Design: Slides from 106 cases of endometrial carcinoma with a serous component were reviewed and grouped into the following categories: Pure ESC-P (n=51), pure ESC-TG (n=12), mixed ESC-TG and EEC (n=7), and ESC-X (n=36). For the mixed ESC-TG, we required that there be more than 25% of the ESC-TG component, which characteristically exhibited a tubuloglandular growth pattern and notably high-grade nuclei. The ESC-X group contained cases with an ESC component admixed with either clear cell, sarcomatous, high-grade EEC or poorly/undifferentiated regions. Immunohistochemistry for p53, estrogen (ER), and progesterone (PR) receptors, and IMP3 was performed. Survival and recurrence rates among the groups were analyzed.
Results: The clinicopathologic features of the ESC-TG group resembled those of ESC-P and ESC-X groups. There was no significant difference in the survival and recurrence rates among the 3 groups. The immunoprofile of the 3 groups are summarized below. Notably, IMP3 was markedly positive in the ESC-TG and ESC-P group and the serous component of the ESC-X group, suggesting that IMP3 may be a marker for serous carcinomas. The combination of positive IMP3 and p53 expression and lack of ER/PR expression best classified ESC-TG as a morphologic variant of ESC-P.
Conclusions: Our data support the concept that there is a group of endometrial cancers composed of tubular glands that are indeed serous carcinomas. ESC-TG display a low-grade, glandular architecture but are characterized by notably high-grade nuclei. ESC-TG and ESC-P have essentially identical clinicopathologic profiles.

	IMP3 (%)	p53 (%)	ER (%)	PR (%)	p value
ESC-TG	90	95	10	5	
ESC-P	92	94	10	8	0.8
ESC-X	77	81	14		0.7

1006 The RNA-Binding Protein IMP3: A Novel Cytoplasmic Marker for Endometrial Serous Carcinoma

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Background: IGF-II mRNA-binding protein 3 (IMP3) is a novel oncofetal RNA-binding protein highly expressed in renal and pancreatic carcinomas. Recently, Zhong *et al* found that the protein expression predicts the metastasis and poor survival in renal cell carcinomas. The aim of this study is to determine if the expression of IMP3 has any diagnostic usage for endometrial carcinomas.
Design: IMP3 expression by immunohistochemistry (IHC) was examined in 298 endometrial samples. These included benign endometrium (n=68), atypical hyperplasia (AH)/endometrial intraepithelial neoplasia (EIN) (n=35), endometrial glandular dysplasia (EmGD) (n=21), endometrial intraepithelial carcinomas (EIC) (n=18), endometrioid carcinomas (EEC) (n=70), mucinous carcinomas (MC) (n=8), serous carcinomas (ESC) (n=51), clear cell carcinomas (CCC) (n=12), and other malignancies (n=15). The benign endometrium included 12 atrophic, 18 proliferative, 14 secretary, 8 menstrual, and 16 gestational enometrium. The carcinomas were either pure form or mixed type with < 10% of a second component . A renal cell carcinoma with known IMP3 expression was used as a positive control. The criteria for positivity was cytoplasmic stains with moderate intensity in at least 50% of target cells.
Results: Among malignant cases, IMP3 expression was mainly found in ESC and its putative precursor lesions: EmGD- 3/21 (14%), EIC-16/18 (89%), and ESC-48/51 (94%) ($p < 0.001$). In contrast, IMP3 expression was significantly lower in non-serous malignancies with 0%, 7%, 0%, 25%, and 33% positive stains in AH/EIN, EEC, MC, CCC, and other malignancies, respectively. IMP3 staining was typically diffuse and strong in ESC, while patchy and moderate in non-serous malignancies. Among benign endometrium, only decidualized stroma (100%) was positive. Trophoblasts in the first trimester chorionic villi were also diffusely positive, which was consistent with previous findings.
Conclusions: We conclude that IMP3 is a new sensitive and specific marker for ESC including serous EIC. Therefore, immunostains with IMP3 antibodies may be of a diagnostic utility in evaluation of endometrial cancinomas, particularly when biopsy material is limited and a concern of ESC arises. Although the significance of IMP3 expression in decidualized endometrial stroma remains unclear, differential diagnosis between decidual changes and ESC is barely problematic.

Head & Neck

1007 Non-Keratinizing Carcinoma of the Sinonasal Tract Is a Clinicopathological and Molecular Entity Different from Keratinizing Squamous Cell Carcinoma

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Background: Sinonasal Non-keratinizing Carcinoma (NKC) of the sinonasal tract has been referred in the past by several terminologies as Schneiderian Carcinoma, Transitional Cell Carcinoma and Cylindrical Cell Carcinoma. The last WHO classification of Tumors of the Head and Neck, includes NKC as a variant of Squamous Cell Carcinoma. The aim of this study is to characterize and compare the clinicopathological features, HPV DNA prevalence and immunophenotypes of NKC and usual Keratinizing Squamous Cell Carcinoma (KSCC).
Design: Sixty-five Squamous Cell Carcinomas of the sinonasal tract were reviewed and the medical records were obtained from patients' files. Thirty-six tumors were selected to perform immunohistochemical studies for p16INK4 (Biocare Medical, Walnut Creek, CA. Clone JC8, dilution 1:100); Ki-67 (Immunotech, Marseille, France. Clone MIB-1, dilution 1:200); p53 (Novocastra Laboratories, Newcastle, UK. Clone BP53-12, dilution 1:50). PCR studies for detecting HPV DNA using both SPF10 and GP5+/6+ techniques were also applied in the same cases.
Results: Forty-eight tumors were KSCC (74%); and 17, NKC (26%). Both tumor types affected predominantly males, in a 3:1 ratio, in the seventh decade of life. NKC had a higher predilection affecting naso-ethmoid structures instead of maxillary sinuses, whereas KSCC ocurred in both sites equally. HPV16 was detected in 8/13 NKC (61%) and 4/23 KSCC (15%). p16 was positive in 8/13 NKC (61%) and 2 KSCC (8%). A high Ki67 positivity (>30%) was observed in 10/13 NKC (67%) and 8/23 KSCC (35%), whereas p53 was highly positive (>30%) in 7/13 NKC (54%) and 16/23 KSCC (70%). KSCC had higher recurrence and metastatic rates (82 and 20% of the cases respectively) than NKC (50 and 7% of the cases, respectively). The overall survival was shorter for KSCC than NKC ($p<0.0012$).
Conclusions: NKC is a distinctive neoplasm of the sinonasal tract with high prevalence of HPV infection. It has a characteristic immunophenotype, frequently expressing p16 and high Ki67 positivity. This tumor type has less tendency to recur and metastasize, and better prognosis than KSCC.

1008 Detection of Human Papillomavirus-16 in Fine Needle Aspirates: A Strategy for Localizing Site of Tumor Origin in Patients Presenting with Metastatic Squamous Cell Carcinoma

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Background: Patients with head and neck squamous cell carcinoma (HNSC) often clinically present with metastases to regional lymph nodes. Fine needle aspiration (FNA) of neck masses is routinely used to establish the presence of metastatic carcinoma and, in turn, to initiate a subsequent workup to determine the site of tumor origin. Human papillomavirus 16 (HPV16) is an important etiologic agent for HNSCs that arise from the oropharynx but less so for tumors from non-oropharyngeal sites. HPV16 detection thus provides a strategy for localizing an important subset of HNSCs, but this approach has not been applied to FNA specimens.
Design: We performed in-situ hybridization for HPV16 on 77 consecutive aspirated neck masses diagnosed as metastatic squamous cell carcinoma. P16 immunohistochemistry was also performed because p16 overexpression may serve as a surrogate marker of HPV-associated HNSC.
Results: HPV16 was detected in 13 (17%) of the 77 aspirates. By site of origin, HPV16 was detected in 10 of 19 metastases from the oropharynx, but in none of 46 metastases from other sites (53% vs. 0%, $p < 0.0001$). HPV16 was not detected in 2 branchial cleft cysts misdiagnosed as metastatic squamous cell carcinoma, but it was detected in 3 of 10 metastases from occult primary tumors. P16 expression was associated with the presence of HPV16: 12 of 13 HPV16 positive metastases exhibited p16 expression, whereas only 4 of 62 HPV16 negative metastases were p16 positive (92% vs. 6%, $p < 0.0001$). P16 expression also correlated with site of tumor origin: 13 of 19 oropharyngeal metastases were p16 positive whereas only 1 of 46 non-oropharyngeal metastases was p16 positive (68% vs. 2%, $p<0.0001$).
Conclusions: HPV16 status can be determined in tumor cells aspirated from the necks of patients with metastatic HNSC. Its presence is a reliable indicator of origin from the oropharynx.

1009 Immunohistochemistry of Molecular Markers in Sinonasal Undifferentiated Carcinomas

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Background: Sinonasal undifferentiated carcinoma (SNUC) is an uncommon and highly aggressive neoplasm of the nasal cavity and paranasal sinuses. Treatment may consist of craniofacial resection and adjuvant chemoradiation. The goal of this study is to expand our knowledge of the immunophenotype of this lesion, focusing on markers potentially mechanistic in the disease process or of possible therapeutic importance.
Design: Our surgical pathology files were searched for cases diagnosed as SNUC. Poorly differentiated carcinomas (PDCs) of the sinonasal cavity and SNUC-like lesions of the larynx served as controls. Immunohistochemistry (IHC) was performed for the following markers: MLH-1, MSH-2, β-catenin, p16, p53, CD117, and EGFR. The immunohistochemical stains for MLH-1 and MSH-2 were scored as intact or lost. The other stains were scored semiquantitatively based on the percentage of tumor cells staining: <5%=0, 5-25%=1+, 26-50%=2+, 51-75%=3+, >75%=4+.
Results: Eleven SNUCs with residual material available for IHC were identified, as were 4 poorly differentiated carcinomas of the sinonasal tract and 2 SNUC-like lesions of the larynx. MLH-1 and MSH-2 were intact in all SNUCs in which concomitant evaluation was possible (10/10). Nuclear staining for β-catenin was not identified in any case. Nuclear and cytoplasmic staining for p16 was observed in 7 of 11 SNUCs (1+ in 5, 2+ in 2). p53 staining was observed in 10 of 11 (1+ in 5, 2+ in 3, 3+ in 1, and 4+ in 1). Four of 11 cases stained for CD117 (2+ in 1, 3+ in 3). SNUCs (and control cases) were uniformly negative for EGFR. MSH-2 was lost in 1 sinonasal PDC; MLH-1 and MSH-2 were intact in the remaining control cases. Control cases were negative for β-catenin nuclear positivity. Four of 4 and 0 of 2 sinonasal PDCs and SNUC-like laryngeal tumors were positive for p16. All control cases marked for p53. CD117 positivity was found in 2 of 4 and 1 of 2 of sinonasal PDCs and SNUC-like laryngeal tumors, respectively.
Conclusions: Aside from p53 immunoreactivity, the most common molecular abnormality idnetified in our SNUC cases was over-expression of p16. Although this finding is non-specific, it suggests that HPV may play a role in the development of at least some SNUCs. The idenitifcation of CD117 immunoreactivity, although potentially pointing to a treatment option, may instead suggest that some of these lesions are poorly differentiated neoplasms of the seromucinous glands. Future directions of study may include more in depth assessments of the role of HPV or CD117 with these lesions.

1010 Clinicopathologic Features of Plexiform Schwannomas Arising in the Head and Neck

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Background: Plexiform schwannoma (PS) is a rare form of schwannoma characterized histologically by a plexiform/multinodular pattern of growth in addition to the usual microscopic features of conventional or cellular schwannoma. A predilection for the head and neck is evident in both PS and conventional schwannomas. The purpose of this study was to assess the frequencies of PS by location, and to analyze clinicopathologic features of head and neck examples.

Design: The files of Mayo Clinic Tissue Registry were searched for plexiform schwannomas occurring between 1991 and 2006. 92 cases (108 tumors) were categorized by site: 26 (24%) cutaneous from a variety of sites; 23 (22%) head and neck (exclusive of the orbit); 16 (15%) soft tissues of the truck; 18 (17%) upper extremity (13 involving hands); 17 (16%) lower extremity (7 involving feet); 2 (2%) viscera (lung, sigmoid colon); 6 (5%) affecting major nerves, and 1 XIIth cranial nerve example. Three cases had known NF2, with an additional case of probable NF 2 (4%). Of the cases arising in the head and neck, pathologic material was available for review in 16 tumors. Histochemical and immunohistochemical stained sections of formalin-fixed, paraffin embedded tissue were reviewed. Microscopic features assessed included encapsulation, cellularity, and mitotic index. Clinical data was available in 8 cases.

Results: Among 23 head and neck cases from 14 females and 9 males (age 7 months – 69 years (mean, 33), locations were as follows: 5 oral cavity; 7 scalp; 7 soft tissues of the neck; 2 parotid gland; 1 ethmoid sinus; 1 in association with CN XII. No patient experienced local recurrence. Two of the cases were associated with NF2, defined by the presence of acoustic schwannomas and multiple schwannomas. Of the latter, 1 patient had an additional PS. Two other cases had additional conventional schwannomas and presumably represent schwannomatosis. A single patient had previously undergone head and neck irradiation. Five tumors were cellular. Of these, 3 exhibited increased mitotic activity and moderate MIB-1 labeling. One tumor showed an epithelioid cytology. Immunohistochemical stains for S-100 protein showed strong and diffuse positivity in all lesions tested.

Conclusions: PS are most commonly found in the skin and subcutaneous tissue. The head and neck is the most frequent specific location. The majority of PS are solitary, sporadic tumors, but multiple tumors can be associated with NF 2 or schwannomatosis, both syndromes involving mutations in the NF2 gene.

1011 Reduced Expression of the Chaperone Protein Calnexin Is Associated with Sentinel Lymph Node Metastasis of Oral Carcinoma

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Background: In oral cancer the presence of nodal metastasis is an important prognosticator. The Major Histocompatibility Complex Class I (MHC I) is a key component of antigen presentation and immune surveillance, and is the end product of a multi-step cellular assembly process. Calnexin is a chaperone protein that plays a key role in the processing of many glycoproteins, including MHC I heavy chain. Although MHC I heavy chain expression is diminished in head and neck cancer, calnexin and other proteins of the MHC I assembly system have not been studied in detail in oral carcinoma. Our aim was to examine the relevance of the MHC I system in early regional metastasis of oral cancer.

Design: MHC I heavy chain, calnexin and twelve other components of the MHC I antigen processing system were evaluated by immunohistochemistry in twenty patients with clinically T2N0 oral carcinoma who underwent staging sentinel lymph node biopsy (SLNB) and synchronous neck dissection. For each patient, a formalin fixed primary tumor sample was analyzed. The percentage of positive tumor cells and intensity of staining (compared to normal adjacent mucosa) were scored on a three point scale for each antibody.

Results: Thirteen tumors (13/20) had regional metastasis detected by SLNB. Decreased expression of calnexin correlated with the presence of nodal disease (p=0.03) and increasing disease stage (p=0.02). None of the other system components, including MHC I heavy chain (p=0.91), showed a significant association with metastasis. No significant correlation between decreased calnexin expression and reduced MHC I heavy chain staining was seen (p=1.00), but thirteen tumors (13/20) did show abnormal expression of MHC I heavy chain.

Conclusions: The lack of correlation between calnexin and aberrant heavy chain expression suggests that decreased calnexin alone does not disrupt the MHC I assembly system in these tumors. However, there is a link between decreased calnexin expression and the presence of regional metastasis and advanced disease stage. Given the broad role of calnexin in the processing of cellular glycoproteins, the association of reduced calnexin and metastasis may represent dysregulation of other important cellular glycoproteins uninvolved in MHC I antigen presentation.

1012 KLF6 Allelic Loss Predicts Poor Survival in Upper Aerodigestive Tract Squamous Cell Carcinomas (UADT-SCC)

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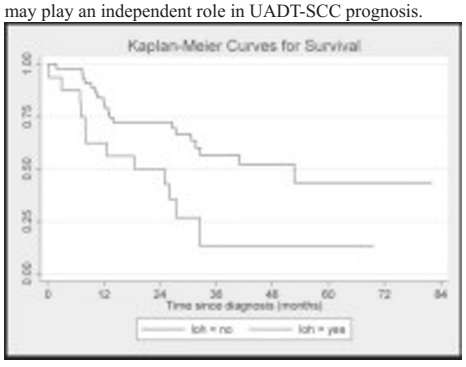
Background: The Krüppel-like factor 6 gene (KLF6) functions as a tumor suppressor gene involved in cell cycle control. We examined KLF6 genotype and RNA expression for UADT-SCC.

