Design: Immunohistochemical staining for Sox9 was performed in 152 ovarian tumors: pure SCT, endometrioid borderline tumor (E-BT), sertoliform endometrioid carcinoma (SEC), FIGO grade 1 endometrioid carcinoma (E-CA), and carcinoid tumor (CT). Extent of staining was based on the percentage of positive cells: 0, <5%; 1+, 6-25%; 2+, 26-50%; 3+, 51-75%; and 4+ 76-100%. Intensity of staining was scored as weak (W), moderate (M), or strong (S).

Results: SOX9 was expressed in the nucleus in 45%, 55%, 39%, 65%, and 10% of SCT, E-BT, SEC, E-CA, and CT, respectively.[Table 1]

Conclusions: Sox9 is expressed in only a subset (45%) of ovarian SCTs, and it is not certain whether Sox9 plays the same role in the pathogenesis of ovarian SCT as it does in Sertoli cell differentiation in normal testes. Sox9 is variably expressed in other ovarian tumors that are in the DDx of SCT and, thus, is not helpful in the IHC distinction of SCT from its histologic mimics.

Table 1. Expression of Sox9 in Ovarian Tumors

Tumors	0*	1+*	2+*	3+*	4+*
SCT (n=36)	56%	11%(3W,1M,0S)	6%(1W,1M,0S)	14%(0W,4M,1S)	14%(0W,1M,4S)
E-BT (n=38)	45%	13%(1W,4M,0S)	21%(1W,4M,3S)	18%(0W,3M,4S)	3%(0W,0M,1S)
SEC (n=13)	62%	23%(0W,3M,0S)	15%(0W,2M,0S)	0%	0%
E-CA (n=26)	35%	4%(0W,1M,0S)	15%(0W,4M,0S)	15%(0W,2M,2S)	31%(0W,2M,6S)
CT (n=39)	90%	3%(0W,1M,0S)	3%(0W,1M,0S)	3%(0W,0M,1S)	3%(0W,0M,1S)

Key: *, result listed as percentage of cases showing each immunoscore for extent of staing (number of cases showing each grade of intensity in parentheses)

947 Comparative Analysis of Alternate Immunohistochemical (IHC) Markers for the Distinction of Ovarian Sertoli Cell Tumors from Endometrioid Tumors and Carcinoid Tumors: A Study of 160 Cases

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Background: Although traditional IHC markers (e.g., pan-cytokeratin, EMA, inhibin, calretinin, etc.) can be useful, they can occasionally have limitations in the distinction of ovarian sertoli cell tumor (SCT) from endometrioid and carcinoid tumors. Alternate markers (e.g., ER, PR, CD10, CK7, etc.) may have potential diagnostic advantages, but their role has not been extensively investigated in this differential diagnosis.

Design: Immunohistochemical stains were performed in the following ovarian tumors: SCT (n=40), endometrioid borderline tumor (E-BT) [n=38], sertoliform endometrioid carcinoma (SEC) [n=13], FIGO grade 1 endometrioid carcinoma (E-CA) [n=27], and carcinoid tumor (CT) [n=42]. Expression in >5% of cells was considered positive.

Results:

	Immunohistochemical Results				
Markers	SCT	E-BT	SEC	E-CA	CT
CK7	15%	100%	85%	100%	24%
ER	8%	87%	85%	89%	2%
PR	13%	84%	77%	93%	2%
CD99	68%	16%	23%	33%	41%
CD10	25%	40%	31%	37%	10%
Synaptophysin	35%	8%	8%	22%	98%
Chromogranin	13%	3%	15%	11%	100%
CD56	48%	16%	15%	30%	57%

The IHC results of Pan-CK, CK8/18, EMA, inhibin and calretinin were not listed in the table.

Conclusions: When traditional IHC markers are problematic in a given case of ovarian SCT versus an endometrioid or carcinoid tumor, adding CK7, ER or PR, and synaptophysin or chromogranin to a panel of markers may be helpful. CD99 and CD10 are neither sensitive nor specific for SCT. In the distinction from CT, use of CD56 should be avoided.

Head & Neck

948 Heparanase Expression in Nasopharyngeal Carcinoma Is Inversely Correlated with Patient Survival

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Background: Heparanase is an endoglycosidase that cleaves heparan sulfate side chains of the major proteoglycans in the extracellular matrix and cell surfaces. Heparanase plays a role in angiogenesis and cancer metastasis and its upregulation was correlated with reduced survival in various malignancies. Heparanase expression was not previously examined in nasopharyngeal carcinoma (NPC).

Design: Immunohistochemical staining for heparanase was performed on sections of 46 NPC patients and correlated with clinical and pathological data. We used the polyclonal antibody which was raised in our laboratory and preferentially recognizes the 50kD active heparanase subunit (J Cell Sci 117:2249,2004).

Results: Positive staining was found in 35% of the cases. Heparanase positive cases had a cumulative 10 years survival of 25%, compared to 70% 10 years survival for heparanase negative cases (p=0.03). 35 patients were males and 11 were females. Median age was 42. Most patients (73%) were diagnosed with stage IV, and most (73%) had undifferentiated carcinoma. 89% were EBV positive (EBER ISH). Median follow up was 7 years. 21 of the 46 patients died of the disease. No significant correlations were found between heparanase staining and gender, age, EBV status, tumor stage and tumor histology (keratinizing, non-keratinizing and undifferentiated carcinoma).

Conclusions: Heparanase expression is associated with decreased survival rate of NPC patients. Heparanase may serve as a prognostic factor in NPC and can be cosidered as a target for development of specific anti-cancer drugs.

949 Frozen Section Evaluation of Head and Neck Margins

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Background: A surgeon's goal is to completely resect a tumor with negative margins. Frozen section (FS) margin evaluation allows a positive margin to be corrected prior to closure and any reconstruction. A final report is later issued following examination of all resected tissues. The final margin status may have prognostic and treatment implications for the patient. FS are costly, they require many resources and are high priority. To analyze how FS is being used and how well it is fulfilling its mission, we reviewed the current practice at medical centers where head and neck cancer surgery is performed and FS are utilized for margin evaluation.

Design: 200 pathologists were surveyed (verbally or by mail- 2 question survey) about their center's process of FS margin evaluation. The survey asked 1:How tissue is procured and evaluated intra-operatively, and 2:How the final pathology margin status is then determined post-operatively from the resected specimen. Our responders represent 100 medical centers (48 states and 4 international). We utilized the membership log of the North American Society of Head and Neck Pathology and the list of the top 50 US Cancer Centers according to US News and World Report (2004 Best Hospitals). Our response rate was 50% (100% for top 50 centers). We analyzed the different methods utilizing the Toyota principles of systems improvement by working backwards from the final product (an adequately resected tumor) stepwise, to reveal possible flaws.

Results: There were no significant trends regarding the first question. Many surgeons send small fragments of tissue from the defect cavity (40%) or from the specimen proper (27%). The second question revealed that most respondents who received small tissue fragments re-sampled all margins for the final pathology report and that there was no indication where the tissue was procured from in relation to the resected specimen (44%). Others did not re-sample margins at all and some were not comfortable with this practice.

Conclusions: There may be more than one ideal method for FS margin evaluation. Many system failures occur in the report of final margins. Most failures are due to lack of anatomic correlation at hand offs between the surgeon and the tissue prosector. Over and under sampling of margins may be occurring. Many respondents expressed frustration in the current system at their institution. There is currently no consensus on how to best submit tissue for FS evaluation.

950 Immunohistochemical Detection of p16^{INK4a} in Dysplastic Lesions of the Oral Cavity

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Background: Mutations in the tumor suppressor gene CDKN2A/p16 have been documented in preneoplastic lesions of the oral mucosa, but whether this corresponds to altered expression of the gene product p16^{INK4a} (p16) is unclear. Furthermore, whether expression of p16 could be a useful marker of dysplasia for use in routine practice remains to be evaluated.

Design: One-hundred twenty biopsy specimens representing various oral cavity sites and degrees of dysplasia were retrieved from the pathology files of Emory University Hospital. Formalin-fixed, paraffin-embedded sections were stained with H&E and with a monoclonal antibody to p16 (Lab Vision Corporation, clone JC2, 1:40 dilution). A blinded review of the H&E slides and the pattern and degree of p16 expression was independently performed by 2 experienced pathologists. A consensus was obtained when diagnoses differed. Morphologic diagnoses were then compared to p16 immunohistochemical expression.

Results: Overall, 62/120 (52%) cases showed no p16 immunoreactivity while the remainder showed p16 expression limited to the basal/suprabasal nuclei and generally confined to the lower one-third of the epithelium (Table). p16 expression was found to be dependent on severity of dysplasia (χ^2 test, p=0.02) with a significant trend toward decreased expression with increasing severity of dysplasia (logistic regression model, p=0.002). However, 12/33 (36%) cases of non-dysplastic squamous mucosa were also negative for p16 expression.

		p16 Expression			
		Negative	Focal Basal Staining	Basal and Suprabasal Staining	Total number of cases
Consensus Grade	No Dysplasia	12 (36%)	18 (55%)	3 (9%)	33 (100%)
	Mild Dysplasia	11 (39%)	12 (43%)	5 (18%)	28 (100%)
	Moderate/Severe Dysplasia	39 (66%)	15 (25%)	5 (8%)	59 (100%)

Conclusions: Decreased immunohistochemical expression of p16 protein in dysplastic lesions, as found in this study, may reflect the biologic events involving loss of p16 gene function in the pathogenesis of oral cancer. However, since a significant proportion of non-dysplastic mucosa also showed no p16 expression, we conclude that p16 immunohistochemistry using a monoclonal antibody is not a useful marker of dysplasia.

951 Ezrin Localization in Head and Neck Squamous Cell Carcinoma. A Tumor Microarray Validation Project

M Brandwein-Gensler, N Schlect, A Thielken, R Mahmood, H Huang, T Belbin, R Smith, M Prystowsky. Montefiore Medical Center/Albert Einstein College of Medicine, Bronx, NY.

Background: Ezrin-Radixin-Moesin (ERM) are linker proteins that connect cell membranes with cytoskeletal actin, regulating cell shape, motility, and proliferation. Our previous microarray expression studies have demonstrated that ERM upregulation are among changes seen with tumor progression. Our previous tissue microarray (TMA) pilot study confirmed that strong ezrin cytoplasmic localization is significantly associated with decreased overall survival. Our aim is to validate this correlation between ezrin expression and tumor progression in a new tumor data set.

Design: TMA were prospectively constructed and included primary SCC, and when available, adjacent mucosa and regional metastases. Immunohistochemistry was performed with monoclonal anti-ezrin (1:100). Ezrin expression was determined with respect to subcellular localization (membranous Fig 1, vs. cytoplasmic Fig 2) and degree of expression and the results compared across clinical and histopathologic variables.
Results: In this prospectively followed group (n = 55), 31 patients (56%) had positive lymph nodes, 15 developed recurrences after a mean of 13.5 months (overall mean follow-up 14.3 months). Cytoplasmic ezrin expression was common (91%); strong (3+) expression was seen in 24% of tumors (Fig 2). Strong ezrin cytoplasmic expression was present in tumors with positive lymph nodes (32% vs. 13%, p=0.12) and higher T stage tumors (30% vs. 7%, p=0.08). Multivariable survival analysis demonstrated that 2+ to 3+ cytoplasmic ezrin expression was associated with recurrence, albeit not significantly (hazard ratio=1.57, p=0.59).
Conclusions: Strong ezrin cytoplasmic localization may indicate the potential for tumor lymph node metastasis. Continued accrual of patients and clinical follow-up are ongoing to confirm this association.

