lesions such as VEMPD. However, the expression pattern of p16 in VEMPD has not been reported. We studied the expression of p16 protein immunohistochemically in 40 cases of VEMPD.
Design: A cohort of 40 cases of VEMPD was searched and retrieved from our hospital archive. Clinicopathologic data was reviewed and tabulated. P16 immunostaining was performed on recuts of all cases. P16 immunostaining pattern was categorized as negative, focal or continuous. Focal pattern is defined as patch and discontinuous staining in less than 30% of cells with weak staining intensity. Continuous pattern is defined as diffuse staining of >90% cells of Pagetoid tumor cells with strong staining intensity.
Results: p16 expression was seen in 36/40 (90%) of VEMPD. Four cases (10%) of VEMPD showed negative p16 staining. Focal p16 staining pattern was observed in 20 (50%) cases. Continuous staining pattern was seen in 16 (40%) cases. VEMPD with dermal invasion, ranging from microinvasion to large nodular mass, was identified in 5 (12.5%) cases. Interestingly, all 5 VEMPD cases with invasion had continuous staining pattern. About 31% (5/16) of VEMPD with continuous p16 staining pattern associated with invasion. In contrast, none of the 24 VEMPD cases with either negative p16 or focal p16 staining pattern was associated with dermal invasion.
Conclusions: We have found that p16 protein is expressed in a vast majority (90%) of VEMPD. Strong and continuous p16 immunostaining pattern is associated with invasive VEMPD. Given overexpression of p16 seen in both VEMPD and usual/classic VIN, p16 immunostaining is not useful in differentiating VEMPD from usual/classic VIN. Our data indicates that p16 tumor suppressor gene is likely involved in the pathogenesis of VEMPD and a continuous pattern of p16 immunostaining may be linked to invasion.

1256 Does Ovarian Hyperthecosis Contribute to the Genesis of Endometrial Polyps, Endometrial Hyperplasia, and Endometrioid Carcinoma in Postmenopausal Women? A Clinicopathologic Study of 238 Cases

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Background: Hyperestrinism due to anovulatory cycles is associated with endometrial polyps (ENPO), hyperplasia (ENHY), and endometrioid adenocarcinoma (EMCA) in pre- and perimenopausal patients. ENPO, ENHY, and EMCA also occur in postmenopausal (PMP) patients but the cause is less clear. Hyperthecosis (HT), the presence of luteinized theca cells in the (non-follicular) ovarian stromal parenchyma, may occur to varying degrees in PMP women. HT largely produces androgens that are converted to estrone in the peripheral adipose tissues. Our aim was to determine if there was a correlation between the presence of ovarian HT and the occurrence of ENPO, ENHY, and EMCA in PMP women.
Design: We selected PMP EMCA specimens with both uterus and ovaries removed in 2011 and matched with a benign control group from 2006 to 2011. EMCA cases were subdivided into FIGO G1, FIGO G2, and FIGO G3. Benign control group was subdivided into endometrial atrophy, ENPO, and ENHY. Archival H&E slides were reviewed to identify the presence of HT. The patient's age was also recorded. We used Chi Square test to compare the frequency of HT and Variance of Analysis to compare the patient's age among different groups.
Results: Our study consisted of 238 PMP women: 108 with an EMCA diagnosis and 130 with a benign diagnosis. Within the benign cases, 71 (54.6%) had atrophic endometrium, 32 (24.6%) ENPO, and 27 (20.8%) ENHY. Among the EMCA cases, 48 (44.4%) were FIGO G1, 46 (42.6%) FIGO G2, and 14 (13.0%) FIGO G3. The frequencies of HT in patients with ENPO (46.9%), ENHY (55.6%), FIGO G1 (43.7%), FIGO G2 (54.3%), and FIGO G3 EMCA (57.1%) were each significantly higher than that in patients with atrophic endometrium (23.9%), supporting an association of HT with ENPO, ENHY, and EMCA in PMP women. No significant difference was seen in patient's age among different groups.
Conclusions: Our study indicates that HT with its resultant risk factor of hyperestrinism may contribute to the pathogenesis of ENPO, ENHY, and EMCA in PMP patients. Although some workers postulate that FIGO G3 EMCA may have a different histogenesis from its lower grade counterparts, our study suggests that EMCA of all FIGO grades may share the common risk factor of hyperestrinism.

1257 Vulvar Bartholin Gland Lesions: A Clinicopathologic Study of 111 Cases

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Background: Bartholin gland is the major vulvar vestibular gland that is normally impalpable and gradually involutes after the age of 30 years. It is histologically composed of simple tubuloalveolar glands with mucin producing alveoli, reminiscent to Cowper s glands in men and minor salivary glands, drain into a central ducts mostly lined by transitional epithelium. Other than Bartholin cyst, very few benign lesions are reported in the literature. This study reviewed the clinicopathologic features of 111 cases of Bartholin gland lesion.
Design: Pathology reports and all available slides of 111 cases of vulvar Bartholin gland lesions from a single Institution (1990-2011) were reviewed. Clinicopathologic features including age, presenting sign or symptoms, extent of surgery, lining epithelium, inflammation, and histopathologic changes of Bartholin gland were evaluated.
Results: Of the 111 patients, the mean age was 41.4 years (range 18-82). The most common presenting symptom was cystic or mass lesion. Surgery ranged from simple biopsy to resection. A cyst was present in 101 cases (91%) (intact 79, ruptured 22). A lining epithelium was identified in 93 of 111 (83.8%) cases. Acute inflammation or abscess was identified in 43 of 111 (38.7%) of the cases. The most common cystic lining epithelium was transitional (33.3%), mixed transitional and squamous (33.3%), squamous (23.7%), or mucinous (9.7%). Bartholin mucous glands were identified in 46 (41%) of cases, with 9 cases of hyperplasia and 1 case of adenoma. In addition, one case of myoepithelioma, adenoid cystic carcinoma, invasive squamous cell carcinoma, and severe squamous dysplasia extending to the Bartholin gland was identified.
Conclusions: Bartholin gland lesion commonly presents as cyst or mass in middle aged women (average of 41.4 years). The most common cystic epithelial linings are squamous, transitional or mixed type. Acute inflammation or abscess is a frequent associated finding in Bartholin cyst, which may be an important histogenetic factor for the cyst formation. Besides Bartholin cyst, other pathologic processes including rare adenoid cystic carcinoma and squamous carcinoma also occur.

Head & Neck

1258 Comparison of p63 and p40 (ΔNp63 Isoform) as Basal and Myoepithelial Markers in Salivary Gland Tumors

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Background: Immunohistochemical staining for p63, a p53 homologue, is frequently used in the clinical setting to confirm squamous, basal or myoepithelial differentiation in a variety of organ sites. There are two main isoforms of p63: TAp63 and ΔNp63, both of which are recognized by the most commonly used antibody to p63, clone 4A4. Recent studies in lung tumors have called into question the specificity of this p63 antibody for squamous differentiation and have demonstrated that an antibody directed towards only ΔNp63 isoforms (p40) has better performance in this respect. We herein performed a similar survey in salivary gland tumors to compare the performance of both p63 and p40 antibodies as markers of basal and myoepithelial differentiation.
Design: Nineteen salivary gland tumors, 15 with adjacent normal salivary tissue, were stained with p63 (BC4A4, prediluted, Biocare) and p40 (5-17, 1:1000, Calbiochem). Tumors were broadly categorized as ductal (n=7; 1 canalicular adenoma, 1 salivary duct carcinoma ex pleomorphic adenoma, 1 mammary analogue secretory carcinoma, and 4 adenocarcinomas NOS), biphasic (n=7; 1 adenoid cystic carcinoma, 2 myoepithelial carcinomas ex pleomorphic adenoma, 1 epithelial-myoepithelial carcinoma, 1 pleomorphic adenoma; 1 basal cell adenoma, and 1 basal cell adenocarcinoma), composed of ductal and myoepithelial/basal elements, purely myoepithelial (n=4; 1 myoepithelioma and 3 myoepithelial carcinomas) and one mucoepidermoid carcinoma (presumed excretory duct phenotype). Nuclear staining was considered positive.
Results: Both p63 and p40 stained the myoepithelial cells surrounding acini and intercalated ducts as well as the basal cells of the striated and larger excretory ducts. All biphasic and myoepithelial tumors showed a similar distribution of reactivity and intensity in the basal and myoepithelial cells for both p63 and p40. In the mucoepidermoid carcinoma, p63 and p40 highlighted epidermoid and intermediate cells in a similar distribution and intensity. However, 3/7 tumors regarded as ductal tumors showed focal p63 positivity, but no p40 expression. These consisted of a minimally invasive adenocarcinoma ex pleomorphic adenoma, one adenocarcinoma NOS, and one canalicular adenoma.
Conclusions: Overall, p40 has a similar distribution to p63 in both normal salivary gland and salivary gland neoplasms. However, p40 does appear to be more specific with respect to a subset of ductal tumors of the salivary gland.

1259 Reduced Expression of the Putative Tumour Suppressor Spinophilin Is an Adverse Prognostic Factor in Squamous Cell Carcinoma of the Head and Neck

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Background: Spinophilin (SPN), a multifunctional intracellular scaffold protein, has been involved in carcinogenesis of lung and other types of cancer. To date, there exist no data about the role of SPN in squamous cell carcinoma of the head and neck (SCCHNs).
Design: In the present study, we evaluated SPN expression in SCCHN tumor tissue by immunohistochemistry in 85 patients who underwent a curative tumor resection. The SPN expression was correlated with clinico-pathological characteristics and multivariate Cox proportional models were used to define its prognostic relevance.
Results: Immunoreactivity for SPN was reduced in 40 (47%) tumors and 9 (10.5%) cases showed complete loss of SPN. Kaplan Meier curve analysis demonstrated that reduced SPN expression is associated with poor survival (p=0.022, log-rank test). Multivariate COX regression analysis confirmed clinico-pathological parameters as independent prognostic factors of survival.
Conclusions: A reduced expression of SPN is frequently found in SCCHNs, which indicate an important role of SPN in the pathogenesis of SCCHNs. Based on our results SPN may represent a novel prognostic factor for predicting patient's outcome in SCCHNs.

1260 Human Papillomavirus Detection and Genotyping in Squamous Cell Carcinomas of the Tongue

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Background: Carcinoma of the tongue is the predominant cancer type of the oral cavity. Human papillomavirus (HPV) has been detected in 99% of carcinomas from the uterine cervix and in many cases of head and neck carcinomas. The carcinogenic influence of genital HPVs in head and neck carcinoma development is controversial. We designed a study that analyzed the presence of HPV in squamous cell carcinomas (SCC) of the

tongue and that determined the implicated genotypes. We compared these data with those obtained using the same methodology in uterine cervix dysplastic lesions of a population from the same geographic area.
Design: Clinical and histological data of 64 patients with SCC of the tongue were collected. Benign lesions of the tongue (30) and cervical dysplasia cases (332) were also processed in parallel to compare the HPV incidence and the genotypes of these lesions with those of carcinoma of the tongue. Paraffin blocks of all the cases were collected, and Polymerase Chain Reaction (PCR) was carried out using the SPF10 set of primers and the Innolypa genotyping methodology.
Results: HPV was detected in 26,2% of the tongue carcinoma cases. All positive cases showed high risk (HR) HPVs, being HPV 56 the most common (42,1%), followed by HPV 18 (26,3%), HPV 16 (10,5%), HPV 66 (10,5%), HPV 39 (5,3%), and HPV 51 (5,3%). None of the control cases of benign lesions of the tongue showed HR-HPV whereas 96,6% cases of cervical dysplastic lesions showed HR-HPV (HPV 16, 52, 51, 53, 31, 18, 33, and 58 were the genotypes detected in these cases).
Conclusions: - HPV incidence in squamous cell carcinoma of the tongue was 26,2%.
- The most prevalent HPV genotype was HPV 56, followed by HPV 18.
- HPV genotypes present in carcinomas of the tongue were markedly different from those found in dysplastic lesions of the cervix from a population of the same geographic area.

1261 Lacking Caspid Protein Expression in p16 Positive Head and Neck Squamous Cell Carcinoma Using HPV-1 Immunohistochemistry

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Background: The incidence of head and neck squamous cell carcinoma (HNSCC) have risen steadily in US and Northen Europe. They are associated with high risk human papillomorirus (HPV). HPV-positive cancer is associated with better patient survival than HPV-negative cancer. Different testing methods have been used for HPV detection, such as in situ hybridization (ISH) or polymerase chain reaction (PCR). P16 immunohistochemistry has been shown to be a sensitive surrogate marker for HPV with a good specificity. Serum HPV caspid proteins, such as major (L1) and minor (L2), have been associated with HPV positive head and neck cancer, and lacking caspid protein expression in uterine cervical intraepithelial neoplasia (CIN) is associated with high grade squamous dysplasia. The caspid protein expression has been shown in head and neck squamous papillomas, but it is not known in HNSCC.
Design: Immunohistochemistry for p16 and HPV-1 was introduced to our laboratory since March 2010. Anti HPV-1 clone K1H8 has been shown immunoreactive with paraffin sections of formalin-fixed HPV infected tissue including HPV 6,11,16,18,31,33,42,51,52,56 and 58. There are total 33 cases of HNSCC with p16 and/or HPV-1 results during March 2010 to August 2012. H&E-stained sections and IHC slides were available in all cases. P16 and HPV-1 were both performed on 20 cases; p16 only in 11 cases; and HPV-1 only in 2 cases. The 33 HNSCC cases include 6 tongue base, 9 tonsils, 5 oral tongue, 7 larynx, 3 neck lymph nodes and 3 posterior oral pharynx. Two cases of laryngeal squamous papilloma were included.
Results: P16 was positive in 15 cases (48.4%), and 13/15 cases showed strong and diffuse positive. The 2 cases with focal p16 positivity were 1 oral tongue SCC and 1 posterior oral pharynx SCC. P16 was positive in 83.3% tongue base SCC, 57.1% tonsil SCC, 66.7% neck metastatic SCC 100% posterior oral pharynx SCC, 20% oral tongue SCC, and 0% larynx SCC. HPV-1 was negative in all 22 cases including the 11 p16 positive HNSCC cases. The two squamous papillomas were positive for HPV-1 but negative for p16.
Conclusions: PCR for HPV L1 gene is one of the gold standard methods for detecting the HPV infection in HNSCC. The value of IHC for HPV caspid proteins was unknown. Our study showed complete lacking caspid protein expression in HNSCC using anti HPV-1. HPV-1 has no diagnostic value for detecting possible HPV infection in HNSCC. HPV-1 is sensitive for HPV associated squamous papillomas. Our study showed that p16 positive rate was high in oralpharyngeal SCC such as tongue base, tonsil and posterior oral pharynx.

1262 P16 Overexpression in High-Grade Neuroendocrine Carcinomas of the Head and Neck Unrelated to HPV-Infection

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Background: High-grade neuroendocrine carcinomas (HGNEC) of the head and neck are infrequent, aggressive neoplasms. Histologically they have the appearance of undifferentiated carcinomas and may have a striking similarity to HPV-associated squamous cell carcinomas of the head and neck, which usually are non-keratinizing carcinomas. P16 is considered a surrogate marker of HPV- associated squamous cell carcinomas of the head and neck. To our knowledge, this is the first report of a series of HGNEC of the head and neck in which a wide immunohistochemical panel including p16 has been performed and HPV has been investigated.
Design: Twelve HGNEC of the head and neck (5 parotid, 3 laryngeal, 2 sinonasal, 1 hypopharyngeal and 1 oral) were reviewed and a panel of immunohistochemical stains was performed: $p16^{INK4a}$ (mtm, Heildelberg, Germany - Roche Diagnostics), cytokeratin7 (clone OV-TL 12/30, Dako, Glostrup, Denmark), cytokeratin20 (clone Ks20.8, Dako), synaptophysin (clone SY38, Dako), chromogranin A (clone DAK-A3, Dako), CD56 (clone 123C3, Dako), neurofilament (clone 2F11, Dako), neuron-specific enolase (clone BBS/NC/Vi-H14, Dako), TTF-1 (clone 8G7G3/1, Dako), p53 (clone DO7, Dako), p63 (clone BC4A4, Biocare Medical, Egham, United Kingdom), and Ki67 (clone MIB-1, Dako). HPV analysis was performed by in situ hybridization (GenPoint HPV DNA Cocktail, Biotinylated, Dako) and by PCR, using the SPF_{10}-DEIA-$LiPA_{25}$ (Labo Bio-Medical Products, Rijswijk, Netherlands). RNaseP/PhHV qPCR was used to evaluate DNA quality and PCR inhibition.
Results: Eight tumors were small cell and four were large cell HGNEC. P16 was strong and diffusely positive in 100% of the neoplastic cells in 9/12 cases and had patchy positivity in 3/12 cases. The positivity of the remaining markers was as follows: cytokeratin7 5/12, cytokeratin20 6/12, synaptophysin 10/12, chromogranin A 9/12, CD56 11/12, neurofilament 11/12, neuron-specific enolase 10/12, TTF1 1/12, p53 11/12, and p63 2/12. All tumors had a proliferative index (Ki67) over 40%. HPV in situ hybridization and PCR were negative in all cases.
Conclusions: HGNEC of the head and neck frequently show a strong, diffuse positivity for p16, but they are not associated with HPV-infection. This must be considered in order to avoid the potential diagnostic pitfall with HPV-associated squamous cell carcinoma, a neoplasm with a better prognosis.

1263 BRAF Mutation Analysis in Primary Mucosal Melanoma of the Head and Neck – A Multi-Institutional Analysis of 66 Cases

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Background: Primary mucosal melanoma is a highly aggressive rare disease of unknown etiology, unlike its cutaneous counterpart in which sun exposure is generally accepted as a major causative factor. Approximately 50% of cutaneous melanomas harbor BRAF mutations involved in the MAP kinase/ERK-signaling pathway. This becomes the oncologic target for a selective and potent inhibitor of mutant BRAF, vemurafenib (Zelboraf). However, BRAF mutation has not been extensively studied in the primary mucosal melanoma of the head and neck primarily due to its low incidence comparing with the cutaneous melanoma.
Design: A multi-institutional collaboration is conducted to collect all primary mucosal melanomas of the head and neck. Unstained slides (5 μm) were cut from the formalin-fixed and paraffin-embedded tissue blocks. All sections contained more than 50% of tumor. BRAF mutation status was analyzed by FDA approved Cobas 4800 BRAF V600 Mutation Test kit. Clinical and pathological parameters were also analyzed.
Results: Seventy-two patients were identified during April 2000 – December 2011. The diagnoses were confirmed on H&E with or without immunohistochemical studies by four pathologists independently (GC, LW, HL, and QY). Invalid results were obtained in six patients after repeat and extraction, and therefore were excluded from the study. In four of the six cases with invalid results, melanin pigments were abundant covering over 50% of the tumor. Among 66 patients with valid results, there were 29 men and 37 women with an average age of 65.2 years (range 39–97 years). The anatomic sites included sinonasal/oral cavity in 53 patients and various sites within the eyes (excluding cutaneous eyelid) in 13 patients. BRAF V600E mutation in exon 15 was identified in 3 patients (4.5%), one of conjunctiva, one of cornea, and one of nasal cavity.
Conclusions: Unlike in cutaneous melanoma, BRAF mutation is much less frequently detected in primary mucosal melanoma of the head and neck (4.5%). This indicates that different etiologic pathways play a role in the pathogenesis of these tumors. The current personalized targeted therapy with vemurafenib (Zelboraf) may not be as effective for this rare deadly disease. Other possible molecular targets in mucosal melanoma need to be investigated.