Design: Paraffin tumor/normal samples were studied in 81 cases of oral SCC. Four microsatellite markers spanning 17 Mb of 10p, and 2 KLF6-specific markers flanking KLF6 gene by 40Kb and 10Kb assessed KLF6 loss. After PCR and electrophoresis, data were analyzed with ABI Genescan and Genotyper software. The peak heights of the two alleles were compared; relative allele ratios ≤ 0.7 in at least two independent reactions was defined as consistent with loss. KLF6 LOH was positive only if at least one of the specific markers, KLF6 M1 and/or KLF6 M2, demonstrated allelic imbalance. RNA extracts of frozen samples from a second cohort with UADT-SCC including extra-oral sites, were studied for KLF6 wild-type (WT) and 3 splice variants (SV) by quantitative real-time PCR. Data were analyzed as continuous and discreet variables for WT and SV1, SV2, and SV3 alone, and as a ratio to WT.

Results: By KLF6 M1/KLF6 M2, 70 cases were informative and KLF6 LOH was present in 27% (19/70) of oral tumors. On survival analysis, KLF6 LOH was positively associated with time to disease progression (DP) (p=0.039,HR 2.5) and overall survival (OS) (p=0.001,HR 4.3) after correction for T&N stage, and treatment. (Graph 1) Cox regression analysis for SV1/WT ratio using a median cutoff of >0.014 revealed an inverse relationship with locoregional recurrence (p = 0.101, HR 0.30) after correlation for TN stage and treatment.

Conclusions: KLF6 loss is a common event (27%) in UADT-SCC, and represents a significant prognosticator in multivariate analyses. Since LOH of the KLF6 locus would be expected to affect all KLF6 transcripts, taken together our LOH and expression data are particularly intriguing. Although preliminary, the observed inverse relationship between KLF6 and SV1 levels with locoregional recurrence and direct relationships of KLF6 (SV1-3) expression with survival highlights the finding that KLF6 isoforms may play an independent role in UADT-SCC prognosis.



1013 Abnormalities in Immunoexpression of Dicer, a Component of RNA-Induced Silencing Complex, Correlate with Aggressive Behavior in Salivary Mucoepidermoid Carcinoma

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Background: MicroRNAs (miR) are small noncoding 18- to 24-nt RNAs that are predicted to regulate up to 30% of protein-encoding genes. miRNA biogenesis requires coordinated processing by the miRNA machinery, of which the Dicer protein is an essential component. Gene array and CGH studies of mammary and salivary type (adenoid cystic carcinoma) malignancies have documented frequent *Dicer* abnormalities, but its role and clinical significance in salivary mucoepidermoid carcinomas (MEC) has not been investigated.

Design: Immunoexpression of Dicer was assessed in 55 tumors: 46 MEC (9 low grade, 14 intermediate grade, and 23 high grade; Brandwein scheme) and 9 adenosquamous carcinomas (AsqCA). Cytoplasmic Dicer positivity was scored semiquantitatively and assigned a numeric category (0-3) relative to expression levels in internal controls: large excretory/striated ducts, or basal/parabasal layers of squamous epithelial surfaces most commonly scored as 2. Dicer levels in epidermoid/intermediate areas of MEC and AsqCA were then correlated with clinical and pathologic parameters. Statistical analyses were performed with SPSS software.

Results: Dicer protein, when expressed, was mainly confined to the epidermoid and intermediate cells of MEC, and the squamous component of AsqCA. Dicer abnormalities (either over or underexpression) with respect to internal controls were more commonly seen in high grade MEC (83%) than in low/intermediate grade MEC (35%) (p = 0.002, Fisher exact, 2 tail) and also more commonly seen in stage III/IV MEC (80%) than in stage I/II MEC (41%) (p = 0.04). Dicer abnormalities were seen frequently in AsqCA (78%) as well. Significant univariate predictors of disease specific survival (DSS) by the Kaplan-Meier method are summarized in Table 1.

Conclusions: Abnormal Dicer immunoexpression correlates with high grade, high stage, and is a univariate predictor of poor disease specific survival in MEC. Hence, the frequent Dicer abnormalities in aggressive MEC suggest a role for miR machinery dysfunction in tumor progression.

Univariate Predictors of DSS

Parameter	Log rank p
Grade: High grade vs Low/Intermediate	<0.001
Stage: III/IV vs I/II	0.003
Age: >50 vs <50	0.013
Dicer: abnormal vs normal	0.034

1014 Immunohistochemical Evaluation of Androgen Receptor, Her-2-neu, and p53 in Benign Pleomorphic Adenomas

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Background: Immunohistochemical stains for androgen receptor (AR), Her-2-neu, and p53 are used as diagnostic markers associated with malignancy in several histologic types of salivary gland tumors. Her-2-neu overexpression has been postulated as a marker that can be used to identify intracapsular and minimally invasive carcinoma ex pleomorphic adenoma. The aim of this study was to determine whether AR, Her-2-neu, and p53 expression can be seen in entirely benign pleomorphic adenomas.

Design: Formalin-fixed, paraffin-embedded tissues from 41 consecutive cases of histologically and clinically benign pleomorphic adenomas were retrieved from our institutional archives. The slides were examined to ensure the absence of significant cytologic atypia, increase in mitoses, or capsular invasion in any case. Immunohistochemical stains for Her-2-neu, AR, p53, and Ki-67 were performed using standard methods. Her-2-neu staining was graded using the Dako Herceptest protocol. Androgen receptor and p53 were analyzed semiquantitatively and graded as negative (<5%), 1+ (5-10%), 2+ (10-50%), and 3+ (>50%). A quantitative Ki-67 proliferative index was determined.
Results: Three of the 41 pleomorphic adenomas exhibited multifocal areas with strong immunoreactivity for Her-2-neu and androgen receptor. The positive staining was mainly confined to the epithelial component, where the tubules had minimal to no atypia. Immunoreactivity for p53 was observed in the epithelial component of 5/41 cases, none of which stained for Her-2-neu and AR. Mean mitotic rate and Ki-67 index were 1 per ten hpf and 2.7% in Her-2-neu and androgen receptor positive cases and 1 per ten hpf and 2.2% in p53 positive cases.
Conclusions: Her-2-neu and androgen receptor expression were identified in 3/41 pleomorphic adenomas (7%). 5/41 pleomorphic adenomas (12%) showed expression of p53. Because these pleomorphic adenomas were entirely benign both clinically and histologically, we suggest that Her-2-neu, AR, and p53 cannot be used to reliably predict early carcinomatous transformation in pleomorphic adenoma.

1015 Diagnostic Dilemma in the New 2005 WHO Histological Classification of Odontogenic Tumors

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Background: The World Health Organization published the "Blue Book" of Pathology and Genetics of Head and Neck tumors in June of 2005. Several changes have been made to the previous 1992 classification of Odontogenic tumors including nomenclature for new "old entities" previously part of cystic lesions and which have been reclassified as tumors to reflect their aggressive nature, while other tumors have been upgraded to a malignant status. There are diagnostic dilemmas due to the similarity of some of these lesions which may have different treatment options. Calretinin, 29-KD EF-hand protein related to other calcium-binding proteins and S100 was tested as a marker to differentiate ameloblastoma from keratocystic odontogenic tumor (KCOT) and to investigate its possible role in the tumorigenesis of ameloblastoma.
Design: 19 cases of ameloblastoma and 9 cases of keratocystic odontogenic tumor (KCOT) represented by formalin-fixed, paraffin-embedded tissue were selected from the University of North Carolina surgical pathology archives as well as one case originally diagnosed as KCOT which recurred a year later as an ameloblastoma. Pleural mesothelioma tissue was used as positive control. Sections were cut from the paraffin-embedded blocks and immunohistochemically stained with Calretinin antiserum 18-0211 using an avidin-biotinylated peroxidase complex method.
Results: All the cases of ameloblastoma (100%) showed positive Calretinin staining restricted to the stellate-like reticulum portion of the tumor. No positive staining (0%) was seen on the epithelial lining of KCOT sections. Furthermore, a case that had originally been diagnosed as KCOT and which recurred one year later as an ameloblastoma showed positivity for Calretinin at both time points, indicating that it was an ameloblastoma both as the primary tumor and the recurrence. Furthermore, gene expression profiling using 41,000 whole genome oligonucleotide microarray analysis showed the presence of CALB2 (calretinin) in ameloblastoma.
Conclusions: There are clinical, diagnostic and therapeutic implications expected to derive from the new nomenclature outlined in the 2005 WHO histological classification of odontogenic tumors. Calretinin appears to be a useful immunohistochemical stain in the differentiation of ameloblastoma and keracystic odontogenic tumor.

1016 Detection of HPV in Metastatic Squamous Cell Carcinoma in Cervical Lymph Nodes: A Tool for Identification of the Site of an Occult Head and Neck Primary

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Background: Many head and neck squamous cell carcinomas (HNSCC) present initially as metastatic disease in neck lymph nodes. In some of these cases the primary site of the tumor remains unknown in spite of thorough examination and multiple endoscopic biopsies of possible sites of origin. Because of known site specificity of HPV-related HNSCC we investigated the utility of detecting HPV in metastatic cervical lymph nodes in determining the possible site of occult primary tumors. Identification of the location of a primary tumor is essential for the proper patient management.
Design: Ninety three cases of HNSCC metastatic to cervical lymph nodes were retrieved from our files. The sites of the primary tumors included the oral cavity (38), larynx / hypopharynx (26) and oropharynx (31). The oropharyngeal tumors were predominantly in the tonsils and base of tongue. The metastatic tumors were classified according to their microscopic features into keratinizing and nonkeratinizing SCC. Additional sections were stained immunohistochemically with p16 antibodies and were evaluated for the presence of high risk HPV by ISH.
Results: The patient's ages ranged from 34 to 80 years. The male to female ratio was 4:1, 7:1 and 9:1 for patients with oral, laryngeal / hypopharyngeal and oropharyngeal carcinomas respectively. HPV was identified in the neck metastasis of 23 of the 93 cases examined (24.7%). The oropharynx was the source of metastasis in 21 of the HPV positive cases (91.3%), while the other sites accounted for only two, ($p<0.0001$). The distribution of the HPV positive metastasis by primary site was 1/35 (2.7%) oral, 1/26 (3.8%) laryngeal / hypopharyngeal and 21/31 (67.8%) oropharyngeal carcinomas. HPV+ SCC were nonkeratinizing with basal cell features and were strongly and diffusely reactive to p16 antibodies. Four of the HPV negative oropharyngeal carcinomas reacted positively for p16 stain, all of which had a hybrid keratinizing/nonkeratinizing phenotype.
Conclusions: Detection of HPV by ISH in cervical metastasis of HNSCC is a strong indication of a primary oropharyngeal origin, with a sensitivity of 91.3% and specificity of 85.7%. Identification of HPV-related tumors may be accomplished not only by ISH but also morphologically and by p16 reactivity. The relationship of ISH-negative tumors, with strong p16 reactivity, to HPV may be elucidated by using a more sensitive technique such as PCR.

1017 Basaloid Squamous Cell Carcinoma of the Head and Neck. A Clinicopathologic Study of 42 Cases

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Background: Basaloid squamous cell carcinoma (BSCC) is a rare and aggressive variant of cancer that mainly arises in the upper aerodigestive tract. The histological characteristics have been well defined since the first description of this entity by Wain et al in 1986. This study reviews the clinical-pathological features and follow-up of a large series of cases occurring in the head & neck region.
Design: Throughout a 31-year period (1974-2005), a total of 42 BSCCs have been diagnosed in the head & neck in our Institution. All the cases were treated with surgery plus radiotherapy and were studied following the same protocol. Follow-up was closed by June 2006.
Results: Males predominated in the series (37M/5F). The average age was 58.5 years (range, 40-85). Tobacco and alcohol consumption was found in more than 80% of the cases. Topographic distribution was as follows: larynx and hypopharynx, 22 cases (52.3%); oropharynx, 13 cases (30.9%); and oral cavity 6 cases (14.2%). One case was found in the neck dissection but a definite site for the primary was not found. Most cases (95.2%) presented as ulcer-infiltrating masses with diameters oscillating between 5 and 55 mm (average, 24.4 mm). The basaloid component predominated in 31 cases (73.8%). Vascular-lymphatic invasion was detected in 5 cases (11.9%). Lymph node metastases were seen in 25 cases (59.6%, mainly levels II and III in the neck dissection), 22 of them (88%) showing the biphasic tumour pattern. Extra-capsular lymph node invasion was demonstrated in 8 cases (32%), 4 of them invading the adventitia of the adjacent jugular vein. Local recurrences appeared in 11 cases (26%) and distant metastases in 6 (14.2%, lung 3, bone 2, liver 1). In 7 cases (16.6%) a second primary tumour was detected (UADT 3, bronchus 3, colon-rectum 1). The 2002 TNM staging was as follows: stage I, 6 cases (14.2%); stage II, 7 cases (16.6%); stage III, 8 cases (19%), and stage IV (21 cases (50%). On follow-up, 22 cases (52.3%) are alive (12 with and 10 without tumour) and 20 (47.6%) died of the disease. Three- and 5-year overall survival (Kaplan-Meier) was 50% and 38.5%, respectively. A significant shorter survival was detected in node positive patients ($p<0.05$, Mantel-Haenzel).
Conclusions: BSCC of the head & neck is a high grade and aggressive tumour showing frequent advanced stage at diagnosis, high percentage of node and distant metastases, frequent second primary tumours and high 5-year tumour-related death. The status of regional nodes is a prognostic factor of survival.

1018 Synergistic Suppression of Head and Neck Cancer Growth by Retinoic Acid and DNA Demethylating Agent, 5-azacytidine

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Background: The use of 13-cis-RA showed initial promise by successfully reversing oral premalignant lesions, however, subsequent relapse of these oral lesions after discontinuing the therapy prohibits the use of 13-cis-RA as an effective antitumor agent. It has been postulated that resistance of head and neck cancers to retinoids is in part due to the decreased expression of various RA receptors (RARs). We hypothesize that treatment of head and neck cancer cells with a DNA demethylating agent, 5-azacytidine (5-AC), will re-activate some of these RAR genes, thus, augment the antitumor effects of RA. In this study, we tested this hypothesis by investigating the in vitro tumor suppression and gene expression profile of RA signal transduction pathway in head and neck cancer cells treated with 13-cis-RA and 5-AC.
Design: cultured head and neck cancer cells were treated without or with 5-AC and/or 13-cis-RA and cell proliferation was determined using CellTiter 96 Aqueous One Solution Cell Proliferation Assay. Gene expression profile of RA signal transduction pathway was analyzed using AffyMetrix oligonucleotide microarray.
Results: 13-cis-RA and 5-AC showed robust, synergistic in vitro tumor suppression (70% suppression with 2 mM 5-AC and 10 mM 13-cis-RA) when compared with treatment with 2 mM 5-AC (28% suppression) or 10 mM 13-cis-RA (30% suppression) alone. By a two-factor analysis of variance (ANOVA) test, 5-AC demonstrated significant interaction effects with 13-cis-RA ($p < 0.001$), supporting a synergy between these two agents. Among 33,000 human genes in AffyMetrix microarray, there are 10 well-characterized RA responsive genes: retinoic acid receptors a, b, and g (RAR a, b, and g); retinoic receptor responders 1, 2, and 3 (RARRES-1, -2, and -3); cellular RA binding proteins 1 and 2; and retinoid X receptors b and g (RXR b and g). In cells treated with 5-AC and 13-cis-RA, only RARRES-1 and RARRES-3 were markedly and synergistically induced.
Conclusions: 13-cis-RA and 5-AC can robustly and synergistically suppresses in vitro head and neck cancer growth and this synergistic tumor suppression is in part due to selective activation of two RA-responsive genes, RARRES-1 and RARRES-3.

1019 Immunoexpression of Estrogen, Progesterone and Androgen Hormone Receptors in Osteosarcoma of Craniofacial Bones

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Background: Sexual hormones play an important role in bone metabolism and probably in Osteosarcoma (OS) pathogenesis. Hormone receptor (HR) expression in long bones has been related with gender, differentiation grade and histologic subtype, and could have a possible direct relation with pathogenesis and evolution. However, there are not studies that support this hypothesis in craniofacial bones. Because ossification type and embriological origin are dissimilar in long and craniofacial bones, it is possible that molecular mechanisms implicated in OS pathogenesis also could be different. The aim of this work was to explore the expression of HR in craniofacial OS.

Design: Twenty-one cases of maxillary OS were retrieved from the files of Surgical Pathology Department of Instituto Nacional de Cancerologia. Clinical data were obtained from medical records (age, gender, site of tumor, clinical outcome). Hematoxylin and Eosin slides were selected and analyzed and immunohistochemistry for Estrogen (ER), Progesterone (PR) and Androgen receptors (AR) by biotin-streptavidin-peroxidase method was performed, using adequate positive and negative controls.

Results: The median age of patients at the time of diagnosis was 35 years (range 16-68), with female predominance (66.7%) in near 2:1 rate. Histologic type was osteoblastic in 52.4% (11/21), fibroblastic in 33.3% (7/21) and chondroblastic in 14.3% (3/21), with predominance of high histologic grade in 85% (18/21) of the cases. AR expression was the most frequent, with 8/21 cases (38%) exhibing weak cytoplasmic staining pattern, followed by nuclear and cytoplasmic ER expression in 6 / 21 cases (28.5%). All tumors tested were PR negative.

