletion of *TP53* was defined as loss in >20% of diploid cells. Amplification of *MDM2* was defined as a *MDM2*:CEP12 ratio of >2.0. Positivity for *MAML2* rearrangement was defined as split signal in >20%.

Results: 63% (10/16) AsqCA were p53 IHC positive, while only 14% (2/14) were MDM2 IHC positive. 52% (9/17) showed loss of *TP53* by FISH while 15% (3/20) showed *MDM2* gene amplification. 75% (6/8) cases with *TP53* loss were p53 IHC positive, though 50% (4/8) *TP53* intact cases were also p53 IHC positive. Only one of two MDM2 immunopositive cases showed amplification. None of the *MDM2* amplified cases showed *TP53* loss. None of the AsqCA tested (n=12) showed *MAML2* rearrangements.

Conclusions: As suggested by clinical and histologic criteria, AsqCA appears to be fundamentally different from MEC at a molecular level as well. They share the high prevalence of p53 alterations seen in conventional SCC. The finding of *MDM2* gene amplification, though rare in AsqCA, is novel, and to date, undescribed in any variant of head and neck SCC.

1144 HPV Is Associated with the Pathogenesis of a Subset of Sinonasal Schneiderian Papillomas but Does Not Specifically Correlate with $p16^{INK4a}$ Immunoreactivity

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Background: Schneiderian papillomas (SP) are rare, accounting for 0.4 to 4.7% of sinonasal tumors. Human papillomavirus (HPV) infections may play a role in the pathogenesis of SP. However, the reported prevalence of HPV in SP is highly variable although HPV6 and 11 are the most consistently detected genotypes. In the cervix, $p16^{INK4a}$ serves as a useful surrogate marker for HPV and its distinct staining patterns are markers of cervical lesion grade. This study aims to determine whether a similar relationship exist in SP and whether HPV status and/or $p16^{INK4a}$ staining defines a morphologic subset of SP.

Design: Twenty-eight specimens (14 inverted, 6 exophytic, 7 mixed exophytic & inverted, and 1 oncocytic) of archival SP were collected from 24 patients (8 female and 16 male). Purified SP DNA extracts were tested for HPV using GP5+/GP6+ PCR with HPV genotypes identified by dot blot hybridization of PCR products. PCR positive specimens were screened for HPV by biotinyl-tyramide-based *in situ* hybridization (ISH), and immunohistochemsistry (IHC) for the HPV L1 capsid protein. IHC for $p16^{INK4a}$ was performed on all specimens. $p16^{INK4a}$ staining patterns were graded according to a four-fold system: focal/sporadic, lower-third, lower two-thirds, or, full-epithelial thickness.

Results: HPV was detected in 16/28 (57.1%) specimens by PCR (HPV6 – 5 SP, HPV11 – 7 SP, HPV16 – 3 SP, HPV16/18 – 1 SP), and in 7 specimens (24.1%) by ISH (all HPV6 or 11 positive). Two (7.1%) samples (both ISH positive) demonstrated HPV L1 staining. $p16^{INK4a}$ staining patterns were observed in 14/16 (87.5%) PCR positive specimens, and in 10/12 (83.3%) PCR negative specimens ($p=1.00$); 1/7 ISH positive specimens (also L1 positive) tested $p16^{INK4a}$ negative. HPV positive samples were not distinguishable by the four $p16^{INK4a}$ staining patterns. HPV was detected by PCR in 10/13 (76.9%) exophytic / mixed exophytic & inverted samples, and in 6/14 (42.9%) inverted samples ($p=0.12$). Among PCR positive samples, HPV was corroborated by ISH in 7/10 (70.0%) exophytic / mixed exophytic & inverted samples, and in 0/6 inverted samples ($p=0.01$).

Conclusions: HPV infections may play a more active role in exophytic type SP than in inverted SP given the absence of ISH or L1 IHC staining in inverted SP. HPV infections detectable by PCR only may be indicative of a more subtle causality or may represent infections that are incidental to SP. $p16^{INK4a}$ IHC staining is not a surrogate marker for HPV among SP.