1264 Comparison and Clinical Validation of Three ERCC1 Antibodies in Head and Neck Squamous Cell Carcinoma (HNSCC)

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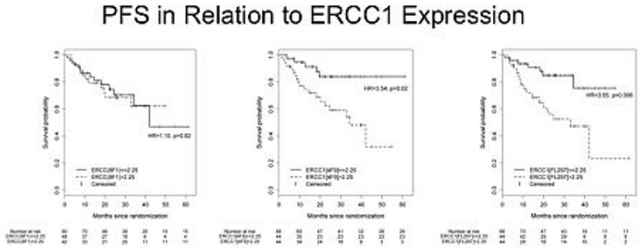
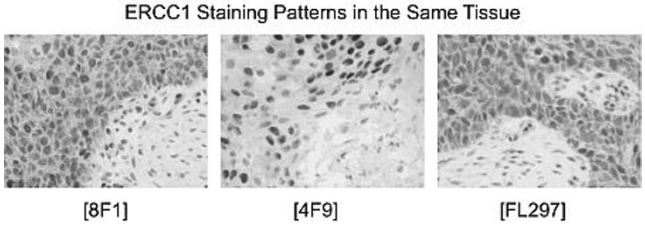
Background: The DNA repair endonuclease excisional cross-complementing group 1 (ERCC1) protein is crucial for repair of interstrand crosslinks induced by platinum chemotherapy. ERCC1 expression predicts clinical benefit from platinum chemotherapy in non-small cell lung cancer and HNSCC, but the specificity of the [8F1] antibody used in the original studies has been questioned. Because new reagents have become available, we evaluated three ERCC1 antibodies for prognostic utility and ease of interpretation.
Design: Optimized immunohistochemistry (IHC) conditions were established against controls for Neomarkers [8F1], OriGene [4F9], and Santa Cruz [FL297] antibodies. IHC was performed on 90 pre-treatment biopsy samples in patients with HNSCC subsequently treated with cisplatin-radiotherapy. ERCC1 expression was described by H-score (weighted percentage of cells demonstrating nuclear staining x staining intensity). The primary endpoint was ability to predict progression free survival (PFS) with a secondary endpoint of qualitative ease of interpretation.
Results:

ERCC1 Expression by Antibody

Antibody	N	Decreased/Normal (H<2.25)	Increased (H>2.25)
[8F1]	90	48 (53%)	42 (47%)
[4F9]	88	40 (46%)	48 (54%)
[FL297]	88	48 (55%)	40 (45%)



The [4F9] antibody demonstrates the crispest nuclear staining.



Conclusions: We found no correlation between [8F1] expression and PFS, but decreased expression by [4F9] and [FL297] antibodies correlated with better PFS, consistent with previous reports that ERCC1 predicts platinum benefit in HNSCC. Nuclear reactivity was the critical interpretive endpoint. The crisp nuclear reactivity of the [4F9] antibody makes it easiest to interpret; thus, it may be preferred for clinical development.

1265 PD1-Infiltrating T Cells: A Good Prognostic Biomarker in HPV Associated Head and Neck Cancer

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Background: The mechanisms underlying the more favorable outcome of HPV-associated head and neck cancer have not been elucidated. This better prognosis may reflect intrinsic features of HPV infected tumor cells or differences in host immune response.

Design: We compared the tumor microenvironment of 32 HPV-positive and 32 HPV-negative head and neck cancers focusing on immunosuppressive cells (regulatory $Foxp3^+$ T cells and PD-1^+T cells). These two groups were matched for various parameters Double immunofluroescence staining and multiparametric cytometry were selected to quantitate immune cells in tumor.

Results: We found that HPV-associated head and neck cancers were associated with a better prognosis compared to HPV negative tumors. They were more heavily infiltrated by regulatory T cells and PD-1 expressing T cells. Surprisingly, levels of PD-1^+T cell infiltration were positively correlated with a favorable clinical outcome in HPV-associated head and neck cancers. To explain this paradoxical result, we showed that these PD-1^+T cells expressed markers of activation and were functional *in vitro* after blockade of the PD-1-PDL-1 axis. One half of PD-1 positive tumor-infiltrating T cells did not express Tim-3 and may represent indeed activated T cells. In addition, in mice, we showed that cancer vaccine increased PD-1 on T cells and concomitantly induced tumor regression. PD-1 blockade synergized with vaccine efficacy.

Conclusions: This study therefore revisits the significance of PD-1-infiltrating T cells in cancer. We provide arguments showing that PD-1 detection could reflect a past anti-tumor immune response, which could be reactivated by PD-1/PD-L1 blockade.

1266 Analysis of PLAG1 & HMGA2 Rearrangement in Salivary Duct Carcinoma and an Examination of the Role of Precursor Lesions

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Background: Salivary duct carcinoma (SDC) is amongst the most common tumors arising in pleomorphic adenoma (PA). Other putative precursors, including low grade cribriform cystadenocarcinoma (LGCCC) and high grade ductal carcinoma in-situ (DCIS) are more controversial. The majority of SDC appear to arise de novo. Recently DCIS was implicated in the majority of SDC. Some LGCCC show microinvasion but only rare examples have a widely invasive component. PA are known to have rearrangement of the PLAG1 or HMGA2 genes in 50-70% of cases. This has been shown to be retained in carcinoma ex PA. A study of PLAG1 and HMGA2 genes in SDC with a variety of putative precursors has not been performed to date.

Design: A TMA was built with duplicate cores of 44 SDC. All cases were reviewed for potential PA and intraductal lesions and were stained with SMA, CK14 and p63. Custom BAC probes were used to evaluate PLAG1 and HMGA2 genes by FISH. A case was considered positive when >20% of cells had a split signal indicating rearrangement of the respective gene.

Results: The patients ranged from 33-86 years old (mean 66.8). SDC ex PA was present in 8 cases. An additional 10 cases had an "obsolete" hyalinized nodule, suspicious for PA. Six SDCs arose in association with a LGCCC-like component. The remaining 20 cases were de novo SDC. A total of 10 cases had PLAG1 (22.7%) and 8 had HMGA2 (18.2%) rearrangment/amplification, respectively. One showed both genes involved. Of these 17 positive cases there were 4 definitive SDC ex PA, 8 SDC with a hyalinized nodule and 5 apparent de novo SDC. There were 6 FISH negative SDC ex PA. A total of 23 SDC ex PA were therefore present in the cohort (52.3%). All 3 cases with testable PA and SDC components were FISH positive in both elements. All 6 SDC ex LGCCC were FISH negative. Myoepithelial staining was present around all LGCCC-like components. It also demonstrated DCIS in 17 other cases. Of these, 11 were present in SDC ex PA or FISH positive apparent de novo SDC. These cases are interpreted as "cancerization" of ducts as it is unlikely to represent a precursor in the presence of a PA. Only 6 FISH negative apparent de novo SDCs showed DCIS (13.6% of the cohort). Pure de novo SDC represented 7 cases (15.9% of the cohort).

Conclusions: A large proportion of SDC arise in PA, which are either visible, show an "obsolete" hyalinized nodule or no trace of PA. A small proportion of SDC arise in LGCCC. Most cases showing DCIS represent cancerization but a minority of cases may show true DCIS.

1267 Poorly Differentiated Oncocytic (Hurthle Cell) Follicular Carcinoma: An Institutional Experience

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Background: According to Turin criteria, poorly differentiated thyroid carcinoma (PDTC) is defined based on growth pattern (solid and trabecular) and high grade features (nuclear pleomorphism, mitoses, and coagulative tumor necrosis). However, the cases reviewed in that study did not include oncocytic follicular carcinoma/Hurthle cell carcinoma (OFC/HCC). OFC/HCC is considered an oncocytic variant (OV) of follicular carcinoma characterized by large size (majority > 4.0 cm) tumors occurring at older age, frequent angioinvasion and distant metastases. In this study, we report our institutional experience with 15 cases of OV-PDTC.

Design: We examined a cohort of 324 cases of OFC/HCC (institutional pathology and consultation files searched from 1/2004 to 7/2012) and identified 15 cases (12 primaries and 3 neck recurrence) of OV-PDTC based on Turin criteria. Histologic slides were reviewed in all cases. Clinical follow-up was obtained from electronic medical records.

Results: The 15 cases of OV-PDTC occurred in 7 male and 8 female patients with average age of 70 yrs (range form 44 to 84 yrs). The primary size was available in 11 cases and 80% were larger than 4.0 cm (average 4.5 cm). In the primary thyroid tumors examined, 6 were encapsulated and 6 were widely invasive; lymph node metastases were seen in 3 and distant metastases in two patients at the time of thyroid resection. Extensive vascular invasion (5-15 foci) was noted in all cases. All tumors showed an admixture of oncocytic or Hurthle cells arranged on solid and trabecular growth pattern and aggregates of cells of small size with minimal eosinophilic cytoplasm; comprising 10-20% of the entire tumor mass. Immunohistochemistry was performed in 7 cases and the poorly differentiated component demonstrated minimal to weak staining for thyroglobulin. Clinical follow-up was available in 11 cases and ranged from 6 to 120 months (average 41 months). Distant metastases (lung, liver, brain, and bone) to one or more sites were seen in 9/11 (82%) patients; 2 (18%) of them had lung metastases at the time of thyroid surgery and 4 patients (36%) subsequently developed cervical lymph node metastases. Two patients had cervical lymph node recurrence only. Two patients died of the disease and 5 are alive either with tumor or free of tumor. The remaining patients are lost to follow-up.

Conclusions: The OV-PDTC is a distinct entity which can be identified on the basis of Turin criteria and the presence of a distinct "small cell" component. It is frequently associated with regional recurrence, distant metastases and can lead to tumor related demise.

1268 Salivary Adenoid Cystic Carcinoma: Something out of Notching?

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Background: Notch signaling is an important mediator of stem cell biology, tumor formation, angiogenesis and cell fate decision. There are 4 Notch receptors (Notch 1-4) and 5 ligands (Jagged-1 and -2,Delta-like (Dll)-1, -3 and -4). Dysregulation of Notch pathway play a crucial role in breast cancer oncogenesis and it could serve as a target for treatment. Little is known about the Notch pathway in salivary adenoid cystic carcinoma (AdCC). To understand the role of Notch in AdCC, we have undertaken a detailed expression analysis of various Notch receptors and ligands in these tumors.

Design: Archival blocks of 199 AdCC were used for this study, with TMA constructed. Immunohistochemical expression of Notch-1, Notch-2, Notch-4, Jagged-1, Delta were analyzed using mouse monoclonal antibodies. Correlations between Notch receptors/ligands expression and clinical and histological parameters were assessed by Pearson's Chi-and M-L Chi-square tests. Survival curves were generated using the Kaplan-Meier method and statistical differences by log rank test.

Results: We report a general increase in the expression levels of Notch-1, Notch-2, Notch-4, Jagged-1, Delta proteins in AdCC (Table 1), while weakly/ undetectable levels in normal tissues.

Markers expression in AdCC

Marker	AdCC (-)ve	AdCC (+)ve
Notch-1	26/175 (15%)	149/175 (85%)
Notch-4	24/164 (15%)	149/164 (85%)
Notch-2	106/161 (66%)	55/161 (34%)
Jagged-1	69/162 (43%)	93/162 (57%)
DLL1/Delta	32/161 (20%)	29/161 (80%)

Subcellular localization for Notch-1, Notch-2 and Notch-4 was predominantly nuclear and cytoplasmic, while cytoplasmic/ membranous for Jagged-1 and Delta-1 ligands. No significant statistical correlation were found between individual markers and clinico-pathological parameters. The combination of Jagged 1 and Notch 2 showed a p value of 0.036 for vital status at last contact within 12 years of presentation; the worst survival was associated with having one of the markers and the best survival was in the group

that had both of them. This may be explained that in a setting that has a ligand (Jagged-1) and Notch-2 together, there is a tumor suppressive effect. On the other hand, Jagged-1 (and Notch- 2) alone may possess tumor-promoting functions and would account for a poor prognostic significance.
Conclusions: High expression of Notch receptors and ligands in AdCC, places Notch signaling as a key player in AdCC pathogenesis. Our data provides the first direct evidence for a relationship between Jagged-1/Notch-2 co-expression and better overall patient survival in human salivary adenoid cystic carcinoma. Targeting the Notch signaling pathway could provide therapeutic benefits.

1269 Comparison of p63 and p40 (ΔNp63) in the Diagnosis of Head and Neck Sarcomatoid Carcinoma

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Background: Sarcomatoid carcinoma (SC) is a variant of head and neck squamous cell carcinoma that includes a mesenchymal-like spindle cell component. SCs are sometimes edematous and hypocellular, mimicking a reactive fibroblastic process. Then again, they may be hypercellular and resemble a true sarcoma. Cytokeratin immunohistochemistry (IHC) is often not helpful in resolving the differential diagnosis: SCs are often cytokeratin negative; and conversely, reactive and neoplastic mesenchymal proliferations may be focally cytokeratin positive. P63 expression is often used as a marker of squamous differentiation, but its usefulness in diagnosing SC is offset by expression in some soft tissue tumors and uncertainty regarding p63 expression in reactive stromal proliferations. P40 is an antibody that recognizes ΔNp63 – a p63 isoform that is highly specific for squamous differentiation. As such, p40 IHC could enhance the diagnosis of SCs.
Design: IHC for both p63 and p40 was performed on 35 head and neck SCs. All of the SCs were biphasic tumors that included a component of conventional squamous cell carcinoma. In addition, 40 reactive stromal proliferations and 139 soft tissue neoplasms were tested. IHC was performed on whole slides (n=110) or tissue microarrays (n=104).
Results: The results are summarized in Table 1. Of the 35 SCs, p63 staining was noted in 20 (57%) cases, and p40 staining was present in 18 (51%) cases. Staining was focal (i.e., ≤5% cells) for p63 and p40 in 5 and 3 cases, respectively. P63 staining was also observed in 40/139 (29%) soft tissue neoplasms and in 12/40 (30%) reactive stromal proliferations. In contrast, p40 staining was observed in only 5/139 (4%) soft tissue neoplasms and 0/40 reactive stromal proliferations.

Table 1

	p63 (%)	p40 (%)
Sarcomatoid carcinoma	20/35 (57)	18/35 (51)
Reactive stromal proliferations	12/40 (30)	0/40 (0)
Soft tissue neoplasms	40/139 (29)	5/139 (4)
Angiosarcoma	3/10 (30)	1/10 (10)
Cellular schwannoma	2/3 (67)	0/3 (0)
Epithelioid sarcoma	1/14 (14)	1/14 (14)
IMT	2/13 (15)	1/13 (8)
Kaposi sarcoma	1/26 (4)	0/26 (0)
LG fibromyxoid sarcoma	5/9 (56)	0/9 (0)
Leiomyosarcoma	5/15 (33)	0/15 (0)
MFH	13/33 (39)	1/33 (3)
MPNST	2/5 (40)	0/5 (0)
Rhabdomyosarcoma	4/9 (44)	1/9 (11)
Synovial sarcoma	3/8 (38)	1/8 (13)

Conclusions: Compared to p63, p40 expression is less likely to be encountered in reactive and neoplastic mesenchymal lesions and thus represents a more specific marker of SC. When it comes to the diagnostic interpretation of spindle cell lesions of the head and neck, p40 staining provides compelling evidence of SC while p63 staining must be viewed with more caution.

1270 Utility of Mammaglobin Immunostaining in the Diagnosis of Mammary Analogue Secretory Carcinoma of the Salivary Glands

JA Bishop, R Yonescu, D Batista, WH Westra. Johns Hopkins Hospital, Baltimore, MD.
Background: Mammary analogue secretory carcinoma (MASC) is a recently described salivary gland neoplasm that is defined by *ETV6-NTRK3* gene fusion. The morphologic features of MASC have been previously described, but they are not entirely specific and overlap with other salivary gland tumors. As a result, diagnosing MASC currently rests on documenting *ETV6* rearrangement, but performing the required molecular tests is not feasible for most surgical pathology laboratories. Mammaglobin immunohistochemistry, on the other hand, is widely available, and has been reported to be frequently positive in MASC. This antibody has yet to be tested against molecular methods on a large number of diverse salivary gland tumors.
Design: Fifteen MASCs confirmed by *ETV6* FISH were tested with mammaglobin immunohistochemistry (clone 304-1A5; DAKO GenPoint, Carpinteria, CA; 1:200 dilution). In addition, tissue microarrays containing a diverse collection of FISH-proven *ETV6* wild-type salivary gland neoplasms (44 adenoid cystic carcinomas, 33 pleomorphic adenomas, 18 mucoepidermoid carcinomas, 8 acinic cell carcinomas, 4 adenocarcinomas not otherwise specified, 3 polymorphous low grade adenocarcinomas, and 2 salivary duct carcinomas) were also tested.
Results: All 15 (100%) MASCs were strongly and diffusely mammaglobin-positive. However, mammaglobin staining was also seen in 2/3 (67%) polymorphous low grade adenocarcinomas, 2/2 (100%) salivary duct carcinomas, 1/8 (13%) acinic cell carcinomas, 2/18 (11%) mucoepidermoid carcinomas, and 2/33 (6%) pleomorphic adenomas. Overall, mammaglobin immunostaining was 100% sensitive and 92% specific for MASC.
Conclusions: Mammaglobin immunostaining is highly sensitive for the diagnosis of MASC. However, caution must be exercised in using mammaglobin immunohistochemistry in place of *ETV6* molecular testing since other salivary gland tumors may be positive. Accordingly, mammaglobin staining should be carefully interpreted in the context of a tumor's histologic features.

1271 Most Non-Parotid "Acinic Cell Carcinomas" Represent Mammary Analogue Secretory Carcinomas

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Background: Acinic cell carcinoma (ACC) is a low grade salivary gland malignancy characterized by serous acinar differentiation. Most ACCs arise in the parotid gland, but ACCs in non-parotid sites, where serous acini are less abundant, have been reported. Given the recent discovery of mammary analogue secretory carcinoma (MASC) – a salivary malignancy defined by *ETV6-NTRK3* gene fusion that histologically mimics ACC – a retrospective re-evaluation of non-parotid ACCs is warranted.
Design: The surgical pathology archives of The Johns Hopkins Hospital were searched for all ACCs arising outside of the parotid gland. For each case, the histologic slides were closely reviewed and immunohistochemistry (mammaglobin, S100 protein) was performed. A confirmatory *ETV6* break-apart FISH assay was attempted on each tumor. Demographic and clinical outcomes data were extracted from patient medical records.
Results: Fourteen carcinomas from non-parotid sites (11 oral, 2 submandibular, 1 sinonasal) diagnosed as ACC were identified. *ETV6* FISH analysis showed that 11 of 14 (79%) cases, including 9 of 11 (82%) oral cavity tumors and 2 of 2 (100%) submandibular tumors, were actually MASCs. The MASCs exhibited uniform eosinophilic cells with vacuolated cytoplasm, microcystic and papillary architecture, intraluminal secretions, and frequent cyst formation. All three FISH-negative carcinomas exhibited overt serous acinar differentiation in the form of basophilic cytoplasmic granules. Immunohistochemistry for mammaglobin and S100 was strongly positive in all 11 MASCs, but negative in the 3 true ACCs. The MASCs occurred in 7 women and 4 men ranging from 20-86 years (mean, 56 years), and usually presented as painless masses. Follow up information was available for 10 of 11 MASCs; only one tumor locally recurred, and no tumors metastasized.
Conclusions: While ACC does rarely arise outside the parotid gland, most non-parotid salivary gland carcinomas with features of ACC are actually MASCs. As a result, the diagnosis of ACC outside the parotid gland should be reserved for cases that exhibit basophilic cytoplasmic granules. Immunohistochemical stains for mammaglobin and S100 are helpful adjunctive tests, and *ETV6* FISH is confirmatory. Like ACCs, extra-parotid MASCs appear to be low grade with limited capacity for aggressive clinical behavior, thus diminishing the impact of tumor misclassification.