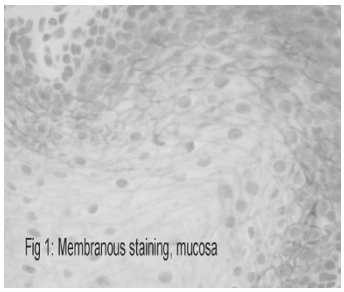
Fig 1: Membranous staining, mucosa

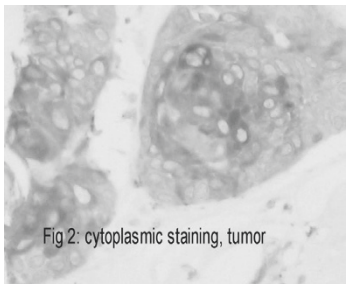
Fig 2: cytoplasmic staining, tumor

952 Rationale for mTOR Effectors as Therapeutic Targets in Head and Neck Squamous Cell Carcinoma (HNSCC)

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Background: HNSCC carries a relatively high mortality rate and a poor prognosis. Recently, we showed that overexpression of phosphorylated (p) nuclear factor-kappaB (p-NF-kB) in squamous cell carcinoma of the tonsil (SCCT) and high grade dysplasia is associated with poor prognosis (Modern Pathology 18: 924, 2005). Because the mammalian target of rapamycin (mTOR) pathway, through immunophilin/mTOR signaling, contributes to the activation of NF-kB; we investigated: a) the expression, state of activation and potential clinical significance of components of the mTOR signal transduction pathway in SCCT patients; and b) the inhibitory effects of rapamycin on the growth and state of activation of mTOR and one of its substrates, p70S6K in two HNSCC cell lines.
Design: Archival biopsy materials from thirty-one patients with SCCT were sudied by immunohistochemistry for expression of phosphorylated (p)-mTOR (Ser 2448), p-p70S6K (Thr 389) and cyclin D1, respectively. Results for SCCT were compared with adjacent non-neoplastic epithelium, when present, and with normal tonsillar epithelium from essentially age matched controls; clinical outcomes were assessed. Two human HNSCC cell lines, SCC-15 and FaDu, were incubated with and without rapamycin to assess its impact on growth and on the expressions of p-mTOR and p-p70S6K by Western blotting.
Results: All three markers were overexpressed in the SSCT when compared to those in the adjacent non-neoplastic epithelium and the normal control tonsillar epithelium ($p<0.05$). A significant relationship existed between p-p70S6K expression in the non-neoplastic squamous epithelium adjacent to the SCCT and death from disease (Hazard ratio= 7.9, 95%CI= [2.1,29.9], p= 0.002). Rapamycin in a dose dependent fashion inhibited growth more in SCC-15, which correlated with a greater reduction in constitutively activated p-mTOR (Ser 2448) and a corresponding reduction in its downstream effector, p-p70S6K (Thr 389) by Western blotting.
Conclusions: Overexpression of constitutively activated mTOR and p70S6K in the mTOR pathway in SCCT and overexpression of p-p70S6K in adjacent non-neoplastic epithelium, portending a worse clinical outcome, have been shown in this study. This coincides with a similar finding in two HNSCC cell lines and with the corresponding inhibition of growth in these cell lines and the accompanying downregulation of p-mTOR and p-p70S6K effected by rapamycin. These data collectively provide a rationale for mTOR effectors as therapeutic targets in HNSCC.

953 E-Cadherin Abnormalities, but Not Epidermal Growth Factor Receptor (EGF-R) Expression Correlates with Adenoid Cystic Carcinoma Recurrence

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Background: Local and distant metastases are common features of adenoid cystic carcinomas (ACCs). However, the underlying events for the aggressive nature of this tumour are poorly understood. Direct interaction between E-cadherin and EGF-R have been demonstrated in experimental studies, but few information on its presence and clinical impact exist in ACCs. **Aim:** To determine CDH1 and EGFR expression in ACCs and correlate with grade, tumour size, metastases diseases, and recurrence.
Design: A hospital-base case control study with blinded histopathological of tumour slides was conducted. Clinical data, follow-up and recurrences were obtained from clinical charts. The main outcome was recurrence of disease in the cases and without recurrence in the control group. Only patients with local recurrence discovered more than 6 month after initial surgery treatment were considered to have postoperative recurrences. Tumour size and residual diseases were obtained from the original surgical pathology reports. Four-3mm tissue cores were punched and arrayed on recipient blocks, including one of remnant tissue. IHQ detection of CDH1 (Dako, clone/NH-38) and EGFR with a standardized system (EGFRpharmDx™), was blindly performed. CDH1 reactivity was graded as normal (100%+ve neoplastic cells), heterogeneous (>25%-75%+ve cells), or negative (<25%+ve cells). EGFR was graded as 0, 1+, 2+ or 3+.
Results: 29 patients with ACC were included, 21 (72%) women and 8 (28%) men with a mean follow-up 61 months. Median patient age at the time of diagnosis was 48 years (range of 20-75 years). Recurrences were observed in 13 (45%), 10 (48%) women and 3 (38%) men (p=0.7). We detected E-cadherin expression on the cell membranes in 17 (77%) ACCs, but its distribution was irregular, as compared to that on normal structures. Abnormal CDH1 expression was observed in 3 (23%) cases, 4 (57%) with recurrence vs 1 (7%) without recurrence (p=0.021), while positive EGF-R expression in 4 (57%) with recurrence vs 8 (53%) without recurrence (p>0.05). No association with differentiation grade, tumour size, metastases diseases was found.
Conclusions: Our results showed that down regulation of E-cadherin expression, but not EGF-R expression, was associated with a more aggressive clinical behaviour of ACCs.

954 Immunoexpression of E-cadherin, MRP-1 /CD9 and EGFR in Acantholytic Squamous Cell Carcinomas of the Head and Neck. A Preliminary Study

AM Cano Valdez, M Nava Villalba, HR Dominguez Malagon. Instituto Nacional de Cancerologia, Mexico; Universidad Nacional Autonoma de Mexico.

Background: MRP-1/CD9 is a member of the transmembrane 4 super family (TM4SF) related with cellular migration, proliferation and metastasis. MRP-1/CD9 is able to interact with other trans-membrane proteins such as integrins to form complexes that help in cellular adhesion. Decreased expression of Motility-related-protein (MRP-1/CD9) is associated with metastatic potential in diverse types of carcinoma. E-cadherin is a calcium-dependent adhesion molecule that plays a crucial role in establishing and maintenance of intercellular connections of epithelial cells and morphogenesis. Changes in expression and function of e-cadherin have been postulated as early events in metastasis and tumor progression. Epidermal Growth Factor Receptor (EGFR) is a member of a family of four receptors [EGFR (HER1 or ErbB1), ErbB2 (HER2/neu), ErbB3 (HER3), and ErbB4 (HER4)], which reside in the cell membrane and its sobreexpression has been related with increased cell division and other aspects of malignant progression of the tumour - angiogenesis, metastasis and an inhibition of apoptosis.
Design: Ten cases of ASCC of the head and neck and ten cases of SCC matched for age, sex and location were studied by immunohistochemistry. The antibodies used were anti e-cadherin ( ) and anti MRP-1/CD9 ( ).The expression was graded as: negative (no signal), weak or strong. Also, the location (membrane or cytoplasm), and the topography (basal or central cells) were recorded.
Results: ASCC showed weak immunoreactivity in 2/9 cases (22%) and 1/9 (11%) for MRP-1/CD9 and E-cadherin, and strong, predominantly membranous positivity in 9/9 cases (100%) for EGFR; while SCC showed strong positive signal in 8/10 (80%) cases for MRP-1/CD9 and 4/10 (40%) cases for E-cadherin, and strong cytoplasmic reactivity for EGFR in 10/10 (100%) cases. Central cells displayed membranous signal for MRP-1/CD9 and E-cadherin in both groups, whereas signal for EGFR was stronger in basal cells.
Conclusions: The results of this preliminary study allowed reaching several conclusions: 1. ASCC presents reduced expression of MRP-1/CD9 and E-cadherin when compared with SCC. 2. There are differences in cellular location of immunoreactivity for EGFR in both groups. The relationship of these findings with a worse prognosis remains to be dilucidated.

955 Risk of Oral Nonmalignant Lesions Associated with Human Papillomavirus Infection, Betel Quid Chewing, and Cigarette Smoking in Taiwan

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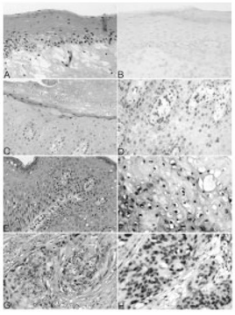
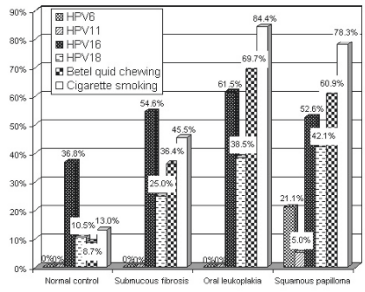
Background: In contrast to previous studies of the association of oral squamous cell carcinoma with human papillomavirus (HPV) 16/18, the associations between nonmalignant oral lesions (chronic inflammation, submucous fibrosis, leukoplakia, and squamous papilloma) and HPV are far less understood. We conducted this study using *in situ* PCR *in situ* hybridization assay that was one of the most sensitive methods for *in situ* viral detection. Other known oral cancer risk factors including betel quid chewing and cigarette smoking were also analyzed.

Design: Oral specimens from 23 patients with submucous fibrosis, 36 patients with leukoplakia, 22 patients with squamous papilloma, and 21 patients without significant lesions were analyzed for the presence of HPV DNA. Their betel quid chewing and cigarette smoking histories were reviewed.
Results: HPV16, HPV18 were frequently identified in all three oral lesions (61.5% and 42.1%), while HPV6 and HPV11 were only seen in squamous papilloma (21.1% and 5.0%). HPV18, betel quid chewing and smoking were significantly associated with leukoplakia and squamous papilloma; while only betel quid chewing and smoking were significantly associated with submucous fibrosis. Multivariate analysis showed betel quid chewing habit remained to be an independent factor for leukoplakia and squamous papilloma.
Conclusions: Our data indicated that betel quid chewing and smoking habits are two important risk factors of these nonmalignant or premalignant oral lesions. While in high risk HPV, only HPV18 but not HPV 16 is a significant risk factor for leukoplakia and squamous papilloma.





956 Retinoblastoma Treated with Suicide Gene Therapy: Study of the Ocular and Systemic Immunologic Response

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Background: Children with retinoblastoma may be resistant to standard therapies and frequently require enucleation of eyes with useful vision. Local or systemic immune responses to the adenoviral vector used in suicide gene therapy are not known and if present it is not known if these responses may produce an anti-tumor effect.
Design: We design and performed an approved study of intra–patient dose escalation of intravitreous injections of an adenoviral vector containing a herpes thymidine kinase gene followed by systemic administration of ganciclovir to treat retinoblastoma . Blood samples were drawn weekly before and after injections and the T cells were examined for evidence of adenovirus–specific reactivity. Histopathology and immunohistochemistry were performed to identify the ocular cellular inflammatory components.
Results: A total of 7 patients were treated with doses greater than 10^{10} viral particles (vp) and 7/7 had complete clinical resolution of their vitreous seeds. Mild inflammation (10^{10} vp) to moderate inflammation, corneal edema, and increased intraocular pressure (10^{11} vp) were noted. No patient had an increase in antibody titer to adenovirus following therapy. In four patients, no changes in the frequency of adenovirus–specific T cell precursors were seen. T-cell response (CD3, CD5, CD43) in the anterior chamber, retina and especially vitreous was prominent compared to an untreated eye with retinoblastoma. Histiocytes (CD68) were found mainly in the uvea, vitreous and retina but not in the control. B-cells (L-26) were seen in the choroid and retina of two patients with marked response to treatment. Plasma cells (CD138) were present in small proportion in anterior segment, uveal tract, vitreous and retina. Only the patient with total response to treatment showed marked staining.
Conclusions: Serology and the study of peripheral lymphocytes show no adenovirus-specific reactivity or changes in the frequency of adenovirus-specific T cell precursors. The results suggest that the type and intraocular location of inflammatory cells may not only play a role in therapy related toxicity but also in anti-tumor response.

957 Oncocytic Change in Pleomorphic Adenoma: Molecular Evidence in Support of an Origin in Neoplastic Cells. A Microarray Comparative Genomic Hybridisation and Chromogenic *In Situ* Hybridisation Study

S Di Palma, MBK Lambros, K Savage, C Jones, A Mackay, T Dexter, M Iravani, K Fenwick, A Ashworth, JS Reis-Filho. Royal Surrey County Hospital, University of Surrey, Guildford, United Kingdom; Institute of Cancer Research, London, United Kingdom; Institute of Cancer Research, Sutton, United Kingdom.