1145 Integration of HPV Testing into the Standard Pathology Assessment of Head and Neck Cancer

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Background: Human papillomavirus 16 (HPV16) has recently been confirmed as a causative agent in the development of a subset of head and neck squamous cell carcinomas (HNSCCs). These HPV16-positive tumors have a clinical profile that diverges from that of HPV16-negative HNSCCs including response to therapy. Accordingly, HPV testing may soon become integrated into the standard pathologic

assessment of HNSCCs. We report a clinical experience of prospective HPV testing in patients with HNSCC.
Design: Pathologic and clinical data was prospectively collected for all patients with HNSCC that had undergone HPV testing at the Johns Hopkins Hospital as part of clinical care during a 51 month period (6/2004 – 9/2008). Standard HPV testing included p16 immunohistochemistry (IHC), HPV16 in-situ hybridization (ISH), and wide spectrum HPV ISH for p16 positive/HPV16 negative cases.
Results: HPV analysis was performed on 195 HNSCCs to facilitate tumor localization (21%) and tumor subclassification (10%), determine patient eligibility for HPV vaccine trials (10%), satisfy patient curiosity (7%), and estimate treatment response (52%). Patients ranged in age from 28 to 82 years (median, 54; mean, 54); and 145 (74%) were male. Overall, 135 (69%) tumors were HPV16-positive. HPV positivity correlated with oropharyngeal site (81% vs. 2%, p < .0001) and male gender (p = .002). Indeed, for men with oropharyngeal carcinoma, the HPV-positivity rate was 85%. The correlation between HPV16 detection and p16 overexpression was 95%: All HPV16-positive cases were strongly p16 positive; however p16 positivity was present in 25% of the HPV16-negative cases. In 45% of these discordant cases, high p16 expression was due to the presence of some other HPV type.
Conclusions: Our prospective experience with HPV testing in the clinical arena confirms HPV16 as an important agent in oropharyngeal carcinomas. HPV status is becoming an increasingly important parameter in the pathologic evaluation of HNSCCs: testing is frequently requested by the oncologist to estimate response to therapy and clinical outcome, and by the pathologist as a tool to aid tumor classification and discern tumor origin. A rational and feasible detection strategy that incorporates both p16 IHC and HPV ISH is able to detect HPV in a remarkably high percentage of oropharyngeal cancers.

1146 A Histologic and Immunohistochemical Study of High-Grade Non-Intestinal Sinonasal Adenocarcinomas

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Background: The WHO currently classifies sinonasal adenocarcinomas (SNACs) that do not resemble typical salivary gland tumors as intestinal or non-intestinal. Non-intestinal SNACs are then further classified as low or high grade based on cellular pleomorphism, mitotic activity and necrosis. Non-intestinal SNACs are somewhat poorly characterized and high-grade non-intestinal (HGNI) SNACs have been only rarely reported. Here we review our experience with these tumors.
Design: The surgical pathology files of two institutions were reviewed for all SNACs. Cases were reviewed and all tumors better classified as salivary gland-type tumors, intestinal-type SNACs (based on histology and / or immunohistochemistry (IHC)) and low-grade non-intestinal SNACs were removed. We recorded all clinical and histologic features. Previous IHC results were noted and additional IHC was performed in select cases.
Results: Twenty-five cases of HGNI SNACs were identified from 20 men and 5 women. Ages ranged 19 to 83 yrs (mean=54.5; median=60). Ten cases involved the nasal cavity and sinuses, 9 involved the nasal cavity only and 6 involved the sinuses only. Surface involvement by tumor was seen in 64% of cases and 24% of cases were associated with Schneiderian papillomas. Many cases were solid and trabecular with occasional small cystic spaces, composed of neoplastic cells with a small amount of amphiphilic cytoplasm. Occasional cases were more nested and composed of larger cells, with more abundant eosinophilic cytoplasm (somewhat akin to salivary duct carcinomas). Most cases had marked cytologic and nuclear pleomorphism, abundant mitotic activity and necrosis, however, these features were not uniform. Tumors lacked CDX2 and CK20 immunoreactivity (aside from rare CK20 immunoreactive cells). Diffuse, strong CK7 immunoreactivity was seen in 43% of cases. S100, p63 and neuroendocrine antigen reactivity were only seen in rare cells in occasional cases.
Conclusions: High-grade non-intestinal SNACs are more common in men and, although they occur over a wide age range, they are much more common in older individuals. Histologically, they show a great deal of heterogeneity, however, the majority appear solid and trabecular and have small cystic spaces. Surface involvement is common and their association with Schneiderian papillomas raises the possibility that the tumors develop from the surface epithelium. Immunohistochemically, the tumors do not show intestinal or myoepithelial differentiation.