1272 *SOX2* as a Potential Therapeutic Target in Squamous Cell Carcinoma of the Head and Neck

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Background: Recently, we identified *SOX2* as lineage specific oncogene in squamous cell carcinomas of the lung (LSCC). Since LSCC are morphologically and clinically related to head and neck squamous cell carcinomas (HNSCC), the aim of our study was to assess whether *SOX2* amplification/over expression occurs in HNSCCs and if *SOX2* could serve as a potential target for HNSCCs.
Design: We assembled a cohort of 503 patients with HNSCCs, including 260 metastases and 141 recurrences. All samples were assessed for *SOX2* amplification by FISH and SOX2 expression by IHC. HPV status was detected by PCR-ELISA. Molecular parameters were correlated with each other and clinico-pathological data. Furthermore, SOX2 expression was modulated in the SCC25 HNSCC cell line via lentiviral vectors carrying shSOX2 and SOX2 over expression constructs to investigate the role of SOX2 in apoptosis resistance.
Results: 20% of primary HNSCCs displayed a *SOX2* amplification and results in SOX2 over expression. In almost all cases, metastatic and recurrent tumor samples shared the same *SOX2* amplification status as the corresponding primary tumor. *SOX2* amplification/over expression was mutually exclusive with HPV infection. Amplification/over expression of *SOX2* was significantly associated with pathological parameters of poor outcome. Efficient modulation of *SOX2* gene and protein expression was obtained in the SCC25 cell line following treatment with lentiviral vectors. Inhibition of SOX2 expression enhanced apoptosis sensitivity of SCC25 cells to apoptosis-inducing agents while SOX2 over expression had the converse effect, inducing therapy resistance.
Conclusions: *SOX2* amplification frequently occurs in primary HNSCC and is a clonal event in metastatic disease. Furthermore, *SOX2* amplification/over expression is associated with worse prognosis, possibly related to enhanced therapy resistance of SOX2 expressing cells. Targeting *SOX2* and related molecular pathways may enhance therapy efficacy in HNSCC. However, *SOX2*-amplified tumors could potentially comprise a subset of HNSCC against which may hold therapeutic efficacy.

1273 Promoter Hypermethylation of the EGFR-Signaling Genes in HNSCC

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Background: Epidermal Growth Factor Receptor (EGFR) signaling pathway appears critically important in the pathogenesis of head and neck squamous cell carcinomas (HNSCC). For the above reason, there has been immense interest in exploring targeted

therapies aiming at EGFR, using either EGFR tyrosine kinase inhibitors (EGFR-TKI) or anti-EGFR monoclonal antibodies (EGFR-mab) for HNSCC. Characterization of epigenetic regulation of EGFR signaling genes (EGFR, E-Cadherin, PTEN and SCOS1) may lead to more effective treatment of HNSCC using EGFR-targeted therapies. Promoter hypermethylation has long been established as one epigenetic mechanism for gene silencing of tumor suppressior genes in human cancer.
Design: Promoter sequences of 4 EGFR signaling genes (EGFR, E-cadherin, PTEN and SCOS1) were analyzed for the presence or absence of CPG island using CpG Plot software and primers were designed for methylation-specific PCR (MSP) to detect promoter hypermethylation in these 4 genes. Genomic DNA samples were extracted from 6 cultured HNSCC cell lines, modified with sodium bisophite, followed by PCR amplification and agarose gel electrophoresis.
Results: All 4 EGFR signaling genes (EGFR, E-cadherin, PTEN and SCOS1) contain a CpG island in their respective promoter sequence, ranging from 233 to 602 basepairs. Following MSP, We detected promoter hypermethylation in 6 of 6 (100%) HNSCC cell lines for the SOC1 gene and in 1 of 6 (16%) cancer cell lines for the ECAD1 gene. No promoter methylation was detected for the EGFR and PTEN genes in any of the cell lines.
Conclusions: The presence of promoter hypermethylation in two important genes (SOC1 and ECAD1) of the EGFR-signaling pathway in HNSCC cell lines supports that the expression of these genes can be silenced by promoter hypermethylation in HNSCC and that DNA Demethylating agent, such as 5-azacytidine, may be use to improve the response of HNSCC to EGFR-targeted therapies by modulating the expression levels of some key EGFR-signaling genes.

1274 Chronic Sclerosing Sialadenitis: A Morphologic Diagnosis with Clinicopathologic Correlation

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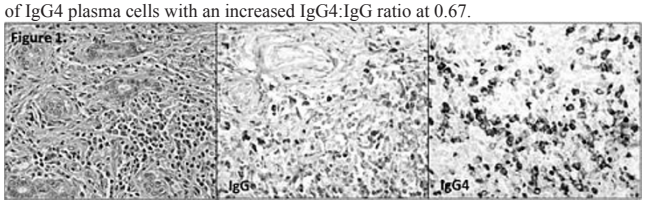
Background: Chronic sclerosing sialadenitis is an uncommon cause of salivary gland enlargement with a distinctive morphologic appearance and an increased number of IgG4 plasma cells. We report a case of chronic sclerosing sialadenitis and compare the morphology of chronic sclerosing sialadenitis to other cases of chronic sialadenitis.
Design: 53 cases of chronic sialadenitis were examined and classified into 5 categories: minimal stromal fibrosis; mild stromal fibrosis; moderate to severe stromal fibrosis; chronic sclerosing sialadenitis; and lymphoepithelial sialadenitis. Immunohistochemical staining for IgG and IgG4 was performed on the chronic sclerosing sialadenitis case.
Results: Twelve cases had minimal stromal fibrosis; 22 cases had mild stromal fibrosis; 15 cases had moderate to severe stromal fibrosis; 1 case had chronic sclerosing sialadenitis; and 3 cases were classified as lymphoepithelial sialadenitis. The chronic sclerosing sialadenitis case demonstrated a characteristic intralobular, hypercellular fibrosis with preservation of the lobular architecture and a dense lymphoplasmacytic infiltrate. Immunohistochemical staining for IgG and IgG4 revealed an increased number of IgG4 plasma cells with an increased IgG4:IgG ratio at 0.67.



In comparison, cases of chronic sialadenitis with moderate to severe stromal fibrosis showed paucicellular fibrosis more pronounced around the ducts. Periductal fibrosis was present in all 15 of the cases, but only 8 cases had intralobular fibrosis. Results are summarized in Table 1.

Table 1. Clinical and Morphologic Findings

	CS with Minimal Fibrosis (n=12)	CS with Mild Fibrosis (n=22)	CS with Moderate to Severe Fibrosis (n=15)	Chronic Sclerosing Sialadenitis (n=1)	Lymphoepithelial Sialadenitis (n=3)
Duct Dilatation	41.6% (5/12)	81.8% (18/22)	86.6% (13/15)	100% (1/1)	66.6% (2/3)
Periductal Fibrosis	50.0% (6/12)	90.9% (20/22)	100% (15/15)	100% (1/1)	100% (3/3)
Intralobular Fibrosis	0% (0/0)	4.5% (1/22)	53.3% (8/15)	100% (0/0)	0% (0/0)

CS - Chronic Sialadenitis

Conclusions: Chronic sclerosing sialadenitis has a distinct morphologic appearance distinguishing this lesion from other inflammatory lesions of the salivary gland. The most prominent of which is the characteristic hypercellular, intralobular stromal fibrosis.

1275 Conventional (Keratinizing) Squamous Cell Carcinoma of the Oropharynx – Extensive p16 Expression Is Associated with Improved Survival

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Background: Tumor HPV status is a strong and independent prognostic factor for survival among patients with oropharyngeal squamous cell carcinomas (SCCs). HPV positive SCC are strongly associated with the nonkeratinizing (NK) phenotype in the oropharynx, which are associated with better survival compared to keratinizing squamous cell carcinomas (K SCCs), despite early nodal metastatic spread. Transcriptionally-active HPV has also been detected in a minority of K SCCs, but it has never been determined if this morphology or the HPV status determines patient outcomes. This study compares clinical outcomes among patients with HPV-positive and negative KSCC.
Design: Materials from patients with oropharyngeal K SCC who were enrolled in an IRB approved radiotherapy database from 1997 to 2011 were retreived from pathology files. All patients received either definitive or postoperative intensity-modulated radiation therapy (IMRT). Cases with any nonkeratinizing features, characterized by nests of oval to spindled cells with hyperchromatic nuclei, scant cytoplasm and indistinct cell borders, were excluded, as were other specific histologic SCC variants. DNA in-situ hybridization (ISH) for high-risk HPV and immunohistochemistry for p16, a surrogate marker for HPV-related carcinomas, were performed. p16 was deemed positive if >75% of cells had nuclear and cytoplasmic staining.
Results: There were 54 cases, 7 (13%) of which were extensively p16 positive. DNA ISH was positive in only 3/6 (50%) of these p16+ patients. Four of the 7 (57%) were treated with definitive IMRT, and 3 with surgery + IMRT. None of these 7 patients developed recurrent disease at 18 to 143 months of follow up. Conversely, 19 of the remaining 47 patients (40%) with negative or partial p16 staining developed recurrent disease. The difference was statistically significant ($p = 0.04$). Kaplan-Meier survival analysis showed significantly better overall survival in the p16 positive group (mean survival 124 months) compare to p16 negative/partial group (mean survival 40 months) (p=0.024).
Conclusions: Diffuse p16 expression in more than 75% of cells identified a small group of patients (13%) with significantly better prognosis. As such, it appears that HPV status is an important prognostic marker to take into account, even in patients with keratinizing squamous cell carcinoma.

1276 Presence and Distribution of Plasmacytoid Dendritic Cells in Head and Neck Lymphoepithelial Carcinoma. Possible Role in the Pathogenesis and Potential Therapeutic Target

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Background: Plasmacytoid dendritic cells (PDCs) are essential part of the immune system through the production of interferon-alpha (IFNα) and antigen presentation. They can be activated by stimulation of "toll-like" receptors (TLRs). The TLR-9 stimulation induces production of IFNα, chemokines and cytokines that promote recruitment of NK cells, activated T lymphocytes and macrophages. It has been suggested that in tumors of viral etiology some components of microorganism interact with elements of tumoral microenvironment and induce TLRs dysfunction. Thus, the limited response of PDCs contributes to reduction of the immune response during chronic viral infections and oncogenesis. The role of PDCs in lymphoepithelial carcinoma (LEC) has not been studied previously.
Design: We reviewed the Surgical Pathology archives of the National Cancer Institute to identify and select those cases diagnosed as H&N LEC. A control group (non-neoplastic lymphoid tissue) was also selected for comparison. Tissue microarrays and immunohistochemistry for CD123, CD4, CD56 TLR-9 and LMP-1, plus in situ hybridization (EBER) were performed. Clinical records were reviewed and data were analyzed through descriptive statistics.
Results: 32 patients with LEC, 22 men and 10 women with a mean age of 49.9 yrs were included. In LEC group, PDCs were identified primarily in the transition between the tumor nests and lymphoid cells. In the control cases, PDCs where found in the interfollicular space and around post-capillary venules. TLR-9 expression by PDCs was observed only in 3 cases of LEC. EBV infection was confirmed by EBER in 18 cases.
Conclusions: This is the first study exploring the role of PDCs in the pathogenesis of LEC. It was observed decreased expression of TLR-9, suggesting dysfunction of these receptors. This limited response of PDCs could contribute to reduce antimicrobial and antitumor immune response, participating in oncogenesis. Therefore, they represent a potential target for new therapeutic strategies based on TLR agonists. At present, drugs with this function have been developed. CpG oligodeoxynucleotides (ODNs) have been tested with promising results as adjuvants in combination with other agents in some tumors. Our results suggest that TLR agonists could be used as adjunctive target therapy in the management of LEC.

1277 Does Close Margin Alone Warrant Postoperative Adjuvant Therapy in Oral Squamous Cell Carcinoma?

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Background: There are major variations between centers regarding postoperative adjuvant therapy for adverse factors in oral squamous cell carcinoma (SCC) with general consensus that close margins alone warrant adjuvant therapy. Our practice has not been to recommend adjuvant therapy for close but uninvolved margins alone. This study examines the implications of close margins in oral SCC using evidence based methods and assesses whether our local failure rate in this cohort was acceptable.
Design: The English literature (1976-2012) was systematically reviewed for best evidence of prognostic implications of close margins in oral SCC for meta-analysis (CMA, Biostat, NJ, USA). A retrospective, single arm, non-inferiority study with local failure as the primary end point and maximum 'acceptable' pre-specified local failure rate of 15% at 5 years (y) as not requiring adjuvant therapy was designed to evaluate clinical feasibility. Margin proximity, tumor differentiation, thickness, patterns of invasion (POI), perineural and lymphovascular invasion (PNI, LVI) were reviewed. Statistical analyses included power analysis, sample size estimation, Log-rank test and Kaplan-Meier survival curves (Stata 11, TX, USA).
Results: Twenty five retrospective (level III) studies reported close but uninvolved margins in 4833 oral SCC patients. Meta-analysis showed higher risk of adverse events in patients with close margins (OR:1.803, 95%CI:1.4-2.3, p 0.00). Funnel plot showed moderate data heterogeneity and asymmetry(I^2:61). Our cohort included 144 patients (M:F=79:65, median age 64y, mean follow up 3.3y) with surgery alone, with histological close (<5mm) margins. The sample size needed to detect 10% detriment

in local control (LC) from 100% with 80% power (p=0.05) was 80 patients with 5y follow up and no censoring. LC in all patients with surgery alone was 91% (95%CI 82%–95%) at 5y. Worse LC was not seen with ordered stratification of close margin. The 5y LC rates for having 0, 1, 2, 3 and 4 additional adverse features of tumor thickness >4mm, infiltrative POI, PNI and buccal mucosa sub-site were 100%, 96%, 83%, and 71%, respectively (p=0.004).

Conclusions: Available best evidence suggests that close margins carry higher risk of adverse events with significant data heterogeneity, chiefly due to variable definitions of close margins, follow up and end points. Our findings indicate that surgery alone without adjuvant therapy offers acceptable LC if the only adverse feature is close margin.

1278 Distinct MiRNA Expression Signature in Olfactory Neuroblastoma

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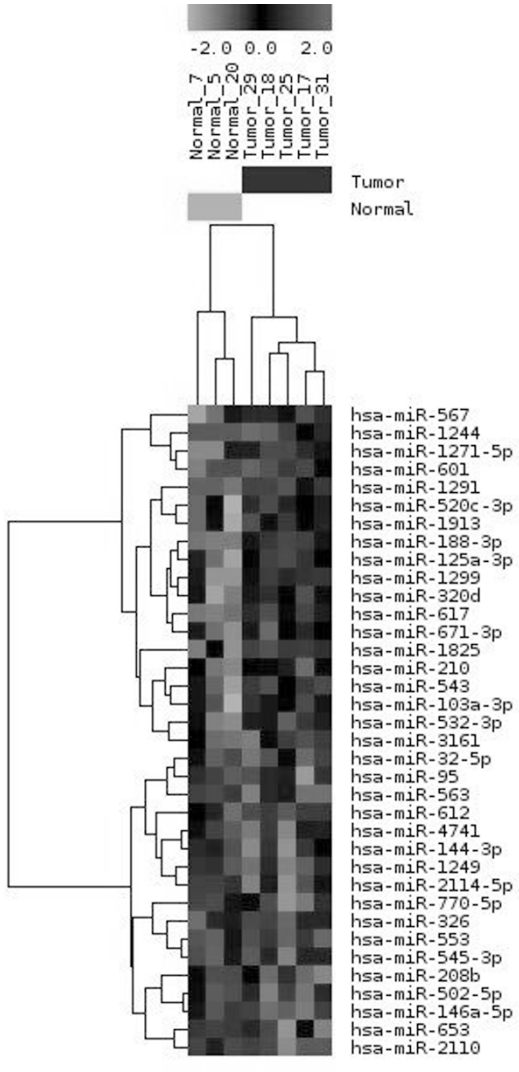
Background: Olfactory neuroblastoma (ONB) is a malignant neuroectodermal tumor that arises from neuroectodermal elements of the olfactory mucosa. Median age for this cancer is 50 years and the 5 year survival is 50 to 66%. MiRNAs have important roles in the posttranscriptional regulation of gene expression. Recently, miRNA profiling has shown that tumors and normal tissues are characterized by specific patterns of miRNA expression, so called signatures that could be used for diagnosis as well as for targeted therapy.

Design: We attempted to identify for the first time a specific miRNA signature of ONB versus normal olfactory neuroectodermal tissue. For this purpose, 5 cases of olfactory neuroblastoma, and 3 cases of normal olfactory neuroectodermal tissue were selected. RNA was analyzed using Nanostring.

Results: We identified 36 microRNAs that were differentially expressed in ONB vs normal tissue. Of 36 genes, 20 were upregulated and 16 were downregulated. 18 of the upregulated genes were expressed more than two times in ONB than in normal tissue whereas 3 of the downregulated genes were 3 times less expressed.

Conclusions: MiRNA profiling of ONB vs normal olfactory neuroectodermal tissue showed a distinct signature represented by 36 different genes that were deregulated. These genes seem to be specific to ONB and differ from the miRNAs identified as deregulated in the literature in adrenal neuroblastomas. That could be represented by a heat map with a "checkered" pattern distinct enough to allow for a specific diagnosis of these tumors.



Of 20 genes that were upregulated, a majority (17) were expressed more than twice compared to the normal control. Of the 18 downregulated genes, 8 were expressed two times less than the normal control. 3 genes were downregulated more than three times than the normal tissue. This specific microRNA signature could be useful for diagnostic purposes, when histology is not conclusive enough to allow for a clear cut diagnosis. Understanding the molecular pathways involved in this type of cancer, with a poor survival rate, will help us design targeted molecular therapies, specifically tailored for the genetic deregulation that is identified.

1279 Investigation of Beta-Catenin Gene and Protein in Ameloblastoma

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Background: Ameloblastoma (AB) is a benign odontogenic tumor with agressive behaviour and high recurrence rates. Wingless Type signaling pathway (WNT) is involved in the development of dental organ and plays a key role by regulating the β-catenin protein proteolysis, which is involved in cell adhesion and proliferation. Thus, WNT signaling pathway in dental development prompted us to study β-catenin gene expression and protein in AB.

Design: Quantitative PCR was performed in 11 cases of AB and 3 cases of non-neoplastic mucosa (NNM) using SYBR™ Green PCR Master Mix to assess the expression of β-catenin gene. Analysis was perfomed according to Pfaffl (2001). Immunohistochemistry for β-catenin protein was performed using EnVision™ System. Immunostaining was evaluated according to location (membrane, nuclear and cytoplasmic) and proportion of positive cells (0, <5%, 1+, 5-25%; 2+, 25-50%, 3+, 50-75%; 4+,> 75%).

Results: A higher expression of β-catenin gene was found in AB compared to NNM, specially in unicystic AB type (p = 0.0173). In NNM, β -catenin protein was observed in membrane of all epitelial cells. Membrane and nuclear immunostainings were more evident in unicystic AB comparing to solid tumor (p = 0.0004 and p=0.047, respectively).