Conclusions: This is the first study that analyze HR expression in maxillary OS. ER and AR with both cytoplasmic an nuclear reactivity was identified, instead of strong nuclear reactvity reported in OS of long bones. These differences may be due to distinctive ossification type and osseous metabolism in craniofacial bones, suggesting the existence of a special mechanism in maxillary OS.

1020 Correlation of Sinus Mucosa Basement Membrane Thickness with Eosinophil Infiltrate in Chronic Rhinosinusitis Patients with and without a History of Bronchial Asthma

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Background: Chronic rhinosinusitis (CRS) is one of the most commonly diagnosed chronic illnesses and is frequently associated with bronchial asthma. Furthermore, studies have demonstrated that the presence of asthma can have a negative impact on the outcome of sinus surgery. It is therefore likely that patients with both CRS and asthma will have sinus mucosa that shows some histopathologic characteristics differing from those seen in CRS patients without asthma. In our study, we examined the relationship between sinus mucosa basement membrane thickness and eosinophil infiltrate in CRS patients with and without asthma.

Design: A retrospective study was performed on 61 CRS cases accessioned from June 2003 to June 2006. Nineteen of these cases had an associated history of bronchial asthma, 42 did not. Two independent evaluators blinded to clinical history gave numerical scores for the observed degrees of basement membrane thickness (0 to 3) and eosinophil infiltrate (0 to 4) on representative hematoxylin and eosin-stained slides from each case. Scores that did not agree were subject to consensus scoring by the same evaluators.

Results: In the CRS cases with a history of asthma, the mean basement membrane thickness score was 2.26 (SD = 0.81), while the mean eosinophil infiltrate score was 3.00 (SD = 1.41). Basement membrane thickness had a strong positive correlation with eosinophil infiltrate (r = 0.78) that was statistically significant ($p < 0.01$). In the CRS cases without a history of asthma, the mean basement membrane thickness score was 1.74 (SD = 1.01), while the mean eosinophil infiltrate score was 2.00 (SD = 1.48). Basement membrane thickness had a weak negative correlation with eosinophil infiltrate (r = -0.10) that was not statistically significant ($p > 0.10$).

Conclusions: In CRS patients with a history of bronchial asthma, sinus mucosa basement membrane thickness had a positive correlation with the number of eosinophils present. In CRS patients without a history of asthma, a relationship between basement membrane thickness and the number of eosinophils present was not shown. A cause and effect relationship between sinus mucosa basement membrane thickness and eosinophil count in CRS patients with asthma is suggested by our findings, however, further studies are recommended.

1021 XIAP Expression in Pleomorphic Adenoma and Carcinoma Ex Pleomorphic Adenoma

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Background: X-linked inhibitor of apoptosis protein (XIAP) is a member of the inhibitor of apoptosis proteins family of caspase inhibitors. Expression of XIAP in various neoplasms has been associated with aggressive behavior. The biological progression from pleomorphic adenoma (PA) to carcinoma ex pleomorphic adenoma (CXPA) has been poorly understood. We studied XIAP expression by immunohistochemistry in PA and CXPA.

Design: Formalin fixed, paraffin embedded representative sections of 14 cases of PA and 7 cases of CXPA (4 invasive and 3 intracapsular) were stained with anti-XIAP (# 610763, BD Biosciences, San Jose, USA) following citrate based antigen retrieval. Granular cytoplasmic staining was considered positive and intensity was assessed from weak (1+) to strong (3+). PAs were morphologically evaluated for cellularity, cytological atypia and mitotic activity.

Results: Of 7 PAs composed mostly of myxohyaline stroma with scattered ductal elements, 2 tumors showed no staining and 5 showed rare (<1%) 1+ positive cells. Of 7 more cellular PAs, of which 5 had sheets of tumor cells comprising more than 50% of the tumor and 2 had sheets comprising more than 80% of the tumor (cellular PA), focal to diffuse 2+ to 3+ staining was observed. Tumor cells with strong staining often exhibited cytological atypia in the form of nuclear enlargement and contour irregularity, prominent nucleoli and eosinophilic cytoplasm. Mitotic activity was occasional seen in cellular areas expressing XIAP. All cases of CXPA demonstrated diffuse 3+ staining in the carcinomatous component and 1+ to focally 3+ staining in cellular areas of the underlying PA.

Conclusions: XIAP is highly expressed in the carcinomatous component of CXPA and in atypical cells within cellular areas of PA. Such atypical cells may represent foci of incipient carcinoma. Expression of XIAP possibly plays a role in the pathogenesis of CXPA.

1022 p63 Expression in Basal Cell Carcinomas and Squamous Cell Carcinomas: A Potential Pitfall in Salivary Gland Neoplasms

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Background: p63 is a recently described homologue of the p53 tumor suppressor gene. p63 is expressed in epithelial basal/stem cells of skin, breast, respiratory mucosa, prostate and uterine cervix and is down-regulated in terminally differentiated cells of these tissues. Studies have shown that p63 is expressed in salivary gland tumors, especially those with myoepithelial differentiation and is subsequently used to aid in the diagnosis of neoplasms in the salivary gland. However, ten percent of malignant salivary gland neoplasms reviewed by the AFIP between 1985 and 1995 were actually metastases. The distinction of primary salivary gland versus metastasis / direct extension from a non-salivary gland site can be at times very difficult and has significant therapeutic and prognostic implications. Our aim in this study is to document expression of p63 in basal cell carcinomas (BCCs) and squamous cell carcinomas (SCCs) from various sites and caution against its sole utility in diagnosing difficult cases of tumors in the salivary gland.

Design: Tissue microarrays were constructed from 47 archival formalin-fixed, paraffin-embedded BCC and SCC cases from the following anatomic locations: skin, oral cavity, larynx, lung, salivary gland, cervix, vulva, penis and rectum. Tissue microarray sections were immunostained with p63 and semiquantitative evaluation (-, 1+, 2+, 3+) and location of staining (full-thickness, lower 2/3, lower 1/3, or rare random cells) evaluation was conducted.

Results: Fourteen of 18 (78%) of BCCs exhibited full thickness staining, while 11 of 29 (38%) SCCs exhibited full thickness staining and 15 of 29 (52%) SCCs showed lower 2/3 or 1/3 staining. There was no difference in staining location based on anatomic site. Also, all cases that had rare random positive cells or completely negative staining (1 BCC and 4 SCCs) were of poorly differentiated tumors. Twenty-two of 29 (76%) of SCCs showed 3+ staining intensity and 14 of 18 (78%) of BCCs also demonstrated 3+ staining intensity with no difference in staining intensity by anatomic site.

Conclusions: Our results suggest that many non-salivary gland tumors with squamous and basaloid differentiation show prominent expression of p63. Also, the differential expression of p63 should not be used to help make the distinction between a salivary gland primary and metastasis or direct extension to the salivary gland. We advise caution in utilizing p63 as the sole indicator to diagnose difficult cases of primary salivary gland neoplasms.

1023 Clinical Testing for Low and High Risk HPV by ISH in Respiratory Papillomatosis

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Background: Respiratory papillomatosis (RP) is thought to often be related to HPV infection. Studies to date have predominantly demonstrated HPV by using polymerase chain reaction (PCR). Today, however, with the available option of Cidofovir treatment and the potential for the HPV vaccine, it may be important to document the presence of HPV with a clinically validated and practical assay. This study describes the use of HPV in situ hybridization (ISH) for identifying low and high risk HPV in respiratory papillomatosis.

Design: Seven clinically well documented cases of long-standing and recurrent respiratory papillomatosis in adults were included in this study. Formalin fixed paraffin embedded tissue samples were utilized from clinical biopsy material. ISH for HPV low and high risk probe sets (Ventana Medical Systems; Tucson) was performed. The slides were examined and categorized as negative or positive. Negative and positive xenograft controls were run with the assay.

Results: All cases demonstrated histologic features of squamous papilloma, typical of respiratory papillomatosis. There was no significant atypia or dysplasia identified in any case. The HPV in situ hybridization assay was successful for all cases studied. All 7 cases of respiratory papillomatosis were found to be positive for the HPV. 6 were positive for low risk HPV and 1 was positive for high risk HPV.

Conclusions: The need for a cost effective, simple, and reliable assay to document HPV in cells and tissues is becoming important in head and neck pathology. This study describes using the new technique of ISH for HPV low and high risk probe sets for the diagnostic workup of respiratory papillomatosis. The assay detected HPV in 100% of the cases studied. ISH HPV assay is considered to be a practical and clinically validated tool to apply to these unusual lesions.

1024 Clear Cell Carcinoma of Salivary and Odontogenic Origin Share Histologic and Immunophenotypic Characteristics

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Background: Clear cell carcinoma (CCC) of the salivary gland and clear cell odontogenic carcinoma (CCOC) are both rare, low-grade malignant neoplasms. Because of their overlapping morphology, these tumors can be diagnostically challenging. This study compares the histologic and immunohistochemical features of CCC and CCOC.

Design: Histologic parameters were delineated in 13 CCC (11 patients) and 7 CCOC (4 patients). Diagnoses were assigned based on the location of the tumor epicenter (extra-osseous = CCC; intra-osseous = CCOC). Immunostains for cytokeratin 5/6 (CK5/6), p63, smooth muscle actin (SMA), and calponin were performed using standard immunohistochemical technique (n=14).

Results: The mean patient age for CCC was 62 years (range 47 to 79 years) while the mean patient age for CCOC was 47 years (range 19 to 76). The CCC group had 3 males and 6 females and the CCOC group had 2 males and 2 females. Sites of involvement for CCC were: palate-3, base of tongue-2, lip-1, anterior floor of mouth-1, mandibular gingiva-1, tonsil-1, nasopharynx-1, and parotid-1. Site distribution for CCOC was: mandible-3 and maxilla-1. All cases were predominantly composed of polygonal cells with cytoplasmic clearing arranged in various combinations of solid, nested, and infiltrative patterns of growth.

Table 1. Histologic features

Feature	CCC	CCOC
Stromal hyalinization	7/12 (58%)	3/7 (43%)
Glandular lumina	5/12 (42%)	3/7 (43%)
Cysts	2/12 (17%)	2/7 (29%)
Squamous metaplasia	2/12 (17%)	2/7 (29%)
Surface/periductal involvement	7/12 (58%)	4/7 (57%)
Perineural invasion	7/11 (64%)	2/6 (33%)
Angiolymphatic invasion	1/11 (9%)	1/6 (17%)

Table 2. Immunohistochemistry results

Stain	CCC	CCOC
Cytokeratin 5/6	6/6 (100%)	2/2 (100%)
p63	7/7 (100%)	2/2 (100%)
Smooth muscle actin	0/10 (0%)	0/3 (0%)
Calponin	0/7 (0%)	0/2 (0%)

Conclusions: Reliable distinction between CCC and CCOC cannot be made on the basis of histologic features or immunohistochemistry. In addition, although both tumors are positive for p63 and CK5/6, their lack of SMA and calponin reactivity distinguishes them from salivary gland neoplasms with myoepithelial differentiation. The CK5/6 and p63 positivity in CCC also may provide some evidence against ductal epithelial differentiation.

1025 Lower Prevalence and Favorable Survival in Human Papillomavirus-Positive Squamous Cell Carcinoma of Tonsil in Taiwan

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Background: Human papillomavirus (HPV) has been known to play a role in the tumorigenesis of head and neck cancers especially the tonsils. High HPV positivity (more than 50%) has been reported in squamous cell carcinoma of tonsil (SCCT) among the Western population. However, the only study from Chinese patients in English literature with limited case number demonstrated absence of HPV in SCCT.

Design: To evaluate the prevalence and clinical significance of HPV positivity of SCCT in Taiwan, 111 patients who underwent either surgical resection with postoperative adjuvant radiotherapy and/or chemotherapy or primary radiotherapy for SCCT between 1992 and 2005 were retrospectively studied. Detection of HPV DNA was performed using the L1 consensus primer set MY09/11 after confirming the presence of β-globin by PCR on the DNA sample extracted from the paraffin-embedded tissue of the tumors. The positive cases were further studied using *in situ* hybridization for the detection of HPV in the tissue of tonsil cancer.

Results: The prevalence of HPV in this series was only 12.6% and the greater risk of HPV was associated with females (OR = 18.48, 95% confidence interval = 5.55 - 61.51), non-smokers (OR = 2.52, 95% confidence interval = 1.41 - 4.52) and none-betel quid chewing group (OR = 2.05, 95% confidence interval = 1.18 - 3.58). The actuarial 5-year disease-specific survival rate correlated significantly with HPV-positive tumor (p = 0.007), the group of female (p = 0.046), and early tumor (T) stage (p < 0.001), but the Cox's regression analysis revealed that only HPV-positive tumor (p = 0.04) and early T stage (p = 0.004) were the independent prognostic factors for survival.

Conclusions: There is a much lower prevalence of HPV positivity for SCCT in Taiwan comparing with the Western population, possibly caused by different life styles. The prognosis of HPV-positive SCCT is better than that of HPV-negative SCCT, suggestive of the difference in pathogenesis.

1026 Expression of p16 and Status of Human Papillomavirus Infection in Carcinoma Associated with Sinonasal Inverted Papillomas

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Background: Carcinoma associated with sinonasal inverted papilloma is uncommon. Human papillomavirus (HPV) infection has been implicated in the pathogenesis of inverted papilloma. HPV infection is associated with overexpression of p16 protein in cervical and head and neck squamous cell carcinoma. To the best of our knowledge, the expression of p16 protein in carcinoma associated with inverted papilloma has not yet been investigated.

Design: Eleven cases of carcinoma associated with sinonasal inverted papilloma were retrieved from the surgical pathology files. An immunohistochemical staining for p16 protein was performed. Tumor DNA was extracted from paraffin blocks and was evaluated for the presence of HPV DNA by a polymerase chain reaction(PCR)-bases-genechip method.

Results: Two patients had moderately differentiated squamous cell carcinoma 162 and 148 months after excision of inverted papilloma with recurrences in the interim. Three cases presented inverted papilloma with focal invasive squamous cell carcinoma and/or carcinoma in situ. Six cases had carcinoma with a pattern similar to that of inverted papilloma. All of the eleven carcinoma specimens showed diffuse intense, full thickness nuclear staining. The adjacent nonneoplastic epithelium revealed basal nuclear staining. HPV DNA was detected in four (36%) of eleven cases. HPV types were 16, 33, 58; 18, 58; 18, 69; and 70 separately.

Conclusions: Overexpression of p16 staining is associated with high-risk HPV infection in carcinoma associated with sinonasal inverted papilloma. It may serve as a surrogate marker for diagnostic aid to identify squamous cell carcinoma associated with inverted papilloma.

1027 Carcinoma Ex Pleomorphic Adenoma: Diagnostic Ancillary Biomarkers in Carcinomatous Transformation

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Background: Carcinoma ex pleomorphic adenoma (CXPA) is the 3rd common malignancy of the salivary gland, developing in either a long-standing primary or a recurrent pleomorphic adenoma (PA). Little about pathogenesis of CXPA and specific indicators for the malignant transformation of PA has been known.

Design: To investigate the modifications associated with acquisition of malignant phenotype, we examined immunohistochemical markers of smooth muscle actin (SMA), p53, p63, Bcl-2, EGFR, VEGF, c-erbB-2, c-kit and Glut-1 in 23 CXPAs and compared expressions of residual PA and carcinomatous elements. In addition, histologic features such as invasiveness, grade and subtype were analyzed.

Results: CXPAs were classified into two groups according to their predominant cellular component: carcinomas with luminal differentiation (69.6%, adenocarcinoma, not otherwise specified 10, mucoepidermoid carcinoma 4, salivary duct carcinoma 2; high grade 9, low grade 7; invasive 4, minimally/non-invasive 12), and carcinomas with myoepithelial differentiation detected by positive reaction for p63 or SMA (30.4%; myoepithelial carcinoma 5, epithelial-myoepithelial carcinoma 2; low grade 7; invasive 3, minimally/non-invasive 4). In CXPA with luminal differentiation, immunohistochemical reactions for p53, c-erbB-2, VEGF and Glut-1 were more common in malignant epithelial cells than in benign components (p=0.012, 0.002, 0.001 and 0.032, respectively) and c-erbB-2 expression was associated with high histologic grade (p=0.041). In CXPA with non-luminal differentiation, only p53 expression was more common in carcinomatous elements (p=0.021). Four each cases showed expression of EGFR and c-kit. Interestingly, c-kit expression was frequent in luminal cells of most residual PA.

Conclusions: The different immunoprofile between carcinomas with luminal and non-luminal differentiations suggests that mechanisms participating in carcinogenesis of CXPA are variable. However, overexpression of p53 protein in both groups implies that p53 mutation is a critical event in malignant transformation. In some CXPAs, selective acquisition of growth factor/receptor such as VEGF, EGFR, c-kit and c-erbB-2 appears to be an additional pathogenetic/progressive mechanism and can be utilized for therapeutic purposes in a minor subset. Expression of Glut-1 in some cases offers a basis for an application of PET to differential diagnosis between CXPA and PA.