1147 Cyclin D1 Expression in Clinical (>1.0 cm) and Micro-Papillary Thyroid Carcinoma

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Background: Cyclin D1 is a G1 cyclin that is essential for the regulation of G1/S transition through the cell cycle. Increased expression of cyclin D1 has been demonstrated in various human tumors including thyroid neoplasms. In this immunohistochemical study, we examined the expression of cyclin D1 in papillary thyroid carcinoma and its variants, especially microcarcinoma.
Design: Sixty-two cases of clinical papillary carcinoma (CLPTC) i.e. measuring >1.0 cm (20 classic variant, 36 follicular variant, 5 tall cell variant and 1 diffuse sclerosis variant), 39 papillary microcarcinoma (MiPTC) and 11 lymph nodes with metastatic papillary carcinoma in 46 patients (32 females and 14 males; age range 21-77 yrs) were examined for cyclin-D1 expression by immunohistochemistry. Twenty-six cases of MiPTC showed multifocal disease. All cases had a significant surrounding benign thyroid parenchyma and a majority demonstrated chronic lymphocytic thyroiditis. The nuclear imunoreactivity was graded semi-quantatively on a sliding scale as 0 = no staining, focal = 0-<25% of the cells showing positive staining and diffuse =>25% of the cells showing positive staining.
Results: Diffuse nuclear staining was seen in 57/62 (92%) CLPTC, 34/39 (87%) MiPTC and 11/11 (100%) metastatic deposits in lymph nodes; strong immunostaining was seen at invasive edge of the tumors with extrathyroidal extension. In particular 32/36 (89%) cases of follicular variant of PTC (FVPTC) showed diffuse staining. Normal thyroid parenchyma showed no immunostaining; focal immunostaining was seen in two cases in foci of chronic lymphocytic thyroiditis.
Conclusions: There is no difference in cyclin D1 gene expression among PTC and MiPTC. According to this study its expression may be of value in diagnostically challenging cases of PTC especially FVPTC in cytology and surgical pathology.

1148 Küttner's Tumor Is a IgG4 Associated Disease

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Background: Autoimmune pancreatitis (AIP) is a inflammatory disease of the pancreas. Recently, the spectrum of this IgG4-associated disease has expanded to include sclerosing lesions of biliary tract, kidney, retroperitoneal soft tissue, and orbit. Some examples of Kuttner's tumor (KT) or chronic sclerosing sialadenitis have shown abundant IgG4 plasma cells; however, this association has not been reported in the Western literature. In this study we explore the association of tissue IgG4 with KT, chronic sialadenitis, and Sjögren's syndrome (SS).
Design: Ten cases of KT received between 1992 and 2008 were retrieved from the department of pathology and were compared with 16 cases of chronic sialadenitis (CS) and 8 cases of clinically proven SS collected during the same period. Immunohistochemistry for CD138, IgG and IgG4 was performed. IgG4-positive plasma cells were quantified per high power field.
Results: Six patients with KT were female and 4 were male. Their median age was 60 years (range 13-76). All 10 KT cases involved the submandibular gland, with bilateral involvement in 2 cases. Morphologically these specimens had marked sclerosis, follicular hyperplasia, and numerous plasma cells. Obliterative phlebitis was observed in 4 cases. A ductocentric pattern of inflammation was seen in one case. The histological features were reminiscent of autoimmune pancreatitis. Nine cases showed increased numbers of IgG4 plasma cells with median of 182/HPF (range 160-608). The one patient whose biopsy lacked IgG4 positive plasma cells showed evidence of cytomegalovirus infection. KT showed significantly higher number of plasma cells ($p<0.05$) than SS and CS. Patients with CS had a median number of 16 (range 2-62) IgG4 positive plasma cells, while SS patients had a median of 1 (range 0-2) cell.
Conclusions: KT shows significant morphological overlap with AIP, and is characterized by IgG4 positive plasma cells. IgG4 emerges as a powerful diagnostic marker for KT. KT is part of the widening spectrum of IgG4 associated diseases.