Background: Cells with oncocytic change (OC) are a common finding in salivary glands and in salivary gland tumours. When found within pleomorphic adenomas (PAs), cells with OC may be perceived as evidence of malignancy and lead to a misdiagnosis of carcinoma ex pleomorphic adenoma (CaExPa). We describe a case of PA with atypical OC, mimicking a CaExPa. Genome-wide molecular analysis was employed to compare the molecular genetic features of the two components and to determine whether the oncocytic cells originated from the PA cells, entrapped normal cells or if these cells constitute an independent tumour.
Design: Representative blocks were immunohistochemically analysed with antibodies raised against cytokeratin (Ck) 5/6, Ck8/18, Ck14, vimentin, p63, a-smooth muscle actin (ASMA), S100 protein, 'anti-mitochondria antibody', beta-catenin, HER2, Ki67, p53 and epidermal growth factor receptor (EGFR). Typical areas of PA and OC were microdissected and subjected to microarray-based comparative genomic hybridisation (aCGH). Chromogenic *in situ* hybridisation (CISH) was performed with 'in-house' generated probes to validate the aCGH findings.
Results: PA cells showed the typical immunohistochemical profile, including positivity for Ck5/6, Ck8/18, Ck14, vimentin, ASMA, S100 protein, p63, EGFR, beta-catenin), whereas oncocytic cells showed a luminal phenotype, expression of anti-mitochondria antibody and reduced beta-catenin staining. Both components showed low proliferation rates and lacked p53 and HER2 reactivity. aCGH revealed a similar amplification in both components, mapping to 12q13.3-q21.1 (*MDM2*, *CDK4*, *SAS*, *HMGA2, GLI1*, *RASSF3*, *DYRK2*, *RAP1B*, *FRS2* and *RAB3IP*), which was further validated by CISH. No HER2 gene amplification or overexpression was observed. The foci of OAM showed an additional low level gain of 6p25.2-p21.31.
Conclusions: Our data demonstrate that the bizarre atypical cells of the present case show evidence of clonality but no features of malignancy. In addition, owing to the presence of a similar genome amplification pattern in both components, we propose that at least in some cases, OC may originate from PA cells.

958 Promoter Methylation of Multiple Tumor-Associated Genes in Head and Neck Cancer

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Background: Epigenetic alteration in association of promoter methylation represents a common and novel mechanism of gene inactivation in human cancer. Previously, we showed that promoter methylation of individual genes, such as *ATM, p16 or MGMT*, had significant negative impact on the overall survival of head and neck cancer patients. In this study, we characterize promoter methylation of multiple tumor-associated genes simultaneously and determine how these genes interact with one another to affect the patient survival.
Design: One hundred consecutive cases of head and neck cancer were collected from the archival paraffin blocks in the Department of Pathology from 1993 to 1998. DNA Samples extracted from tissue sections were modified with sodium bisulfite, followed by methylation-specific PCR using primer sets for 6 tumor-associated genes (DAPK, ATM, ECAD, hMLH1, MGMT, and p16). The results were then correlated with complete clinical follow up of these 100 patients.
Results: Promoter methylation at DAPK, ATM, ECAD, hMLH1, MGMT, and p16 was detected in 25%, 25%, 74%, 34%, 20%, and 27% cases respectively. Promoter methylation of at least one gene was seen in up to 87% cases. Appling recursive partitioning method, *ATM, p16* and *MGMT* were found to be strongly associated with patient survival. Two patterns of promoter methylation were observed: type I (*ATM-/p16+* or *ATM-/p16-/MGMT-*) and type II (*ATM-/p16-/MGMT+* or *ATM+*). The overall 5-year survival rate for the patients with type I pattern is 48% (95% CI: 37% to 49%). By contrast, the overall 5-year survival rate for the patients with type II pattern is only 25% (95% CI 13% to 49%) The difference in survival between these two groups is statistically very significant ($p = 0.0074$). By multivaraiate analysis, type II methylation pattern remains as significant and independent predictor for worse 5-year survival ($p = 0.0048$).
Conclusions: 1) Aberrant promoter methylation occurs frequently in head and neck cancer (87%); 2) Among 6 tumor-associated genes analyzed, methylation at *ATM or MGMT* gene has much more significant impact on reduced overall 5-year survival in patients with head and neck cancer.

959 Proteomic Study on the Crosstalk between Tumor Cells and Stromal Fibroblasts

LY Fang, MH Wu, WF Chiang, CY Hsiao, SH Hsieh, YL Chen, YT Jin. National Cheng Kung University Medical Center, Tainan, Taiwan.

Background: The interactions between tumor cells and surrounding stroma play a critical role in carcinogenesis, but the molecular mechanisms have not been well characterized.
Design: Proteomic profiling was performed to systematically analyze the protein expression changes induced by tumor-stromal fibroblasts interactions on a transwell coculture system. The proteome changes of each cell types were analyzed by 2-D gel electrophoresis and LC-Q-TOF peptide mass fingerprinting.
Results: Our analysis successfully identified a set of proteins that are potentially involved in such interactions, many of which have been previously implicated in tumor invasion, metastasis, and angiogenesis. A number of tumor-associated proteins

including cofilin-1, tumor protein, translationally-controlled 1 (TCTP, histamine-releasing factor), calgranulin B, fatty acid binding protein-5 (FABP-5) and galectin-1 were consistently found to be significantly altered in their expression levels in oral cancer cells (OSCC) cultured with human gingival fibroblasts, compared with that of cultured cancer cells alone.

Conclusions: A set of proteins that are up-regulated in such interactions have been also found in fibroblasts such as calmodulin, galectin-1, Mn-superoxide dismutase (Mn-SOD), and Cu, Zn-SOD. We also characterized the role of the galectin-1, a protein identified as markedly induced in both cancer cells and fibroblasts in our coculture system, in the oral cancer cells–stromal fibroblasts interactions and oral cancer progression.

960 Laminin γ2 Chain Is a Marker of Dissemination in Carcinoma Ex-Pleomorphic Adenomas

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Background: Laminin γ2 chain (Lamγ2) is involved in tumor invasion and metastastic behavior. Our aims were:1) To compare Lamγ2 expression in pleomorphic adenomas and myoepitheliomas (PA) and carcinomas ex-pleomorphic adenomas (Ca-ex-PA); 2) To evaluate its correlation with tumor dissemination in Ca-ex-PA.

Design: The series includes 91 salivary glands tumors: 61 PA and 30 Ca-ex-PA (21 invasive, 5 microinvasive and 4 *in situ*). ABC peroxidase method and anti-laminin (Chemicon) monoclonal antisera was used in formalin-fixed and paraffin-embedded tissue. We evaluated Lamγ2 expression in cells and extracellular matrix. Follow-up of patients with malignant tumors was reviewed: 14 patient had metastases; 7 died of disease, mean follow up was 42 months.

Results: Two types of staining were found: at the basement membrane (BM) and at the cell cytoplasm. In PA, Lamγ2 was expressed in the BM in all but one case; cytoplasmastic positivity was found in very few cells of one of the cases. In Ca-ex-PA, Lamγ2 was present at the BM in 24 cases (80%) and at the cytoplasm of 13 cases (43,3%). Lamγ2 intracytoplasmatic expression was significantly associated with the occurrence of metastases (9 out 14 cases, $p<0.031$).

Conclusions: 1) Laminin γ2 chain is highly expressed at the basement membrane of both PA and Ca-ex-PA 2) At the cytoplasmatic level laminin γ2 was predominantly found in malignant tumors and statistically significantly associated with metastatic behavior of Ca-ex-PA which can be of prognostic value.

961 DNA Repair Gene, hOGG1, in Head and Neck Squamous Cell Carcinoma (HNSCC): Analysis of Loss of Heterozygosity in Invasive Carcinoma and Adjacent Normal Mucosa

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Background: Tobacco and alcohol, two major known head and neck cancer risk factors, produce reactive oxygen species in cells and oxidative lesions in DNA. Among various oxidative DNA lesions, 8-oxoguanine is by far the most abundant and mutagenic, If not sufficiently repaired. The gene encoding human 8-oxoguanine DNA glycosylase 1 (hOGG1), capable of excision repair of 8-oxoguanine, has been cloned and mapped to the short arm of chromosome 3 (3p25-26), a region showing frequent loss of heterozygosity (LOH), in HNSCC.

Design: A total of 66 pairs of invasive SCC and adjacent nonneoplastic squamous mucosa were included in the study. DNA samples from tumor, adjacent nonneoplastic squamous mucosa and paired stromal tissues were obtained from tissue sections collected using laser-capture microdissection (LCM). These DNA samples (198) were subjected to PCR amplification using 6 fluorescent-labeled microsatellite makers, followed by fragment analysis using ABI PRISM 3100 Genetic Analyzer. The hOGG1 gene is located between two telomeric markers (3S1297, 3S1304) and 4 centromeric markers (3S1289, 3S1300, 3S1261, 3S1274), used for analyses.

Results: Using a strict criteria, in which only cases showing LOH with both telomeric and centromeric markers were considered to be LOH for the hOGG1 gene, we identified 32 out of 66 cases (48%) of invasive SCC with hOGG1 LOH at both telomeric or centromeric markers and among these 32 cases, 24 (75%) showed hOGG1 LOH in the adjacent nonneoplastic squamous mucosa. In 26 cases (39%), both tumors and adjacent nonneoplastic mucosa showed LOH at either telomeric or centromeric markers and this indicated that hOGG allelic loss in tumor and adjacent hyperplastic mucosa was possible but could not be determined with absolute certainty. In 8 cases (12%), The nonneoplastic mucosa displayed retention of heterozygosity in the remaining 8 cases (12%).

Conclusions: Loss of heterozygosity (gene loss) of the hOGG1 gene frequently occurs in nonneoplastic squamous mucosa (75%), adjacent to the invasive SCC. This result indicates that the deletion of hOGG1 gene is an early event in head and neck squamous carcinogenesis.

962 Metastatic Squamous Cell Carcinomas of the Head and Neck to Lung: Immunohistochemical and Molecular Analysis

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Background: Smoking is strongly associated with head and neck and pulmonary squamous cell carcinomas (SCC). Therefore, in patients with a history of head and neck SCC, a lung metastasis can be difficult to differentiate from a new lung primary tumor. In this study, we analyzed immunohistochemistry (IHC) of SCC of head and neck metastatic to the lung and compared the results with molecular analysis.

Design: Paraffin blocks were retrieved from 28 cases of 14 patients. IHC was performed for p16, p63, K903, CK5/6, CK7 and CK20. For molecular analysis, 4-8 microdissection targets from neoplasm and non-neoplastic controls were removed under stereoscopic guidance and analized by PCR for LOH at 1p, 3p, 5q, 9p, 10q, 17p, 17q, 21q, 22q and point mutation determination in k-ras-2 . The tumors were classified as de novo or metastasis based on 3 levels of concordance: (1) marker affected - tumors were considered concordant if 50% or more of the same markers were mutated, (2) same gene copy affected, and (3) temporal sequence of mutation.

Results: IHC analysis was performed in 27 tumors. The table below shows the percentages of positivity for the different sites:

	p16	p63	K903	CK5/6	TTF-1	CK7	CK20
Head & neck	14	100	93	100	0	21	7
Lung	8	100	100	100	0	23	23

The immunohistochemical pannel was very similar for all tumors, and non-contributory in determining metastases versus separate primaries status. In contrast, molecular analysis demonstrated that 4/12 of the lung SCCs represent metastases from the head and neck primary and 8/12 SCCs represent de novo carcinomas.

Conclusions: IHC was not useful separating de novo tumors versus metastasis in patients with SCC of both head and neck and lung origin. Molecular analysis should be considered the gold standard for this distinction.

963 Overexpression of Nitric Oxide Synthase Isoforms (EcNOS and iNOS) in Squamous Cell Carcinoma of the Tongue

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Background: Nitric Oxide (NO) is a small, highly reactive free radical that is present at very low levels in many normal physiological processes throughout the body. A decade ago, we hypothesized that NO would be over produced in tumors and would play an important role in human tumor biology. Since then previous work from our laboratory has shown that NO is over produced by many human tumors and that the exposure to this free radical can 1) promote tumors, 2) increase cell division, and 3) result in increased gene expression of Glutathione-S-Transferase-pi (GST-pi). We have previously mapped the expression pattern of Nitric Oxide Synthetase (NOS) in carcinomas of most of the upper aerodigestive tract and have found that ecNOS is the dominant isoform expressed by these tumors.

Design: In this study we analyzed 49 primary human squamous cell carcinomas arising in the tongue using standard immunohistochemical methods. Intensity of the immunostaining was graded from 1 to 3.

Results: Both the ecNOS and iNOS isoforms were expressed to some degree in all of these tongue carcinomas, unlike in tumors from other parts of the upper aerodigestive tract that we have studied so far. Expression of the isoforms was found to be (immunostaining intensity: number of cases), ecNOS: negative: 23, 1+: 20, 2+: 5 and 3+: 1; iNOS: negative: 1, 1+: 11, 2+: 19, and 3+: 18. There was an inverse correlation between the expression of ecNOS and iNOS.

Conclusions: The over expression of NOS in squamous cell carcinomas of the tongue is consistent with our hypothesis and our previous findings in other tissues. However, the inverse correlation of expression between the ecNOS and iNOS isoforms in a given tissue is unique. It suggests that in carcinomas of the tongue the NO biology is different from that in other areas of the upper aerodigestive tract. Further studies are indicated to determine if there are clinical differences between those cases which present with the over expression of one isoform versus the other, and if co-expression of these isoforms results in a different clinical course.