1028 Comparative Study of the Expression of Ki67, p53, MMP-1 and E-Cadherin in Verrucous Hyperplasia and Verrucous Carcinoma of the Oral Cavity

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Background: Verrucous carcinoma (VC), a variant of squamous cell carcinoma (SCC), is characterized by locally invasive growth and rare metastases. A significant problem in the diagnosis of VC is a lack of reproducible criteria to distinguish it from verrucous hyperplasia (VH), which can resemble VC clinically and histologically. This problem is compounded with poorly oriented tissue sections and biopsies failing to demonstrate lesional margins. Immunohistochemistry has been shown to be helpful in the distinction between pseudoepitheliomatous hyperplasia and SCC in the head and neck. This study explores the role of immunohistochemistry in the distinction between VC and VH in the oral cavity.

Design: Twenty-eight cases of VH and thirty-two cases of VC from the oral cavity were studied. Diagnoses were confirmed by 2 pathologists. Formalin-fixed, paraffin-embedded archival material was used for immunohistochemistry (avidin-biotin immunoperoxidase technique). For Ki67, p53 and E-Cadherin the fraction of the epithelium displaying staining was recorded. For MMP-1 staining of stromal cells was graded as present or absent.

Results:

Table 1

	Ki67 VH %	Ki67 VC %	p53 VH %	p53 VC %	E-Cadherin VH %	E-Cadherin VC %
Absent	3.6	0	32.1	9.4	0	0
Basal layer	53.6	6.3	64.3	34.4	0	0
Basal and Suprabasal	42.9	78.1	3.6	53.1	0	0
Diffuse	0	15.6	0	3.1	100	100

% - percent of cases displaying staining pattern

Ki67 and p53 demonstrated highly significant differences between VH and VC groups ($p < 0.001$). There was a marginally significant difference in staining of adjacent stroma cells with MMP-1 ($P < .05$), with expression found in 35.7% of VH cases and 68.8% of VC cases.

Conclusions: Ki67, p53, and matrix metalloproteinase-1 demonstrated significant staining trends. Although a properly oriented hematoxylin-eosin-stained section including normal marginal tissue is considered to be the gold standard for differentiation of VH and VC, this immunohistochemistry panel may serve as a useful diagnostic adjunct in difficult cases.

1029 Silver-Stained Nucleoli Diameter Measured by Computer Morphometry as a New Prognostic Factor in Sinonasal and Oral Malignant Melanomas

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Background: Sinonasal and oral malignant melanomas (SOMM) represent a group of rare, highly aggressive malignant tumors with poor 5-year survival rates. It has been difficult to establish clear prognostic factors due to late presentation of the disease, limited number of cases and poor clinical outcomes. In our previous study we demonstrated that matrix metalloproteinases MMP2, MMP14, and MMP9 could be used as a prognostic markers in patients with SOMM. The mean longest nucleoli diameter measured on silver-stained sections in uveal melanoma cases was determined as prognostic factor for survival, but no such studies have been performed on SOMM. We investigated the correlation between nucleoli diameter, MMPs-expression, and clinical outcome of patients with SOMM.

Design: Sixteen cases of SOMM, including 11 sinonasal and 5 oral, were studied. The mean and maximal AgNOR-stained nucleoli diameters were assessed using computer morphometry techniques. Up to five representative microscopic fields (x 400) including at least 50 nucleoli per case were chosen for evaluation. Median patient age was 66 years. Clinical follow-up was available for all cases with a mean follow-up period of 50 months. Nine of 16 patients developed metastases, 11 died of disease, 5 were alive by the end of the study; 15 patients received surgery, 9 radiotherapy, 6 chemotherapy and 1 immunotherapy.

Results: The maximal nucleolar diameter (MaxND) was significantly correlated with patient survival. In the group with MaxND equal or larger than 8 µm, 5 out of 5 patients died by the end of follow-up (median survival time was 13 months). By comparison, in the group with MaxND less then 8 µm, 6 out of 11 patients died by the end of follow-up (median survival time was 61 months) ($p=0.0009$. MMP2 and MMP13 showed negative correlation with MaxND ($R=-0.54$, $p=0.032$ and $R=-0.49$, $p=0.049$, respectively). No association was found between nucleoli diameter and MMP9 and MMP14 expression.

Conclusions: Our study demonstrates for the first time that MaxND along with previously reported MMP2, MMP14 and MMP9 expression level could be used as prognostic markers in patients with SOMM and the findings could in the future, aid in the development of new treatment modalities.

1030 A Report on Seven Cases of Hybrid Central Odontogenic Fibroma/Giant Cell Lesions (hCOF/CGCL) of the Jaws and Immunohistochemical Evaluation for PTHrP and RANK

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Background: Central odontogenic fibroma (COF) is characterized by poorly to cellular fibroblastic proliferation and a variable odontogenic epithelial (OE) component. Central giant cell lesions (CGCL) are osteolytic myofibroblastic proliferations characterized by osteoclast-like multinucleated giant cells (MGC). Rare examples of hCOF/CGCL have been described. Two pathogenetic theories exist based on clinicopathologic characteristics. One regards the CGCL component as reactive to the COF while the other regards the CGCL as inducive of a COF-like proliferation. The possibility of colliding tumors seems unlikely. We present seven additional cases, one in a patient with cherubism, and the immunohistochemical (IMH) findings for PTHrP (parathyroid hormone-related protein) and RANK (TRANCE receptor).

Design: Seven patients (six males, one female) with hCOF/CGCL , 15 to 73 years old (mean: 37.14) were retrieved. All lesions occured in the mandible. FFPE sections were stained for PTHrP and RANK. The staining patterns in hCOF/CGCL were qualitatively evaluated and compared with 2 cases each of COF and CGCL.

Results: The COF component predominated in 3 cases, the CGCL component in 3 while in one the ratio was 50:50. Zones of collagen fibers featuring a whorling pattern and containing multiple nests of OE were present. In four cases there were hyalinized deposits in OE. Some foci of MGC contained few OE that were revealed by CK19 IMH. Cytoplasmic PTHrP and RANK staining was observed in both OE and MGC. Four cases showed equal staining intensity for PTHrP while in 3 MGC were more prominently stained. COF and CGCL featured staining of OE and MGC, respectively. In four lesions and in some areas OE failed to stain for RANK, a pattern seen in one of two COF. MGC in CGCL stained consistently.

Conclusions: In hCOF/CGCL, OE and MGC stain for PTHrP and RANK although staining of OE may vary. One can hypothesize that epithelial cells can induce osteoclastogenesis and formation of CGCL. In COF where OE featured cytoplasmic PTHrP and RANK without presene of MGC another protein such as osteoprotegerin may hinder the presence of MGC. The possibility of CGCl inducing a COF-like lesion cannot be totally ruled out. This is the also the first report of hCOF/CGCL in cherubism.

1031 DNA Methylation: A Comparative Study of Methodologies in Salivary Gland Neoplasia

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Background: Hypermethylation of CpG islands in tumor suppressor genes is an epigenetic change recognized to lead to reduction or silencing of gene transcription. Identification of methylated CpG islands may be performed by methylation specific polymerase chain reaction (MSPCR), a semiquantitative method, or by the newer quantitative method, pyrosequencing. Pyrosequencing uses a DNA sequencer and additional primers designed to detect methylated CpG regions following the initial bisulfite modification and DNA amplification. Although both methods detect methylation status, comparative studies to assess the sensitivity and degree of gene methylation have not been discerned.

Design: Tumor suppressor genes, *RARb, RASSF1,* and *MGMT* were analyzed for methylation status in 64 malignant salivary gland tumors by MSPCR (data previously reported) and reanalyzed for methylation status by the newer quantitative pyrosequencing method. Additionally, the methylation status of four salivary gland tumor cell lines (A253, HSG, HSY, and RET) were also evaluated.

Results: MSPCR showed methylation in 25 malignant salivary gland tumors, and pyrosequencing detected methylation in 28 tumors. MSPCR and pyrosequencing demonstrated a similar number of methylated tumors at the *RASSF1* gene. No tumors were methylated at the *MGMT* gene by pyrosequencing versus 4 tumors detected by MSPCR. Methylation at the *RARb* gene was present in 26 tumors by pyrosequencing versus 10 tumors by MSPCR. The additional tumors with *RARb* methylation by pyrosequencing were identified in all four tumor types studied: acinic cell carcinoma, adenoid cystic carcinoma, mucoepidermoid carcinoma and salivary duct carcinoma. The *RARb* gene methylation identified by pyrosequencing most often showed low levels of methylation (15-20%) when not detected by MSPCR. All four salivary gland tumor cell lines were highly methylated at the *RARb* gene. A253 was also methylated at the *MGMT* gene and RET methylated at the *RASSF1* gene.

Conclusions: 1) Gene methylation may be detected by both MSPCR and pyrosequencing techniques. 2) The methylation status in tumors most frequently correlated between MSPCR and pyrosequencing when high levels of gene methylation were present. 2) MSPCR remains a good screening tool for detection of highly methylated genes. 3) Pyrosequencing is a more sensitive technique for lower levels of methylation in tumor suppressor genes which may play a role in salivary gland pathogenesis.

1032 Overexpression of P16 ^{INK4a} and Positivity of Human Papillomavirus In Situ Hybridization Predict Favorable Outcome in Tonsillar Squamous Cell Carcinoma

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Background: To understand the biology of human papillomavirus (HPV) and find out clinically accessible methods to determine its prognostic significance in primary tonsillar squamous cell carcinoma (TSQC).

Design: This study included 75 patients with primary TSQC diagnosed or treated in National Taiwan University Hospital for whom archival tumor tissue were available. Immunohistochemical stains of P16 ^{INK4A}, HPV in situ hybridization, and nested polymerase chain reaction (PCR)-based genechips were performed to detect HPV infection and determined the serotype. Patient's clinical data were compared with their HPV infection profile confirmed by above mentioned methods. Real-time PCR was also performed in the HPV16 positive lesions to demonstrate viral integration status.

Results: The HPV positive rates of PCR-based genechips, the overexpression of P16 ^{INK4A}, and the positivity of HPV in situ hybridization were 73%(55/75), 53%(40/75), and 44%(33/75), respectively. Both the overexpression of P16 ^{INK4A} and HPV in situ hybridization positivity were associated with favorable prognoses (P=0.015 and 0.001, respectively). Presence of HPV infection is also an independent prognostic factor regardless of the results of P16 ^{INK4A} immunostain or HPV in situ hybridization according to multivariate analyses (P=0.016 and 0.001, respectively). The positivity of PCR-based genechips was not statistically significant.

Conclusions: Primary TSQC with HPV infection is associated with a better outcome, and immunohistochemical stains of P16 ^{INK4A} and HPV in situ hybridization may serve as clinically accessible markers.

1033 The Distinct Expression of Molecular Markers in Hashimoto's Thyroiditis and Papillary Carcinoma of Thyroid

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Background: Focal atypical nuclei and nuclear alterations resembling papillary carcinoma are found in thyrocytes of Hashimoto's thyroiditis(HT) with or without papillary carcinoma(PC). However, the association between PC and Hashimoto's thyroiditis is not clear whether it is incidental or genetically related.

Design: We evaluated the expression of galactin-3(GAL-3), MUC-1, cytokeratin 19(CK19) and fibronectin(FN) in 18 PC with HT, 18 PC, 10 HT, and 15 nodular hyperplasia(NH).

Results: Diffuse and strong cytoplasmic and nuclear expression of GAL-3, CK19 Muc-1 and FN were seen in neoplastic cells of all cases of PC with HT and PC. with one negative Muc-1 stain. In the 28 cases revealing the thyrocytes showing PC-like nuclear alterations(PCN), positive expression were found in 25, 22, 28, and 23 cases for GAL-3, MUC-1, CK19, and FN, respectively. Other thyrocytes without PCN were negative for these stains. Interestingly, thyrocytes with PCN of all cases showed more diffuse and strong positive stainining for CK19 than others. There were weak and focal positive findings in 10, 14, 0, and 1 cases for GAL-3, MUC-1, CK19 and FN in 15 NH and 18 normal thyroid tissue, respectively.

Conclusions: The thyrocytes with PCN show immunophenotypic changes quite similar to PC. Therefore, It suggests the possible existence of early neoplastic transformation of thyrocytes in Hashimoto's thyroiditis toward PC. Particularly, CK19 is more predictive molecular factor showing malignant change than others.

1034 Low Grade Sinonasal Sarcoma with Neural and Myogenic Features: A Distinctive Subset of Primary Sinonasal Sarcomas

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Background: A wide spectrum of varied neoplasms occurs in the sinonasal region, and several appear unique to this location. We have recently encountered examples of a distinctive spindle cell sarcoma of the sinonasal tract showing histologic features reminiscent of adult-type fibrosarcoma or synovial sarcoma, but with neural and myogenic features by immunohistochemistry.

Design: Fifteen cases were retrieved from the consultation files of the authors (9) and retrospective review of institutional files (6). Tumors were studied for the expression of the following markers by immunohistochemistry: S-100, SMA, MSA, desmin, myogenin, AE1/AE3, CD34, and ER/PR. Reverse transcriptase polymerase chain reaction (RT-PCR) for the synovial sarcoma fusion genes *SYT-SSX1* and *SYT-SSX2* was done in 7 cases. Inclusion criteria were the characteristic histology of a highly cellular spindle cell neoplasm with uniform elongated nuclei and an infiltrative growth pattern, and at least focal expression of S-100 protein.

Results: There were 10 females and 5 males with an age range of 24 to 74 years (mean, 51). None had a history of neurofibromatosis. Tumors most frequently involved the ethmoid sinus (11 cases) and/or nasal cavity (8). Histologic features noted in addition to those above included lack of nuclear pleomorphism, indistinct cytoplasm, prominent vascular pattern, associated benign respiratory epithelial proliferation, bone infiltration, and low mitotic rate. S-100 expression was typically spotty or patchy. Tumors were also positive for SMA (12/13), MSA (9/10), and CD34 (4/13). Expression of either SMA or MSA was detected in all but one case studied (12/13). Focal weak EMA expression was present in 3 (of 14), and a single case (of 14) was focally positive for AE1/AE3. All cases were negative for desmin (10), myogenin (8), ER/PR (6), and the 2 most common synovial sarcoma fusion transcripts (7). Limited follow-up was available (8 cases). Multiple local recurrences were noted in 5, but no patient was known to have metastasis or disease-related death.

Conclusions: We believe this constellation of findings indicates a unique biphenotypic sinonasal neoplasm that differs from typical adult-type fibrosarcoma, synovial sarcoma, MPNST, leiomyosarcoma, or cellular schwannoma elsewhere in the body. The pathologic features and clinical follow-up are most consistent with a low grade sarcoma. Identification of additional cases will help better elucidate the line of differentiation of this tumor, and further define its natural history.

1035 Characterization of Renal Cell Carcinoma Metastatic to the Thyroid

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Background: Renal cell carcinoma (RCC) metastasizing to the thyroid can histologically mimic a primary thyroid neoplasm or metastatic clear cell carcinoma from other sites. Morphology alone is not always sufficient to establish a definitive diagnosis. Diagnostic immunohistochemistry in this setting can be extremely useful, yet has not been well studied. We studied the clinicopathologic findings and immunohistochemical profile of RCC metastatic to the thyroid gland.

Design: Sixteen cases of metastatic RCC to the thyroid were studied. Immunohistochemical staining was performed with antibodies for RCC markers [CD10, EMA and S-transferase S-alpha (GST-alpha)], and thyroid marker TTF-1 thyroid marker.

Results: The patients' median age was 63 years (range 54 to 90). There were 7 females and 9 males. All 16 renal cancers were of clear cell type. A history of RCC was documented in 14 of 16 patients prior to thyroidectomy, while in 2 patients the diagnosis of RCC was made after evaluation of the thyroid. The Fuhrman nuclear grade in 13 available primary RCC was of grade 2 in 7 cases, grade 3 in 1 and grade 4 in 5. 12 patients presented with multiple thyroid nodules and 4 with a solitary mass/nodule. Metastatic RCC was suspected preoperatively in 8 (50%), while it was an unexpected finding in the other 8 cases, in which thyroid surgery was performed for thyroid or parathyroid lesions. Thyroid FNA was performed in 7 patients, with cytologic findings suspicious for RCC in 3 cases. CD10 was positive in 12 (80%) and EMA was positive in 8 (53.3%) cases, and 15/16 (93.8%) cases showed positive staining for either CD10 or EMA. Adjacent thyroid tissue was negative for both markers. TTF-1 was negative in RCC but positive in the thyroid tissue in all cases. One RCC was negative for all three markers. GST-alpha showed diffuse positive staining in metastatic RCC but the adjacent thyroid tissue also exhibited appreciable positive staining for this marker.

Conclusions: Clear cell type is the predominant type of RCC that metastasizes to the thyroid. A single RCC immunomarker is inadequate for confirming the diagnosis of metastatic RCC. Use of a panel of markers, including CD10, EMA and TTF-1, is recommended to distinguish metastatic RCC from other thyroid or metastatic clear cell lesions. GST-alpha is of little value in this setting.