Conclusions: The expression of β-catenin gene in ABs suggests the participation of the WNT pathway in the development of this tumor. In solid AB, the loss of membrane expression β-catenin, indicates that this protein could contribute to the greater invasiveness of this histological subtype of AB. In addition, in unicystic AB, the function of cell adhesion appears to be retained, while the nuclear staining of β-catenin suggests that this molecule could be involved in tumor growth.

1280 Salivary Duct Carcinoma: Actionable Somatic Mutations Identified by Massively Parallel Sequencing

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Background: Salivary duct carcinoma (SDC) is a highly aggressive malignant epithelial tumor that comprises about 9% of salivary gland malignancies arising most frequently in the parotid. Most SDC patients present with locally advanced disease, and currently the prognosis is poor with a median survival of 3 years. A comprehensive mutational profiling of SDC is necessary in order to identify actionable genetic alterations and to potentially expand the range of treatment options for SDC patients.

Design: Ten primary surgically resected SDC cases were selected for targeted DNA sequencing of 189 cancer-related genes. Upon the histological review, 40 um of formalin-fixed paraffin embedded (FFPE) tumor and the matching normal tissue sample (available in 8 cases) were subjected for DNA extraction followed by sequencing library construction and hybridization-based capture of 3230 exons and 37 intronic intervals. Deep sequencing was performed, yielding an average coverage of >750X for uniquely-mapping reads. Sequence data were analyzed for single nucleotide variants, small insertions and deletions, fusion genes and amplifications.

Results: Eight of ten (80%) of SDC harbor actionable somatic mutations and/or amplifications including 4 *PIK3CA* mutations (40%), 1 *BRAF* V600E mutation (10%), and 3 cases with *ERRB2* amplifications (30%), one of which also harbors *ERBB2* S310F activating mutation. In three cases, putative loss-of-function mutations were detected in *NF1* gene. Additional study cases are pending deep sequencing.

Conclusions: The majority of SDC cases harbor mutations potentially targetable with currently established therapies. Massively parallel sequencing of a large panel of cancer-related genes is a powerful method for detection of actionable genetic alterations and could be used to select SDC patients eligible for clinical trials. Almost one third of SDC have potentially significant genetic alterations in *NF1* tumor suppressor gene, and further studies are necessary to elucidate the role of *NF1* in the pathogenesis of SDC.

1281 Genetic Changes Associated with Distant Metastasis in HPV-Positive Oropharyngeal Squamous Cell Carcinoma

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Background: The incidence of oropharyngeal squamous cell carcinoma (OPSCC) has been on the rise in the past few decades. While the role of HPV in tumor biology has been extensively studied and is well established, the genetic changes underlying disease progression, which is mostly in the form of distant metastasis, are largely unknown. We sought to determine if DNA sequence mutations could differentiate patients with HPV positive OPSCC who develop distant metastasis (DM) with those who do not (NDM).

Design: Nine cases were selected from an existing large database, all of which were positive for p16 immunohistochemistry and HPV type 16 E6/E7 by RNA in situ hybridization. Five of these had documented DM, and 4 had no disease recurrence (minimum clinical follow up of 4 years). DNA was extracted from formalin-fixed

paraffin-embedded (FFPE) tissue. Exon enrichment was performed using Agilent v3 capture reagents, and DNA was sequenced on a HiSeq 2000 using 2 x 101 bp reads. The resulting data was aligned to the human hg19 reference and variants called using the Genome Analysis Toolkit. Variants preset in dbSNP (version 132) were removed from the analysis.

Results: On average, 55M reads were generated in each case, resulting in >50x average exome coverage. Non-SNP single nucleotide variants (SNVs) were identified in 1260 genes across all cases. There were 220 genes mutated solely in the DM cases and 60 in just the NDM cases. The most likely biologically significant genes within the DM group that were not present in the NDM group were MST1 (4/5; 80%), EPHB2 (2/5; 40%), ASPM (2/5; 40%), FGFR2 (2/5; 40%) and CTDSPL (2/5; 40%). Many of these are tumor suppressor proteins. Non-synonymous mutations in genes previously identified in OPSCC were present in both the DM and NDM cases and included TP53 (3/9; 30%) and PIK3CA (1/9; 11%).

Conclusions: Using whole exome sequencing on formalin fixed tissue, we found several potential novel genes mutated in OSCC patients with DM that were wild type in NDM patients. The function and significance for many of these are not well characterized, particularly for head and neck cancer, but some are established tumor suppressor proteins, and some may be useful as prognostic biomarkers or for future targeted therapies.

1282 Oral Mucosa and Soft Tissue Alterations Associated with Osteonecrosis of the Jaw

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Background: Osteonecrosis of the jaw (ONJ) is a complication of radiation (RON) and bisphosphonate (BON) therapy for head and neck cancer, plasma cell neoplasms (PCN) or bone metastases. We noted epithelial and plasma cells alterations mimicking the underlying disorder and aim to assess their frequency and better describe these morphologic findings.

Design: Patients with an ONJ diagnosis were identified in our database. Charts and histologic slides were reviewed. Mucosal changes, including pseudoepitheliomatous hyperplasia (PEH), cytologic atypia, epithelial islands within the bone marrow space (bone pseudoinvasion-BPI) were recorded. Presence of acute, chronic lymphoplasmacytic infiltrate (LPI) and dense plasma cell infiltrate (PCI) were recorded, the latter when PCs >50% of total LPI. PC features such as hyperchromasia, binucleation, anisocytosis, exocytosis and Russell bodies were also recorded.

Results: There were 36 samples from 32 patients with ONJ, 21 men and 11 women, mean age 59 (range 38-83), 23 had RON and 9 BON. The mandible was involved in 30 (23 RON and 7 BON) and maxilla in 7 (3 RON and 4 BON) patients. Mucosa was seen in 30/36 and bone fragments in 21/36 samples. Underlying disease was head and neck squamous cell carcinoma (HNSCC) in 20, PCN in 5 and other diseases in 7 patients. PEH was present in 24 of 30 cases (80%), 12 from HNSCC patients. Reactive epithelial atypia was seen in 22/30 cases and considered severe in 15 (68%), the latter was associated with erosion and re-epithelialization; severe dysplasia suspected in 2. Residual SCC was present in 2 samples. BPI was present in 11 of 21 (47%) samples, 4 from HNSCC patients. Acute inflammation was seen in 84% and LPI in 94% of cases; of these, 17 (54%) had dense PCI, seen in all samples from PCN patients. Atypical PCs were seen in 23 cases, 6 from PCN patients; in 2, further workup was negative. Hyperchromatic, large PCs with binucleation and Russell budies were frequent but no Dutcher bodies, prominent nucleoli or mitotic figures were present. *Actinomyces-like* organisms were more common in the bone fragments from BON patients (100%, 8/8 samples) when compared to RON patients (54%, 7/13 samples), (p-value: 0.04).

Conclusions: PEH and bone pseudoinvasion are common neoplastic mimickers of mucosa in ONJ samples, the latter change heretofore not described. Atypical plasma cells are also common in ONJ but histologic examination allows separation from PCN in most cases. Rare cases require additional workup for definitive diagnosis.

1283 Transcriptionally Active HPV Infection and Salivary Adenoid Cystic Carcinomas

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Background: The rate of high-risk Human Papillomavirus (HR-HPV)-mediated carcinogenesis has been steadily increasing over the past three decades with respect to oropharyngeal cancer. The issue of HPV as a potential contributor to salivary malignancies is currently under investigation. We have previously demonstrated transcriptionally active HPV16/18 in 43% of mucoepidermoid carcinomas. HR-HPV has been detected by Boland et al in salivary adenoid cystic carcinomas (ACC) studied by in-situ hybridization. Here, we assess the detection rate of transcriptionally active HPV16/18 in ACC.

Design: 41 patients with confirmed diagnosis of ACC were studied. This is a retrospective cohort from 2004 to 2012, the female to male ratio was 1:1, and mean age was 59 years (range 29-93). Samples were procured from paraffin blocks by sterile technique, tumor selection was morphologically guided to avoid including any overlying squamous mucosa. Nested PCR was performed on the extracted DNA with Gp5+/6+ consensus primers including appropriate internal, positive, and negative controls. All tumors positive by PCR were further studied by nested real-time PCR for type-specific cDNA; RNA was extracted and reverse transcription was performed. DNAase was used to remove any residual DNA. Primer pairs for HPV16E6, HPV16E7, HPV18E6, and HPV18E7 were used and appropriate controls were included. ACC samples were designated as HPV positive only if both concordant type-specific E6 and E7 cDNA sequences were detected.

Results: HPV genome (untyped) was detected in 20/41 (48.8%) of patient samples. HPV16E6/E7 was detected in 12/41 (29.3%) and HPV18E6/E7 was detected in 2/41 (4.9%) of ACC. One ACC was HPV16/18 double positive. Six ACC were consensus primer positive and HPV16/18 negative, suggesting infection by other HPV types; HPV typing in ongoing. No association was seen between HPV status and patient gender, age, and tumor site; there was no association between HPV genome detection and specimen age. However, HPV cDNA was significantly less likely to be detected in specimens prior to 2010, as compared to specimens from 2010 and later ($p = 0.0171$, Fisher's two-tailed exact test).

Conclusions: HPV genome has been detected in almost half of 41 ACC studied by nested PCR using consensus primers; these data do not address the issue of passenger versus driver infection. Transcriptionally active HPV16/18 was detected in almost one third of ACC. RNA degradation may account for the decreased transcript detection rate for older specimens. These data do not establish causality, but suggest a potential for HR-HPV to contribute to ACC-carcinogenesis.

1284 Distinguishing between Dysplasia and Reactive Atypia in Laryngeal Mucosa: Utility of p53, Ki67, CD44 and p16 Immunohistochemistry

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Background: Dysplasia and inflammatory reactive atypia show overlapping histologic features. Distinction between these is of clinical significance and can be particularly difficult in small biopsies with procedural artefacts. This study analyses the differential immunohistochemical staining of p53, Ki67, CD44 and p16 in these entities.

Design: Laryngeal and oropharyngeal biopsies from 2004-2012 were reviewed and immunostained with p53, CD44, Ki67 and p16. All slides were independently reviewed by 2 pathologists. Descriptive statistics and crosstabulations were performed (SPSS 11.5, IL, USA).

Results: There were 121 biopsies from 64 patients (M:F 46:18, median age:73 years) including non-neoplastic entities (NN) (48%), low grade dysplasia (LGD) (13%), high grade dysplasia (HGD) (17%), HGD with foci suspicious for invasion (8%), and invasive squamous cell carcinoma (SCC) (14%). Patchy p53 staining limited to the basal cell layer was seen in 36% of NNs. 71% of LGDs showed p53 in the lower third of the mucosa and in 30% focally involved the mid-portion of the mucosa. Full thickness p53 was seen in 88% of HGDs with and without foci suspicious for malignancy. In cases suspicious for invasion, p53 highlighted the microinvasive nests. Uniform full thickness p53 staining was seen in 100% of SCC. Thus p53 immunostaining significantly correlated with presence of dysplasia and malignancy (p=0.00). Ki67 staining was seen in the basal cell layer and lower thirds of 95% of NNs and 71% of LGDs. 75% of HGDs showed suprabasal Ki67 involving either 2/3rds or the entire thickness of the mucosa. Suprabasal ki67 staining was seen in 30% of SCCs. Thus Ki67 expression was not discriminatory in LGDs and reactive atypia. However, suprabasal expression of Ki67 was frequently seen in HGDs (p=0.04). CD44 membranous staining of low to moderate intensity was seen involving the lower and mid thirds of the mucosa in 91% of NNs and 86% of LGDs. Strong, full thickness membranous staining with CD44 was seen in 38% of HGDs and 67% of SCC. Thus strong membranous full thickness CD44 staining correlated with HGD and invasive malignancy (p=0.012). Patchy, focal, nuclear and cytoplasmic immunostaining with p16 was seen in 29% of cases with LGDs and 13% of cases with HGDs. Immunostaining with p16 was not of discriminatory value (p=0.51).

Conclusions: Increased expression of p53, suprabasal staining of Ki67 and strong full thickness CD44 are of diagnostic utility in distinguishing between dysplasia and reactive atypia in morphologically difficult cases.

1285 Oral Dysplasia Adjacent to Small T1 Oral Squamous Cell Carcinomas with Superficial Invasion

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Background: Oral squamous dysplasia (OD) grade is important for management decision making but is an imperfect predictor of malignant potential in certain lesions. In other anatomic sites (e.g., uterine cervix), grading of dysplasia relies heavily on the presence and localization of mitotic activity. We aim to assess squamous dysplasia, analyze the presence of mitoses and other histologic parameters in mucosa adjacent to small superficially invasive oral squamous cell carcinoma (OSCC).

Design: Pathology database was reviewed retrospectively for T1 OSCC with available slides from resection and excisional biopsies specimens only. Tumor width and depth were measured on the slides. Areas immediately adjacent to the invasive tumors was evaluated for dysplasia, presence and localization of mitoses and presence of erosion. Any mitotic figure irrespective of atypia were recorded as follows: present in basal/parabasal, mid and superficial layers. Dysplasia was graded on both sides of the invasive tumor and highest grade of dysplasia was recorded using WHO criteria as mild, moderate, severe and carcinoma in-situ. A binary system of low (LGOD; mild) and high grade dysplasia (HGOD; moderate, severe OD and CIS) was also used. Fisher exact test was used for statistical analysis.

Results: Twenty-six patients, 17 men and 9 women (mean age 58, range 38-80) with T1 OSCC from tongue (n=23), palate (n=2) and gingiva (n=1) were included. Clinical presentation was a mass in 8/26, ulceration/erosion in 2/26 and leukoplakia in 9/26. Tumors width ranged from 0.1 to 1.5 cm (mean 0.6; median 0.5) and its depth from 0.05 to 0.8 cm (mean 0.18; median 0.1). Highest grade of dysplasia was mild in 9/26 (35%), moderate in 7/26 (27%) and severe OD/CIS in 10/26 (38%) patients. Mitoses were present in mid and superficial layers exclusively in HGOD (7/17) and not seen in LGOD (0/9) (p-value 0.03). However, mitoses were absent all together in 5/9 LGOD and 5/17 HGOD. No significant relationship between presence of mitoses, tumor depth or width and erosion in the dysplastic mucosa was present.

Conclusions: Mucosa immediately adjacent to these small OSCC were associated with mild dysplasia in one third and moderate dysplasia in one fourth of cases and mid/superficial layers mitoses were absent in most cases. These findings provide

indirect evidence that criteria for grading dysplasia used by surgical pathologists in other epithelia will underestimate the malignant potential of a significant subset of oral dysplastic lesions.

1286 Adenoid Cystic Carcinoma of Salivary Glands: Clinicopathologic Study with Emphasis on Myb Protein Expression

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Background: Adenoid cystic carcinoma (ACC) is the second most common primary salivary gland malignancy which shows ductal and myoepithelial differentiation and different growth patterns. Recently, a t(6;9) translocation resulting in the MYB-NFIB fusion gene has been reported in ACC. In addition, overexpression of Myb protein has been demonstrated by IHC including both MYB-NFIB translocation positive and negative tumors. The aim of this study to assess prognostic factors in ACC with emphasis on Myb expression.

Design: 34 cases of ACC were retrieved from the pathology file. Myb immunostain was performed on 31 cases with <5% nuclear staining considered as negative. The cases were subjected to a detailed histopathologic analysis.

Results: Median patient age was 53.5 (24-79) with female predilection (20F/14M). Tumors were located in minor (21,62%) and major (13,38%) salivary glands. Mean size of tumor was 2.9 cm. The majority of tumor showed increased mitoses (56% with ≥3 mitosis/10HPFs), apoptosis (85%), and open chromatin pattern (60%). Tumor necrosis was identified in 6 cases (17%). The predominant growth pattern was cribriform in 70% of cases and solid in 8%. Peirneural invasion (PNI), vascular invasion and positive margins were noted in 79%, 0.8% and 75%, respectively. Median FU was 27 months. Two patients developed local recurrences, four had distant metastases, and four had LN metastases. None of the patients who had tumors with mitoses ≤ 3/10 HPFs developed distant metastases, while 4/18 (22%) patients with tumors showing >3 mitoses/10 HPFs developed distant metastases. Solid growth pattern did not correlate with recurrence. Myb stain was positive in 19/31 (61%) ACC. Myb staining was categorized as follows: 0 (39%), 5-50% (16%), and ≥50% (45%). Myb expression did not significantly correlate with the following: recurrences, distant or LN metastases, solid growth pattern, tumor necrosis, or PNI. However, MYB correlated with increased mitotic rate (>3/10 HPFs) (P<0.03).

Conclusions: Myb expression was found in 61% cases of ACC. MYB staining correlated with increased mitotic rate (>3/10 HPFs) (P<0.03). None of the patients who had tumors with mitoses ≤ 3/10 HPFs developed distant metastases which is suggestive that mitotic rate might be useful to grade ACC. Additional cases of ACC will be studied to further investigate the results.

1287 Solitary Fibrous Tumor of Head and Neck; a Diverse Clinicopathologic Spectrum

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Background: Solitary fibrous tumors (SFT) are typically benign, circumscribed mesenchymal tumors of CD34-positive fibroblastic lineage with prominent hemangiopericytoma-like branching vascular pattern, occurring mostly in middle-aged adults. These are very rare in head and neck region, and malignant examples are exceptional.

Design: We identified 9 cases of SFT of the head and neck from the pathology archives and studied their clinicopathologic characteristics. IHC for CD34, vimentin, S100, BCL2, SMA, desmin, keratins, CD68, EMA, GFAP, neurofilament, synaptophysin, and ki67 were performed. Primary meningeal hemangiopericytoma, synovial sarcoma, fibrosarcoma, and sinonasal glomangiopericytoma were excluded. Other lesions excluded especially in mucosal sites were neurofibroma, schwannoma, leiomyoma, and spindle cell myoepithelioma.

Results: The age range was 28 to 83 years (mean 54); 8 were males and one female. Two were intracranial (pons, frontal meningeal), 4 soft tissue (nasopharynx, parapharyngeal, inferior orbital, parotid), and 3 oral tumors (2 buccal mucosa, 1 floor of mouth). Histologic examination showed a spindle cell neoplasm with alternating hypo- and hypercellular areas, with intervening ropy collagen, patchy branching vascular pattern, diffuse CD34 and significant BCL2 positivity. Malignant cases exhibited significant pleomorphism, foci of necrosis, and mitotic activity of more than 4 per 10 high-power fields (up to 23). Ki67 labeling index in malignant SFT varied from 5 to 25 percent. One case in parapharyngeal region appeared to arise from a benign / low-grade SFT by dedifferentiation. Two cases recurred after 24 (pons) and 7 (parotid) years. There was no evidence of metastasis.

Conclusions: SFT of head and neck can occur in diverse locations, are mostly benign, non-recurring tumors with characteristic histology and diffuse CD34 positivity that can involve meninges, mucosal sites (oral, respiratory), skull base, parotid or soft tissue. Malignant examples, may occur de novo or by dedifferentiation, show overt anaplasia, increased mitotic activity, and can develop late recurrences.