964 Immunohistochemical Characterization of Selected Odontogenic Tumors: A Case for Myoepithelial Differentiation?

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Background: Many odontogenic tumors and cysts are thought to arise from remnants of invaginated dental lamina, which contribute to formation of the enamel organ through complex interactions with the underlying neural-crest derived mesenchyme. The dental lamina is derived from oral epithelium and would therefore be expected to share its immunohistochemical characteristics. We recently noted that the lesional cells in a calcifying epithelioid odontogenic tumor (CEOT) show cytoarchitectural features reminiscent of myoepithelial-derived tumors, showing epithelioid cells arranged in reticular, microcystic, pseudoglandular and cribriform patterns within a prominent hyalinized extracellular matrix. Moreover in 1983 NG El-Labban *et al* used electron microscopy to demonstrate a population of myoepithelial-like cells in CEOT. We therefore characterized the expression of myoepithelial-associated epitopes in several odontogenic tumors and cysts, odontogenic rests, and oral epithelium.

Design: 3 CEOTs, 3 ameloblastomas, 2 dentigerous cysts, an odontogenic keratocyst (OKC), 2 odontogenic rests, and 3 oral epithelia were immunostained for myoepithelium-associated epitopes (p63, calponin, S100, CK5/6, GFAP) and CK7.

Results: All of the entities tested were immunoreactive for CK5/6 and p63. Calponin reactivity was noted in two of three CEOTs and three of three ameloblastomas. CK7 reactivity was noted in two of three CEOTs (one focal, one diffuse). GFAP reactivity was noted focally in one of three CEOTs. Interestingly, the calponin negative/diffusely CK7 positive CEOT was of peripheral rather than central type. No entity was immunoreactive for S100.

Conclusions: In keeping with a likely common developmental origin, oral squamous epithelium, odontogenic rests, dentigerous cysts, a representative OKC, CEOTs, and ameloblastomas show a common CK5/6 and p63 positive immunoprofile. Both CEOTs and ameloblastomas show immunohistochemical overlap with myoepithelial lesions as they express a combination of CK5/6, p63 and calponin; one of three CEOTs also

shows focal GFAP immunoreactivity. Our findings suggest a degree of myoepithelial differentiation in CEOTs and potentially ameloblastomas; there is a need for a bigger series to further elucidate these findings. Awareness of this immunohistochemical profile is crucial for accurate identification and differential diagnosis of oral biopsy specimens, particularly if the odontogenic nature of the lesion is not suspected.

965 Laryngeal Squamous Cell Carcinoma Does Not Exhibit BRAF Mutations: A Comparison to Anaplastic Thyroid Carcinoma

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Background: Anaplastic thyroid carcinoma can be difficult to diagnose in biopsy specimens as many of the typical immunohistochemical stains for thyroid derivation are negative in these undifferentiated tumors. Frequently, these aggressive tumors involve multiple structures of the neck, including lymph nodes, larynx, and strap muscles. One of the most significant differential diagnostic considerations is whether the tumor is a poorly differentiated squamous cell carcinoma that is secondarily involving the thyroid or an anaplastic thyroid carcinoma as these tumors are treated very differently. BRAF mutational status, a marker for both well-differentiated papillary carcinoma (~40% of tumors) and anaplastic thyroid carcinomas (~50% of tumor), was examined as a potential aid in the differential diagnosis between high-grade squamous cell carcinomas and anaplastic thyroid carcinoma.

Design: High-grade laryngeal squamous cell carcinomas and anaplastic thyroid carcinomas were microdissected and DNA was extracted from normal and tumor tissues. PCR was performed for exon 15 of the BRAF gene and the amplicons were subjected to automated cycle sequencing using the BigDye Terminator kit (ABI, Foster City, CA). The sequence was analyzed for the T1799A substitution.

Results: 25 high stage laryngeal squamous cell carcinomas with invasion beyond the thyroid cartilage were included in one experimental group for this study. The other experimental group included 7 definitive, well-characterized anaplastic thyroid carcinomas. No BRAF mutations were seen in the laryngeal squamous cell carcinoma group (0/25, 0%). 5/7 (71 %) of anaplastic thyroid carcinomas were positive for the BRAF T1799A mutation.

Conclusions: The high rate of BRAF mutations that has been reported for anaplastic thyroid carcinomas was supported by our data. Furthermore, no mutations in BRAF were found in any of the laryngeal squamous cell carcinomas that were studied. These data suggest that BRAF mutational analysis can be useful as an adjunctive test in biopsies of bulky neck tumors in which the differential diagnosis includes anaplastic thyroid carcinoma and high-grade squamous cell carcinoma.

966 Adenofibroma of the Head and Neck? A New Entity Involving the Mucoserous Glands

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Background: Fibroepithelial neoplasms of the breast, gynecologic tract, and other organs are well recognized and relatively common. However, these neoplasms are rare in the head and neck. We have encountered three such lesions involving mucoserous glands in our consult service, which, to our knowledge, have not been previously described.

Design: The clinical and histologic features of these cases are reviewed. Histochemical and immunohistochemical stains were performed to evaluate the epithelial and stromal components of each neoplasm.

Results: The patients, 2 females and 1 male, ranged from 67 to 76 years of age. The neoplasms were located in sites containing mucoserous glands (minor salivary glands), namely the oral vestibule, retromolar trigone, and nasal cavity. Tumors ranged from 1.5 cm to 2.5 cm. Histologically, all three neoplasms were well circumscribed, non-encapsulated, and consisted of a bland, spindle cell proliferation with an integral epithelial component that accounted for 25% to 50% of the tumor volume. All three cases showed randomly distributed, dilated ductal structures, often with periductal stromal hyalinization. In addition, the nasal tumor contained glands having a non-lobular appearance with a predominance of PAS-positive, diastase-resistant cytoplasmic granules, suggestive of serous differentiation, as well as a minor population of mucinous glands. Mucinous glands were also present in the oral vestibule tumor, but the retromolar trigone tumor only showed the dilated ductal structures. CD 34 reactivity within the spindle cells was noted in all 3 cases (2 tumors diffusely, 1 tumor focally). Positivity for CD 99 and bcl-2 was identified in the spindle cells of both cases tested and smooth muscle actin positivity of these cells was noted in 1 of 3 cases. The ductal/glandular components were positive for cytokeratin in all cases and were surrounded by an outer layer of myoepithelial cells (positive for p63, smooth muscle actin, and S-100). The circumscription, as well as integral epithelial component distinguished these neoplasms from a solitary fibrous tumor with glandular entrapment. The three tumors were treated by excision. Acquisition of follow-up data is in progress.

Conclusions: Three cases of a rare, hitherto unreported, fibroepithelial neoplasm involving mucoserous glands of the head and neck are described. Since the spindle cell component tended to predominate, we prefer the term adenofibroma rather than fibroadenoma.

967 Aberrant Epigenetic Alterations Are Acquired Early during Head and Neck Squamous Carcinogenesis

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Background: Epigenetic alteration in association with promoter methylation represents a common and novel mechanism of gene inactivation in human cancer. We have previously shown that epigenetic alterations in association with promoter hypermethylation in critical tumor suppressor genes play significant roles in the biologic behavior of this tumor. To explore the possibility that promoter methylation occurs at the early stage of tumor development, we characterize promoter methylation of 4 tumor-associated genes (ATM, p16, MGMT and hMLH1) in invasive head and neck cancer and adjacent non-neoplastic squamous mucosa.

Design: A total of 52 pairs of invasive squamous cell carcinoma and their corresponding nonneoplastic squamous mucosa were included in this study. DNA samples from tumor, and paired non-neoplastic squamous mucosa were obtained from tissue sections collected using laser-capture microdissection (LCM). These DNA samples (104) were subjected to sodium bisulfite modification, followed by methylation-specific PCR (MSP) using gene-specific and methylation-specific primer sets for 4 tumor-associated genes (ATM, p16, MGMT and hMLH1).

Results: Aberrant promoter methylation in *ATM*, *p16*, *MGMT*, *and hMLH1* genes was detected in 5/52 (9%), 12/52 (23%), 7/52 (13%), and 13/52 (25%) cases of invasive SCC respectively. Promoter methylation was detected in at least one of these four genes in 24 of 52 cases (46%). Except for two cases in which *p16* promoter methylation was seen in primary tumors but was not detected in the corresponding nonneoplastic mucosa, the remaining cases (50/52; 96%) showed complete concordance between primary tumor and adjacent nonneoplastic squamous mucosa, with promoter regions of these four genes being methylated in both primary tumor and adjacent non-neoplastic mucosa or unmethylated in both tumor and adjacent non-neoplastic mucosa.

Conclusions: Aberrant promoter methylation of tumor-associated genes occurs frequently in invasive head and neck cancer (46%). The presence of promoter methylation in nonneoplastic squmous mucosa, adjacent to invasive cancer, indicate that epigenetic alterations of these tumor-associated genes are acquired very early during head and neck squamous carcinogenesis.

968 Cartilagenous Choristoma of the Tonsils

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Background: Cartilagenous choristoma of the tonsils is comprised of mature or immature benign cartilage in a background of fibroconnective tissue, and may predispose to chronic tonsillitis or cause difficulty in surgical excision of the tonsils. In this study, we evaluated the incidence of cartilage as well as skeletal muscle, fat, seromucinous gland and bone (bone choristoma) in routine tonsillectomy specimens.

Design: In Group 1, a retrospective study of H&E sections of 1172 tonsillectomies were independently assessed for the presence of non-native normal tissues. In Group 2, a prospective study of 45 totally embedded tonsillectomies was performed.

Results: In Group 1, 590 (50.4%) patients were males and 582 (49.6%) were females. The mean age was 11.45 years (range 16 months-68 years). 22 (1.9%) tonsillectomies had cartilage (15 females and 7 males), 1142 (97.6%) had skeletal muscle, 504 (43%) had fat and 809 (69%) had seromucinous glands. No bone was identified in any case (0%). In Group 2, 24 (53.3%) patients were males and 21 (46.7%) were females, with a mean age of 9.2 years (range 2 years-35 years). One (2.2%) tonsillecomy had cartilage (25 year old female), 45 (100%) had skeletal muscle, 31 (68.9%) had fat and 44 (97.8%) had seromucinous glands. No bone was identified in any case (0%). In all choristoma cases in Groups 1 and 2, cartilage was typically identified in a background of fibroconnective tissue.

Conclusions: The incidence of cartilaginous choristoma in both patient groups was not significantly different and was comparable to that previously reported. Females had twice the incidence of tonsillar choristoma compared with males. The incidence of skeletal muscle, fat and seromucinous glands was much higher in the totally embedded Group 2 tonsillectomies and significantly higher than previously reported. Bone choristoma, a lesion described in the literature, was not encountered in either group of this study.

Table 1

Cases	Male	Female	Bone	Cartilage	Skeletal Muscle	Fat	SM. Glands
1172 Group 1	590 (50.4%)	582 (49.6%)	0 (0%)	22 (1.9%)	1142 (97.6%)	504 (43%)	809 (69%)
45 Group 2	24 (53.3%)	21 (46.7%)	0 (0%)	1 (2.2%)	45 (100%)	31 (68.9%)	44 (97.8%)

Table 2: Incidence of Cartilaginous Choristoma in Group 1

Positive Cases	Males	Females	Age Range	Mean Age
22	7 (31.8%)	15 (68.2%)	3 yrs - 50 yrs	13.3 yrs

969 Sclerosing Odontogenic Carcinoma. A Previously Unpublished Variant of Odontogenic Carcinoma

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Background: Odontogenic carcinomas are extremely rare odontogenic tumors. They can be classified into five major categories: (a) ameloblastic carcinoma (primary, dedifferentiated, peripheral), (b) malignant epithelial odontogenic ghost cell tumor (a.k.a. dentinogenic ghost cell carcinoma) (c) primary intraosseous squamous cell carcinoma (solid or cystogenic), (d) clear cell odontogenic carcinoma and (e) metastasizing ameloblastoma. We describe three cases of odontogenic carcinomas that share histologic features not previously reported in the literature.

Design: The tumors involved one 72-year-old male and two females, 73-year-old and 46-year old, respectively. Histological preparations from diagnostic material were reviewed. Immunohistochemical evaluation included cytokeratin 5/6, 7, 8/18 (CAM5.2), 19, high molecular keratin and ε-cadherin.