1036 Moesin Expression Correlates with Pattern of Invasion in Upper Aerodigestive Tract Squamous Cell Carcinoma (UADT-SCC)

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Background: Our previous studies demonstrate that over-expression/mislocalization of ezrin and moesin are associated with poor prognosis. We have also demonstated the predictive value of pattern of invasion (POI) with respect to local recurrence and overall survival. Here, we study the association between POI, ezrin and moesin and outcome data.

Design: Tissue microarray blocks were constructed from samples of 66 patients with primary UADT-SCC. To evaluate POI, we studied whole sections from 11 cases with non-aggressive POI (patterns 1, 2, 3) & 11 cases with aggressive POI (patterns 4, 5). Immunostains for ezrin and moesin were performed, slides were scored for intensity on a scale of 0 to +3.

Results: Moesin staining differed with respect to POI. For tumors with aggressive POI, mean cytoplasmic expression was 1.5 (median 2.0), mean membranous expression was 1.2 (median 2.0), whereas for tumors with nonaggressive POI, mean cytoplasmic expression was 0.8 (median 1.0), mean membranous expression was 0.2 (median 0). On Kaplan Meier (KM) analysis, disease specific survival (DSS) was decreased for any membranous moesin staining ($p = 0.0061$). Moesin expression was not significantly associated with DSS or disease progression (DP) after regression analysis. No correlation was seen with ezrin expression and POI. On KM analysis, DSS and DP were signficantly decreased with +3 ezrin cytoplasmic staining, ($p = 0.0010$, $p = 0.0230$). Time to distant metastasis alone was also decreased for strong cytoplasmic ezrin ($p = 0.057$). On Cox regression analysis, strong cytoplasmic ezrin remained significantly associated with DSS adjusted for age, T stage, N stage, smoking ($p = 0.019$, HR 8.7,CI = 1.5 - 50.2).

Conclusions: Moesin expression directly correlates with POI on whole tissue sections. We see a relationship between DSS and membranous moesin ($p = 0.0061$), however, no association was maintained after regression analysis. We continue to accrue patients and followup this cohort. We confirm that strong cytoplasmic ezrin is significantly associated with DSS when corrected for confounders. Interestingly, significance was lost for cytoplasmic ezrin and LRR with regression analysis. Is ezrin mislocalization and over-expression associated with hematogenous metastases rather than lymph node metastasis or local invasive capacity? The lack of association with ezrin expression and POI supports this view.

1037 High Prevalence of HPV DNA in Squamous Cell Carcinoma and Squamous Papilloma of the Tonsils

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Background: Human papillomavirus (HPV) is recognized as the major etiologic factor in cervical cancer and is also associated with carcinomas of the anogenital region and head and neck. The HPV detection rate in squamous cell carcinoma (SCC) of the tonsils ranges from 25-100% and approximately three-quarters of cases are caused by HPV-16. HPV has also been demonstrated in a subset of squamous papillomas (SP) of the tonsils, most commonly types 6 or 11. In addition, HPV has been identified in 5-10% of normal tonsillar tissue samples.

Design: Formalin-fixed, paraffin-embedded (FFPE) specimens of tonsillar SCC or SP (2000-mid 2006) were retrieved from Fletcher Allen Health Care archives. Related specimens such as lymph nodes with metastatic SCC, or the contralateral, tumor-free tonsil removed at the time of surgery were also collected. DNA extracts from FFPE tissue blocks were tested for HPV using the GP5+/6+ PCR assay and dot-blot hybridization analysis for 37 HPV types.

Results: A total of 85 unique tonsil specimens from 57 patients were assessed for HPV. Among 30 SCCs of the tonsil, 83% (25/30) were HPV-positive, and the most prevalent subtype was HPV-16 (84%), followed by types 52 (8%), 18 (4%), and 11 (4%). The average age of the patients with HPV-positive SCC was 53.9 years, versus 64 years for virus-negative tumors, although this difference was not significant (P=0.1909). SCCs with basaloid features were positive (3/3) for HPV-16. One of three (1/3) tonsil specimens with carcinoma *in situ* and no invasive component was positive for HPV-16, and a high-grade carcinoma with nasopharyngeal features was positive for HPV-18. The frequency of lymph node metastases in tonsillar SCC was 47% (14/30), and 93% (13/14) of the lymph node metastases were HPV-positive(in addition to HPV positivity in the tonsil SCC). Dual HPV infection was present in 3 cases, including types 35/52 and 6/18, and 11/16. The frequency of HPV positivity in SP was 38% (9/24), and the frequency of subtypes was HPV-16 (78%), 6 (11%), and 11 (11%). Among 4 tumor-free tonsil specimens, one case (1/4) was HPV-positive.

Conclusions: In this study, HPV was identified in 83% of squamous cell carcinomas, strengthening the hypothesis that HPV plays a significant role in the etiology of these tumors. Interestingly, HPV may play a lesser role in squamous papillomas, 38% of which tested HPV positive.

1038 p63 Immunohistochemistry Differentiates Salivary Gland Oncocytoma and Oncocytic Carcinoma from Metastatic Renal Cell Carcinoma

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Background: Metastatic renal cell carcinoma (RCC) to head and neck sites can pose diagnostic challenges as they often resemble primary salivary oncocytic lesions since both tumor types may show morphology ranging from clear to oncocytic. Immunohistochemical panels have been reported to help with this differential but are not entirely specific or sensitive. We have noticed anecdotally that p63 routinely stains salivary gland oncocytomas but not metastatic RCC and thus elected to formalize this observation in a study of its utility in this differential.

Design: 26 salivary gland oncocytomas, 9 salivary gland oncocytic carcinomas (CA) and 15 head and neck metastatic RCC with documented renal primaries (7 major salivary gland, 3 nasal, 2 thyroid, 1 maxillary, 1 oral, and 1 paratracheal) were evaluated. Morphologic features included: cytoplasmic tinctorial quality (clear vs. oncocytic), growth pattern, presence of lumina/pseudolumina, blood lakes and stromal vascularity. Tumors were stained with antibodies to p63, renal cell carcinoma marker (RCCm), CD10, and vimentin using standard immunohistochemical staining.
Results: 8 oncocytomas (31%) and one oncocytic CA (11%) had clear cell features while 6 metastatic RCC (40%) had oncocytic features. All oncocytomas had scattered true lumina with secretions while 8 metastatic RCC (53%) had scattered morphologically similar pseudolumina accompanied by blood lakes. 7 oncocytomas (27%) and 5 oncocytic CA (56%) had prominent stromal vascularity, compared to metastatic RCC of which all had prominent stromal vascularity. Within the context of this study, p63 was 100% specific and sensitive for primary salivary oncocytic lesions, staining the periphery of tumor nests in all oncocytomas and oncocytic CA. RCCm was 100% specific for RCC only but only 40% sensitive. CD10 and vimentin showed specificities of: 62% and 77% respectively and sensitivities of: 71% and 71% respectively with regards to identifying metastatic RCC.
Conclusions: Primary oncocytic lesions and metastatic RCC show considerable morphologic overlap though a careful consideration of morphologic parameters along with a clinical history are often sufficient to separate these tumors. However, p63 positivity effectively distinguishes primary salivary oncocytic lesions from metastatic RCC. While RCCm, CD10, and vimentin performed adequately in this respect, they were significantly less reliable than p63.

1039 Sinonasal Rhabdomyosarcoma in Adults: A Report of 12 Cases

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Background: Rhabdomyosarcoma (RMS) is considered the most common head and neck sarcoma in children. Its occurrence in adults is rare.
Design: We report a series of 12 sinonasal RMSs in adults seen at the Hospital of the University of Pennsylvania.
Results: Twelve adult patients with sinonasal RMS were diagnosed between 1988 and 2006. Ages ranged from 18-86 years with a mean of 40 years. All patients were diagnosed by biopsy. Sites of involvement included the nasal cavity and the ethmoid and maxillary sinuses. Three patients showed extension into the orbit. One of these patients also showed extension into the facial skin as well as the parotid gland. Two patients had a history of radiation to the area including a 55 yo male with a history of congenital retinoblastoma as a child and a 26 yo female with a history of radiation for acne at age 17. Histologically, 8 lesions were classified as alveolar subtype, 2 were classified as embryonal and 2 could not be further classified. All cases had positive reactivity for at least one muscle marker (desmin, myogenin, or myoD1) by immunohistochemistry. One tumor had diffuse positivity for AE1/3 cytokeratin. Frozen tissue was available for molecular analysis for PAX3/PAX7-FKHR fusion transcripts in 6 patients. PAX3-FKHR or PAX7-FKHR fusion transcripts were identified in 4 cases confirming a diagnosis of alveolar RMS. The remaining 2 cases were negative supporting a diagnosis of embryonal RMS. Following biopsy, 5 patients had further tumor debulking procedures. Eight patients were treated with chemotherapy and radiation. Treatment was unknown in four patients. Metastases beyond the sinonasal tract were seen in 4 patients (cervical lymph nodes, brain, scalp and lung). Three patients are alive with no evidence of disease (one patient 10 years post diagnosis of alveolar subtype), 2 patients are alive with disease, 3 patients died of disease (all 3 with alveolar subtype) and 4 patients are lost to follow up.
Conclusions: While sinonasal RMS is uncommon in adults, it should be considered in the differential diagnosis of small round cell tumors in this site. RMS can occur at any age, even the elderly. Molecular studies for PAX3/PAX7-FKHR on fresh frozen tissue can aid in the diagnosis of alveolar RMS. While immunohistochemistry for muscle markers can aid in the diagnosis of RMS, diffuse cytokeratin reactivity can occasionally be seen and care must be undertaken not to render a diagnosis of carcinoma. Chemotherapy, radiation therapy, and surgery can benefit some patients with sinus RMS, alveolar subtype.

1040 Clinicopathologic Differences in Oral Cavity and Sinonasal Mucosal Melanomas

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Background: Mucosal melanomas of the head and neck are rare, comprising < 0.7% of all melanomas and carry a poor outcome. We compare the gross and histologic features of oral cavity melanoma (OCM) and sinonasal melanoma (SNM) and correlate these differences with clinical behavior.
Design: A retrospective analysis of 20 cases of SNM and 8 cases of OCM treated at Emory University Hospital between 1986-2006. The diagnosis of melanoma was confirmed in all cases by either immunohistochemistry, Fontana-Masson staining or the presence of melanin. Both the gross growth pattern (polypoid/flat) and the histologic growth pattern were recorded. Tumor thickness was measured with an ocular micrometer from either the most superficial layer of mucosal epithelium, or ulcer base of the mucosa. All cases were reviewed and evaluated for ulceration, necrosis, melanin, giant cells, vascular invasion, perineural invasion and invasion to deep structures (bone, cartilage, muscle). The pathologic findings were correlated with clinical stage and survival for each anatomic site.
Results: Statistically significant differences were noted between the pathologic features of OCM and SNM. All OCM occured in the hard palate or maxillary alveolar mucosa, were pigmented and had a flat growth pattern with an *in situ* or pagetoid component, and an average thickness of 3.9mm vs. SNM, which were polypoid (20/20), with tumor depths >10mm (18/20), and little or no melanin pigment (14/20), or pagetoid component (1/20) (p<.001). Necrosis and surface ulceration were common features of SNM compared to OCM (p<.002). OCM were more likely to exhibit an epithelioid growth pattern (6/8), whereas SNM more often had sarcomatoid-like features (10/20) or neuroendocrine-like features (7/20) (p<.05). These significant pathologic differences did not correlate with disease specific survival: OCM and SNM (median survival, 17 months vs 12 months).
Conclusions: SNM exhibit more aggressive pathologic features compared to OCM. Amelanosis, and sarcomatoid and neuroendocrine-like histologic features are more common in SNM. Despite statistically significant pathologic differences between OCM and SNM, these did not have a significant impact on prognosis. A poor outcome was noted in both groups with only 2 patients, both with Stage I OCM, surviving more than 5 years, but with disease.

1041 Epithelioid Hemangioendothelioma of the Head and Neck: A Clinicopathologic and Immunohistochemical Study

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Background: Epithelioid Hemangioendothelioma (EHE) is a rare vascular tumor commonly reported in soft tissue, bone, liver and lung. Only 16 cases have been reported in the Head and Neck (HN) region.The treatment and behavior of these lesions, remain unclear. We studied five new HN cases to define the diagnostic and clinicopathologic factors that may impact on the differential diagnosis and patient outcome. We also studied the tumors for the expression of Podoplanin, a transmembrane mucoprotein expressed in lymphatic endothelium.
Design: Six cases with the diagnosis of EHE were retrieved from the files of the Pathology Department at MDACC, in the last 15 years, however, one of the cases was excluded from the study since it did not fulfil the diagnostic criteria. The clinical and pathologic features of five cases were examined. We performed immunohistochemical analysis for CD 31, Podoplanin and Pankeratin expression. Also included in this study was a case of angiolymphoid hyperplasia with eosinophilia.
Results: The patients comprised 2 males and 3 females who ranged in age from 4 to 71 years (mean age of 46 years). The patients presented with lesions in the gingiva, submandibular region, nasal cavity and tongue and ranged in size from 0.7 to 2.5 cm. Light microscopic features were similar to EHE at other sites. All tumors had an infiltrative pattern with cords and nests of epithelioid to spindled cells. A consistent feature was the presence of small intracellular lumen formation. Immunohistochemical studies showed uniform and strong cytoplasmic reactivity of the tumor cells for Podoplanin and CD31. Angiolymphoid hyperplasia with eosinophilia showed strong cytoplasmic reactivity for CD31, however, staining for Podoplanin was weak and focal. All of the cases were negative for Pankeratin. One patient presented with lymph node metastasis and three develped local recurrences.Recurrence was not seen after wide local excision in follow-up period ranging from 9 months to 10 years.Unlike other sites, EHE of HN has not been associated with mortality or distant metastases.
Conclusions: Hemangioendothelioma of the HN is a: 1. Low grade malignancy with a tendency for local recurrence and regional regional lymph node metastasis, 2. Complete excision with negative margins is the treatment of choice, 3. The expression of Podoplanin in these tumors may suggest an aberrant up-regulation associated with neoplastic transformation, 4. Podoplanin may not be a useful marker in the differential diagnosis of potentially malignant vascular neoplasms.

1042 Role of Prothymosin alpha in the SOX4 Pro-Survival Pathway in Adenoid Cystic Carcinoma

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Background: Sox 4, a transcriptional regulatory protein, is known to be up-regulated in adenoid cystic carcinoma(ACC).Work in our laboratory has shown that down regulating Sox4 in an ACC cell line causes apoptosis, suggesting it confers a pro-survival phenotype in cancer cells. We have performed RNAi knockdown experiments in a cell line that identifies prothymosin alpha (PTMA) as a target of Sox4 gene regulation. PTMA is known to negatively regulate apoptosome formation and is a likely mediator of the Sox4 pro-survival pathway. The present study is undertaken to study the expression of PTMA vis a vis SOX4 expression in salivary gland adenoid cystic carcinoma(ACC).
Design: A tissue microarray of 45 cases of ACC and normal salivary gland tissue was made from paraffin embedded formalin fixed tissue obtained from the Adenoid Cystic Carcinoma Registry at The University of Virginia. Immunohistochemistry was performed on this TMA for PTMA and SOX4. The slides were manually read for percent and intensity of staining(cytoplasmic and nuclear for PTMA and only nuclear for SOX4).
Results: Both Sox4 and PTMA proteins are significantly up-regulated in ACC relative to normal salivary gland tissue. There is a strong positive correlation between Sox4 and PTMA expression (P=<0.0251). The immunohistochemical results were correlated with mRNA levels by RT-PCR analysis of a subset of frozen ACC and normal salivary gland tissue.
Conclusions: Our results suggest that Sox4 and PTMA play a role in ACC tumorigenesis, and are consistent with a functional relationship between Sox4 and PTMA protein levels.

1043 Prognostic Impact of p53 and p63 Immunoexpression in Oral Squamous Cell Carcinoma

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Background: Abnormalities or inactivation in p53 protein are frequent in human cancer. P63, a homologue of the p53 gene, has been described as a nuclear transcriptional factor with diverse functions in the cell cycle which ranges from apoptosis to cell differentiation. P63 has also been associated with the development of various

epithelial neoplasms. The role of p53 and p63 proteins in the prognosis of oral squamous cell carcinoma (OSCC) is still debatable. This study aims to investigate the immunoexpression of these proteins and its relationship with some clinicopathologic parameters of prognostic significance in OSCC.
Design: The following data were retrieved from 106 medical files: primary site of the lesion, degree of histologic differentiation, recurrences, metastasis, disease-free survival (DFS) and overall survival (OS). For immunohistochemical staining, the paraffin-embedded blocks were cut into 3-µm sections, deparaffinized, and rehydrated. A standard avidin-biotin-peroxidase method was used. After the reactions, the sections were developed for 5 minutes with DAB. Light Mayer's hematoxylin was applied as a counterstain. The slides were then dehydrated in a series of ethanols and mounted with Permount. Only nuclear staining of tumoral cells was considered. Tumors with 10% or more positive nuclear staining in neoplastic cells were considered positive for p53 analysis. A semi-quantitative assessment of p63 expression was performed in tumoral cells and recorded as: 0 – no stained cells; 1 – 1-25% positive cells; 2 – 26-50% positive cells; 3 – 51-75% positive cells, and 4 – more than 75% positive cells. The intensity of immunostaining was also evaluated as absence, weak, moderate, or strong. Statistical analysis was performed using the Graph Pad Prism v.4 software.
Results: OSCCs were more frequently positive for p63 (87.8%) than p53 (52.8%). There was no significant relationship between p53 expression and recurrences or histologic differentiation, however, p53 expression was significant higher in metastatic disease (p=0.002). Positivity for p53 correlated with a low OS (p=0.046). There was no relationship between intensity and quantitative expression of p63 according to histologic differentiation, recurrences or metastasis. The tumors with strong intensity for p63 immunostaining had OS significantly higher (p=0.008). There were no significant differences between DFS and p53 or p63 immunoexpression.
Conclusions: In OSCCS, p53 overexpression and decreased intensity in p63 immunoexpression are associated with metastases and poor outcome.