1288 TOP2A Overexpression as a Poor Prognostic Factor in Patients with Nasopharyngeal Carcinoma

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Background: Despite the advances in diagnostic imaging and treatment modalities, the risk stratification and final outcomes in patients with nasopharyngeal carcinomas (NPC) still remain suboptimal. Through data mining from published transcriptomic database, *TOP2A* was first identified as a differentially upregulated gene in NPC tissues, which encodes topoisomerase 2 alpha implicating cell division via selective cleavage, rearrangement, and re-ligation of DNA strands. Given the roles of TOP2A in prognostication and as the frontline therapeutic target in common carcinomas, such as breast cancer, we explored the significance of TOP2A immunoexpression status in a well-defined cohort of NPC patients.

Design: TOP2A immunohistochemistry was retrospectively performed and analyzed using H-score method for biopsy specimens from 124 NPC patients who received standard treatment without distant metastasis at initial diagnosis. Those cases with H-score larger than the median value were construed as featuring TOP2A overexpression. The results were correlated with the clinicopathological variables, disease-specific survival (DSS), and distant metastasis-free survival (DMFS).

Results: TOP2A overexpression was significantly associated with AJCC stages III-IV (p=0.019) and univariately predictive of adverse outcomes for DSS (p=0.0078) and DMFS (p=0.0003). In multivariate comparisons, TOP2A overexpression still remained prognostically independent to portend worse DSS (p=0.047, hazard ratio=1.732) and DMFS (p=0.003, hazard ratio=2.569), together with advanced AJCC stages III-IV.

Conclusions: TOP2A expression is upregulated in a subset of NPCs and its increased immunoexpression significantly correlated with advanced stages and tumor aggressiveness, justifying the potentiality of TOP2A as a prognostic biomarker and a novel therapeutic target of NPC.

1289 Periostin Overexpression as a Poor Prognostic Factor in Patients with Nasopharyngeal Carcinoma

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Background: Despite the advances in diagnostic imaging and treatment modalities, the risk stratification and final outcomes in patients with nasopharyngeal carcinomas (NPC) still remain suboptimal. Cell adhesion molecules play critical functional roles in the cross talk with tumor microenvironment to implicate carcinogenesis. Focusing on cell adhesion-associated genes, we performed data mining from published NPC transcriptomes and first identified *POSTN* as a significant upregulated gene encoding periostin. This secreted cell adhesion protein is known to be overexpressed in other carcinomas with metastasis- promoting function. We therefore explored the significance of periostin immunoexpression status in a well-defined cohort of NPC patients.

Design: Periostin immunohistochemistry was retrospectively performed and analyzed using H-score method for biopsy specimens from 124 NPC patients receiving standard treatment without distant metastasis at initial diagnosis. Those cases with H-score larger than the median value were construed as overexpressed. The results were correlated with the clinicopathological variables, disease-specific survival (DSS), local recurrent-free survival (LRFS) and distant metastasis-free survival (DMFS).

Results: Periostin overexpression was significantly associated with N_{2-3} status (p=0.004) and AJCC stages III-IV (p=0.006) and univariately predictive of adverse outcomes for DSS (p=0.0002), LRFS (p=0.0138) and DMFS (p=0.0028). In multivariate comparisons, periostin overexpression still remained prognostically independent to portend worse DSS (p=0.003, hazard ratio=2.311) and LRFS (p=0.024, hazard ratio=3.187), together with advanced AJCC stages III-IV.

Conclusions: Periostin expression is upregulated in a subset of NPCs and its increased immunoexpression significantly correlated with advanced disease and tumor aggressiveness, justifying the potentiality of POSTN as a prognostic biomarker of NPC.

1290 Identification of Sox2 as a Marker for Ameloblastic Carcinoma

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Background: Recent interrogations of the cancer genomes of multiple solid tumors reveal prominent amplifications of the gene encoding Sox2. In fact, Sox2 is pivotal in maintaining the renewal potential of stem cells and inhibiting their differentiation. Ameloblastic carcinoma is a malignancy of odontogenic origin with a dismal prognosis. Separation of this entity from its benign counterparts may be challenging in specimens where atypical features are present. In this study, we aim to assess the diagnostic value of Sox2 in differentiating ameloblastic carcinoma from ameloblastoma.

Design: 4 ameloblastomas (AB), 3 atypical ameloblastomas (AA), 3 ameloblastic carcinomas (AC) (the tissue block of 1 consult AC was unavailable for additional staining), and 3 dentigerous cysts (DC) were included. Immunohistochemical studies were performed on tissue blocks with antibodies against Sox2, CD56, CD138, SMA, Calretinin, and β-catenin. Immunostains were interpreted by 3 pathologists, and scores were recorded based on the areas of stains (1-4 depicting 0-25%, 26-50%, 51-75%, and 76-100%, respectively) and intensity of stains (0-3 depicting negative, minimal, intermediate, and strong staining, respectively) in the cells of interest. Kruskal–Wallis one-way ANOVA was employed to compare the ameloblastic groups.

Results: Sox2 showed strong diffuse nuclear positivity in the 2 stained AC compared to few to none scattered positive cells in either AB or AA (P=0.078). Sox2 highlighted the basal proliferative zone of epithelium in DC. None of the previously reported ameloblastic markers could separate AC from its benign counterparts. Both CD56 and CD138 were variably positive in all ameloblastic and DC epithelium except 1 AB specimen marking as CD56-, CD138+. Scattered positive cells with β-catenin nuclear staining were present in 2 AB specimens. Although previously suggested as a sensitive ameloblastic marker, Calretinin was only positive in 5 of 10 cases.

Conclusions: Sox2 is a novel marker that readily differentiates AC from AB with a distinct diffuse nuclear staining in the former. The P value was not significant due to

the limited sample size. However, we have initiated a broader collaboration to further characterize Sox2 in AC. Previous studies suggest diffuse Sox2 stain correlates with a poor prognosis and a more aggressive clinical course in multiple malignancies including oral squamous cell carcinoma. Strong Sox2 expression in tumor cells may recapitulate one of the hallmarks of cancer, loss of differentiation coupled with uncontrolled renewal.

1291 A Quantitative Histomorphometric Classifier Identifies Aggressive Versus Indolent p16 Positive Oropharyngeal Squamous Cell Carcinoma

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Background: Human papillomavirus-related (p16 positive) oropharyngeal squamous cell carcinoma (OSCC) represents a steadily increasing proportion of head and neck cancers and has a favorable prognosis. However, approximately 10% of patients develop recurrent disease, mostly distant metastasis, and the remaining patients often have major morbidity from treatment. Better knowledge about which patients have more aggressive tumors versus more indolent ones is critically important. We recently identified cellular anaplasia and multinucleation as associated with disease recurrence, and the current work sought to find a computer-aided histomorphometric classifier that could detect changes to predict tumor behavior simply from automated digital image analysis of H&E slides.

Design: Using a tissue microarray cohort of p16 positive OSCC cases with clinical follow up, digitally scanned H&E images were marked binarily according to tumor recurrence versus none. Each nucleus was identified via an automated computerized image analysis algorithm. Then, using a novel cluster cell graph that measures the spatial distribution and clustering of cells, a series of topological features defined on each node of the subgraph (i.e., local graph metrics such as clustering coefficients, ratio of connected components and skewness of edge lengths) were analyzed and a random forest decision tree classifier developed and trained. Then, over 25 runs of 3-fold cross validation using subsets of the cases for independent training and testing, the classifier was validated.

Results: There were 160 p16+ patients on the array, of whom 19 (11.9%) developed recurrent disease. Classifier results are listed in Table 1. The classifier was correct in 140 cases (87.5%), had a 47.8% positive predictive value, and 94.2% negative predictive value.

Table 1

	Classifier +	Classifier -	Total
Recurrence	11 (47.8%)	8 (5.8%)	19
No Recurrence	12 (52.2%)	129 (94.2%)	141
Total	23	137	160

$p<0.0001$

In univariate analysis, patients with a positive classifier had poorer overall, disease free, and disease specific survival ($p<0.001$ for each).

Conclusions: Based only on tiny H&E punches, a computer-aided morphometric classifier can strongly predict tumors at low likelihood of recurrence. With further validation, this may be a very useful in practice to select patients for de-escalated therapies versus those who should receive more aggressive treatment.

1292 Human Papillomavirus Is Uniformly Retained in the Distant Metastases of Primary Oropharyngeal Squamous Cell Carcinomas

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Background: High-risk human papillomavirus (HPV) is both causative and prognostic in the majority of oropharyngeal squamous cell carcinomas (OpSCCs). Distant metastases in such tumors are relatively uncommon (<10% of patients). No studies have thoroughly evaluated distant metastases to see if the HPV is consistently retained. The aim of this study was to evaluate for transcriptionally-active HPV in matched primary OpSCCs and their distant metastases.

Design: Surgical pathology files were searched and 20 matched pairs of primary OpSCCs and their metastases, for which tissue specimens were available, were retrieved. Two study pathologists reviewed all cases to confirm the diagnoses and to evaluate histologic features. Real-time PCR (RT-PCR) for detection of E6/E7 mRNA for all high-risk HPV types and p16 immunohistochemistry (IHC) were performed.

Results: Cases were from 1996 to 2012. Distant metastases were found in lung (65%), bone (20%), non-regional lymph nodes (10%) and pericardium (5%). Histologically, 15 primary tumors were nonkeratinizing (NK), 3 NK with maturation, one basaloid, and one keratinizing (K) SCC. Seventeen (85%) of the metastases had the same histologic type as the primary tumor (p=1.0). p16 was extensively positive in 19 of the primary tumors, and p16 results were identical for all matched pairs. The single pair that was negative for p16 was a K SCC. All 20 matched pairs were concordant for HPV status by RT-PCR with 19 of 20 positive for high risk HPV. Three different types of HPV were identified: HPV-16 in 17 pairs and HPV-18 and HPV-35 in a single pair each. All 19 HPV positive tumors were p16 positive. HPV transcriptional activity was assessed relative to control genes and there was no significant difference between primary tumors (mean 5.8 units) and their distant metastases (mean 5.7 units).

Conclusions: The distant metastases from HPV-related primary OpSCCs uniformly retain HPV and p16 status, and HPV transcriptional activity. They also retain similar morphology. This argues that p16 IHC can be utilized to differentiate metastatic OpSCCs from separate, new, primary SCCs in other organs. These findings also have major treatment implications for any future agents that would specifically target HPV proteins or associated molecules in such patients.

1293 Mucosal Microcystic Adnexal Carcinomas of the Head and Neck: A Report of 4 Cases

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Background: Microcystic adnexal carcinoma (MAC) is a rare neoplasm occurring predominantly in the centrofacial skin of middle-aged patients. MACs demonstrate a stereotypic morphology with minimally atypical cells arranged in angulated ducts percolating through densely sclerotic stroma. Despite their bland appearance, these tumors show extensive invasion, local destruction, and are prone to recurrence. Their identification is frequently straightforward in the facial skin, but they pose significant diagnostic difficulties when they arise in alternate locations. On our consultation service, we have encountered several diagnostically challenging cases of MAC occurring in the head and neck. To our knowledge, there has only been a single report (Schipper et al, 1995) of this tumor occurring in the tongue and one report from the parotid gland (Hunt et al 1995). We know of no prior reports of MAC arising in the nasopharyngeal area or clivus.

Design: The University of Virginia Department of Pathology internal and consultative files were searched for all cases of MAC arising in the head and neck between 12/2003 and 9/2012.

Results: Four consultation cases were identified (Table 1). Each case demonstrated infiltrating cords, islands, and tubules comprised of minimally atypical cuboidal cells with round to oval nuclei and fine chromatin. Nucleoli were rarely prominent and mitotic figures were infrequent. Desmoplastic stroma was consistently evident in the background. Perineural invasion was identified in two cases, and three cases showed invasion of the skeletal muscle. One case closely abutted a minor salivary gland.

Table 1

Case #	Patient Age	Patient Sex	Tumor Location	Presentation
1	41	F	Base of tongue	Tongue mass
2	47	F	Anterior tongue	Tongue lesion
3	73	M	Nasopharynx, clivus	Diplopia for 8 months
4	54	F	Floor of mouth	Floor of mouth lesion

Conclusions: MAC may rarely arise in the nasopharynx and oropharynx. Including MACs on the differential diagnosis for gland-forming lesions of these sites is critical because while their remarkably bland histology lends itself to misclassification as benign, their behavior is locally aggressive. Desmoplastic stroma is a useful diagnostic aid and perineural and skeletal muscle invasion may be seen but are not requisite for diagnosis. MACs in this location may also mimic malignant salivary gland tumors including adenoid cystic carcinoma, polymorphous low grade tumor, and low grade adenocarcinoma, non-intestinal type.

1294 Comparing Biomarkers for Tissue Detection of HPV-Associated Oropharyngeal Cancer

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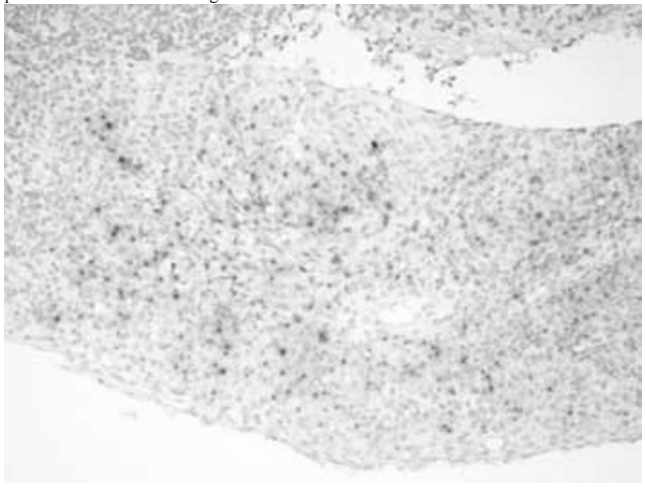
Background: Human papillomavirus (HPV)16 infection-associated oropharingeal squamous cell carcinomas (OPSCC) have relevant prognostic and therapeutic implications. Several algorythms have been proposed for their identification, however only recently has the virus transcriptional activity been recognized as the marker of virus-induced oncogenesis and the gold standard for test validation. In situ hybridization (ISH) for detection of genotype-specific mRNA of HPV E6/7 genes allows to assess on routine bioptic samples HPV oncogene transcription, avoiding the need of fresh tissue. This study was aimed at comparing the diagnostic accuracy of different current tests against HPV E6/7 mRNA ISH.

Design: 21 consecutive paraffin-embedded OPSCC were tested with ISH for HPV16 E6/7 mRNA and high risk (HR) HPV DNA, p16 immunostain, HPV DNA amplification with SPF10 primers and viral genotyping, and HPV16 E6 gene amplification. Test sensitivity and specificity were assessed with mRNA ISH as the gold standard.

Results: mRNA ISH identified viral transcripts in 8 of 21 cases, all positive by p16 stain, DNA ISH, HPV DNA and E6 gene amplification. All tests but DNA ISH were positive in some mRNA-negative cases.



The table summarizes test and statistic analysis results.

Table 1

Test	N. positive (%)	N. negative (%)	Sensitivity	95% CI	Specificity	95% CI
HPV16 mRNA ISH	8 (38)	13 (62)				
p16	12 (57)	9 (43)	100	63.1-100	69.23	38.6-90.9
SPF10 HPV DNA	20 (95.2)	1 (4.8)	100	63.1-100	7.69	0.2-36.0
SPF10 HPV 16	10 (47.6)	11 (52.4)	62.50	24.5-91.5	61.54	31.6-86.1
HPV16 E6 DNA amplification	15 (71,4)	6 (28.6)	100	63.1-100	46.15	19.2-74.9
HR HPV DNA ISH	8 (38)	13 (62)	100	63.1-100	100	75.3-100

Conclusions: Our results further validate the current diagnostic algorithm for HPV-associated OPSCC identification, which includes p16 stain and DNA ISH in p16-positive cases. The 100% correspondence of mRNA and DNA ISH results allows to reserve cumbersome mRNA ISH for research rather than diagnostic goals. The low specificity of PCR-based assays confirms the observation that HPV DNA amplification does not discriminate passenger (transcriptionally inactive) from oncogenic, transcriptionally active HPV infections. Moreover, integrated HPV16 can be missed when using primers amplifying the L1 region, which is lost upon viral integration.

1295 Positive SOX10 Expression in a Broad Range of Salivary Gland Tumors

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Background: SOX10 is a novel immunohistochemical marker that has shown diagnostic utility in melanocytic and nerve sheath tumors. However, the specificity in head and neck sites has not been fully explored. This is of particular importance due to the morphologic overlap between melanomas and other head and neck tumors such as salivary gland neoplasms. Previously, SOX10 expression has been reported in a small number of pleomorphic adenomas of the parotid gland. We wished to more fully explore SOX10 expression in the spectrum of salivary gland tumors.

Design: SOX10 immunohistochemistry was performed on tissue microarrays comprising 229 benign and malignant salivary gland neoplasms. Strong diffuse nuclear staining was scored as positive, while weak or subset staining in <50% of cells was scored as negative. Stains for p63 and smooth muscle heavy-chain myosin were also performed, and correlation with SOX10 staining was determined.

Results: Multiple salivary gland tumor types show a high proportion with strong SOX10 positivity: pleomorphic (38/48) and monomorphic adenomas (10/10), polymorphous low-grade adenocarcinoma (14/16), adenoid cystic carcinoma (81/84) and acinic cell carcinoma (22/23). While it has been previously shown that SOX10 highlights myoepithelial cells in various organ sites, staining was seen beyond the myoepithelial component of these tumors, including diffuse staining in luminal-type epithelial cells. Furthermore, SOX10 staining showed poor correlation with the expression of the myoepithelial markers p63 (r=0.074, p=0.26) and smooth muscle myosin (r=0.148, p=0.03). Virtually no staining was seen in certain tumor subtypes, including oncocytoma (0/12), mucoepidermoid carcinoma (1/24) and salivary duct carcinoma (0/3), suggesting a possible role for SOX10 as an aid in subclassification of salivary gland tumors.

Conclusions: SOX10 is expressed in a broad range of salivary gland tumors, limiting its utility in head and neck sites as a marker of melanocytic and nerve sheath differentiation. However, it may serve as a useful diagnostic tool to differentiate between common subtypes of salivary gland tumors.

1296 CD34 and a-SMA Distinguish Verrucous Hyperplasia from Verrucous Carcinoma

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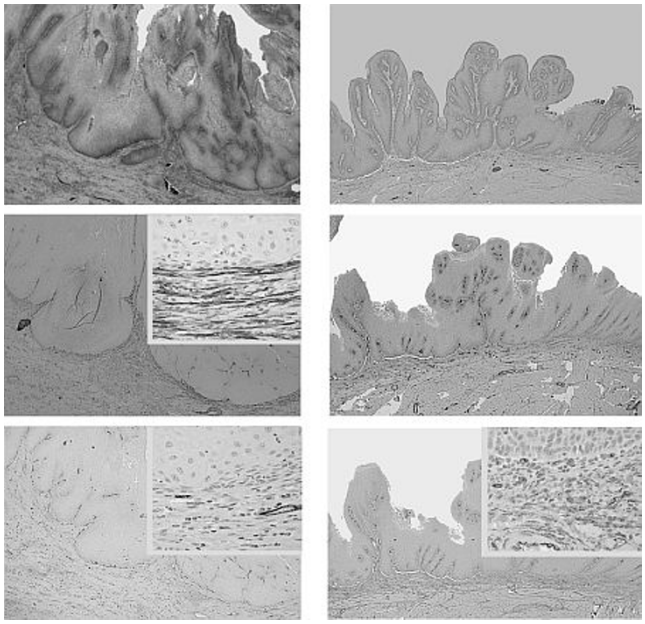
Background: Distinguishing between verrucous carcinoma (VC) and verrucous hyperplasia (VH) is a diagnostic challenge. The determination relies heavily upon whether the lesion lies above or below a line connecting the uninvolved epithelium on either side of the lesion. This feature may be difficult or impossible to evaluate due to failure of the surgeon to excise widely enough or technical difficulties with embedding and cutting. Epithelial features do not clearly resolve the issue, as both lesions are devoid of cytologic criteria for malignancy. Previous studies have demonstrated key differences in the stroma of benign and malignant squamous lesions. Stroma associated with invasive carcinoma is characterized by a loss of CD34+ fibrocytes with a gain of SMA+ myofibroblasts; opposite reactions are seen in uninvolved stroma. We employ these principles to determine whether VC and VH can be distinguished.