1149 Amplification and Overexpression of HER-2/neu in Salivary Duct Carcinoma and Salivary Duct Carcinoma Ex Pleomorphic Adenoma of the Salivary Gland

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Background: Salivary duct carcinoma (SDC) is an aggressive malignant tumor associated with high mortality. Recently, the indications for use of trastuzumab (Herceptin) as adjuvant therapy for SDC, similar to breast cancer, has been discussed. The object of this study is to clarify the relationship between *HER-2/neu* protein overexpression in immunohistochemistry (IHC) and gene amplification detected by fluorescence in situ hybridization (FISH) in SDC.
Design: We studied five cases of pure SDC and nine cases of SDC ex pleomorphic adenoma (PA) (13 male and 1 female, age 37 to 85, Ave. 62 yr) diagnosed between 1997 and 2007. Formalin-fixed, paraffin-embedded tissue from 14 cases were evaluated by IHC and FISH for *HER-2/neu* status.
Results: Of the 6 cases (43%) scored 3+ by IHC and all of them showed gene amplification by FISH. 4 cases (29%) scored 2+ and half of them showed amplification. 3 cases (21%) scored 1+ and 1 case (7%) scored 0 showed no amplification. 8 of 10 cases (80%) scored more than 2+ by IHC showed *HER-2/neu* gene amplification consequently.

table 1

case	histology	Age	Gender	Outcome	HER2-IHC	HER2-FISH
1	SDC	66	M	Dead	3+	Amplified
2	SDC	85	M	Dead	0	Not Amplified
3	SDC	60	M	Dead	3+	Amplified
4	SDC	62	M	Dead	3+	Amplified
5	SDC	78	M	Dead	2+	Not Amplified
6	SDC ex PA	37	M	Alive	2+	Amplified
7	SDC ex PA	77	M	Alive	1+	Not Amplified
8	SDC ex PA	72	M	Dead	1+	Not Amplified
9	SDC ex PA	55	M	Alive	2+	Not Amplified
10	SDC ex PA	70	M	Dead	1+	Not Amplified
11	SDC ex PA	55	M	Dead	2+	Amplified
12	SDC ex PA	41	F	Alive	3+	Amplified
13	SDC ex PA	66	M	Alive	3+	Amplified
14	SDC ex PA	50	M	Alive	3+	Amplified

Conclusions: Clinically, most patients with SDC are in advanced stage and are metastasized when they visit clinics, and it is difficult to perform curable surgical treatment on them and some are inoperable. Our data demonstrate that Trastuzumab has a possibility of adjuvant therapy on the patients with FISH-positive *HER-2/neu* SDC and may play a role for improvement of their prognosis.

1150 *C-kit* Gene Mutations Are Frequently Present in Primary Adenoid Cystic Carcinoma of the Salivary Gland

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Background: The *c-kit* proto-oncogene encodes a transmembrane receptor tyrosine kinase (KIT), which plays an important role in the development of mast cells, hematopoetic stem cells, germ cells, melanocytes and cells of Cajal in the gastrointestinal

tract. KIT is expressed in many human neoplasms, and its activation through gain-of-function mutations has been shown to be the underlying genetic event in gastrointestinal stromal tumors (GISTs), germ cell tumors and hematologic malignancies. KIT is also expressed in adenoid cystic carcinomas (ACC). Tyrosine kinase inhibitors were used by some to treat ACC of the salivary gland (ACCSG) with variable outcomes, but none of the cases evaluated had *c-kit* mutations. The aim of our study was to identify *c-kit* mutations in primary ACCSG.
Design: We identified 14 cases of ACCSG (13 primary, 1 cervical lymph node metastasis) from the pathology files of our institution. KIT protein expression was evaluated by immunohistochemistry (IHC) using formalin-fixed paraffin-embedded tissue. Mutational analyses of the *c-kit* extracellular domain (exon 9), juxtamembrane domain (exon 11) and the tyrosine kinase domains (exons 13 and 17) were performed by polymerase chain reaction, T/A cloning, clonal selection and subsequent DNA sequencing.
Results: All 14 cases demonstrated strong KIT expression by IHC. Molecular analysis was successful in 10/14 cases, and *c-kit* missense point mutations were detected in 7/10 cases (70%) including 7 in exon 11, 2 in exon 9, 2 in exon 13 and 2 in exon 17. Eight silent point mutations were detected in 5 cases. Two cases contained missense mutations in multiple exons (exons 11 and 9, exons 11 and 17). Different mutations were found in the primary tumor and the cervical lymph node metastasis of 1 patient. Point mutations in similar domains described in GISTs were detected in our study, including Pro551Leu and Lys558Glu (5' end of exon 11), Leu576Phe (3' end of exon 11), Val643Ala (exon 13) and Asn822Ser (exon 17). Additional novel point mutations in exons 9, 11, 13 and 17 were also identified.
Conclusions: This study is the first to report *c-kit* gene mutations in primary ACCSG. These potential gain-of-function mutations in exon 11, and less frequently in exons 9, 13 and 17, may play a role in ACCSG. Identification of such activating mutations in ACC may be of prognostic value and may also be predictive for response to KIT tyrosine inhibitor (imatinib) treatment.