1044 The Role of Claudin-1 in the Invasion and Metastases of Oral Squamous Cell Carcinomas

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Background: Better management of Oral Squamous Cell Carcinoma (OSCC) requires better prognostic biomarkers of tumor behavior and metastases, because up to 23 % of occult, metastatic disease are missed with routine physical and imaging examinations. Recently in a cDNA microarray study in our lab, we reported Claudin-1 (CLDN1) gene as significantly over-expressed in OSCC patients with lymph node metastases.
Design: To analyze CLDN1's role in the invasion and metastases of OSCC, we used cell lines established from primary OSCCs in three functional assays including the matrigel cell invasion assay (BD Biosciences, Ontario, Canada), the wound-healing migration assay, and the clonogenic assay. We examined the relative expression of CLDN1 in each cell line by measuring the mRNA and protein levels by Quantitative Real Time Polymerase Chain Reaction (Q-RT-PCR) and Western blot techniques, respectively. To prove the specificity of our results we used short interfering RNA molecules (siRNA) designed to specifically knock-down CLDN1 gene expression in all examined cell lines. We then used knocked-down cell lines in matrigel invasion assays. Conversly, we up-regulated CLDN1's expression in one cell line with low levels of CLDN1 by transfecting it with a cloned vector carrying the CLDN1 gene. We then used the up-regulated cells in a matrigel invasion assay. Each of the above experiments was done in triplicates.
Results: Primary OSCC cell line with highest expression of CLDN1 migrated more quickly and with a statistically higher number of cells compared with other cell lines with lower expression of CLDN1 using the matrigel cell invasion assays. Clonogenic assays indicated that cell line with highest expression of CLDN1 showed statistically significant increase in number and size of colonies formed in semi-solid media compared to other cell lines with lower expression of CLDN1. In the scratch wound-healing migration assay, cells with highest expression of CLDN1 migrated more quickly, whereas cell lines with lower expression of CLDN1 migrated more slowly and only partially covered the induced wound within the same time frame. CLDN1's knock-down using siRNA resulted in significantly fewer number of invading cells in all examined cell lines compared with control. Conversly, up-regulating CLDN1 resulted in significantly higher number of invading cells than control.
Conclusions: Our results suggest that over-expression of CLDN1 in OSCC increases the potential for invasion and metastases. This also suggests that CLDN1 may be used as a prognostic biomarker of invasive, aggressive OSCC.

1045 COX-2, MMP-11 and CK19 Expression in Intraductal and Invasive Salivary Duct Carcinoma

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Background: Salivary duct carcinoma (SDC) is a relatively uncommon neoplasm characterized by extensive local invasion and high incidence of metastasis. Histologically it shows ductal differentiation without myoepithelial participation and resembles intraductal and invasive ductal carcinoma of breast. The incidence and possible transformation of intraductal SDC to its invasive form is not well studied. COX-2 has been associated with angiogenesis and tumorigenesis, and MMP-11 is involved in tissue remodelling. Both are overexpressed in invasive ductal carcinoma of breast. There has been conflicting reports regarding the expression of CK19 in intraductal and invasive SDC. The aim of this study is to investigate the incidence of intraductal disease within invasive SDC and to characterize the expression of COX-2, MMP-11 and CK19 in intraductal and invasive SDC.
Design: We studied 17 conventional SDC, 5 SDC arising in carcinoma ex-pleomorphic adenoma (Ca-ExPA), 2 pure low-grade intraductal SDC, 1 pure high-grade intraductal SDC, and 1 intraductal SDC with de-differentiation. All cases were formalin-fixed paraffin embedded. Stains for CK14, SMA, Calponin, CK19, COX-2 and MMP-11 were performed in all cases.
Results: The mean age of the patients was 64 (range 33-83). There were 8 females and 18 males. Most tumors arose in parotid (24) and 2 in submandibular gland. The mean tumor size was 2.8cm (range 0.5-6.5cm). Foci of intraductal carcinoma were identified in 9 of 17 conventional SDC. CK14, SMA and calponin confirmed the presence of myoepithelial cells surrounding these foci. Interestingly 4 of 5 SDC arising in Ca-ExPA also showed tumor cells surrounded by myoepithelial cells. All SDC tumor cells were strongly positive for CK19 and COX-2. MMP-11 was expressed by fibroblasts surrounding tumor cells with a trend for increased expression around invasive SDC.
Conclusions: The presence of an intraductal component in SDC is relatively common (53%) but is not a prominent component despite architectural features suggesting in-situ disease. The presence of myoepithelial cells surrounding intraductal SDC needs to be confirmed by immunohistochemistry and should not be based on morphology alone. Unlike previous reports, CK19 is expressed by intraductal and invasive SDC. High MMP-11 expression in stromal cells may be considered a marker of invasion and metastatic potential. The high expression of COX-2 in SDC suggests that it may play a role in tumorigenesis as is the case in other adenocarcinomas.

1046 Hormonal Regulation of Head and Neck Venous Malformation (VM)

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Background: Vascular malformations consist of venular malformation (Port-Wine Stains), venous malformation (VM), arteriovenous malformation (AVM) and lymphatic malformation (LM). VM is a congenital malformation made up of ectatic veins. VM is almost always present at birth but steadily progressive with advancing age. Most VM occur in the head and neck region, particularly the buccal mucosa and the tongue. Frequently VM shows growth acceleration with changes of hormonal levels, such as at puberty, during pregnancy or use of birth control pills. Without early surgical intervention, this lesion will result in significant cosmetic and functional loss, such as disfiguration due to ulceration, bleeding and infection, visual obstruction, or airway obstruction.The molecular mechanisms underlying the development and progression of this vascular lesion are poorly understood. In this study, we performed immunohistochemical staining for estrogen receptor (ER) and progesterone receptor (PR) on head and neck VM in order to elucidate how the growth of this vascular malformation is regulated by these two hormones.
Design: A total of 9 cases of head and neck VM were collected from the Department of Pathology. Immunohistochemical (IHC) studies were performed with antibodies against ER and PR.
Results: 9 cases of head and neck AVM consist of 7 females and 2 males with an average age of 41.8 year ranging from 26 to 55 years. PR was diffusely expressed in 3 of 9 (33.3%) cases and focally expressed in 2 of 9 (22.2%). The 3 cases with diffuse PR expression were all derived from female patients. Both endothelium and smooth muscle fibers of the malformed vasculature were positive for PR expression. ER expression was uniformly negative in all 9 cases.
Conclusions: up to one third of head and neck VM show diffuse PR expression, indicating that progesteron and/or its receptor may play significant role in the development and/or progression of head and neck VM in a subset of patients. ER has minimal, if any, effect on the progression of head and neck VM.

1047 A Differentiation Based Immunohistochemical Classifier That Is Prognostic for Head and Neck Tumor Patients

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Background: Gene expression profiling of tumors has revealed novel tumor subtypes within cancers derived from the same tissue and similarities between subtypes originating from different tissues. For example, the 'basal' subtype of breast cancer expresses a signature in common with squamous cell carcinoma of the lung and a subtype of head and neck carcinoma (Chung et al., Cancer Cell, 2004). We have endeavored to translate gene expression signatures into IHC assays for distinguishing tumor subtypes.
Design: Tissue arrays were constructed from NSCLC tumor patients seen at the CCIH and the UAB, and head and neck patients seen at Stanford University. A five antibody classifier (TRIM29, CEACAM5, NCSTN, ABCG2, TLE3) trained on the CCIH cohort for distinguishing adeno from squamous carcinoma of the lung, was validated on the independent UAB cohort. Cox proportional hazard terms and classifier cut-offs were pre-specified using the CCIH NSCLC data and this model tested for classification of the head and neck patients.
Results: The classifier trained to separate adenocarcinoma from squamous carcinoma of the lung divided the head and neck patients into two distinct groups with statistically significant outcome differences. The sensitivity of the test for detecting patients at high risk of death due to disease was 83% and the specificity was 57%.
Conclusions: The clinically significant similarities defined by gene expression patterns or IHC staining patterns between carcinomas arising in different tissue origins suggests that there is a differentiation based classification of carcinoma that transcends tissue of origin. This classifier, as well as classifiers trained to directly predict outcome in head and neck tumors, may help select between therapeutic options for treating this notoriously aggressive tumor.

	DOD	Recurrence
'Squamous'	24/48 (50%)	21/43 (49%)
'Adeno'	5/37 (14%)	8/36 (22%)
HR (95% CI)	5.31 (2.02-14) p=0.0007	3.01 (1.33-6.82) p=0.008

1048 EGFR Expression in Intraoral and Major Salivary Gland Acinic Cell Carcinoma

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Background: Although acinic cell carcinomas (ACC) are low-grade lesions, they have a tendency to recur or metastasize even after very long periods of time. The initial management of ACC is surgical resection with possible adjuvant radiation therapy and patients with locoregional recurrence or metastatic disease may be treated with systemic therapy. Targeting erbB1/EGFR and erbB2/HER2 has shown promising results in the treatment of malignancies, including salivary gland malignancies. Since no overexpression of HER2 was found in ACC in a recent study we have assessed the presence of EGFR overexpression in ACC, which has to our knowledge not yet been studied.

Design: The records of the Oral Pathology and Surgical Pathology departments of our institution were searched for tumors diagnosed as acinic cell carcinomas over a period of 20 years (1986-2005). The slides were reviewed, the diagnoses confirmed and clinical and histologic information was extracted from the reports and assessed from the slides. Immunoperoxidase stains for EGFr (H11, Dako), Ki67 (MIB1, Dako) were performed. EGFR overexpression was scored according to the EGFR pharmDx Interpretation Guide and tumors with 2+ or 3+ staining were considered positive for overexpression. The percentage of nuclei staining for Ki67 was visually assessed in two fields at the periphery of the tumor.

Results: 38 cases were identified; with paraffin blocks available on 22 cases. Eleven intraoral (IO) palate (n = 4), buccal region (n = 3), the upper lip (n = 3), lower lip (n= 1), and the retromolar region (n = 1). and 11 major salivary gland (MSG) tumors (all parotid). The mean age was 43.3 (13-65) for IO ACC and 40.5 (23-73) for MSG ACC and the M/F gender distribution was 1:2 in MSG ACC and 1:7 in IO ACC. MSG ACC tended to be larger and a higher proportion of IO ACC than MSG ACC had a cystic component. Immunoperoxidase stains showed a higher proportion of IO tumors showing 2/3+ staining for EGFR compared to MSG tumors (72% vs 36%), while MSG ACC cases had a higher mean Ki67 index.

Conclusions: A relatively higher proportion of IO ACC showed EGFR over-expression compared to MSG ACC. MSG ACC have a higher Ki67 index compared to IO ACC. This information may be useful in designing therapy protocols for inoperable recurrent and metastatic ACC.

1049 Teratocarcinosarcoma of the Nasal Cavity and Paranasal Sinuses: Three Cases Assessed for Chromosome 12p Status

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Background: Sinonasal teratocarcinosarcoma (SNTCS) is a rare malignant neoplasm occurring predominantly in males, with approximately 40 cases reported in the literature. Histologically, these tumors are characterized by the presence of epithelial elements admixed with mesenchymal components. Amplification of 12p is an event usually associated with bona fide germ cell neoplasms, including mediastinal and testicular teratomas. The histogenesis of SNTCS remains uncertain and no genetic studies have been reported to date.

Design: Three cases, two from the archives of Memorial Sloan-Kettering Cancer Center and one from St. Luke's-Roosevelt Hospital Center, were evaluated by fluorescent in situ hybridization (FISH). These involved a 61 year old man with right anterior ethmoid sinus and nasal mass; a 73 year old man with right maxillary sinus, sphenoid sinus, and right nasal mass; and a 65 year old woman with a sinonasal mass. FISH evaluation for chromosome 12p status was performed in paraffinized, formalin-fixed tissue from all cases, employing a commercially available chromosome arm specific painting probe (Metasystems, Inc.).

Results: Microscopic examination revealed multiple admixed epithelial and mesenchymal elements in all three cases. Immunohistochemical studies highlighted neuroectodermal structures (synaptophysin and chromogranin positive); epithelial structures including squamous and glandular elements (cytokeratin and EMA positive); and mesenchymal areas, including frank sarcoma (vimentin positive), all of varying proportions. A malignant germ cell component was not present morphologically in any of the cases, although focal labeling for AFP was identified in one case. This focus was negative for PLAP and B-HCG. Molecular genetic evaluation of all three cases demonstrated only two copies of chromosome 12 in each case.

Conclusions: Although the histogenesis of SNTCS remains uncertain, we have identified an absence of 12p amplification in three cases. Our findings suggest that 12p amplification, if it occurs at all in this setting, would be the exception and that teratocarcinosarcoma is basically a somatic type neoplasm exhibiting divergent differentiation rather than a germ cell neoplasm.

1050 Olfactory Neuroblastoma with Divergent Differentiation: From Ganglioneuroblastoma to Carcinoma

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Background: The olfactory neuroepithelium is a specialized mucosal surface capable of pluripotent differentiation. As such, olfactory neuroblastomas (ONB) may rarely show areas of epithelial or ganglioneuronal differentiation. We examine the phenotypic diversity of ONB in six tumors spanning the extremes of divergent differentiation and propose a subclassification scheme.

Design: Six ONB with divergent differentiation were evaluated. Inclusion criteria were: 1) A submucosal lobular round cell proliferation with rosette formation and prominent vascular stroma 2) Positivity for synaptophysin, chromogranin or neuron specific enolase (NSE) 3) At least focal S100 protein sustentacular staining and 4) histologic evidence of either glandular, squamous, or ganglioneuronal differentiation. In addition to those mentioned above, the following histochemical/immunohistochemical stains were used to confirm divergent differentiation: mucicarmine, cytokeratin, p63, Neuronal nuclear protein (NeuN), PGP 9.5, and CD99. Clinical parameters were obtained when available.

Results: Clinical parameters were unavailable for one case. The mean age was 41.5 (range: 4-83). The male:female ratio was 2:3. Staging was unavailable in two cases. One patient was Kadish Stage A, two patients were Stage B, and one patient was Stage C. Three main morphologic/immunophenotypic patterns of divergent differentiation were seen: 1) Olfactory Ganglioneuroblastoma: A prominent ganglioneuromatous component (20%) with ONB, Hyams grade I-II (n=1). Both components were positive for NeuN, PGP 9.5, and S100 (both tumor cells and sustentacular cells). 2) Mixed Lineage Olfactory Neuroblastoma: A prominent, distinct adenocarcinoma/adenosquamous (10-40%) component with ONB, grade III-IV (n=3). Only epithelial components were keratin and mucicarmine positive. 3) Olfactory Carcinoma: Scattered adenosquamous foci (<5%) blending with ONB, grade III/IV (n=2). All components were diffusely positive for cytokeratin and in one case, p63. Both cases were also CD99 positive.

Conclusions: While ONB with divergent differentiation is rare, pattern based substratification may have biologic significance though validation with additional cases is needed. Pattern 1 is associated with a lower grade ONB component while Patterns 2 and the newly described Pattern 3 are of higher grade.

1051 Increased Tryptase-Positive Mast Cells and Angiogenesis during Lip Carcinogenesis

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Background: Angiogenesis is associated with tumor growth and invasion in a number of malignancies, including squamous cell carcinoma (SCC) of the lip. Tryptase is a mast cell (MC)-derived protease of pro-angiogenic and pro-inflammatory effects which is significantly increased in premalignant lip lesions such as actinic cheilitis (AC) and lip cancer, at both the peritumoral (PT) and intratumoral (IT) stroma. The aim of this study was to determine if MC-derived tryptase is associated with increased angiogenesis during lip carcinogenesis.

Design: Human biopsies of normal lip (n=14), AC (n=39) and lip SCC (n=12) were processed to determine co-expression of tryptase and CD34 (endothelial cell marker) by double immunohistochemistry. Microvascular density (MVD) and tryptase-positive MCs were counted at the subepithelial areas in normal lip and AC, and at PT and IT stroma in lip SCC (20 counting fields/sample, 400x). Results were analyzed for statistical differences with JMP IN 4.0.4.