Design: Nine VH, 14 VC, and 14 conventional infiltrating keratinizing squamous cell carcinomas were retrieved from the surgical pathology files. Tissue stroma was assessed for reactivity with anti-CD34 antibody (Novocastra, NCL-END, mouse IgG, 1:25 dilution) and anti-α-SMA antibody (DAKO, M0851, mouse IgG, 1:100 dilution). Tumor-free stroma was also assessed in 12 of these cases.

Results:

Table 1. Stromal Staining Patterns Among Groups

Groups	n	SMA+	CD34+
Tumor-free	12	0.0%	100.0%
Verrucous hyperplasia	9	0.0%	100.0%
Verrucous carcinoma	14	92.9%	14.3%
Squamous cell carcinoma	14	100.0%	0.0%



Upper left: Classic VC. **Middle left**: A network of slender, bipolar SMA+ myofibroblasts ensheathes the rete pegs. **Bottom left**: Stromal cells are negative for CD34. **Upper right**: Classic VH. **Middle right**: Stromal cells are negative for SMA. **Bottom right**: The delicate CD34+ meshwork is seen in continuity with non-tumoral stroma at the right. Only one outlier VC case stained exactly opposite to the other VC cases. This case had no uninvolved adjacent epithelium to evaluate.

Conclusions: The present series suggests a discriminating role for the use of CD34 and α-SMA to distinguish VH from VC with very high sensitivity and specificity. The presence of a single outlier VC case, albeit without accompanying normal adjacent epithelium, raises the possibility of reconsidering the current histologic diagnostic criteria for VC.

1297 Mammaglobin and S100 Immunoreactivity in Salivary Gland Tumors Other Than Mammary Analogue Secretory Carcinomas

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Background: Mammary analogue secretory carcinoma (MASC) of the salivary glands is a recently described entity with histopathologic and immunophenotypic features similar to secretory carcinoma (BSC) of the breast. These tumors exhibit a lobulated growth pattern with microcystic and glandular architecture and stain strongly for mammaglobin (MG), vimentin, and S100. Similar to BSC, MASC also harbors a balanced translocation t(12;15)(p13;q25) that results in *ETV6-NTRK3* fusion. Immunohistochemistry (IHC) for MG and S100 have been shown to effectively differentiate MASC from its morphologic mimics, especially acinic cell carcinoma. However, limited data exists on the MG and S100 reactivity of other salivary gland tumors, such as polymorphous low grade adenocarcinoma (PLGA), adenoid cystic carcinoma (AdCC), mucoepidermoid carcinoma (MEC), adenocarcinoma, NOS (ANOS) and mucinous adenocarcinoma (MAC).

Design: IHC for MG and S100 was performed on PLGA, AdCC, MEC, ANOS and MAC. Staining strength (weak, moderate, strong) and extent (1+ = 1-25%, 2+ = >25-50%, 3+ = >50-75%, 4+ = >75%) were evaluated. Cases with >1+ intensity and >1+ extent for both MG and S100 were analyzed by FISH using the ETV6 (12p13) breakapart probe.

Results: Fifteen cases each of PLGA, AdCC, and MEC, 2 cases of ANOS and 1 case of MAC were identified. The clinical parameters are summarized in table 1. Positive staining for both S100 and MG was seen in 9/15 PLGA, 2/15 AdCC, 0/15 MEC, 0/2 ANOS and 0/1 MAC (IHC results summarized in table 2); and the 9 PLGAs and 2 AdCC were further analyzed by FISH. There was no correlation between S100 and MG positivity in PLGA or AdCC for both intensity (PLGA p=0.06, AdCCp=0.83) and extent (PLGA p=0.86, AdCC P=0.63). Seven PLGA were negative for the 12p13 translocation by FISH, and two PLGAs were non diagnostic. Both AdCCs were also negative for the translocation

		PLGA (n=15)	AdCC (n=15)	MEC (n=15)	ANOS (n=2)	MAC (n=1)
Age	Range (Mean)	45-75 (62)	32-101 (55)	15-75 (48)	58-70 (64)	64
Sex	M:F	6:9	5:10	6:9	1:1	0:1
Location	Parotid gl	0	1	4	2	1
	Submandibular gl	0	3	0	0	0
	Minor salivary gl	15	10	10	0	0
	Lacrimal gl	0	1	1	0	0

		PLGA (n=15)	AdCC (n=15)	MEC (n=15)	ANOS (n=2)	MAC (n=1)
S100 intensity	>= 2+	13	10	3	0	0
S100 extent	>= 2+	14	2	0	0	0
MG intensity	>= 2+	15	7	8	2	1
MG extent	>= 2+	10	3	7	2	1

Conclusions: MG and S100 positivity in salivary gland tumors is not specific to MASC and occurs in a majority of PLGA and a minority of AdCC cases, indicating the need for caution in diagnosing MASC based on IHC in small biopsy material in the absence of cytogenetic confirmation.

1298 Well-Differentiated Squamous Cell Carcinoma with Verrucous Features – A Variant of Verrucous Carcinoma with Extremely Favorable Prognosis

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Background: Verrucous carcinomas (VC) of the head and neck (HN) are tumors which recur locally but do not metastasize, when not associated with any frank cellular dysplasia or an invasive squamous cell carcinoma (SCC) component. However, VC with dysplasia or focal invasion are treated just as conventional SCC. There has been no study on differences in biologic behavior between VC, VCs with dysplasia or limited invasion, and those with extensive invasion.

Design: All institutional HN VC cases were reviewed and categorized into 3 groups: pure VC, well differentiated SCC with verrucous features (SCC-VF), and SCC arising in VC (SCC-VC). SCC-VF was defined as VC with frank dysplasia or with minor invasive SCC (<2mm in depth). SCC-VC was defined as VC with major invasive SCC (>2mm). Cases were also matched with conventional SCC based on location, T and N stage. Clinical features were compared using one way ANOVA with multiple comparisons.

Results: Of 49 cases, 16 were VC, 20 SCC-VF and 13 SCC-VC. Forty nine conventional SCC served as matched controls. Follow up was 1-145 months (median 32) for the cases, and 21-134 months (median 51) for controls. Only 1/16 (6.3%) VC cases and 2/20 (10%) SCC-VF cases recurred locally versus 7/13 (53.8%) SCC-VC. All VC and SCC-VF cases were node negative at presentation whereas SCC-VC had nodal metastases in 2/13 (15.4%) cases. These differences were statistically significant (Table 1). No patients with VC or SCC-VF died with disease versus 5/13 (38.46%) patients with SCC-VC. Regional nodal recurrence and distant metastases were absent in pure VC and very uncommon among other groups and hence the differences were not statistically different. Stage matched conventional SCC cases were not statistically significantly different from any of the groups.

	VC versus SCC-VF	SCC-VF versus SCC-VC	VC versus SCC-SC	VC versus matched SCC	SCC-VF versus matched SCC	SCC-VC versus matched SCC
Local recurrence	0.14	**0.0175**	**0.0031**	0.96	0.31	0.79
Overall disease recurrence	0.14	**0.0073**	**0.0031**	0.94	0.21	0.84
Nodal positivity	0.36	**0.0109**	**0.0009**	0.99	0.83	0.96
Regional recurrence	0.69	0.32	0.20	0.95	0.66	0.87

Conclusions: Our findings suggest that well differentiated SCC with verrucous features (i.e. VC with dysplasia or limited invasive SCC) is very similar in clinical behavior to pure VC, and different than more extensively invasive SCC arising in VC.

1299 Mammary Analogue Secretory Carcinoma (MASC) of the Salivary Gland: Revisiting Our Acinic Cell and Poorly Differentiated Carcinomas Using Immunohistochemistry and FISH Studies

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Background: Mammary analogue secretory carcinoma (MASC) is a recently described entity with unique morphological, clinical and genetic characteristics. These tumors were previously diagnosed as acinic cell carcinomas (ACC) or poorly differentiated carcinomas, among others. A few institutions have reviewed these cases and reported their experience.

Design: All cases of ACC and poorly differentiated carcinomas of the salivary glands in a ten year period were reviewed. There were a total of 27 cases, including 11 cases of acinic cell carcinomas and 16 cases of poorly differentiated carcinomas. The hematoxylin and eosin stained slides were reviewed and tumors that morphologically fit in this new category- MASC- according to the recent literature were selected. Those features included epithelial cells with abundant vacuolated cytoplasm, minimal nuclear pleomorphism, papillary and microcystic architecture with cellular hobnailing and eosinophilic secretions. This process narrowed down the initial number to 12 cases (7 ACC and 5 poorly differentiated adenocarcinomas) on which S-100, mammoglobin (MMG) and DOG1 immunostains were performed. The cases that were immunoreactive for S-100 and nonreactive for DOG-1 were then submitted for FISH analysis for the characteristic t(12;15)(p13;q25) with ETV6-NTRK3 fusion.

Results: Six (6) cases demonstrated positivity for S-100 protein and negativity for DOG1 by immunohistochemistry. FISH studies confirmed ETV6-NTRK3 rearrangement in three (3) cases, initially diagnosed as ACCs. The three cases stained positive for MMG by immunohistochemistry. One (1) additional case had amplification of the ETV6 gene, and 1 case had deletion of ETV6. The remaining case had no cytogenetic abnormalities detected. Among the three tumors with t(12;15)(p13;q25), two patients were male (2/3). The two cases on which ETV6 gene was amplified (1) and deleted (1) were also from male patients.

Conclusions: MASC is a new diagnostic entity that should be in the differential diagnosis of salivary gland tumors that mimic ACC. They differ from conventional ACC morphologically, immunohistochemically and molecularly. S-100 protein is positive, as opposed to the majority of ACC. They predominantly affect male patients. Positivity for MMG and S-100 and negativity for DOG1 are useful screening tools prior to further confirmatory FISH studies. The novel finding of deletion and amplification of ETV6 in MASC, with absent ETV6-NTRK3 rearrangement, may indicate an additional pathogenetic pathway of this neoplasm.

1300 Expression of p21 in Oropharyngeal Squamous Cell Carcinoma Is Associated with HPV Status but Is Not an Independent Prognostic Marker

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Background: The incidence of oropharyngeal squamous cell carcinoma (OPSCC) has been increasing over the past three decades. High risk human papillomavirus (HPV) is associated with OPSCC, and patients with HPV have a better prognosis. This finding has prompted research into other potential prognostic biomarkers, one of which is expression of the tumor suppressor protein p21.

Design: Immunohistochemistry was performed for p21 on a tissue microarray cohort of OPSCC cases with known clinical follow-up and HPV status (by RNA in situ hybridization [ISH]). Expression of p21 was assessed visually by a study pathologist (MEP) and also by digital image analysis using Aperio's membrane algorithm, version 9. Expression of p21 was frequent and extensive and was therefore dichotomized as >= versus < 75% staining.

Results: There were 214 patients identified, the majority of whom were men (90%) of Caucasian race (88%) who smoked (71%). The mean age at diagnosis was 55. HPV RNA was detected in 81% of the tumors tested. All tumors had some p21 expression, with a mean of 60% by visual analysis and 52% by digital analysis. By visual analysis, 40 patients (19%) had extensive p21 staining (75% or more); by digital analysis, 12 patients (6%). Pearson's correlation between visual and digital analysis was strong (r=0.7). Diffuse p21 staining was more frequent in HPV negative patients (p=<0.001), more frequent in patients with T3 and T4 tumors (p=0.04), and less frequent in those undergoing surgical treatment (p=0.03). Kaplan-Meier survival analysis showed that diffuse p21 expression, lack of HPV, high T stage, and non-surgical treatment were each individually associated with decreased overall survival (p<0.05). Diffuse p21 expression was not associated with disease free or disease specific survival in univariate analyses. In multivariate Cox regression analysis for overall survival, high T stage (HR 2.6; 95%CI 1.5, 4.7) and lack of HPV (HR 2.9; 95% CI 1.5, 5.4) remained significant predictors of patient demise, but p21 expression and treatment type were no longer statistically significant.

Conclusions: Although diffuse p21 expression may suggest more aggressive tumors in patients with OPSCC, it is not an effective independent prognostic biomarker when controlling for well-established risk factors such as T-stage and HPV status.

1301 The Absence of Human Papillomavirus E6/E7 mRNA Transcripts in Squamous Cell Carcinomas of the Oral Tongue: HPV Is Not a Relevant Agent in Squamous Cell Carcinomas of the Oral Tongue Including Those That Are Non-Tobacco Related

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Background: The human papillomavirus (HPV) is an important cause of many head and neck squamous cell carcinomas (SCCs), but its role in cancer of the lateral tongue remains controversial. Indeed, detection rates of HPV in SCCs of the oral tongue have varied from 0% to 100%, largely reflecting differences in detection methodologies. The suspicion of an HPV-related lateral tongue SCC may be heightened in young patients and in patients who have never smoked. A recently described RNA in-situ hybridization (ISH) method for detecting HPV E6/E7 mRNA is sensitive, specific, and allows for accurate detection of HPV in routinely processed formalin-fixed tissues.

Design: We evaluated 107 SCCs of the lateral tongue for the presence of HPV using p16 immunohistochemistry and an RNA in situ hybridization (ISH) assay targeting high risk HPV E6/E7 mRNA. The ISH was performed using the RNAScope VS system on Ventana's Discovery XT. The study population included 41 patients who had never smoked, and 9 patients under the age of 40.

Results: HPV E6/E7 mRNA transcripts were detected in only 1 of 107 (0.9%) SCCs. P16 expression was detected in 10 of 107 (9.3%) SCCs. The one HPV-positive SCC was also p16 positive, while 9 of 10 p16-positive SCCs were negative for HPV E6/E7.

Conclusions: HPV is not detected in the vast majority of lateral tongue SCCs. Accordingly, routine HPV testing is not necessary for SCCs of the lateral tongue, and even selective testing is unwarranted in those patients without traditional risk factors such as smoking. Furthermore, p16 staining is not a reliable marker of HPV positivity at this particular anatomic site.

1302 Comprehensive Genomic Profiling of Relapsed and Metastatic Adenoid Cystic Carcinomas (ACC) by Next Generation Sequencing (NGS) Reveals New Routes to Targeted Therapies

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Background: ACC of the head and neck (HN) and upper respiratory tract features a highly variable clinical course with systemic chemotherapy of limited value for patients with metastatic disease. The recent discovery of a *MYB-NFIB* gene fusion in ACC led us to hypothesize that comprehensive clinical NGS could reveal actionable genomic alterations (GA) and potentially expand treatment options for ACC.

Design: DNA was extracted from 4 formalin-fixed paraffin embedded sections cut at 10 microns from 8 primary HN ACC and 1 tracheal ACC. NGS was performed on hybridization-captured, adapter ligation libraries derived from 2 primary parotid tumors, 1 recurrent lesion in the mouth and 6 metastatic lesions, including 4 lung and 1 each in a lymph node and bone. The exons of 182 cancer-related genes were fully sequenced using the Illumina HiSeq 2000 at an average sequencing depth of 776X All classes of genomic alterations (GA) were evaluated including point mutations (mut),

insertions, deletions, copy number alterations, and select gene fusions/rearrangements. Actionable GA were identified as impacting targeted anti-cancer therapies on the market or in registered clinical trials.
Results: The 6 male (67%) and 3 female (33%) ACC patients had a mean age of 58 years. Four (44%) tumors were Stage III and 5 (56%) were Stage IV time of NGS. Three of nine (33%) ACC featured a total of 12 GA (average of 4.0 GA per tumor). Three of three (100%) of ACC with GA were Stage IV. Mut in *TP53* was identified in 2/3 (67%) ACC with GA. GA in *BAP1*, *KDM6A*, *KDR*, *KIT*, *PDGFRA*, *PTCH1*, *ARID1A*, *EPHA6*, and *KDM6A* were each identified once in one of the 3 ACC with GA. Of the 3 ACC with GA, 2 (67%) had GA potentially associated with clinical benefit of targeted therapies including potential entry into 11 CT. Two of nine (22%) of ACC had actionable GA including one ACC that featured amp of *KIT* and *PDGFR* genes which directed the patient to tyrosine kinase inhibitor therapy (imatinib, dasatinib) and one ACC with a *PTCH1* mutation which potentially directed the patient to an FDA-approved hedgehog pathway inhibitor (vismodegib) used to treat cutaneous basal cell carcinoma.
Conclusions: Although GA in ACC are a relatively uncommon occurrence possibly reflecting their generally well-differentiated histologic appearance, deep NGS of ACC can discover actionable GA that could influence therapy especially in advanced stage tumors that are difficult to characterize by other diagnostic methods in a single comprehensive test.

1303 ProExC and p16 Are of Limited Diagnostic Utility in the Classification and Grading of Dysplastic Lesions of Upper Aerodigestive Tract

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Background: Upper aerodigestive tract (UADT) malignant and premalignant squamous lesions exhibit some similarities to squamous lesions seen in the cervix. Presence of HPV in head and neck squamous cell carcinoma has been shown to to have clinical and prognostic implications. HPV surrogate markers, p16 and ProExC, are beneficial in distinguishing cervical high grade squamous intraepithelial lesions from their mimicks. The aim of our study was to evaluate the role of p16, ProExC and Ki-67 in the diagnosis and grading of dysplastic lesions of UADT thus potentially identifying those lesions most likely to progress.
Design: Formalin fixed paraffin blocks from 79 UADT biopsy cases containing a range of dysplastic and non-dysplastic squamous epithelium were retrieved from files. Sections were stained with H&E, ProExC, Ki-67 and p16. Three pathologists with a special interest in Head and Neck pathology reviewed the H&E slides and a consensus diagnosis was reached in all cases. Using the WHO classification, dysplasia was divided into low (mild) and high (moderate and severe) grade. Immunohistochemical studies were scored: ProExC and Ki-67 were considered positive when strong nuclear reactivity was seen in >50% of the thickness of the epithelium. ProExC patterns of between 25-50% of cells and Ki-67 staining <50% of cells was also recorded but not considered positive. p16 was considered positive when there was cytoplasmic/nuclear and cytoplasmic staining in >90% of the epithelial cells.
Results: There were 29 non-dysplastic cases, 22 low grade and 28 high grade dysplasia cases. Positive staining for Ki-67, ProExC and p16 was observed in 79%, 64% and 7% of high grade lesions respectively. These were negative in 98%, 98% and 100% of non high grade lesions. An additional 32% of high grade lesions showed ProExc staining in 25-50% of cells. Ki-67 demonstrated the highest test performance as it was more sensitive than ProExC for confirming the presence of high grade dysplasia with equivocal specifities, although not statistically significant in these small numbers (p>0.05). Ki-67 had the highest positive predictive value (96%). When used in panels of two, Ki-67 and ProExC had the highest sensivity and 100% positive predictive value. Ki67 and ProExC also showed high concordance (92%).
Conclusions: Ki-67 is of diagnostic utility in the segregation of low and high grade lesions in the UADT. While p16 and ProExC are well established markers in HPV related high grade lesions at other sites our study shows no diagnostic utility for these markers in the classification of UADT dysplasia.