Results: Two cases occurred in the mandible and one in the maxilla. The tumors presented radiographically as expansile radiolucencies. Histologically, they were characterized by small nests and thin cords of small cuboidal or polygonal epithelial cells, featuring, only focally, cytoplasmic clearing. Pleomorphism was not remarkable and mitoses were not encountered. The most striking feature of malignancy was the tendency of the neoplastic nests and cords to infiltrate striated muscles and peripheral nerves. Sclerosis

of the stroma was also remarkable. Immunohistochemically, all tumors stained for cytokeratins-5/6, cytokeratin-19, high molecular weight keratin and ε-cadherin. Focal staining for cytokeratin-7 was seen in one case. The staining in that case was focal and only in areas of clear cells. Cords and nests that comprised the majority of tumor were negative. All tumors were negative for cytokeratin-20 and CAM5.2. The patients were surgically treated and one of them received adjuvant radiation. Microscopic tumor nests and cords were found far beyond of what surgically appeared as tumor free margins. All patients are free of disease 3, 2 and 10 years, following treatment, respectively.
Conclusions: The origin of these tumors is most likely odontogenic. Therefore, we propose the name sclerosing odontogenic carcinoma. Since current classifications of odontogenic tumors do not include such example it is important that pathologists are aware of this variant.

970 WHO Classification of Lymphomas of Orbit and Ocular Adnexa and Location of Involvement in MALT Type Lymphomas Correlate with Outcome

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Background: Lymphomas involving the ocular adnexa and orbit are not uncommon. Due to their location they pose a challenge in determining optimal treatment. According to the WHO classification, various lymphomas are separate diseases with distinct clinical behavior. However, the behavior of some lymphomas occurring in the skin, particularly in the head and neck region, is very different from their nodal counterparts. Long range follow-up and clinico-pathological correlation is necessary to determine the biological behavior of the lymphomas of orbit and ocular adnexa.
Design: The biopsies from 119 cases of lymphomas diagnosed during the period of 1971 to 2004 were reviewed in conjunction with results of ancillary studies. The cases were reclassified according to the WHO classification, using additional immunohistochemical stains. The presenting features, treatment, and final outcome were noted from chart review where possible.
Results: The vast majority of lymphomas (92.4%) were derived from B-cells and in decreasing order of frequency they were: Marginal zone lymphoma (MZL) (50.4%), follicular lymphoma (FCL) (13.4%), diffuse large B-cell lymphoma (9.2%), mantle cell lymphoma (MCL) (5.6%), small lymphocytic lymphoma (5%), lymphoplasmacytic lymphoma and atypical B-cell infiltrate (2.5% each), and plasmacytoma and lymphoblastic lymphoma (1.7% each). One case of lymphocyte predominant Hodgkin Lymphoma and 8 cases (6.7%) of T-cell lymphoma were observed. Bilateral involvement was seen in 14% and lymphadenopathy was documented in 39%. Most patients with lymphadenopathy were treated with chemotherapy and most patients without lymphadenopathy received radiation or surgical excision. Overall outcome was similar in the two groups. Adverse outcome (death due to lymphoma or persistent disease) occurred in 64% patients with FCL and MCL, compared to 20.9% of MZL. Adverse outcome in MZL was seen with orbital, eyelid, or lachrymal gland location but not with subconjunctival location.
Conclusions: Over 90% of lymphomas in the orbit and ocular adnexa are B-cell lymphomas, with marginal zone lymphomas being the commonest. Overall outcome is affected by the type of lymphoma, in a fashion similar to the same lymphoma occurring in the lymph node. In cases of MZL, a conjunctival location at presentation carries a favorable prognosis.

971 Expression of Matrix Metalloproteinases in Sinonasal and Oral Malignant Melanomas

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Background: Sinonasal and oral malignant melanomas (SOMM) are rare malignancies accounting for less than 2% of all melanomas. Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes required for extracellular matrix degradation in a variety of physiological and pathological processes including wound healing, embryo implantation, tumor invasion and metastases. We studied the correlation between MMPs expression and clinical outcome.
Design: Seventeen cases of SOMMs, including 12 sinonasal and 5 oral, were studied. The expression of MMP2, MMP9, MMP13 and MMP14 was assessed immunohistochemically on paraffinized sections. The level of MMPs expression was scored based on extent of tumor staining (0, negative; 1, <25%; 2, 25-75%; 3, >75%). Median patient age was 66 years. Clinical follow-up was available for all cases with a mean follow-up period of 49 months. Ten of 17 patients developed metastases, 12 died of disease, 5 were alive by the end of the study; 16 patients received surgery, 10 radiotherapy, 7 chemotherapy and 1 immunotherapy.
Results: MMP2 expression was seen in 15 of 17 cases (88%). Eight patients exhibited low staining (<25%); more extensive MMP2 expression (≥25%) was seen in 7 patients. Three of 8 patients with MMP2 staining <25% survived by the end of the follow-up period (mean survival time 54 months), as opposed to the group with ≥25% expression, where only one patient was alive and mean survival time was shorter (39 months). One of 2 patients with negative MMP2 staining was also alive. The vast majority (9 of 10) of patients with metastases showed MMP2 expression. Positive MMP14 immunoreactivity was seen in 6 of 17 cases (35%). A significant correlation was found between MMP14 expression and poor patient survival. Thus, higher survival rates were seen in MMP14 negative tumors versus the MMP14 positive tumors (45 and 0%, respectively). MMP9 expression was seen in 5 cases (29%) and MMP13 in 6 cases (35%); no correlation was found with patient survival. The oral malignant melanomas showed the longest survival rates (24-171 months) as opposed to the sinonasal group (5-67 months). Moreover, spindle or mixed cell types exhibited higher survival rates as compared with the epithelioid cell variant.
Conclusions: In this study we have identified distinct MMPs immunostaining patterns that might be useful to predict a more aggressive clinical course. Tumor location, cell type and MMP2 and MMP14 expression appear to be associated with a poorer prognosis.

972 Epithelial Expression in Olfactory Neuroblastoma: An Immunohistochemical Analysis

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Background: Olfactory neuroblastoma (ONB) is a rare malignancy of neural crest origin whose typical immunohistochemical profile includes reactivity for NSE and S100. While originally described as negative for epithelial markers, recent studies have begun to observe that some ONBs express keratin, specifically CAM5.2, and rarely EMA. The goal of this study was to further investigate the prevalence and degree of epithelial positivity in a large number olfactory neuroblastomas.
Design: A retrospective search of the computerized database from the pathology department of Vanderbilt University Medical Center was conducted for all cases interpreted as ONB. The available hematoxylin and eosin-stained slides and immunostains were reviewed by the authors to confirm the diagnosis and to identify the block containing the largest amount of tumor. Tissue which had been decalcified or previously frozen was not selected for examination. Immunophenotypic analysis was performed using antibodies against AE1/AE3, CAM 5.2, EMA and CEA. Scoring was based on the percentage of positive tumor cells and the intensity of staining.
Results: A total of twenty-eight olfactory neuroblastomas were identifed from the pathology archives. Eleven cases represented consultation material and did not have the formalin-fixed paraffin-embedded tissue or unstained slides requisite for immunohistochemical evaluation. Thus 17 cases comprised the study cohort. All samples were uniformly negative for CEA. Immunostaining for cytokeratin AE1/AE3 and CAM5.2 labelled the same 6 cases (35.3%); however, the degree of reactivity was weaker and more focal with AE1/AE3 than with CAM5.2. Four cases(23.5%) displayed weak to moderate membranous staining for EMA in less than 10% of tumor cells.
Conclusions: Our results confirm that reactivity for CEA is absent in olfactory neuroblastomas and that approximately 35.3% of ONBs stain for low molecular weight keratin (CAM5.2), a percentage within the range reported by others. Unlike previous smaller investigations, we found the rate of AE1/AE3 expression equal to that for CAM5.2 and a higher percentage of cases staining for EMA. Such epithelial reactivity is important to note for a tumor that has a fairly nonspecific antigen profile and whose differential diagnosis includes tumors such as sinonasal undifferentiated carcinoma, which is also reactive for epithelial markers.

973 Molecular Evidence for the Same Clonal Origin of Multifocal Papillary Tumors in Patients with Papillary Thyroid Carcinoma

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Background: Patients with papillary thyroid carcinoma often have two or more distinct papillary tumors at thyroidectomy. Whether these multifocal papillary lesions are clonally related or whether they arise independently is unknown as previous studies have shown conflicting results. Molecular analysis of microsatellite alterations and X-chromosome inactivation status in separate tumors in the same patient can be used to define the genetic relationships among the multiple coexisting tumors.
Design: We examined 22 female patients who underwent thyroidectomy for thyroid carcinoma. All patients had multiple separate papillary carcinomas (2 to 6), 2 of which had lymph node metastases at the time of thyroidectomy. Genomic DNA samples were prepared from formalin-fixed, paraffin-embedded tissue sections using laser-capture microdissection. Loss of heterozygosity (LOH) assays for 3 microsatellite polymorphic markers for putative tumor suppressor genes on chromosomes 3p25 (D3S1597), 9p21 (D9S161), and 18p11.22-p11 (D18S53) were performed. In addition, X-chromosome inactivation analysis was performed on the tumors from all patients.
Results: Twenty of 22 (91%) cases showed allelic loss in one or more of the papillary lesions in at least one of the three polymorphic markers analyzed. Concordant allelic loss patterns between coexisting papillary tumors were seen in 13 of 22 (59%) cases at one or more loci and in 3 of 22 (14%) cases at two or more loci. A concordant pattern of non-random X chromosome inactivation in the multiple coexisting papillary lesions was seen in all informative cases.
Conclusions: Our data suggest that the multifocal tumors in patients with papillary thyroid carcinoma often arise from the same clone. Thus, intrathryoid metastasis may play an important role in the spread of papillary thyroid carcinoma, a finding that has important therapeutic, diagnostic, and prognostic implications.

974 BCL-2, Ki67 and P53 Expression in Oral Necrotizing Sialometaplasia and Squamous Cell Carcinoma

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Background: Necrotizing Sialometaplasia (NS) is an uncommon inflammatory reactive condition of the salivary glands affecting mostly the hard palate region. Although first described last century by Abrams et al (1973), it is still an active area of study due mainly to the fact it may be a potential pitfall for a diagnosis of malignancy in the head & neck region and unnecessary radical surgery. Despite the presence of some characteristic features that help distinguish NS from Squamous Cell Carcinoma (SCC) on the hematoxlin & eosin slides, some cases of NS with extensive pseudoepitheliomatous hyperplasia and reactive atypia are often difficult to differentiate from invasive SCC. To the best of our knowledge, the use of immunohistochemical staining to differentiate NS from SCC has not been utilized.
Design: To differentiate NS from SCC, immunhistochemical stains (BCL-2, Ki67 and P53) were utilized. Fourteen cases of NS and eleven cases of invasive SCC (based on

H&E slides) of variable sites in the head & neck region were randomly selected from our surgical pathology archived formalin fixed-paraffin embedded tissue blocks. Each case was stained with BCL-2, Ki67 and P53 antibodies. The cases were reviewed, including the H&E and the immunostained slides, by two pathologists.

Conclusions: Distinguishing between NS and SCC can occasionally pose a diagnostic challenge as a result of their overlapping histologic appearance. While some cases of NS can be focally positive for P53, the NS cases are generally negative for BCL-2, Ki67 and P53. Invasive SCC cases are generally positive for P53 and Ki67, approximately half of the SCC cases are positive for BCL-2. Our results indicate that use of Ki67 and P53 immunohistochemical staining as an adjuvant to histopathologic examination may be helpful in differentiating NS from SCC.

	RESULTS					
	BCL-2		Ki67		P53	
	+	-	+	-	+	-
NS (14 cases)	0	14	0	14	2 (focal)	12
SCC (11cases)	6	5	11	0	11	0

975 Immunohistochemical Separation of Differentiated Thyroid Tumors

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Background: The distinction between follicular variant of papillary thyroid carcinoma (FVPTC) and follicular adenoma (FA) can be very difficult. Similarly, distinguishing usual papillary thyroid carcinoma (PTC) from the more aggressive tall cell variant of PTC (TCVPTC) or FA from follicular carcinoma (FC) may be challenging when based only on histopathologic features.

Design: A total of 142 thyroid tumors including FVPTC (n=45), PTC (n=43), TCVPTC (n=6), FA (n=27) and FC (n=21) were examined using tissue microarrays. Immunohistochemistry was done with antibodies to HBME1, galactin-3 (Gal-3), cytokeratin 19 (CK19), CITED1, matrix metalloproteinase 11 (MMP-11), fibronectin (FN), S100A4, thyroid peroxidase (TPO) and Ki-67. The immunostaining results were evaluated independently by two pathologists. Follow up was available in all cases (2 to 30 years).