1151 Striated Duct Adenoma: A Report of 3 Cases of a Distinctive Lesion of Salivary Gland

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Background: Benign tumors of salivary gland can be separated into pleomorphic adenoma, basal cell adenoma, myoepithelioma and others based on their cell constituents and architectural patterns. Most of these benign salivary gland tumors contain a myoepithelial/basal cell component, with the exception of canalicular adenoma. Normal striated ducts in salivary gland show a single ductal layer with only patchy or absent basal/myoepithelial cells. To our knowledge there has been no benign neoplastic ductal lesion described that is composed primarily of striated ducts with no myoepithelial contribution.
Design: Three cases of striated duct adenomas were collected from the consultation files of the authors and reviewed. Each case was stained with various keratins, p63, actin and S100.
Results: The cases were from parotid (2) and oral cavity (1). They ranged from 0.9-2.4 cm. Two patients with clinical data were females of 47 and 57 years of age. All 3 cases were encapsulated/circumscribed masses. The tumors were made up entirely of back-to-back ducts with virtually no intervening stroma. The ducts were of varying size with large caliber lumina containing eosinophilic secretions. Interspersed ducts formed cysts up to 1 mm in diameter. The ductal cells were eosinophilic, had visible striations and bland basally oriented nuclei. All lesions contained abundant large vessels, some of which were staghorn in shape. One case contained psammoma bodies and focal papillary formations. None of the cases showed the typical epithelial beading pattern with abundant stroma seen in canalicular adenomas. One case had striated duct hyperplasia (SDH) in the background parotid. All 3 cases and SDH were positive for keratins and S100. Normal salivary elements were S100 negative. No basal/myoepithelial contribution was found with p63 or actin in the tumors or in the background SDH: a pattern identical to normal striated ducts and unlike acini, intercalated and excretory ducts (which all contain basal or myoepithelial cells). CK5/6 in one case demonstrated strong positivity with basal accentuation, which was again identical to the background striated ducts.
Conclusions: Striated duct adenoma is a rare unilayered ductal lesion with virtually no stroma and no myoepithelial component. These neoplasms recapitulate normal striated ducts morphologically and immunohistochemically. Striated duct adenoma is distinct from all other benign salivary gland lesions, including canalicular adenoma.

1152 Intercalated Duct Lesions of Salivary Gland: A Re-Appraisal of 30 Cases of a Putative Precursor Lesion

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Background: Intercalated duct lesions (IDL) are benign ductal proliferations that have been described rarely in association with epithelial-myoepithelial carcinoma (EMC) and are also seen in association with other salivary gland tumors. They have been proposed as a putative precursor lesion, but a detailed review of IDL's has not been performed.
Design: 30 cases of IDL were identified and reviewed and immunostaining with CK7, ER, PR, lysozyme, S100, CK14 and calponin was performed. Due to the morphologic resemblance to basal cell adenoma (BCA), 8 of these tumors were stained for comparison.
Results: The patients with IDL ranged in age from 19-80 yrs (average 53.2). There was a 1.8:1 female predominance. The majority were parotid lesions (25/30 - 83%) with the remaining cases in submandibular gland. Most cases (24/30 - 80%) were small nodular lesions ranging from 1-8 mm (average 3.2 mm). The remainder were diffuse/multifocal lesions. 16 IDL's (53%) were seen in conjunction with another salivary gland tumour, the most common being BCA/BC adenocarcinoma (7 cases). The IDL's showed a spectrum from irregular ductal proliferations to round encapsulated "adenomas" with hybrid forms. 1 case showed a direct transition from IDL to a BCA. Focal acinic differentiation was common. Most cases had an indistinct myoepithelial layer on H&E, however some demonstrated a focal clear cell myoepithelial layer. Others had periductal hyalinization. Immunohistochemically, IDL's stained diffusely for CK7 (100%) and S100 (71%) and focally for lysozyme (100%) and ER (88%). They were negative for PR. Normal intercalated ducts also showed consistent CK7, lysozyme and focal ER staining but were S100 negative. A thin myoepithelial layer was highlighted with CK14 and calponin in all IDL's (100%) and in normal intercalated ducts. In contrast, BCA's were larger (average 2.1 cm), did not show significant ER or lysozyme staining and had a more prominent neoplastic basal/myoepithelial component with these markers.
Conclusions: IDL's have a variety of growth patterns. They share features with normal intercalated ducts. They are distinct from BCA's which have a biphasic neoplastic population. However transition to and association with BCA's is common and appears more common than with EMC. Their frequent occurence in association with salivary tumors lends credence to their role as a precursor lesion.