1304 Do Small Oncocytic Follicular Carcinoma/Hurthle Cell Carcinoma Exist? An Institutional Experience

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Background: Oncocytic follicular carcinoma / Hurthle cell carcinoma (OFCA/HCC) is a rare tumor of the thyroid gland; when compared to non-oncocytic follicular carcinoma; it is more frequently associated with an aggressive clinical course. Usually OFCA/HCA are large, however, tumors measuring 2.0 cm can occur. In this study, we report our institutional experience with 119 cases of OFCA/HCA diagnosed between 2000-2012.
Design: One hundred nineteen cases of HCC diagnosed between 2000-2012 were retrieved from electronic pathology files at University of Pennsylvania Medical Center employing the search terms of "Hurthle cell", "carcinoma" and "thyroid". The data points recorded for this study included patient's age, sex, size of tumor, method of diagnosis (fine needle aspiration vs. biopsy vs. resection), lymph node status at time of resection, and clinical follow-up.
Results: The case cohort included 37 males and 82 females (average age 55 yrs, median 56 yrs). Pre-operative fine-needle aspiration (FNA) was performed in 73/119 cases (61%) and was diagnosed as consistent with "Follicular neoplasm with oncocytic features / Hurthle cell neoplasm" in 58 cases. Twenty five (21%) tumors measured ≤ 2.0 cm (including 4 measuring ≤ 1.0 cm) in 20 females and 5 males (average age 54 yrs); and 94 (79%) measured > 2.0 cm in 62 females and 32 males (average age 56 yrs). Definite angioinvasion was present in 48/119 (40%, 8 with >5 foci of angioinvasion) and lymph node metastases in 7/119 (6%) cases. Of these, 8/48 (17%) tumors with angioinvasion (2 with >5 foci of angioinvasion) and 1 with lymph node metastasis measured ≤2.0 cm. Clinical follow-up was available in 40 (40/119 34%) cases (range 1-137 months); 10/40 (25%) developed lymph node metastases, 4 developed tissue recurrence and distant metastases were seen in 12/40 (30%) cases. One case with distant metastasis to lung and 1 case with lymph node recurrence measured 1.1 cm and 2.0 cm respectively.
Conclusions: In our experience, OFCA/HCC can present as small (≤2.0) tumors with sex and age distribution similar to large (≥ 2.0) tumors. Importantly, small tumors can be associated with angioinvasion (8/25 32%), lymph node and, rarely, distant metastases. Both large and small OFCA/HCC can be easily detected as oncocytic tumors by FNA.

1305 The Variable Pathologic Presentations of Medullary & Micro-Medullary Thyroid Carcinoma: An Institutional Experience

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Background: Medullary thyroid carcinoma (MTC) is a rare tumor which can present in both sporadic and familial forms. The pathologic diagnosis of MTC can be difficult due to variability in its clinical presentation, size and morphology. In this study, we report our institutional experience with 45 cases of medullary thyroid carcinoma diagnosed between 2000-2007.
Design: Forty-five cases of MTC diagnosed between 2000-2007 were retrieved from electronic pathology files at University of Pennsylvania Medical Center employing the search terms of "medullary", "carcinoma" and "thyroid". The data points recorded for this study included patient's age, sex, family history, size of tumor, method of diagnosis (fine needle aspiration (FNA) vs. biopsy vs. resection), pre- and post-operative calcitonin and CEA levels, presence of concomitant follicular derived thyroid carcinoma, lymph node status at time of resection, and clinical follow-up.
Results: The cohort included 17 males and 28 females (average age 53 yrs, median 51 yrs); of these, 6 had a history of multiple endocrine neoplasia II. Pre-operative FNA was performed in 33/45 cases (33%); 15 were diagnosed as consistent with MTC, 8 as suspicious for MTC and 10 as follicular neoplasm. Pre-operative calcitonin was available in 17 (range 4.1-1709 mg/L and CEA in 2 cases (range 3.4-20.9 mg/L) Based on size, 20 tumors were classified as micro-MTC (average size 0.35 cm) and 25 as macro-MTC (average size 2.8 cm); of these, 15 MTC (9 micro and 6 macro-MTC) occurred with other thyroid malignancies (papillary carcinoma 12, follicular carcinoma 2, and anaplastic carcinoma 1 case). Bilateral MTC were noted in 11 cases, including 4 cases of MENIIB. Lymph node metastases were present at primary resection in 18 (18/45 40%) cases; of these, 6 were from micro-MTC and 2 showed metastases only from concomitant papillary thyroid carcinoma. Clinical follow-up was available in 21 (21/45 47%) cases (range 1-146 months). In 4 patients the calcitonin levels rose or remained elevated postoperatively; of these, 2 had regional lymph node recurrence and 1 developed distant metastases to lung and liver and subsequently died of disease. No tumor recurrence or distant metastases were seen in cases of micro-MTC.
Conclusions: In our experience, MTC is a heterogeneous disease due to its clinical presentation, pathology, and follow-up. Its preoperative diagnosis can be accomplished by FNA. Sporadic micro-MTC carcinoma is an indolent tumor and can occur with other malignant tumors of the thyroid gland.

1306 Sonic Hedgehog Pathway (HHIP, PTCH1 and SHH) VEGF-A in Oral Squamous Cell Carcinomas: Association with Expression of VEGF-A and Microvessels

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Background: Sonic Hedgehog (SHH) signaling has been implicated in tumor development and progression by stimulating angiogenesis and its molecular mechanisms are little known in oral squamous cell carcinomas (OSCC). The aim of this study was to characterize the transcripts expression involved in the SHH pathway (SHH, PTCH1, HHIP, VEGF-A) and microvessel density OSCC.
Design: A total of 50 OSCC, eight non-tumor epithelium adjacent to oral cancer (NTEA), and two oral mucosa obtained from healthy individuals were included. After RNA extraction, complementary DNA was obtained using high-capacity transcriptase. Quantitative PCR reactions were using Taqman Gene Expression Assays (SHH, PTCH1, HHIP and VEGF-A). Cq (ΔΔCQ) relative quantification method was perfomed for gene expression analysis. Fifty six paraffin-embedded specimens of OSCC were used for tissue microarray (TMA) and by immunohistochemistry, VEGF and CD34 were studied.
Results: *HHIP* and *PTCH1* transcripts were detected in OSCC and NTEA. Seventeen (34%) OSCC cases were negative for *SHH* gene expression. POSITIVE correlation between *SHH* and *HHIP* (rs=0.29; p=0.044, Spearman test) and *SHH* and *PTCH1* (rs=0.49; p=0.0003, Spearman test), *SHH* and *VEGF-A* (rs=0.37; p=0.0086; Spearman test) gene expression were observed in OSCC. Higher levels of VEGF-A expression seemed to increase the microvessel density of OSCC (rs=0.55;p=0.0050; Sperman test). Higher levels of transcripts and VEGF-A protein were related to OSCC with perineural invasion and those moderately differentiated. Finally, increased expression of VEGF-A was detected in NTEA (p = 0.034, Mann Whitney test).
Conclusions: THE positive correlation between the gene expression of *SHH* and *VEGF-A* indicates that pathway SHH could participate in tumor angiogenesis, and VEGF-A gene expression was the only factor that contributed to a greater MVD in our series. In addition, increased expression of this gene and protein VEGF-A was related to more aggressive behavior of OSCC. The expression of *HHIP*, *PTCH1* and *VEGF-A* in NTEA also indicates that SHH pathway can be activated in the tumor margins and might suggest existence of a field cancerization. At the same time, increased gene expression of *VEGF-A* in this location suggests that adjacent cells to oral cancer could actively participate in the secretion of important growth factors to the tumor development.

1307 GATA3 Immunoexpression in Salivary Gland Neoplasms

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Background: GATA3 is a zinc finger transcription factor that regulates the normal development of many tissues and cell types. Recent studies have shown that immunohistochemical nuclear staining for GATA3 among tumors is almost entirely limited to carcinomas of breast and urothelial origin. However, to our knowledge salivary gland tumors have not been tested with this antibody. Given that breast tissue and salivary gland tissue share a similar embryologic origin, we sought to determine whether GATA3 staining may be seen in salivary gland neoplasms.

Design: GATA3 immunohistochemistry (clone L50-823, BioCare, Concord, CA) was performed on a diverse collection of 164 primary salivary gland neoplasms (122 on tissue microarrays and 42 on whole slides), and the staining patterns were recorded.

Results: The results are summarized in Table 1. Staining for GATA3 was observed in 81/164 (49%) of salivary gland tumors. The background benign salivary was also usually weakly positive in acini and ducts. Some staining was seen in almost every tumor type tested, but it was particularly common in tumors with an "oncocytic" histologic appearance. Notably, GATA3 was 100% sensitive for two tumors that histologically mimic breast cancer: salivary duct carcinoma (25/25) and mammary analogue secretory carcinoma (15/15). Overall, most GATA3 tumor staining was weak or moderate in intensity, though salivary duct carcinoma (17/25 or 68%) and mammary analogue secretory carcinoma (9/15 or 60%) often showed strong positivity.

Table 1

Tumor type	GATA3 staining (%)
Acinic cell carcinoma	1/7 (14)
Adenocarcinoma, NOS	0/2 (0)
Adenoid cystic carcinoma	9/41 (22)
Epithelial-myoepithelial carcinoma	1/2 (50)
Mammary analogue secretory carcinoma	15/15 (100)
Mucoepidermoid carcinoma	11/27 (41)
Oncocytic carcinoma	1/2 (50)
Oncocytoma	5/5 (100)
Pleomorphic adenoma	13/34 (38)
Polymorphous low grade adenocarcinoma	0/4 (0)
Salivary duct carcinoma	25/25 (100)
Total	81/164 (49)

Conclusions: GATA3 immunostaining is relatively common in salivary gland neoplasms. As a result, a salivary gland origin must be considered in the differential diagnosis of a GATA3-positive carcinoma of unknown primary. In addition, GATA3 immunohistochemistry cannot be used to rule out metastatic breast cancer in the setting of a salivary gland tumor that has the appearance of breast carcinoma. Finally, although GATA3 is not specific for any salivary gland tumor, strong staining may be helpful to confirm the diagnosis of salivary duct carcinoma or mammary analogue secretory carcinoma.

1308 CRTC1/3-MAML2 Fusions Are Not Seen in Metaplastic Warthin Tumors and Metaplastic Pleomorphic Adenomas of Salivary Glands

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Background: The recurrent translocations t(11;19) and t(11;15) resulting in CRTC1-MAML2 or CRTC3-MAML2 fusion oncogenes, respectively, are identified in a large proportion of mucoepidermoid carcinomas (MEC) of the salivary gland and have impact on prognosis. However, there are conflicting data on specificity of this translocation, in particular, on its ocurrence in Warthin tumor (WT) of the parotid gland. It was speculated that extensive squamous metaplasia in WT could explain the presence of t(11;19) translocation in a subset of WTs.

Design: We have evaluated 15 cases of metaplastic WT and 8 cases of pleomorphic adenomas (PA) with squamous and/or mucinous metaplasia, extensive enough to mimic MEC. Detections of CRTC1-MAML2 and CRTC3-MAML2 fusion transcripts were performed using nested RT-PCR. 20 cases of low-grade fusion positive MEC were included for comparison.

Results: None of 15 analyzed metaplastic WT showed positivity for fusion transcripts CRTC1-MAML2 or CRTC3-MAML2. We did not detect above mentioned transcripts in any case of PA with extensive squamous/mucinous metaplasia, as well. All 20 cases of low grade MEC revealed CRTC1-MAML2 translocation, however, CRTC3-MAML2 translocation was not detected in any case.

Conclusions: We present our experience with the CRTC1-MAML2 and CRTC3-MAML2 translocations in a cohort of salivary gland tumors with extensive squamous and mucinous metaplasia. In contrast to low grade MEC, nor metaplastic pleomorphic adenomas neither metaplastic Warthin tumor harbored translocations t(11;19) and t(11;15).

1309 Solitary Fibrous Tumor of the Head and Neck: Institutional Experience of Two Referral Centers

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Background: Solitary fibrous tumors (SFTs) uncommonly present in the head and neck, and to date have been reported in limited numbers in the literature. Additionally, a number of lesions previously diagnosed in the head and neck as hemangiopericytomas (HPC), giant cell angiofibroma (GCA), and orbital fibrous histiocytoma (OFH) have been recently appreciated to be within the expanded spectrum of SFTs. Thus, we performed a comprehensive review of head and neck SFTs in order to more fully characterize their features and natural history.

Design: We performed a comprehensive search and review of lesions diagnosed as SFT, HPC, GCA, and OFH from 1985-2011 at the University of Michigan Health System (UMHS) and from 1982-2007 at the University of Pittsburgh Medical Center (UPMC). Cases were reviewed to confirm diagnoses, and data for clinical, histologic, immunohistochemical, and outcome parameters were recorded.

Results: A total of 47 cases with archival material were identified and reviewed (27 UMHS; 20 UPMC), with 21:26 male:female ratio and mean age 48.6±16.9y (range 15-92). Anatomically lesions involved the orbit (38.3%), sinonasal tract (23.4%), skull base (21.3%), oral cavity (14.9%), salivary or subcutaneous sites (each 10.6%), and parapharynx (4.3%), with 8/47 cases extending to multiple sites. Original diagnoses included SFT (62%), HPC (29.8), and OFH (8.5%). Predominant histologic pattern on review was classic SFT-like in 63.8%, cellular (former HPC-like) in 31.9%, and GCA-like in 4.3%. Nuclear pleomorphism/atypia was high in 21.3%, low in 27.6%, and absent in 51.1% of cases; necrosis was present in 17.5% and infiltrative grown in 45.2%. Immunostain for CD34 was positive in 29/34 (85.3%), BCL2 in 13/14 (92.9%), and CD99 in 10/12 (83.3%) cases with archival material. S100 was negative in 28/29 (96.5%), and both cytokeratins and SMA negative in 21/22 (95.4%). Follow-up data were available for 27 cases (median, 114mo.). Ten tumors recurred (37.0%), including one orbital case which metastasized to lung and parotid.

Conclusions: SFTs present in a wide anatomic distribution in the head and neck, and include a significant minority of cases showing cellular morphology, a feature associated with adverse outcome in a subset of SFTs from other anatomic sites. While the high rate of local recurrence identified likely reflects referral bias, it underscores the biologically intermediate malignant potential of these tumors and the importance of their recognition and need for complete excision and long term follow-up.

1310 Association between Stromal Myofibroblasts and Molecular, Clinical, and Histopathological Features of Premalignant and Malignant Lip Lesions

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Background: Tumor stroma composition is an important prognostic factor in several neoplasias. An increased density of stromal myofibroblasts is associated with higher tumor aggressiveness and mortality in oral squamous cell carcinoma (SCC). Lip SCC is a type of oral cancer characterized by altered p53 and COX-2 expression as well as by an activated stroma both in its premalignant and malignant stages, and includes increased angiogenesis, inflammatory infiltration and fibroblast density. Since myofibroblasts have not been characterized during lip carcinogenesis, the aim of this study was to assess myofibroblast density and its association with molecular and clinicopathological features of both the premalignant lip lesion, actinic cheilitis (AC) and lip SCC.

Design: Sixty two AC samples (11 with dysplasia) and 54 lip SCC samples were processed for immunohistochemical detection of myofibroblasts with the marker smooth muscle actin (SMA). Immunohistochemical detection of p53 and COX-2 was also performed. Samples were scored 1 if SMA+ cells corresponded only to blood vessels, 2 if SMA+ myofibroblasts were <50% of the stroma, and 3 if SMA+ myofibroblasts were >50% of the stroma. Scores (based on intensity and extension) were also obtained for p53 and COX-2 expression at the epithelial or tumor compartments of each sample.

Results: Myofibroblasts were significantly increased in lip SCC (55%) as compared to AC (13%) ($P<0.0001$, Fisher´s Exact test). In lip SCC samples, 44% had score 1, 24% score 2, and 31% score 3. In AC samples, 86% had score 1 and 13% score 2, with no score 3. Female patients with lip SCC showed higher stromal myofibroblast density than males ($P<0.05$). Myofibroblast density had no association with TNM stage or tumor differentiation, however, it was associated with epithelial dysplasia in AC ($P<0.05$). Stromal myofibroblast density was also associated with p53 expression both in AC and lip SCC ($P<0.0001$, t-test), but no association was found with COX-2 expression.

Conclusions: Myofibroblast density increases as lip cancer progresses, and it is associated with gender (higher in females) and p53 expression. This suggests that stromal myofibroblasts are important markers of malignancy during lip carcinogenesis.

Supported by CONICYT Chile, grant FONDECYT 1090287.

1311 Advantages of Three-Grade Histopronostic Classification in Therapeutic Management of Primary Epithelial Parotid Carcinoma

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Background: The treatment of primary parotid carcinomas involves tumor staging and histological grading in two grades: low and high grade malignancy. We took recent publications in this area into account and assessed the benefits of a three-grade histological classification for identifying a group of tumors with an intermediate malignancy grade.

Design: A 20-year standardized single center treatment history, including total parotidectomy, neck dissection and radiotherapy, was assessed retrospectively. The histological review of 113 consecutively treated parotid malignancies identified 98 suitable cases for univariate and multivariate survival analysis.

Results: Treatment involved total parotidectomy in 91.6% of cases, partial or total facial nerve resection respectively in 16.7% and 13.5%, neck dissection in 83.3% and postoperative radiotherapy in 70.8%. Forty-one tumors (36.3%) were classified as low-grade carcinomas (13 acinic cell carcinomas, 10 low-grade mucoepidermoid carcinomas, 7 ex pleomorphic adenoma intracapsular or minimally invasive carcinomas, 4 basal cell adenocarcinomas, 2 low-grade adenocarcinomas NOS and 1 polymorphous low-grade adenocarcinoma), 28 cases (24.6%) as intermediate-grade (14 adenoid cystic carcinomas with a cribriform/tubular pattern, 5 intermediate-grade mucoepidermoid carcinomas, 4 acinic cell carcinomas with a KI-67 index of over 10%, 3 ex pleomorphic adenoma invasive carcinomas with an intermediate-grade component, 2 intermediate-grade adenocarcinomas NOS) and 44 cases (39%) as high-grade carcinomas (19 high-grade adenocarcinomas NOS, 11 high-grade mucoepidermoid carcinomas, 5 salivary duct

carcinomas, 4 ex pleomorphic adenoma invasive carcinomas with a high grade histology, 3 solid adenoid cystic carcinomas and 2 dedifferentiated acinic cell carcinomas). The 5-year overall survival, disease specific and recurrence free survival rates were 79.4%, 83.5% and 70.8%, respectively. Univariate analysis confirmed the classical prognostic factors, while multivariate analysis identified the clinical stage and grade, especially when analyzing three groups, as the most important prognostic factors ($p<0.005$). In the Cox models studied, a one-grade elevation increased the risk of non-specific death by 2.6-fold and the risk of disease recurrence by 2.3-fold.

Conclusions: This study highlighted the possible prognostic significance of intermediate grade tumors. A three-grade histological classification could provide a more accurate risk stratification and management of patients with salivary gland carcinomas.