Results: Distinction of FVPTC from FA could be done with multiple biomarkers including HBME1, Gal-3, CITED1, CK19, FN and S100A4 ($p<0.0001$). PTC and TCVPTC could be distinguished from each other by MMP11 ($p<0.05$). FA could be separated from FC by HBME1, FN ($p<0.05$) and Ki-67 ($p<0.0001$). TPO was expressed by most FA (96%), FC (76%), and FVPTC (67%), but was only detected in 21% of PTC and in 0% of TCVPTC.

Conclusions: These results indicate that a combination of multiple antibodies can be used in the differential diagnosis of various borderline thyroid lesions when histopathological examination is equivocal. The application of a panel of antibodies including HBME1, Gal-3, S100A4, FN, TPO, and CITED1 along with Ki-67 was most useful.

976 IgG4-Related Sclerosing Sialadenitis Is Histologically Distinct from Other Types of Sialadenitis

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Background: Sclerosing sialadenitis is one of the major complications of autoimmune pancreatitis (AIP). Numerous IgG4+ plasma cells are noted in both of the conditions, indicating that they are pathogenetically related. IgG4-related sclerosing sialadenitis (IG4-SS) could be seen without AIP, and probably represents at least some "Kuttner tumors". Given the steroid responsiveness, recognition of IG4-SS is clinically important, but histologic features of IG4-SS are not fully described in comparison with other types of sialadenitis.

Design: We reviewed 197 patients with major salivary gland resection for which the final pathologic diagnosis was inflammation. Pathology reports and/or clinical charts, as well as all the H&E-stained slides were reviewed. Cases with insufficient clinical information, mucocele, granulomatous sialadenitis, or histologically mild inflammation without sialoliths were excluded. Immunostaining for IgG and IgG4 was done for severely inflamed lesions.

Results: 156 patients remained for the analysis. 146 had sialolithiasis. Submaxillary gland(s) was resected in every case. Seven of 10 patients without sialoliths were diagnosed to have IG4-SS. The average age was 66.7 years (range 57-75); 6 were female. Four patients had bilateral diseases with (2 cases) or without involving other salivary/lacrimal glands. Another patient had retroperitoneal fibrosis. None of the patients had AIP. Histologically, dense lymphoplasmacytic infiltration with numerous lymphoid follicles were surrounded by thick collagen bands, giving rise to a lobulated inflammatory mass. Salivary gland structure was obliterated, although some residual epithelial cells were found to be intermingled within the inflammatory cells. IgG4+ plasma cells were numerous. In chronic sialadenitis with sialoliths, average age of the patients was 45.6 years (range 14-85). Histologically, salivary gland architecture was preserved. Edema and lymphoplasmacytic infiltration were common, and sometimes remarkable in the lobules. Fibrosis was seen between the lobules. On rare occasions, fibrosis was so conspicuous that it simulated IG4-SS. Occasional findings were neutrophils in the duct system, periductal dense inflammation and/or fibrosis, and lymphoid follicles. IgG4+ plasma cells were generally scarce, and IgG4/IgG ratio was always lower compared to IG4-SS. Three cases without sialoliths were similar to cases with sialoliths.

Conclusions: IG4-SS is a distinct inflammatory process of the salivary gland. It could be diagnosed histologically with an aid of immunostaining for IgG4.

977 Genetic and Epigenetic Alteration Profiles for p16 in Head and Neck Squamous Cell Carcinoma in Young Adults

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Background: The typical head and neck squamous cell carcinoma (HNSCC) patient is the over 55 year old male smoker. However, in recent times, there has been a worldwide increase in incidence of HNSCC in young adults (under 40 years old), many of them 'never' smokers and many of them females. Functional inactivation of p16 is a common event in classic HNSCC and it has been suggested that exposure to tobacco smoke can increase the predisposition for genetic and epigenetic alterations (e.g. deletion or DNA methylation) of p16 gene. The purpose of this study is to evaluate inactivation and expression patterns of p16/INK4A in HNSCC with a particular focus on the not so typical HNSCC patient (the young adult nonsmokers).

Design: Twenty-five samples of HNSCC (10 under 40 yrs old, 15 over 40 yrs old) were collected prospectively. CGH microarray, methylation-specific PCR and immunohistochemistry were used to evaluate the genetic and epigenetic profile of p16 (CDKN2A).

Results: Overall, 48% of the samples showed p16 inactivation (either by deletion or methylation). Deletion of p16 was only detected in the older cohort (46%), and was completely absent in the young cohort. In contrast, methylation of p16 was more prevalnet in the young adults (30%) and only occurred in 13% of the older group. Strong p16 staining was evident in equal numbers in the young cohort and in the older cohort (20%). Methylation was a more common event than deletions in the nonsmokers and in females, while the reverse was true for the smokers and males.

Conclusions: Our results indicate that inactivation of the p16 gene is a frequent event in HNSCC. However, while deletion is the main mechanism of inactivation in male smokers, methylation is a more common event in female nonsmokers. This suggests that specific modes of inactivation of p16 in HNSCC are related to specific patient profiles. This most likely reflects different aetiologies or exposure patterns, which need to be further explored.

978 Proteomic Analysis of Saliva in Oral Squamous Carcinoma: A Potential Diagnostic and Screening Application

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Background: Saliva is a rich source of proteins and enzymes of protective and digestive functions. It also contains plasma protein components that may reflect systemic disease effects. Saliva is a readily accessible secretion and can be used in the diagnosis and early detection of patients with head and neck squamous carcinoma.

Design: Saliva from five normal volunteers and three patients with oral squamous carcinomas were collected and centrifuged to eliminate cellular contents. Specimens were subjected to affinity purifications column to remove major known proteins such as albumin, immunoglobulin G, trypsin, transferring and haptoglobin. After depletion, each sample was divided and analyzed as follows: 1) initial In-solution digestion liquid chromatography/tandem mass spectrometry (LC-MS/MS), 2) LC-MS/MS after In-solution digestion and peptide separation by anion exchange chromatography and 3) SDS-PAGE and In-gel digestion followed by liquid chromatography/tandem mass spectrometry (LC-MS/MS).

Results: Method #1 identified between 14 to 44 different proteins in the five normal samples. Method #2 identified between 21 to 118 proteins and method #3 identified between 64 to 163 proteins. Common protein present in all five samples were 4,11 and 26 for methods #1, 2 and 3 respectively. Among the protein identified by all methods are: Chromosome 6-open-reading frame 58, bactericidal permeability=increasing protein-like and HRPE 773 proteins. Saliva from three patients with OSCC was analyzed using method #3. Unique proteins identified were 33, 14 and 33 for each sample. The numbers of common proteins in all samples were 34. In comparison to normal saliva, patients with SCC, two proteins were unique: a-1-B-glycoprotein and complement factor-B. Common proteins in SCC but in one or two normal saliva are: Annexin-1, ceruloplasmin, fibrinogen gamma, hemopexin, keratin-1 and 10, matrix melloproteinases 9 and proapolipoprotein.

Conclusions: 1) A set of common proteins are identified among all normal salivas. 2) Inter-methodological differences exist between specimens. 3) A panel of known proteins distinguish normal from HNSC specimens. 4) Saliva is an easy and accessible source for diagnosis and early detection of HNSC.

979 WHO Classification of Intraocular Lymphoma by Cytologic and Flow Cytometry Evaluation

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Background: Intraocular lymphoma, considered as part of central nervous system (CNS) lymphoma, is a rare type of non-Hodgkin lymphoma (NHL). The incidence of intraocular lymphoma has increased in the past 20 years. Intraocular lymphoma often presents as chronic uveitis resistant to corticosteroid therapy. Diagnosis and classification of intraocular lymphoma can be challenging because of the sparse cellularity and characteristics of the vitreous specimens. The goal of the current study is to examine if intraocular lymphoma can be adequately diagnosed and classified according to WHO classification scheme based on cytologic and flow cytometry evaluation.

Design: We reviewed cytologic preparations of 161 consecutive vitrectomy specimens including vitreous taps and washings retrospectively from 1999 to 2005. In addition, the flow cytometry reports were reviewed for the cases diagnosed as lymphoma for appropriate immunophenotyping. Clinical information of the patients diagnosed with lymphoma was obtained from the medical records.

Results: Of the 161 specimens, 18 cases (11.2%) were diagnosed as lymphoma. Five patients had prior history of systemic lymphoma and three had prior history of CNS lymphoma. Of the 18 patients, 15 had additional samples submitted for 4-color flow cytometry analysis. Due to low cellularity of most of the specimens, the flow cytometry study was performed by using a limited panel of antibodies composed of CD19, CD20, CD5, CD10, Kappa and lambda light chain. Two of the 15 specimens did not have enough cells for immunophenotyping by flow cytometry. Of the 18 cases, 15 cases (83.3%) can be adequately diagnosed and classified according to the WHO classification. These included 10 cases of diffuse large B-cell lymphoma, 1 extranodal low grade marginal zone B-cell lymphoma (MALToma), 1 precursor B-lymphoblastic lymphoma and 3 T-cell lymphoma-NOS. Of note, all 10 cases of diffuse large B-cell lymphoma were CD10 negative.
Conclusions: The vast majority of intraocular lymphoma cases can be adequately classified according to the WHO classification with the combination of cytologic and flow cytometry evaluation. Diffuse large B-cell lymphoma is the most common subtype of NHL in this site, in contrast to ocular adnexal lymphoma in which MALToma is the most common subtype. Virtually all diffuse large B-cell lymphomas in this site lack CD10 expression suggesting a non-germinal center B-cell origin.

980 Mutational Profiling of Mucoepidermoid Carcinoma: Relationship to Grade and Prognosis

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Background: Mucoepidermoid adenocarcinomas occur in both major and minor salivary glands and are typically divided into three categories: low, intermediate, and high grade. Most grading schemes are based on the percentage of the cystic component present in the tumor; low grade tumors have a high cystic content and high grade tumors have a low cystic content. Although grading criteria are controversial, prognosis is related to the grade of the tumor. Molecular mutations are not well described and no attempts to relate grade or prognosis to mutational profiles have been made.
Design: Mucoepidermoid carcinoma cases with long-term follow-up were retrieved from the archives and graded using conventional histologic criteria. Tumors were microdissected, DNA was extracted, and PCR was performed for 15 different tumor suppressor genes for loss of heterozygosity (LOH) analysis. Analysis was performed in a semi-quantitative manner using automated fragment analysis. Fractional allelic loss (FAL) was calculated and the tumors were compared for grade and prognosis.
Results: 31 mucoepidermoid carcinoma cases were studied (9 low grade, 11 intermediate grade, and 12 high grade). Patient ages ranged from 7 to 85 years (mean 51). 31/31 patients were treated with surgery and 14/31 received radiation therapy. Six of 29 patients with lymph node sampling had metastases. During the follow-up period (mean 44 months), 14 patients died of disease (DOD) and 17 had no evidence of disease (NED). In low grade tumors, 2/9 are DOD, in intermediate grade 2/11 are DOD, and in high grade tumors, 8/12 are DOD. The gene loci that showed the highest rates of LOH were 3p, 9p, and 17p. The mean FAL for low grade tumors was 23.5% (range 0-71%), for intermediate grade tumors it was 37% (range 0-66%), and for high grade tumors it was 53% (range 22-88%). The FAL for patients who are DOD was 48% and for NED was 32%.
Conclusions: Mucoepidermoid carcinoma grading is not reliably reproducible and does not always correlate with prognosis. Thus, additional markers for tumor grade and prognosis would be desirable. Several loci appear to be involved in tumorigenesis (3p, 9p, and 17p). The mean FAL of mucoepidermoid carcinomas correlates with the histologic grade of the tumors, however the ranges of FAL overlapped for individual tumors. The mean FAL was higher in patients who are DOD than in those who were NED, which suggests an association with prognosis.