Results: A significant increase in MC-derived tryptase was found in AC and lip SCC as compared to normal lip (P<004, Kruskal-Wallis and Wilcoxon). However, MVD was only significantly increased in lip SCC as compared to normal lip and AC (P<0.002, Wilcoxon), with higher MVD at the PT stroma than at the IT stroma (P<0.0007). In addition, a significant association was found between MVD and MC-derived tryptase in lip SCC and normal lip (P<0.005, R square=0.29).

Conclusions: The results suggest that MC-derived tryptase could be stimulating angiogenesis at both the tumor invasion front and at the intratumoral stroma in lip SCC. Therefore, MCs may be playing a significant role in tumor progression and invasion during lip carcinogenesis. Supported by CONICYT, Chile, grant FONDECYT 1050581 and grant from the University of Concepcion Research Foundation, DIUC 203.103.014-1.0.

1052 Examination of the Antigen Processing Machinery and Related Proteins in Head and Neck Squamous Cell Carcinoma

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Background: Head and neck squamous cell carcinomas (HNSCCs) comprise over 45,000 cases per year in the US. The disease often recurs, sometimes after serious morbidity secondary to treatment. Some data suggest that escape from the immune system via the loss of HLA class I or related antigens may play a role in tumorigenesis and progression. Such losses may explain the lack of tumor response to T-cell based immunotherapy. We investigated the expression of various HLA class I antigens, including proteins that comprise the antigen processing machinery (APM), in a tissue microarray of HNSCC and compared the results to tumor site, histologic subtype, and patient survival.

Design: A tissue microarray with 34 HNSCCs was stained using monoclonal antibodies to the HLA class I complex (HLA class I heavy chain and beta-2-microglobulin), the related protein MICA and complex assembly proteins, and APM (calnexin, calreticulin, ERp57, tapasin, TAP1, TAP2, LMP2, LMP7, LMP10, Delta, MB1, and Z). The staining score included quantity (0: no staining; 1: 1-25% of neoplastic cells showing immunoreactivity; 2: 26-75%; 3:76-100%) X quality (1: weak staining; 2: strong). Results were compared to survival, histologic subtype, and site.

Results: Decreased expression was common for many of the proteins. No tumors showed complete antigen loss for MICA, calnexin, calreticulin, TAP2, LMP2, LMP7, Delta, MB1, and Z. 6% (15% membranous) showed complete loss for HLA class I heavy chain; 6% (18% membranous) for beta-2-microglobulin; 9% for ERp57; and 3% each for tapasin, TAP1, and LMP10. Decreased staining for tapasin and Delta correlated with worse survival (p<0.05), while decreased staining for tapasin, TAP1, Beta-2-microglobulin and MICA correlated with a basaloid histologic type (p<0.05). Decreased staining for MICA was associated with a non-oral site (p<0.05).

Conclusions: Although decreased expression of HLA class I and related antigens is not uncommon in HNSCCs, complete loss is infrequent. Decreased antigen expression of some APC proteins correlated with a worse prognosis, basaloid histologic type, and tumor location. Studies of HLA antigens in a large number of HNSCC of various stage, histologic type, and location appear warranted. Whether reduction or complete loss of these antigens correlates with response to therapeutic modalities needs to be investigated.

1053 5'-Methylthioadenosine Phosphorylase (MTAP): A Potential Therapeutic Target for Upper Aerodigestive Tract Squamous Cell Carcinomas (UADT-SCC)

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Background: The polyamine biosynthetic pathway is involved in replication, transcription and translation. 5'-methylthioadenosine (MTA), a late byproduct of polyamine synthesis, is processed by MTAP. Inhibition of MTAP causes MTA accumulation and feedback inhibition of polyamine biosynthesis. MT-DADMe-ImmA is a transition state analogue that is a potent MTAP inhibitor. We report on MT-DADMe-ImmA sensitivity in cell lines from UADT-SCC, and a tissue microarray (TMA) survey of its potential therapeutic target expression, MTAP in UADT-SCC.

Design: FaDu, Cal27, HeLa, and A549 (which is drug resistant) cell lines were treated with either 20 mM MTA or 1 mM MT-DADMe-ImmA, alone or in combination, and compared with untreated controls. TMAs were constructed with triplicate cores from 58 patients from tumor, mucosa, and metastases; there were 15 anterior oral cavity (AOC), 19 oropharyngeal (OP), and 24 laryngeal (Lx) and 12 metastatic tumors. IHC for MTAP was performed (1:100) on untreated cell lines and TMA slides. Staining intensity was scored from 0 to +3.

Results: FaDu and Cal27 demonstrated growth inhibition and apoptosis in 5 and 8 days, respectively, with MT-DADMe-ImmA treatment. HeLa and A549 were resistant. IHC on these cell lines for MTAP revealed uniformly cytoplasmic (C) > nuclear (N) staining for HeLa and A549. The majority of Cal27 cells had N > C staining. MTAP was positive in all TMA specimens with N and C expression. The mean tumors scores per site were: **AOC**: 1.8 N, 1.6 C / **OP**: 1.7 N, 1.8 C / **Lx**: 1.8 N, 1.7 C. Mean mucosal scores were 1.9 N, 1.4 C, and mean metastasis scores were 2.1 N, 2.1 C. N > C staining was present in 33.3% (5/15) AOC, 15.8% (3/19) OP, 25% (6/24) Lx tumors, and 33.3% (4/12) metastatic tumors. For mucosa, N > C staining was seen in 72.5% (29/40) samples.

Conclusions: Preliminary cell line studies reveal that MT-DADMe-ImmA may have therapeutic potential for squamous carcinomas. We hypothesize that N>C MTAP expression correlates with cell proliferation and may predict sensitivity to MTAP inhibitors. If so, then MTAP inhibitors may have therapeutic potential in up to a third of UADT-SCC. Further studies appear warranted.

1054 Nasopharyngeal Carcinoma: Criteria Supplement to WHO Classification

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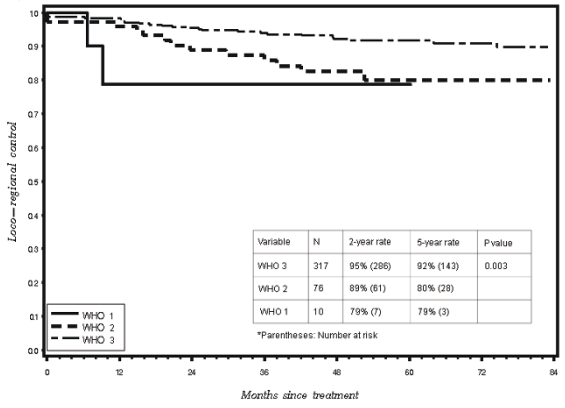
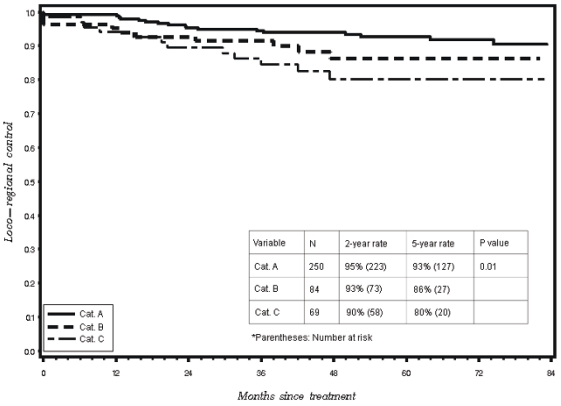
Background: The nasopharyngeal carcinoma (NPC) classification has been established by World Health Organization (WHO). The criteria to classify NPC are vague. Subclassification of the NPC into type 2 or 3 has been considered optional because of no clinical significance. Recently, we have proved the WHO types 1 and 2 are poor prognostic factors. The present study aims to develop well-defined histologic criteria to classify NPC.

Design: Between 1990 and 2002, 403 patients' slides were reviewed. The WHO type was recorded from pathology report. Two criteria were selected: N/C ratio and architecture. The N/C ratio was defined as high if there was no differentiation. The N/C ratio was defined as low if there was differentiation in at least 20% of tumor area. The differentiation was defined by increased cytoplasm dispersed or in zones. The architecture was defined as without nesting if there was no obvious cell nest present under low magnification. The architecture was defined as with nesting if there was more than 50% tumor area showing obvious nesting under low magnification. The reviewed pathology was grouped into three categories: (A) high N/C tumor; (B) low N/C tumor without nesting; (C) low N/C tumor with nesting.

Results: For WHO types, 79% patients were of type 3, 19% of type 2, 2% of type 1. After review, 62% patients were of category A, 21% of category B, 17% of category C. With a median follow-up of 59 months, 78% patients were free of disease, 22% had relapsed. The five-year loco-regional control probability was 89%. The loco-regional control rates according to WHO types and the reviewed categories were all statistically significant (p=0.003 and 0.01).





Conclusions: The criteria we used are simple and easy to follow. We recommend the identification of the three WHO types adopting the criteria we used in our present study.

1055 RNA-Binding Protein IMP3 Expression in Neoplasms of the Schneiderian and Squamous Mucosa of the Head and Neck

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Background: The Schneiderian and squamous epithelium of the upper aerodigestive tract may give rise to neoplastic lesions that may be *benign* [fungiform papillomas (FP) and squamous papillomas (SP)], *locally aggressive* [inverted papilloma (IP)] or *malignant tumors*. These lesions may have vastly different outcomes and management. The benign lesions have a small potential for malignant transformation, an event that is generally preceded by multiple recurrences. Detection of malignancy in these lesions is critical. IMP3, a recently described member of the insulin growth factor family, is an oncofetal RNA binding protein that is over-expressed in carcinoma of lung, pancreas and kidney. Its expression profile in benign and malignant epithelial neoplasms of the head and neck has not been studied in detail.

Design: Immunohistochemistry was performed on 4 µm thick formalin fixed paraffin embedded tissue sections from 59 head and neck tumors: 22 Schneiderian papillomas (10 FP, 12 IP), 24 SP, and 13 squamous cell carcinomas (SCC), using mouse monoclonal antibody to IMP3 protein (L523S, Corixa corporation, Seattle, WA, USA) after heat induced antigen retrieval (citrate buffer, pH 6.1) in a Dako autostainer. Positive staining was defined as dark brown cytoplasmic and /or membrane staining in tumor cells.

Results:

table 1

	FP	IP	SP	SCC
IMP3	0/10 (0%)	0/12 (0%)	1/24 (4%)	12/13 (92%)

All Schneiderian papillomas were negative for IMP3. Of the 24 SP, only one SP with moderate dysplasia showed IMP3 expression in 25%-35% of cells. Twelve of the 13 SCC [9 in-situ and 4 invasive] showed positive IMP3 expression. Nine SCC (6 in-situ, 3 invasive) showed diffuse and strong immunostaining (>50% positive cells) while 3 SCC (2 in-situ, 1 invasive) showed focal immunostaining (5%-15%). Upon follow-up (range 3-4 years), 4/8 IMP3 positive CIS developed invasion and 3/4 IMP3 positive invasive SCC showed disease progression.

Conclusions: IMP3 expression is highly specific for in-situ and invasive SCC of the head and neck. It is not expressed in benign proliferative lesions of the squamous and Scheiderian mucosa. Its association with moderate to severe dysplasia may help detect malignant transformation. There may be an association between IMP3 expression and disease progression, however, further studies are needed to evaluate its prognostic significance.

1056 p16 Protein Experession in Neoplasms of the Schneiderian and Squamous Mucosa of the Head and Neck

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Background: The head and neck mucosa may give rise to the Schneiderian *fungiform (FP)* and *inverted papillomas (IP)*, *squamous papillomas (SP)* and *squamous cell carcinomas (SCC)*. The tumor suppressor gene protein p16 is an inhibitor of Cyclin Dependent Kinases that regulate the G1-S phase of the cell cycle. Its expression is dependent on negative feedback from the retinoblastoma gene product and is suppressed in most malignancies. However, oncogenic mechanisms that inactivate the retinoblastoma gene, e.g. high risk HPV induced E7 oncoproteins, enhance the expression of p16, as seen in cervical dysplasia and malignancy. In a recent study, HPV DNA was detected in 61% of head and neck cancers. Using p16 as an indirect marker of HPV oncoprotein E7, we investigate its frequency in benign and malignant epithelial neoplasms of the head and neck.

Design: Immunohistochemistry was performed on 4 µm thick formalin fixed paraffin embedded tissue sections from 62 head and neck tumors: 10FP, 12 IP, 27 SP and 13 SCC (9 in-situ and 4 invasive including 2 papillary SCC). The anti-p16 mouse monoclonal antibody (clone 6H12, Vector, Burlingame, CA) was used with Dako's Envision system after heat induced antigen retrieval (citrate buffer, pH 6.1) in a Dako autostainer. Positive p16 expression was defined as strong nuclear and cytoplasmic staining in tumor cells. An adenocarcinoma in-situ of the cervix was used as positive control.

Results:

table 1

	FP	IP	SP	SCC
p16	0/10 (0%)	0/12 (0%)	3/27 (11%)	2/13 (15%)

p16 expression was seen in only 5 of 62 tumors (8%). All Schneiderian papillomas were negative for p16. Three SP including one with moderate dysplasia, showed weak p16 expression in the basal and suprabasal squamous cells. Two invasive papillary SCC showed strong and diffuse p16 expression in all tumor cells. The remaining SCC (85%) were negative for p16.

Conclusions: p16, a surrogate marker of HPV induced oncoprotein E7, is expressed in a very small proportion of head and neck tumors. This is in contrast to the higher reported incidence of HPV DNA detection in head and neck lesions. It appears that only a small proportion of such infections may follow the carcinogenic pathways requiring the formation of the oncoproteins E6 and E7. The majority of head and neck neoplasms show downregulation of p16.

1057 Angiostatin Receptor Annexin-II Is Expressed in Juvenile Nasopharyngeal Angiofibroma: Implications for Therapy

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Background: Juvenile nasopharyngeal angiofibroma (JNA) is a rare benign, but locally aggressive vascular and mesenchymal neoplasm that typically affects boys and adolescent males. The treatment of choice is surgical, with radiation therapy reserved for advanced cases. Both treatment modalities are troubled by potential complications including recurrence, hemorrhage, facial growth retardation, and cranial nerve dysfunction. Given its highly vascular nature, a potential alternative therapy for JNA may be offered by angiostatin, a potent angiogenesis inhibitor that has caused complete regression of human tumors in animal models. Angiostatin's effects appear to be mediated by annexin-II, a cell surface signal transduction protein on endothelial cells. In the current study, we examined annexin-II expression in JNAs by immunohistochemical staining.

Design: Formalin-fixed paraffin-embedded sections of 22 JNAs were immunostained with anti-annexin-II. Staining intensity in tumor endothelium and stromal cells was evaluated semi-quantitatively utilizing a four tier scale as follows with non-neoplastic endothelium serving as positive control: 0=negative staining; 1+ staining less intense than control; 2+ equal to control; 3+ staining more intense than control. Negative controls stained appropriately.

Results: Annexin-II immunoperoxidase staining demonstrated diffuse cytoplasmic positivity in endothelial and stromal cells in all cases of JNA. In 21 of 22 cases (95%) the staining intensity of the endothelium was equal to endothelial staining in control endothelium (2+). 1 of 22 cases (5%) had 1+ endothelial positivity. In the stromal cells, the staining results were 82% 2+, 14% 3+, and 4% 1+. Similarly intense annexin-II staining was also noted in overlying respiratory and squamous epithelium, and in stromal fibroblasts and epithelia of a variety of control tissues tested.

Conclusions: JNAs demonstrate diffuse, robust expression of angiostatin receptor annexin-II by immunohistochemistry, suggesting that angiostatin or angiostatin-analogue therapy may theoretically offer benefit in the treatment of this anatomically challenging neoplasm. However, the widespread, similarly intense expression of annexin-II in epithelia and stroma in a variety of tissues may indicate a side effect risk potentially limiting the efficacy of such treatment.

1058 Intraoperative Frozen Section Clarifies the Thyroid Fine Needle Aspirate Diagnosis of Follicular Neoplasm: Reduction of Total Thyroidectomies for Benign Lesions

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Background: Thyroid nodules are common in the adult population and the majority are benign. Fine needle aspiration (FNA) accurately identifies nodules which require surgical intervention by categorizing them as benign or malignant. However, in 15-30% of cases, an FNA interpretation of "follicular lesion" or "follicular neoplasm" is rendered, in which case the differential diagnosis includes nodular hyperplasia, follicular adenoma, follicular carcinoma, and follicular variant of papillary thyroid carcinoma (PTC). The role of intraoperative frozen section (FS) in evaluating follicular lesions is controversial and widely downplayed in the literature, given the accuracy of FNA and the limitations of FS in evaluating features of malignancy. We examined the benefit of FS in follicular lesions, compared to no FS, in terms of patient outcome and specimen handling.