1312 EGFR Expression and KRAS and BRAF Mutational Status in Intestinal-Type Sinonasal Adenocarcinoma

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Background: Intestinal-type adenocarcinoma (ITACs) of the nasal cavity and paranasal sinuses are rare and aggressive tumor, with an estimated 5-year survival of 40%. Interestingly these tumors show morphologic and phenotypic features that are usually indistinguishable from colorectal cancer (CRC). These resemblances make reasonable to foresee that both tumor types may share equivalent genetic alterations. Since *KRAS* and *BRAF* mutations could be negative predictive factors for anti-EGFR therapies in CRC, we sought to determine the incidence of *KRAS* and *BRAF* mutations in ITACs. In addition we determine the prognostic impact of both *KRAS/BRAF* mutations and epidermal growth factor receptor (EGFR) expression and their potential association with clinicopathological features of ITACs.

Design: Paraffin embedded tumor samples of 43 ITACs were analyzed for *KRAS* exon 2 and *BRAF* exon 15 mutations and for EGFR expression through immunohistochemistry. Patients' information was extracted from medical records.

Results: 27 tumors (63%) showed EGFR positivity and 30% exhibited high expression level (+2/+3). *KRAS* mutations (p.G12D, p.G12C, p.G13D) were detected in 21 cases (48.8%). *BRAF* mutation (p.V600E) was identified in 3 specimens (6.1%). Using multivariate analysis, EGFR expression (+1, +2, +3) was significantly correlated with stage ($p<0.001$) and histotypes ($p<0.001$). No correlation was found with clinico-pathological parameters and *KRAS* and *BRAF* mutational status. Finally, no significant correlation was found in the disease free and overall survival between tumors with or without *KRAS* and *BRAF* mutations and EGFR expression.

Conclusions: Our data confirm the morphologic and phenotypic similarities at the genetic level between CRC and ITACs showing deregulation of *KRAS/ BRAF* and EGFR. No significant differences have been observed in clinico-pathological based on *KRAS/ BRAF* genotype and no prognostic value of *KRAS/BRAF* mutations were found. Despite multimodality treatment including surgery, radiotherapy and chemotherapy, ITACs show poor survival and new therapeutic approached are needed to improve this prognostic. A possibility may be offered by anti-EGFR therapies that have been developed in CRC. Our results indicate that *KRAS* mutations and EGFR expression are frequent in ITACs. So that EGFR directed molecular treatment could be investigated in a subset of patients affected by ITACs and *KRAS* mutation analysis could be used to preclude patients from receiving such treatment.

1313 Absence of BRAF and NRAS Mutations in Chronic Lymphocytic Thyroiditis and Primary Thyroid Lymphomas

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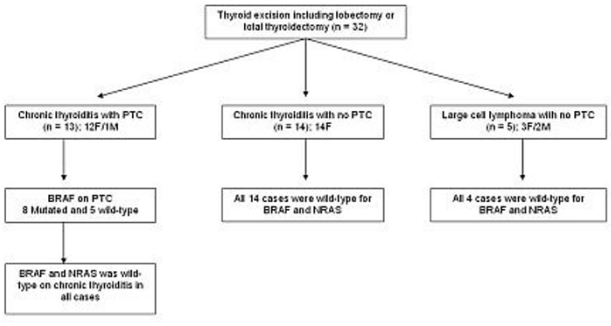
Background: Primary thyroid lymphomas are rare and account for 1-5% of all thyroid malignancy. A recent article described BRAF and NRAS mutation in 24% and 8%, respectively, of diffuse large B cell lymphomas of the thyroid. Chronic lymphocytic thyroiditis is presumed to be a precursor lesion for both papillary thyroid cancer (PTC) and lymphomas of the thyroid. There is scarcity of data on study of above mutations in these precursor lesions.

Design: A total of 32 cases of thyroid excisions including lobectomy and or total thyroidectomy were retrieved from our files with diagnosis of chronic thyroiditis with no PTC (n = 14); chronic thyroiditis adjacent to PTC (n = 13); and primary thyroid lymphomas with no PTC (n = 5).



Histological sections with the target areas of interest were identified and manually microdissected on 10µm sections for BRAF and NRAS mutation analysis. The isolated DNA was analyzed using a PCR based assays that amplified the region of the BRAF gene encompassing codon 600 or NRAS codon 61. Fluorescently labeled probes hybridized to those regions and a thermal melting procedure distinguished non mutated, wild type sequences from mutated sequences.

Results: The patients included 29 females and 3 males with a mean age of 45 years (range = 28-72 years). Primary thyroid lymphomas cases included DLBCL (n = 4) and Burkitt's lymphoma (n = 1). No BRAF and NRAS mutation was identified in either chronic lymphocytic thyroiditis or in primary thyroid lymphomas. However, BRAF was mutated in 61% of PTC.

Conclusions: We found no evidence of BRAF and NRAS mutations in 27 cases of chronic thyroiditis in our study. These results do not support that chronic lymphocytic thyroiditis is a precursor to BRAF or NRAS mutated primary thyroid lymphomas, or that such mutations are a late event in that progression.

1314 Immunohistochemical Expression of p40 in Sarcomatoid Squamous Cell Carcinoma of the Head and Neck

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Background: Sarcomatoid or spindle cell squamous carcinoma (SCSC) of the head and neck is an uncommon histologic subtype that may present considerable diagnostic difficulty on routine histology. Conventional squamous cell carcinomas are known to express p63, however this marker is not entirely specific for squamous differentiation. p40 has been recently shown to be as sensitive and more specific than p63 for squamous cell carcinoma in the diagnosis of non-small cell lung cancer. To the best of our knowledge, there do not appear to be any studies in the English medical literature examining the utility of p40 staining in SCSC. We sought to investigate the diagnostic utility of p40 immuohistochemistry in this aggressive variant.

Design: We searched our archives for all cases of SCSC of the head and neck region diagnosed from January 2008 to July 2012. Hematoxylin and eosin stained slides were reviewed. Five (5) head and neck sarcomas were selected as controls, comprising two high-grade sarcomas, a soft tissue malignant myoepithelioma, a monophasic synovial sarcoma and a chondroblastic osteosarcoma. p63 and p40 immunohistochemistry was performed on all cases and controls.

Results: Twenty-two (22) cases of SCSC were identified. In these cases, p63 was expressed in more cases than p40 (18/22 vs 13/22). Thirteen (13) cases showed strong and diffuse positivity for p63, 5 showed focal or rare cells positive and 4 cases were negative. Interestingly, none of the cases showed stronger staining for p40 than p63. The results of immunochemical staining are summarized in Table 1.

p63 Staining	p40 Staining	
Strong and Diffuse		
13 cases	Strong and Diffuse	9 cases
	Focal	2 cases
	Negative	2 cases
Focal Staining		
5 cases	Strong and Diffuse	0 cases
	Focal	2 cases
	Negative	3 cases
Negative		
4 cases	Strong and Diffuse	0 cases
	Focal	0 cases
	Negative	4 cases

Among the sarcoma controls, only the monophasic synovial sarcoma showed focal positivity for p63; all other sarcomas were negative. Of note, the chondroblastic osteosarcoma displayed nuclear positivity for p40; however, this entity should be easily distinguished from sarcomatoid squamous cell carcinoma on morphologic grounds. All other sarcomas were negative for p40.

Conclusions: p63 and p40 are both expressed in SCSC. p40 is relatively underexpressed, and is less sensitive than p63 in these tumors. Due to the small size of available cases, specificity could not be accurately evaluated. Based on our findings there appears to be no advantage to using p40 over p63 in the diagnosis of SCSC.

1315 Topographic Distribution of PTC by Mapping in Coronal Sections of Thyroidectomy Specimens

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Background: Papillary thyroid carcinoma (PTC) is commonly a multifocal and bilateral disease with significant potential for lymph node metastasis. Mapping of PTC foci has not been studied. The purpose of this study is to map PTC foci in coronally-sectioned thyroidectomy specimens.

Design: One hundred consecutive thyroid specimens (thyroidectomy or lobectomy followed by completion thyroidectomy) were sectioned in the coronal plane to identify and characterize PTC foci. Using current histologic criteria for diagnosis, the specimens were divided into 3 groups: encapsulated variant (EV) (either with follicular or papillary architecture), usual variant (UV), and tall cell variant (TCV). Each group was further substratified according to the maximum tumor diameter: <1 cm, <2 cm, and ≥2 cm.

Results: Patient ages ranged from 23 to 76 years (54 ± 12) with a female-to-male ratio of 3:1. The predominant tumor was located in the right lobe, isthmus, and left lobe in 52%, 8%, and 40%, respectively, with sizes ranging from 3 to 60 mm (18.8 ± 6.6). The histologic variants TCV, EV, and UV accounted for 17, 24, and 59 cases, respectively. Three topographical patterns can be distinguished: (a) solitary nodule (n=24) with most cases being <1 cm; (b) predominant mass with adjacent satellite nodules (n=21) exhibiting varying degrees of fusion; and (c) similar to (b) but with discrete, isolated foci throughout the thyroid gland (n=55). For the latter group, most of the predominant tumor masses measured >2 cm. Topographical patterns (a), (b), and (c) were identified in 58.8, 33.3, and 10.2%; 5.9, 33.3, and 20.3%; and 35.3, 33.3, and 69.5% of the TCV, EV, and UV groups, respectively. For topographical pattern (c), bilateral involvement

was seen in 72.7% of cases. Of the TCV, EV, and UV groups, 23.5, 20.8, and 52.5%, respectively exhibited bilateral involvement. The rate of bilaterality was statistically significant between tumors <1 cm and tumors <2 cm and ≥2 cm (p-values = 0.03 and 0.028, respectively).

Conclusions: TCV, EV, and small UV tend to be unifocal. Large UV are more often multifocal and bilateral. Secondary tumor nodules are most often located at the periphery of the main tumour and merged in a significant number of cases. Although intra-thyroidal lymphangitic spread cannot be excluded in this study, the topographical pattern of multifocality and the histopathology favor satellite tumor development in a zonal pattern around the main tumor mass with frequent superimposed field effect resulting in a random location throughout the thyroid gland.

1316 The Indoleamine 2,3-Dioxygenase (IDO) Pathway Is Constitutively Activated in HPV-Mediated Oropharyngeal Carcinoma

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Background: Overall cancer-specific immunity is impacted by the degree of immune tolerance mediated by both cancer and HPV, balanced against the degree of successful local adaptive immunity. Indoleamine 2,3-dioxygenase (IDO) is a tryptophan-catabolizing enzyme which promotes immune tolerance. IDO activation in dendritic cells results in CD8+ apoptosis and differentiation of naïve CD4+ into $T_{reg,}$ thus down-regulating adaptive immunity. The IDO pathway has not yet been investigated in the context of HPV-mediated oropharyngeal cancer (OPC). The rationale for this stems from the fact that there are a number of constitutive HPV-mediated pathways which promote immune dysregulation. We investigate the IDO pathway in head and neck cancer cell lines and OPC with known HPV status.

Design: We studied 8 cancer cell lines from the head and neck: 1HPV16+ OPC cell line and 7 HPV-negative cell lines. Two of these HPV-negative cell lines were transfected with HPV16 E6/E7 vectors. IDO mRNA was measured by RT-PCR; activated GCN2 (a downstream IDO target) was measured by Western blot. We also studied 31 OPC specimens for IDO protein by IHC; the HPV16/18 status was previously established by nested RT-PCR for type-specific E6 and E7 transcripts.

Results: Constitutive IDO and GCN2 activation was present only in the HPV16+ OPC cell line, but not in any HPV-negative cell lines. Both HPV transfected cell lines demonstrated new IDO and GCN2 activation.

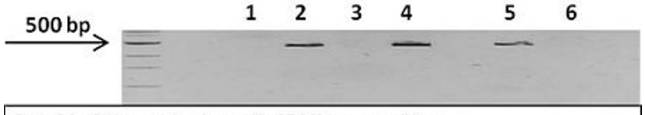

Fig.1. IDO mRNA expression detected by RT-PCR in tumor cell lines.

Lane 1:UAB4 (HPV-negative); lane 2: UAB4 transfected HPV16E6E7; lane 3: UAB3 (HPV-negative); lane 4: UAB3 transfected HPV16E6E7; lane 5: UAB1 (HPV16+); lane 6: negative control (no template).

IDO mRNA expression was detected (463 bp) in UAB1 (lane 5) and transfected UAB3 and UAB4 (lanes 2 and 4).

In the OPC cohort, 15/31 tumors (48%) were HPV16/18 positive. IDO tumor expression was seen in 93% (13/14) of HPV16+OPC, and 17% (3/17) of HPV-negative OPC. Tumor IDO expression was significantly associated with HPV-mediated OPC ($p < 0.001$).

Conclusions: These data support the idea that the IDO pathway is constituitively activated in HPV-mediated OPC. Thus IDO activation is yet another potential promoter of immune tolerance impacting the overall balance of local cancer immunity.

1317 IgG4-Related Disease Is a Rare Cause of Recurrent Mastoiditis and Can Be Mimicked by Severe Otitis Media

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Background: IgG4-related disease (IgG4-RD) is a recently recognized entity that causes progressive fibrosis and formation of mass lesions. It may present as a single focus or widespread disease in multiple sites, including in the head and neck where it has been shown to involve salivary glands, and lacrimal glands, among others. IgG4-RD can be diagnosed histologically by its hallmarks of storiform fibrosis, prominent lymphoplasmacytic (LPC) infiltrate, and obliterative phlebitis, as well as elevated serum IgG4. However, infections have been reported to elicit a histologic response that may mimic IgG4-RD. Our group recently reported a case of IgG4-related mastoiditis. We sought to elucidate the frequency of IgG4-RD as a previously-unrecognized cause of mastoiditis.

Design: We searched the pathology archives for all cases of mastoiditis between 2008 and 2011 and identified 162 cases of chronic otitis media (OM), and received in consult one case with histologic features of IgG4-RD. These were assessed for histologic evidence of IgG4-RD, and immunohistochemistry (IHC) was performed in cases with characteristic histologic findings.

Results: We identified 9 institutional cases with the histologic appearance of IgG4-RD, two of which were positive for IgG4+ plasma cells via IHC. The other 7 showed rare IgG4 positive plasma cells (<10/ HPF). Case #1 was a 33yr old male with recurrent otitis media episodes that resolved with antibiotics. A mastoid MRI showed possible cholesteatoma and he was found intraoperatively to have extensive granulation tissue surrounding the ossicles. Histology and IHC showed granulation tissue with an extensive polyclonal LPC infiltrate with 113 IgG4+ cells/hpf. Case #2 was a 68yr old male who suffered acute delirium and CT findings of coalescent mastoiditis and an osteopenic skull base, concerning for mastoiditis leading to meningitis. Intraoperative cultures grew *S. pneumonia*. Histology and IHC showed extensive fibrosis and a LPC infiltrate with 88 IgG4+ cells/hpf. Both cases are felt to represent chronic infection. The consultation case, diagnosed with IgG4-related mastoiditis, was a 50yr old female with 9 years of culture-negative serous OM, causing cerebritis and forming a progressive mass-like lesion requiring surgical excision. Histology & IHC showed storiform fibrosis and LPC infiltrate with >300 IgG4+ cells/hpf.

Conclusions: Our results show that IgG4-RD is an extremely rare cause of recurrent mastoiditis/OM, and severe infection may show histologic and IHC features that mimic IgG4-RD.

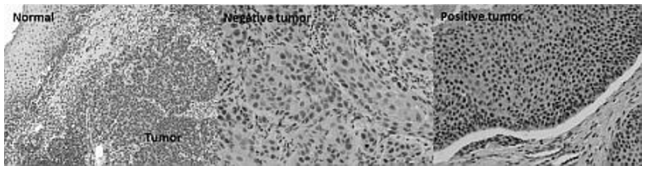
1318 High Immunohistochemical Expression of Translesion DNA Synthesis Enzyme Polymerase n in Head and Neck Squamous Cell Cancer Is Associated with Platinum-Based Chemotherapy Resistance

W Zhou, X Liu, P Chu, S Loera, B Wenig, K-M Chou, Y Yen. St. Luke's - Roosevelt Hospital and Beth Israel Medical Center, New York, NY; City of Hope Comprehensive Cancer Center, Duarte, CA; Indiana University, Indianapolis, IN.

Background: The development of cancer drug resistance in mucosal derived squamous cell carcinoma of the head and neck (HNSCC) is a persistent problem limiting the successful treatment of locally advanced malignancies. Recent evidence from basic research indicates that translesion DNA synthesis enzyme polymerase η (pol η; XPV gene) negatively influence the effectiveness of cisplatin by bypassing cisplatin induced DNA adducts, but little is known as to whether the immunostaing level of pol η in HNSCC patient samples is ultimately relevant to tumor chemotherapy sensitivity.

Design: Immunohistochemistry staining for pol η was performed on surgical specimens of sixty-four cases of mucosal derived, locally advanced HNSCC. The correlations of expression level of pol η with patient demographic, tumor staging, histological differentiation, and clinical outcomes were investigated. Expression of pre and post-treatment pol η in relation to chemoresponse was further evaluated in 49 cases treated with platinum-based chemotherapy.

Results: Nuclear Pol η expression was limited at basal layer of benign squamous mucosa. Higher nuclear pol η expression was detected in 65% of SCC. Pol η staining level was negatively associated with tumor grade, but not significantly differed by gender, age, Tobacco/alcohol history, tumor stage and metastatic status. Complete response rate was observed significantly higher in negative/low baseline Pol η staining group (44%, p=0.03). No survival significance was found to be related to Pol η staining level, although there is a trend showing patient with high Pol η expression tends to have shorter overall survival.



Conclusions: Our data, for the first time, provide evidences that in situ pol η staining might be a novel marker of chemoresponse in HNSCC patients receiving platinum-based therapy.

Hematopathology

1319 c-Myc Expression Correlates with Plasmablastic Morphology

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Background: Plasmablastic (PB) differentiation can be seen across a spectrum of plasma cell neoplasms. While it is the defining feature in plasmablastic lymphoma, it can occur secondarily in the plasmablastic variant of myeloma, where it portends an aggressive prognosis. *MYC* gene rearrangement has been well-established in both, thus supporting a biologic link between these entities. Still, the significance of MYC dysregulation in plasma cell neoplasms remains poorly understood, and the association between MYC expression and PB morphology has not been well studied. A recently available c-Myc antibody affords an opportunity to examine associations between MYC expression, plasma cell morphology, and proliferative status.

Design: 63 cases were retrospectively identified. Decalcified specimens were excluded from analysis, limiting our study to extramedullary plasma cell myeloma (PCM, n=24), plasmablastic lymphoma (PBL, n=12), and plasmacytoma (PC, n=27). Each case was assessed for the percentage of tumor cells with PB morphology, previously defined as cells with increased N:C ratio, fine chromatin, large nucleolus, diminished hof region, and decreased cytoplasm (Greipp, 1998). In addition, immunohistochemical (IHC) stains for c-Myc (Epitomics) and Ki-67 were performed, quantified, and compared, respectively, to the PB morphology percentage by linear regression analysis.

Results: PB morphology showed strong correlation (R^2=0.92) with c-Myc expression across all plasma cell neoplasms **(Figure 1)**, as well as within each diagnostic category, yielding the following R^2 values: PC=0.93, PCM=0.92, and PBL=0.89. In contrast, comparison of PB morphology with Ki-67 had an inferior correlation overall (R^2=0.50) and individually: PC=0.75, PCM=0.38, and PBL=0.41.