981 Epithelial-Myoepithelial Carcinoma: A Review of the Morphologic Spectrum and Immunophenotype of 32 Tumors

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Background: Epithelial myoepithelial carcinoma (EMCa) is rare (1% of all salivary gland tumors). In order to further define its morphologic spectrum and characterize its immunophenotype, we report our experience with 32 EMCa.
Design: Thirty-two cases (31 patients, 1987-2005) were retrieved from our archives. We evaluated cytologic and architectural features. In 29 cases, the expression of cytokeratins (AE1/AE3, CAM 5.2, and Pankeratin), p63, smooth muscle actin (SMA), calponin, smooth muscle myosin heavy chain, vimentin, S100, GFAP, Ki-67, p53, bcl-2, and c-kit were evaluated.
Results: The majority of tumors showed a characteristic nodular/multinodular pattern of infiltration and areas of biphasic tubular histology. Tumor characteristics are summarized below. Unusual growth patterns included biphasic papillary (4/32, 13%), basaloid (1/32, 3%) and "Verocay" like (1/32, 3%). Variant morphologies in the myoepithelial cells included spindled (3/32, 9%), squamous (2/32, 6%), "ancient" change (4/32, 13%). Unique epithelial components included sebaceous differentiation (1/32, 3%) and dedifferentiation (1/32, 3%). The epithelial cells were positive for one or more broad-spectrum keratins. P63, S100, and SMA had the best performance for myoepithelial staining. The average Ki-67 reactivity was 15% (range 5-30%). Bcl-2 was positive in 6/8 cases (75%) and c-kit was positive in 10/11 (91%) cases. p53 was only strongly expressed in the dedifferentiated tumor.
Conclusions: EMCa has an ever-increasing morphologic spectrum, including some new variants described above. Most are low grade, though some histologically aggressive tumors including a dedifferentiated case were found. The differential diagnosis includes adenoid cystic carcinoma (ACC) and other well differentiated salivary gland neoplasms. Broad-spectrum cytokeratins are useful in selectively staining the epithelial cells, while p63, S100 and SMA are the most useful myoepithelial markers. Ki-67, bcl-2 and c-kit are not useful in distinguishing EMCa from ACC. Diffuse p53 immunoreactivity is rare, and may be involved in tumor progression.

Tumor Characteristics	
Epithelial:myoepithelial ratio	0.62 (range:0.05-1.5)
Cystic change	9/32 (28%)
Calcifications	6/32 (19%)
Perineural invasion	10/32 (31%)
Angiolymphatic invasion	3/32 (9%)
Necrosis	5/32 (16%)
Nuclear atypia	Mild 21/32 (66%), Moderate 10/32 (31%), Severe 1/32(3%)*
Mean mitotic activity /10 high power fields	4.2 (1.8 epithelial, 2.3 myoepithelial)

* Dedifferentiated case

982 Distinction of High Grade Neuroendocrine Carcinomas of the Head and Neck from Basaloid Squamous Cell Carcinoma

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Background: Primary high grade neuroendocrine carcinomas (HGNECA) of the head and neck have a predilection for the supraglottic larynx. Most have small cells with a morphology akin to small cell carcinoma of the lung. Others have larger cells and more differentiation with rosette formation and trabeculae. These tumors typically have an aggressive clinical course and surgery is often not feasible or indicated given the high rate of metastasis. Basaloid squamous cell carcinoma (BSCC) is an aggressive squamous cell carcinoma variant, typically in the supraglottic larynx, pyriform sinus, and oropharynx. These show early metastasis to regional nodes and treatment typically includes surgical resection, neck dissection and radiotherapy just as in ordinary squamous cell carcinoma. Chemotherapy for HGNECA is more aggressive than for BSCC. It is important to distinguish these tumor types, but often quite difficult histologically, particularly on small biopsy specimens and where neuroendocrine markers are weak or negative. We studied several immunostains for utility in this setting.
Design: A database search yielded 12 cases of HGNECA and 11 cases of BSCC. The cases were re-reviewed and only those tumors with classic, typical histologic features were utilized. Tumors were stained for p63, cytokeratin 34ßE12, cytokeratin 5/6, synaptophysin, chromogranin-A, S-100 and smooth muscle actin (SMA). Slides were reviewed by both pathologists blinded to the diagnoses. Staining was graded as follows: No staining – 0, 1-25% of tumor cells staining – 1+, 25-50% of tumor cells – 2+, 50-75% - 3+ and 75-100% - 4+.
Results: All BSCC were positive for 34ßE12 (average staining intensity of positive cases 3.7) and for CK5/6 (3.2) while only 1 of 12 (8%) HGNECA were positive for CK5/6 (1.2), and only 2 of 12 (17%) HGNECA positive for 34ßE12 (0.5). 10 of 12 (83%) HGNECA were positive for p63 (2.8). 10 of 11 (91%) of BSCC were positive for p63 (3.4). 5 of 12 (50%) HGNECA were positive for synaptophysin (1.5) and 10 of 12 (83%) for chromogranin-A (2.3). All BSCC were negative for neuroendocrine markers. All HGNECA were negative for S100 and SMA while 5 of 11 (45%) BSCC were positive for S-100 (0.6), and 2 of 11 (18%) BSCC for SMA (0.1).
Conclusions: In addition to neuroendocrine markers, cytokeratins 34ßE12 and CK 5/6 are useful to distinguish head and neck HGNECA from BSCC. Neuroendocrine markers are consistently negative in BSCC. p63, while strongly positive in squamous cell carcinomas, is also positive in HGNECA, and so is not useful in this setting.

983 Clonal Nature of Sclerosing Polycystic Adenosis of Salivary Glands Demonstrated by Using the Polymorphism of the Human Androgen Receptor Locus (HUMARA) as a Marker

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Background: Sclerosing polycystic adenosis (SPA) is a recently described, rare lesion of the salivary glands of uncertain nature that bears a resemblance to epithelial proliferative lesions of the breast. The true nature of the lesion is unknown, but it is generally believed to represent a pseudoneoplastic sclerosing and inflammatory process. Local recurrence has been described in about one third of the cases. Recently, superimposed dysplastic changes ranging from low-grade dysplasia to carcinoma in situ have been reported in SPA. Although no metastases and/or disease related patient deaths were documented, these clinical and histopathological features suggest that SPA could represent a neoplastic lesion.
Design: In order to examine the clonality, we applied the HUMARA assay to fragments of salivary gland lesions consistent with a diagnosis of sclerosing polycystic adenosis (SPA) from nine female and two male patients. Clonality analysis was performed according to the previously described method based on the digestion of genomic DNA with methylation-sensitive restriction enzymes followed by PCR amplification of the CAG repeat at the human androgen receptor gene (HUMARA) locus at the X chromosome.
Results: The two male cases were non-informative, as they are homozygous for the HUMARA locus, and the samples derived from three female patients were unsuitable for clonality gene analysis because of low quality of DNA. All the remaining six female patients could be studied, and were found clonal as indicated by HUMARA gene testing. Our results demonstrate a significant loss of intensity of one allele after *Hpa*II digestion in these six specimens. Clonality ratios ranged between 1.5 and 15.5. As the final clonality ratio ≥1.5 has been used as a criterion of clonality, the samples of salivary gland SPA derived from all these six female patients were considered clonal.
Conclusions: The clonality assay using the polymorphism of the HUMARA locus indicated that SPA is clonal in nature, and thus it most likely represents a neoplastic process.

984 Mdm-2 and p21 Expression in Actinic Cheilitis

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Background: Actinic Cheilitis (AC) is a pre-malignant lesion of the lip vermillion caused by overexposure to ultraviolet (UV) radiation. It has been shown that UV light alters cell cycle proteins in the lip, with increased expression of the proapoptotic mediator p53, and decreased expression of the anti-apoptotic bcl-2. The cell cycle markers murine double minute (mdm-2) and p21, have also been involved in UV-induced carcinogenesis, and are considered indicators of malignancy. mdm-2 has been associated with p53 and p21 degradation, whereas, p21 expression is modulated by p53. However, their specific role in UV-induced lip carcinogenesis has not been elucidated.

Design: Normal oral mucosa (n=6), normal lip (n=7), and AC (n=13) biopsies were stained immunohistochemically for p21 and mdm2, to determine their expression and distribution. Positive and negative cells were counted at 40x magnification by two calibrated observers. A minimum of 1000 cells were counted for each marker at the basal, parabasal and suprabasal layers. Results were expressed as percentage of positive cells/ 1000 cells (mean ± SEM). Differences among groups were examined using one-way ANOVA and Tukey-Kramer tests.

Results: Nuclear staining of p21 was observed in the parabasal and suprabasal layers of the epithelium of AC, normal lip, and oral mucosa samples. The percentage of p21-positive cells in AC was significantly reduced as compared to normal lip and oral mucosa ($p<0.05$), with no differences between normal lip and oral mucosa. mdm2 expression was predominantly nuclear with some degree of faint cytoplasmic staining in each AC sample. mdm2 was higher in AC as compared to normal lip ($p=0.0045$, Wilcoxon and Kruskal Wallis) and oral mucosa ($p=0.0009$) with no significant differences between normal lip and oral mucosa.

Conclusions: The results showed that in AC there is an increase in mdm-2 expression and a decrease in p21 in the epithelium, as compared to normal lip and oral mucosa. This suggests that mdm-2 may be stimulating p21 degradation in AC, leading to altered keratinocyte apoptosis. Supported by grants DIUC 204.103.105-1.0, 203.103.014-1.0, 203.103.011-1.0 and Fondecyt 1050581.

985 E-Cadherin Expression Is Increased in Salivary Duct Carcinoma

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Background: Salivary duct carcinoma (SDC) is a rare, aggressive salivary gland malignancy characterized by infiltrative, rounded tumor nodules that suggest expanded salivary ducts, and strongly resemble ductal carcinoma in-situ (DCIS) of the breast. Like other salivary gland neoplasms, SDC is presumed to arise from ducts. E-cadherin (E-cad) is a cell-cell adhesion protein involved in differentiation and maintenance of normal epithelia. In a variety of neoplasms, reduced or absent E-cad expression is associated with tumor invasiveness, dedifferentiation, and worse prognosis. In breast cancer, E-cad additionally distinguishes itself as a diagnostic aid due to its differential expression in in-situ and invasive ductal and lobular carcinomas. We examined E-cad staining in SDC to clarify its histogenetic origin and correlate expression with its aggressive behavior.

Design: Paraffin embedded excisions of 19 cases of SDC, 11 cases of breast DCIS/ invasive ductal carcinoma, 8 cases of breast lobular carcinoma in-situ/invasive lobular carcinoma, 11 normal salivary glands, and 10 cases of normal breast were stained with antibody to E-cad. Staining intensity was graded as strong, moderate, or weak, and distribution as diffuse, patchy, focal or absent.

Results: E-cad diffusely stained epithelial cell-cell boundaries in ducts and acini or lobules of normal salivary gland and breast; in breast, staining was uniformly strong, while in salivary gland it was weak to moderate. Diffuse, strong E-cad staining was seen in in-situ and invasive ductal lesions, while 88% of lobular lesions were E-cad negative. Ninety-five percent of SDC were E-cad positive, the majority diffusely so. Staining was variable within each tumor, but was predominantly moderate to strong in intensity. No significant difference in staining was observed between large, rounded "intraductal" tumor nodules and small, infiltrative appearing tumor nests. E-cad staining was consistently stronger in SDC than in residual normal salivary gland on the same slide.

Conclusions: E-cad expression in SDC supports its ductal histogenetic origin and furthers its resemblance to DCIS of the breast. However, unlike a subset of ductal breast carcinomas and other epithelial malignancies in which loss of E-cad expression is correlated with particularly aggressive behavior, SDC is a highly aggressive tumor whose E-cad expression is not only maintained, but is enhanced compared to normal ducts. These findings suggest that in this tumor, other genetic alterations may be correlated with its poor prognosis.

986 Low-Grade Cribriform Cystadenocarcinoma of Salivary Gland Is a Low-Grade Intraductal Carcinoma Not a Variant of Cystadenocarcinoma

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Background: Low-grade cribriform cystadenocarcinoma (LGCCC) is a rare neoplasm of salivary gland initially described as low-grade salivary duct carcinoma (LGSDC). Considerable controversy remains regarding the long term clinical behaviour, nomenclature, and relation of this neoplasm to conventional salivary duct carcinoma. To further our understading of this neoplasm, we describe the microscopic and immunophenotypic features of three additional cases, including one with a longstanding clinical course which developed a high-grade invasive carcinoma.

Design: All three neoplasms were routinely fixed in formalin. Selected blocks were stained with antibodies to AE1:AE3, cam 5.2, ck7, ck14, ck19, ck20, high-molecular weight keratin (HMWK), BRST-2, E-cadherin, Her2Neu, estrogen (ER) and progesterone receptors (PR), S100, calponin, smooth muscle actin (SMA), muscle specific actin (MSA), and p63.

Results: There were 2 females and 1 male. The age range was 50-73. One tumor had been present for 9 years before causing paresthesias and rapidly increasing in size. The parotid gland was involved in all cases. All the tumors showed a low-grade non-invasive component comprised of round smooth cysts showing micropapillae, "roman arches" and cribriform structures. The tumor cells displayed extensive apocrine differentiation with eosinophilic granular cytoplasm and apical "snouts". Foci of pale cells with vacuolated cytoplasm were also present. Only one case showed a high-grade invasive carcinoma which was also present in cervical lymph nodes. The ductal cells of all tumors were positive for AE1:AE3, cam 5.2, CK7, CK19, HMWK, BRST-2, and E-cadherin. They were negative for ck20, ER, PR, and Her2Neu. The intraductal component was surrounded by a thin layer of myoepithelial cells positive for ck14, calponin, p63, and actins. Only one case expressed S100. The invasive carcinoma lacked myoepithelial cells, was E-cadherin negative and focally expressed ck20.