1153 Global Epigenetic Alterations in Head and Neck Squamous Cell Carcinoma

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Background: Head and neck cancer is the most common epithelial malignancy of the upper aerodigestive tract. Despite multimodality treatment regimens of combined surgery and chemoradiation therapies, the overall 5-year survival for head and neck cancer has not exceeded 50% for the past 3 decades. Recently, there is growing evidence that in addition to widespread genetic abnormalities, epigenetic alterations in association with promoter hypermethylation play significant roles in the development and progression of human cancer. In this study, we analyze a global methylation pattern in 4 HNSCC cancer cell lines as compared to normal oral keratinocyte cell line using Illumina DNA methylation microarray.
Design: 4 HNSCC cell lines (CRL1623, HEp2, SQ20B and UMSCC1) treated with or without 5-azacytidine, a DNA demethylating agent and a normal oral keratinocyte cell line (HOK16B) were used for extraction of genomic DNA, followed by sodium bisulfite modification and global promoter methylation analysis using Illumina BeadArray (HumanMethylation27) containing 28,544 target CpG sites, representing a total of 14,956 genes.
Results: Among 14,956 genes, there were 1230 genes that showed increased promoter methylation by 2-fold or greater in all 4 HNSCC cell lines CRL1623, HEp2, SQ20B and UMSCC1) as compared to the normal keratinocyte line (HOK16B). Following the treatment of 5-azacytidine, 42 out of these selected 1230 genes showed decreased methylation in all 4 HNSCC cell lines by at least 10%. Thus, these 42 genes are likely those whose expression is commonly regulated by epigenetic mechanism in HNSCC but not in normal oral keratinocytes. Using these 42 genes for cluster analysis, 4 HNSCC cell lines were clustered closely to one another and the promoter methylation pattern for HNSCC cell lines was distinctively different from that in normal oral keratinocytes.
Conclusions: Using the powerful Illumina DNA methylation microarray, we have identified a small subset of genes that frequently undergo promoter methylation in HNSCC. This information should be invaluable in helping us to identify novel epigenetic markers for early cancer detection or molecular targets for epigenetic therapy in the treatment of HNSCC.

Hematopathology

1154 Flow Cytometric Panel Including CD10, CD19, CD38, CD58 and CD45 Increases Diagnostic Accuracy for Distinguishing Hematogones from Precursor B-Cell Acute Lymphoblastic Leukemia (Pre B-ALL)

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Background: Hematogones are benign precursor B-lineage lymphoid population that are seen in increased numbers in the bone marrow during of childhood, post bone marrow transplant, immune deficiency and autoimmune disorders. They typically show expression of dim CD45, CD10, CD34, CD19, TdT and variable CD20. However, this phenotype is also seen in patients with precursor B-cell acute lymphoblastic leukemia (pre B-ALL) causing a significant problem in distinguishing residual pre B-ALL from hematogones in a regenerating marrow. Earlier studies suggested flow cytometric analysis using the above markers as being useful for differentiating hematogones from pre B-ALL, however, in our experience the use of these limited panel of markers is insufficient and may hinder correct interpretation of bone marrow samples. Recent studies showed that CD58 is commonly expressed in pre B-ALL but not in hematogones and expression of CD38 is different in the two populations. We generated a 5-color antibody panel including the markers mentioned above to assess the feasibility of using a novel panel to distinguish pre B-ALL from hematogones.
Design: Total of 37 cases including 27 cases of pre B-ALL (diagnostic and residual/relapsed cases) and 10 cases of hematogones were analyzed by five color flow cytometery. Two separate tubes with following markers (Tube1:CD38-PE, CD10-FITC, CD19-ECD, CD20-PC5, CD45-PC7 and tube2: CD58-PE, CD10-FITC, CD19-ECD, CD34-PC5, CD45-PC7) were used simultaneously with the standard acute leukemia panels. Results were correlated with the bone marrow histologic findings and cytogenetic analysis.
Results: Hematogones typically showed expression of bright CD38, moderate (mod) CD19, mod CD10, variable CD20 and no CD58; in contrast pre B-ALL showed weak to mod CD38, mod to strong CD19, mod to strong CD10 and mod CD58. Without the combination of both CD38 and CD58 together, some cases would have been difficult to interpret.