Design: 167 patients with thyroid nodules termed "follicular lesion", "follicular neoplasm", or "suspicious for follicular neoplasm" by FNA, who underwent surgery at a single institution, were divided into FS or no FS groups. The FS and final diagnoses, extent of surgery, nodule size, and number of blocks submitted of the nodule were tabulated.

Results: 93 of 167 cases (56%) had intraoperative FS. 65% of FS clarified the FNA diagnosis: 13% of FS were called benign, 39% favored benign, 9% favored malignant, and 4% were called malignant. Only 35% of FS echoed the FNA diagnosis of "follicular lesion/neoplasm". The distribution of final diagnoses was similar between the two groups (approximately 55% follicular adenoma, 20% non-neoplastic, 15% PTC, and 10% follicular carcinoma). Mean nodule size and blocks submitted per centimeter of nodule were not statistically different between the two groups. The rate of total thyroidectomy was 36% in the FS group and 57% in the no FS group. 10 out of 93 patients (11%) in the FS group had a total thyroidectomy for benign disease, versus 22 out of 74 (30%) in the no FS group.

Conclusions: By providing additional information beyond the FNA interpretation in 65% of cases, FS was associated with a significantly reduced rate of total thyroidectomy compared to not performing FS. FS thus appears to offer substantial benefit to patients with follicular lesions.

1059 Epithelial-Mesenchymal Transformation and Invasion in Head and Neck Squamous Carcinomas

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Background: Squamous carcinoma (SCC) is a phenotypically heterogeneous tumor with a broad spectrum of differentiation. Epithelial cell modification may result from alteration of the homeostatic equilibrium between fundamental cellular pathways and local host environment leading to epithelial-mesenchymal transition (EMT). However the role of adhesion and motility related genes in EMT's association with invasion and progression of head and neck(HN) SCC remain poorly defined. In an effort to identify these events, we investigated several adhesion and metastasis associated markers that may play a role in EMT in SCC invasion and progression.

Design: Seven HNSCC cell lines and tissue specimens from 21 patients with invasive SCC were used to determine the differential role of Src, p-Src, EGFR, p-EGFR, E-Cadherin(E-cad) and vimentin in this entity. In vitro and immunohistochemical methods were used in the analysis. The in vitro assays included siRNA for c-Src, cell migration and proliferation assays. The biologic effects of a Src inhibitor, PP2 were also examined in vitro. Marker expression and tumor differentiation were also compared.

Results: Src, E-cad and EGFR were differential expressed in the majority of the cell lines with elevated p-Src associated with decreased E-cad levels and increased EGFR and vimentin expression. 4 of 7 cell lines showed high p-SRC compared to normal tissues. In-vitro inhibition of Src, via both Src inhibitor, PP2 and siRNA derived clones, were associated with decreased cell proliferation (50%), and were 2-3 times less motile. Variable phenotypic expression was noted by immunohistochemistry of primary tumors. A subset showed differential expression with low p-Src and intense E-cad, and saromatoid SCC had low E-cad and high p-SRC. High p-src was found in poorly differentiated and sarcomatoid SCC and was associated with high vimentin expression.

Conclusions: Our study indicates that 1) p-Src, E-cad and EGFR are differentially expressed in a subset of these tumors and appears to play a major role in EMT and invasion in HNSCC, 2) The conversion of the epithelial to sarcomatoid phenotype is characterized by loss of E-cad, elevated p-Src and the acquisition of high vimentin, 3) Inhibition of Src decreased cellular proliferation 4) These factors may be targeted for therapeutic intervention in patients with progressive disease.

1060 HPV, p16, and bcl-2 Expression in Oropharyngeal Squamous Cell Carcinoma

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Background: Inactivation of the cell cycle inhibitor gene p16 may be involved in high risk human papillomavirus 16/18 (HPV)-related carcinogenesis because E6 and E7 oncoproteins may impair p16 and, indirectly, bcl-2 functions. Prior studies have demonstrated a possible association of high-risk human papilloma virus 16/18 (HPV) with oropharyngeal squamous cell carcinoma (OPSCC) and that P16 protein overexpression may be a surrogate marker for HPV expression. We sought to explore the relationships of HPV, P16 and bcl-2 expression in pathogenesis and clinical behavior of OPSCC.

Design: Archival formalin-fixed, paraffin-embedded tissue sections from 39 cases of OPSCC from 2002 to 2005. Expression of HPV 16/18 was assessed by in-situ hybridization. P16 and bcl-2 were assessed by immunohistochemisty (IHC). Any expression of strong nuclear HPV staining was scored as positive. Any expression of strong nuclear and cytoplasmic staining for P16 and bcl-2 was considered positive. SAS statistical analysis was performed to determine any association with expression of HPV, P16, and bcl-2 in regard to histologic grade, location, clinical stage, and outcome.

Results: Of the 39 cases, 29 were male and 10 were female. Thirty-five (87%) patients presented clinically as Stage 4, 4 (10%) as Stage 3, and 1 (3%) as Stage 2. All patients received radiation and chemotherapy as primary treatment modalities. Of the 39 cases reviewed 24 (61.5%) were positive for HPV and 31/39 (79.5%) were positive for P16 expression. A statistically significant correlation with the expression of P16 and HPV ($p=0.037$) was noted. No correlation was found with expression of HPV, P16 and bcl-2 and clinicopathologic parameters such as histologic grade, location, and outcome with the exception of bcl-2 expression and histologic grade. Bcl-2 expression was statistically ($p=0.02$) associated with poorly differentiated and undifferentiated OPSCC compared to bcl-2 negative lesions.

Conclusions: We demonstrate an association with HPV infection and P16 staining. P16 staining should be considered as a simple and potentially reliable immunohistochemical stain for diagnosing cases of HPV induced OPSCC. No association between HPV infection and bcl-2 overexpression was found suggesting that viral oncoproteins may not deregulate bcl-2 expression.

1061 Thyroid Transcription Factor 1 (TTF-1) Immunoreactivity in Low-Grade Nasopharyngeal Papillary Adenocarcinoma (LGNPPA): A Report of Three Cases

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Background: LGNPPA is uncommon primary nasopharyngeal malignant neoplasm with indolent biological behavior. The morphologic features of this tumor are rather distinct but can be similar to those of thyroid papillary carcinoma (TPC). TTF-1 is a nuclear transcription factor necessary for development of thyroid and pulmonary tissue. TTF-1 is expressed in thyroid carcinomas and most lung carcinomas. The presence of TTF-1 reactivity in LGNPPA is unusual. We report three cases of LGNPPA showing TTF-1 immunoreactivity, and discuss the significance of this finding and the potential origin for this "aberrant" expression.

Design: The 3 cases were identified by a database search of our files for all nasopharyngeal tumors. The clinicopathologic features were analyzed. Representative paraffin blocks were immunohistochemically stained with antibodies against cytokeratins (AE1/AE3, CAM5.2, OSCAR, CK5/6, CK7, CK8, CK19, CK20), thyroglobulin, TTF-1, p63, calponin, carcinoembryonic antigen (CEA), chromogranin and synaptophysin.
Results: Of our 3 cases, 2 occurred in women ages 13 and 18, and 1 in a man age 64. Patients complained of nasal obstruction and examination revealed nasopharyngeal-based exophytic lesions. The light microscopic features were those typically seen in LGNPPA. All three tumors showed diffuse (nuclear) immunoreactivity for TTF-1, as well as cytokeratin immunoreactivity (AE1/AE3, OSCAR, CAM5.2, CK7, CK8, CK19). No immunoreactivity was seen for thyroglobulin, CEA, p63, calponin, chromogranin and synaptophysin.
Conclusions: TTF-1, although considered a dedicated marker of thyroid and lung carcinomas, may be found in neuroendocrine carcinomas of non-pulmonary origin. In addition, we report herein the presence of TTF-1 immunoreactivity in LGNPPA, thereby extending the list of tumors that may stain with TTF-1. In conjunction with light microscopic features overlapping with those of TPC, the presence of TTF-1 may result in an erroneous diagnosis of metastatic TPC. The presence of surface epithelial derivation and absence of thyroglobulin staining should allow for a diagnosis of LGNPPA differentiating it from TPC even in the presence of TTF-1 staining. The origin for the "aberrant" TTF-1 expression remains uncertain but the embryologic development of the thyroid gland from the primitive pharynx may provide a mechanism to explain this phenomenon.

Hematopathology

1062 Different Chemokine Receptor Expression Profile in Extragastric and Gastric MALT-Lymphomas

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Background: Chemokine receptors mediate migration and activation of lymphocytes through binding of their ligands and contribute to the development of hematopoietic neoplasms. Strong expression of CXCR5 was detected in transformed B cells in *Helicobacter (H.) pylori* positive gastric MALT lymphomas. Recently, *Chlamydia (C.) psittaci* was identified as the causative infectious agent of ocular adnexal MALT lymphomas. The aim of this study was to identify the expression pattern of all 19 currently known chemokine receptors in extragastric and gastric MALT-lymphomas. Furthermore, extragastric MALT lymphomas were investigated for the presence of *C. psittaci* DNA and their chemokine profile compared to their uninfected controls.
Design: 16 extragastric and 5 *H. pylori* positive gastric formalin-fixed, paraffin-embedded MALT lymphoma samples were processed for RNA and DNA isolation. Extragastric sites of lymphoma origin were salivary gland, thyroid gland, skin and ocular adnexa. Semiquantitative Real Time PCR was performed on a GeneAmp® 5700 Sequence Detector. Expression of 19 chemokine receptors was determined in triplicate and the number of cycles was compared to the reference gene HPRT and peripheral blood, based on calculations of 2-deltadeltaCT. The samples were further analyzed for presence of *C. psittaci* DNA via Real-time PCR performed on the LightCycler instrument.
Results: Comparing the mean values of chemokine receptor expression of extragastric to *H. pylori* positive gastric MALT lymphomas, CXCR1 (23 times down regulated, p=0.002), CXCR2 (170 times down regulated, p=0.029), CX3CR1 (22 times up regulated, p=0.002) and XCR1 (de novo expressed, p=0.002) were significantly differently expressed. 10 of 16 (63%) extragastric and none of 5 *H. pylori* positive gastric MALT lymphomas tested positive for *C. psittaci* DNA. No difference in the chemokine receptor expression profile between *C. psittaci* infected vs. uninfected extragastric MALT lymphoma samples could be observed.
Conclusions: *C. psittaci* infection is associated with a significant proportion of extragastric MALT lymphomas other than ocular adnexa. Further, differently expressed chemokine receptors might be responsible for the various sites of origin of MALT lymphomas. Thereby, the expression profiles of CXCR1, CXCR2, CX3CR1 and XCR1 seem to be the key determinant in the homing of malignant B-cells either into the stomach or into extragastric sites.

1063 DRAQ-5 Based No-Lyse, No-Wash Bone Marrow Aspirate Evaluation by Flow Cytometry

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Background: Flow cytometry (FC) is a powerful tool for objective phenotyping of hematolymphoid neoplasia. Analysis of bone marrow aspirates and peripheral blood specimens by flow cytometry typically requires an erythrocyte lysis or gradient separation method to remove erythrocytes prior to analysis which may result in the loss of certain populations, in particular nucleated erythroid cells. This results in FC analysis not reflecting the true composition of nucleated cells. DRAQ-5 is a novel, far-red fluorescing DNA specific dye that penetrates live cells and is excitable using a 488-nm laser. We sought to develop a method where we could evaluate bone marrow aspirates (BMAs) by FC without specimen manipulation/ lysis by exploiting DRAQ5 fluorescence as a gating parameter to analyze nucleated events.
Design: We analyzed a total of 30 normal and abnormal BMAs (15 males, 15 females) from patients with a variety of diagnoses on an FC500 flow cytometer utilizing a DRAQ5 based no-lyse, no-wash FC protocol (DRAQ 5 protocol) in combination with CD71 FITC and CD45 PE antibodies to determine the percent of different major cell populations present in the BMA (nucleated RBCs, blasts, myeloid, monocytic and lymphs). These were compared to blinded morphologic differential counts performed on Wright stained slides prepared from the same specimen and to differential counts obtained by conventional erythrocyte lysis FC analysis.
Results: Light scatter and fluorescence staining of the cells in the DRAQ5 protocol were comparable to conventional FC analysis and allowed discrimination of the major bone marrow cell populations in normal and abnormal specimens. The correlation coefficient between DRAQ5 protocol differential counts and morphologic counts were: 0.97 nRBCS; 0.97 myeloid; 0.94 blasts; 0.42 monocytes; 0.58 lymphs. Correlation coefficients between conventional FC and morphologic differentials were: 0.84 nRBCS; 0.89 myeloid; 0.98 blasts; 0.40 monocytes; 0.52 lymphs. NRBC counts were significantly different between the conventional FC and morphology ($p<0.001$); no significant difference was observed for the DRAQ5 protocol.
Conclusions: The DRAQ5 protocol is a simple method to quantify the major cell populations in the BMA. The method more accurately quantifies nucleated erythroid cells compared to conventional FC and thus allows for quantitation of blasts/ abnormal cells that reflect the morphologic nucleated cell differential.

1064 CXCR4 Expression in Follicular Lymphomas

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Background: CXCR4 is a chemokine receptor that is involved in lymphocyte trafficking, stem cell mobilization and is associated with tumor cell invasiveness. We previously have shown that in diffuse large B cell lymphomas CXCR4 is a poor prognostic factor. It has also been shown that CXCR4 is one of several preferentially overexpressed peptides in follicular lymphomas and also it has been demonstrated that CXCR4 is a chemotactic factor for follicular lymphoma cells. We investigated the expression of CXCR4 in follicular lymphomas and correlated its expression with prognosis.
Design: Patients with follicular lymphoma (n=218) followed up at University Hospital, Zurich were selected to generate a tissue array. Patient mean follow up was 60 months. Immunohistochemical studies were done utilizing CXCR4 (fusin h-118; sc-9046 Santa Cruz). In addition, CXCR4 expression was correlated with Fak, MLK3 and CARP-1. The cases were scored according to staining intensity and percentage tumor cell staining. We scored the staining by multiplying staining intensity with percent cells staining. A cutoff value of 100 was utilized to classify the cases as expressors vs. non-expressors.
Results: In normal lymphoid tissues, CXCR4 had a weak diffuse staining in the germinal centers which was both cytoplasmic and nuclear. In the interfollicular areas there were scattered staining lymphocytes. CXCR4 staining was present in 143/218 cases of follicular lymphoma. The staining was predominantly nuclear but also cytoplasmic. CXCR4 expression did not have any prognostic value in the follicular lymphomas tested. We also analyzed a subgroup of diffuse large cell lymphomas of follicular origin and found that expression of CXCR4 was a poor prognostic factor with marginal significance. CXCR4 expression had a strong correlation with Fak and CARP-1 expression.
Conclusions: CXCR4 is frequently expressed in follicular lymphomas. Its expression correlates with expression of Fak. However, unlike that of transformed follicular lymphomas, or diffuse large B cell lymphomas in general, its expression is not a poor prognostic factor.

1065 Loss of Carp-1 Expression Is a Poor Prognostic Factor in Follicular Lymphomas

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Background: Carp-1 is a recently described apoptosis and cell cycle arrest inducing peptide that presumably acts as a tumor suppressor. We have previously shown that its expression strongly correlates with apoptotic activity in diffuse large B cell lymphomas and is inversely associated with pAkt expression. We have also demonstrated its loss of expression in a variety of solid tumors that are high grade. Since the follicular lymphomas are associated with resistance to apoptosis acquired by the t(14;18) translocation, we hypothesized that CARP-1 expression would have a biological and prognostic value.
Design: A tissue array was generated utilizing 220 cases of follicular lymphoma followed up at University Hospital Zurich, with a mean follow up of 60 months. CARP-1 antibody was generated by immunizing rabbits to synthetic CARP-1 peptide. The cases were represented in duplicate in the array. The cases that demonstrated at least 25% staining were considered positive for expression. We also correlated CARP-1 expression with Fak, CXCR4 and MLK3 expression, all of which were prognostic markers in diffuse large cell lymphomas.
Results: CARP-1 expression was present in 172 of 220 cases. Its expression correlated with Fak expression. we have previously demonstrated Fak to be a good prognostic factor in diffuse large cell lymphomas, and associated with a germinal center cell phenotype. In addition, CARP-1 expression showed a strong correlation with CXCR4 expression. The patients expressing CARP-1 had a better overall survival compared to non-expressors (106 vs. 57 months mean survival; p=0.0312). We also tested a follicular lymphoma cell line (WSU-FSCCL1) to observe the in vitro effects of CARP-1. In vitro transduction of CARP-1 and its shorter fragments induced apoptosis which was associated with translocation of Nur77 to the cytoplasm. Immunoprecipitation studies demonstrated binding and colocalization of Nur77 and CARP-1 in several lymphoma cell lines, suggesting Nur77 is involved in apoptosis induced via CARP-1.
Conclusions: Loss of CARP-1 expression is associated with a poor prognosis in follicular lymphomas. CARP-1 is a candidate as a prognostic marker and a potential target for therapy in follicular lymphomas.