Conclusions: LGCCC is a non-invasive, low grade intraductal neoplasm with an intercalated duct phenotype and apocrine differentiation which only occasionally develops an invasive component. The designation "low-grade intraductal carcinoma" of salivary gland or "salivary intraductal neoplasm of low malignant potential" rather than the current designation "LGCCC" or "LGSDC" probably reflects more accurately the behaviour of these lesions.

987 Expression of Apolipoprotein A-II in Head and Neck Squamous Cell Carcinoma by Immunohistochemistry

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Background: Apolipoprotein A-II is a biomarker for early detection of certain cancers. Elevated levels of apolipoprotein A-II in the sera of patients with head and neck squamous cell carcinoma has been demonstrated by SELDI immunoassay recently. To further investigate tumor tissue expression of apolipoprotein A-II, we have subsequently detected this biomarker in HNSCC cells by immunohistochemistry.

Design: Twenty three HNSCC cases were retrieved from our departmental file. Ten cases of benign squamous mucosa were used as control samples. All tissue was formalin fixed and paraffin embedded. Immunohistochemical staining was performed using primary goat anti-human apolipoprotein A-II antibody (1:11,000, polyclonal, Biodesign International, Cincinnati, OH), by a streptavidin-biotin kit (BioGenex, San Ramon, CA). Apolipoprotein expression was considered to be positive if cellular membrane staining was present.

Results: Tissue expression of apolipoprotein A-II in HNSCC tumor cells was identified in 69.5% (16/23) of cases. Of these, 13% were classified as diffuse (3/23), 17% patchy (4/23), and 39% focal (9/23). The density of positivity was scored from weak to strong. Five tumor cases showed no staining (21.7%). Ten cases of benign squamous mucosa demonstrate basal cells with membranous staining. No staining was seen in sub-epithelial soft tissue or salivary glands.

Conclusions: This study supports the prior findings of apolipoprotein A-II expression in HNSCC serum by SELDI immunoassay. Our study shows that this biomarker can be detected in tumor cells by immunohistochemistry. These preliminary results may be critical to facilitate the early detection and diagnosis of HNSCC in the future.

988 Differential Role for p63 Gene Isotypes (DeltaN and TA) in Head and Neck Squamous Tumorigenesis

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Background: The p63 gene plays an important role in stratified squamous epithelium maintenance and differentiation. The gene manifests several isotypes with different functions. The two main isotypes, TA and DeltaN are produced by two distinct promoters and exert oppossing cellular effects. TA is homologous to the p53 gene and DeltaN plays a paradoxical p53 function. We hypothesize that p63 isotype expressions rather than the full length protein reflect the biological effect of this gene in HNSC.

Design: The expression of p63 isotypes by RT-PCR and quantitative RT-PCR were performed on 18 HNSCC cell lines and paired normal and tumor tissues from 45 patients with oral squamous carcinoma.

Results: Cell-lines:

•All 18 cell lines showed high expression of DeltaN isotype. Five cell lines showed co-expression of both TA and DeltaN; with lower expression of TA. TA expression was higher in cell lines with p53 mutation than those with wild type ($p=0.05$)

Tumors and Normal:

•Normal mucosa was either negative or manifested low expression (≥ 5).

•Of the 34 tumors, (75 %) showed high DeltaN and 16 (35.5%) had high TA ($p=0.003$). Only 12 (26.6%) of the cases showed high co-expression of both isoforms.

•High DeltaN was correlated with high stage, perineural invasion and outcome ($P=0.02$).

Conclusions: We conclude that: 1) p63 isotype expressions are significantly different in both cell lines and tumors. 2) The TA isotype overexpression was associated with p53 mutations in cell lines and suggest a compensatory effect for this isotype. 3) DeltaN is the dominant isotype in HNSC carcinoma. 4) High DeltaN expression is associated with aggressive clinical outcome.

989 Hormonal Profiling in Salivary Duct Carcinoma: Potential Stratification for Therapy

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Background: Salivary duct carcinoma (SDC) is an uncommon neoplasm of lethel outcome. We previously reported that novel estrogen receptor coactivator PELP-1 and ER-β and not α are co-expressed in the majority of these tumors. Moreover, recent data indicate that AR expression may counterbalance the growth stimulatory effect of ER-β in mammary carcinoma. We analyzed the incidence, relationship and potential use of hormonal markers to stratify patients with SDC for therapy.

Design: Eighty-three SDCs with available tissue blocks from our files from 1980-2005 formed the materials for this study. Immunohistochemical staining for ERbeta and α, AR, and PELP-1 were performed. Two independent reviewers using a 0 to 3+ scale for each biomarker independently evaluated immunoreactivity of tumor cells for nuclear staining (ERbeta, ER-a and AR), and cytoplasmic staining intensity (PELP-1). Nuclear staining in ≥ 10% of cells were considered positive.
Results: Patients comprised of 52 males and 26 females (2:1) with a mean age of 60.7 years. SDC arose in the parotid gland in 63 (76%), submandibular gland in 8 (10%), and minor salivary glands in 4 (5%). 49% of patients had lymph node metastases at diagnosis with overall 5 year survival of 20% (8 of 40). None of the tumors reacted to ERα. All cases showed expression for at least one of the remaining markers. AR expression was identified in both men and women, though the percentage of positive tumors was higher in men than women (75% vs. 38%). In comparison, ERβ was positive in 100% of SDC from women and in 90% of men. Overall, 94% of tumors expressed ERβ, 94% expressed PELP-1, and 60% AR. Co-expression of all three biomarkers was present in 58% of cases. No correlation between biomarkers, clinicopathologic features and patient survival was found.
Conclusions: Our results indicate: 1) the hormonal co-activator PELP-1 and ERβ are expressed in the majority of tumors, 2) co-expression of ERβ and androgen receptors are found in more than 50% of the tumors, 3) a subset of tumors that lack AR expression and ERβ positivity was noted in (40%) of the cases and may signify a loss of AR inhibitory effect on ER-β and 4) patients may be substratified based on the differential expression of these markers for hormonal therapy.

990 Basaloid Salivary Gland Neoplasms: Imbalance of the Apoptotic/ Proliferative Homeostasis in Pathogenesis

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Background: Basaloid tumor salivary glands encompass benign and low-grade malignant tumors.]The histologic and biologic distinction between these lesions can be difficult. Moreover, they occasionally manifest overlapping cellular features with solid adenoid cystic carcinoma (ACC) and basaloid squamous carcinoma (BSC). We hypothesize an unbalanced apoptotic/proliferation ratio in favor of a cell death defect underlie the pathogenesis of the entity. NFkB is a novel transcriptional factor with vital role in regulating both cell growth and death. Surrogate markers of cell proliferation (Ki-67) and anti-apoptotic transcriptional factors (BCL-2) and NFkB can be used to assess the status of these pathways in different tumors.
Design: Paraffin blocks from 13 basaloid adenomas and 16 basal cell adenocarcinomas formed the materials for this study. For comparison, we included 12 solid ACC, and 11 BSC. Immunohistochemical analysis for Bcl-2, NFkB, and Ki67 were performed on each tumor. Bcl-2 (cytoplasmic) and NFkB (nuclear) expression were graded 0-3+. Proliferation index (PI) was defined as % of Ki-67 nuclear staining in 500 tumor cells.
Results: Basaloid adenomas (BA) showed high expression (2 or 3+) of Bcl-2 and NFkB in 11/13 (84%) and low Ki-67 (0.2%) in 13/13 (100%). Basaloid adenocarcinoma showed high Bcl-2 expression in 7/16 (44%) (p-value=0.01) and low NFkB in 2 cases. The proliferation index for solid ACC and BSCC were 10-38% and 26-68% respectively and were significantly different from basaloid adenomas and adenocarcinoma (p>0.0001). High expression was also present in all ACC and six BSC for Bcl-2 and NFKB (ACC 11/12 and BSC 9/11).
Conclusions: 1) High expression of Bcl-2 and NFkB and low Ki-67 in basaloid adenoma supports apoptotic suppression as the primary mechanism for their tumorigenesis, 2) relative elevation of Ki-67 (6%) may portend malignant transformation in basaloid tumors, 3) proliferation is the dominant pathway in the oncogenesis of solid ACC and basaloid squamous carcinomas and 4) these markers may be used along with the histologic evaluations to assess their biological progression and response to therapy.

991 Assessment of p16 Expression in Oral Hyperkeratoses that Progressed to Dysplasia

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Background: Progression of a small subset of oral leukoplakias to epithelial dysplasia is recognized. Further characterization of such precursor lesions is of both clinical and prognostic interest. Recent evidence suggests that alterations of the cell cycle regulatory gene CDKN2A may be involved in the development of certain dysplastic lesions. Overexpression of p16 protein is observed in the setting of CDKN2A inactivation, and has been previously documented in oral dysplasias. We sought to examine the p16 immunostaining pattern in oral hyperkeratoses that ultimately progressed to dysplasia.
Design: A retrospective search of all oral dysplasias accessioned over a 1-year period was performed. Selected cases were required to have an initial, biopsy-confirmed diagnosis of hyperkeratosis and a follow-up diagnosis of dysplasia, at the same site. Immunohistochemical staining with p16 was performed employing standard techniques, with appropriate controls. Immunoreactivity was assessed by evaluating staining intensity (scored 0 [negative] to 3 [strong]) and localization (basal, middle, upper third of the epithelium).
Results: Our search yielded 115 cases of which 5 cases fulfilled the aforementioned criteria. Follow-up diagnoses in these cases included 2 mild, 2 moderate, and 1 severe dysplasia. All 5 initial hyperkeratoses exhibited immunoreactivity for p16 (1 to 2 in intensity, basal and rarely middle third of the epithelium). Similarly, all 5 follow-up dysplasias demonstrated p16 positivity (1 to 2 in intensity). In the dysplasias, staining localization often correlated with the distribution of atypical cells. One case of mild dysplasia that progressed to severe dysplasia was selected as a positive control case; both biopsies exhibited p16 staining, with intensity increasing from 1 (initial lesion) to 2 (follow-up). Two cases of hyperkeratosis that remained hyperkeratoses at follow-up served as negative control cases; all were p16-negative.
Conclusions: Our results indicate that there may be a trend towards p16 positivity in oral hyperkeratoses and their associated dysplasias compared to non-progressing hyperkeratoses, suggesting that p16 alterations may be present and detectable even at the precursor stage of dysplasia development. We believe p16 stains may be informative in cases that are clinically worrisome yet display only benign features histologically. In such cases, p16 positivity may provide further support for more vigilant clinical observation and discretionary follow-up biopsies. Further investigations are ongoing.

992 Immunohistochemical Detection of XIAP in Head and Neck Squamous Cell Carcinoma

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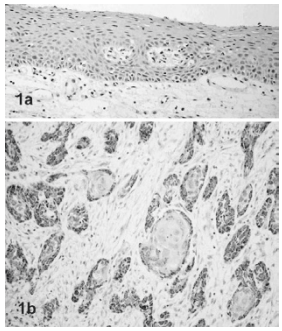
Background: The X-linked inhibitor of apoptosis (XIAP) is the most potent member of the family of IAPs, a group of structurally related caspase inhibitors. Increasing evidence implicates XIAP in the aggressiveness of certain tumors, including renal carcinomas and hematopoietic malignancies. We examined the expression of XIAP in squamous cell carcinoma of head and neck.
Design: 5 μm sections from 42 routinely processed specimens of head and neck squamous carcinoma were subjected to citrate-based antigen retrieval, followed by incubation with monoclonal anti-XIAP antibody (BD Biosciences) and Envision Plus reagents (Dako). Granular cytoplasmic staining was considered positive. Extent and intensity of staining were recorded.
Results: Normal squamous epithelium was either non-staining (n=14, fig 1a) or displayed generally weak basal staining (n=9). Carcinoma in situ was non-staining (9 of 15 cases) or displayed generally weak staining (6 of 15 cases). XIAP staining was positive in 26 of 42 carcinomas (62%). The majority of the non- and weakly-staining carcinomas were well to moderately differentiated. In contrast, intense and extensive staining was most frequently found in poorly differentiated carcinomas (fig 1b) and/or in infiltrative cell nests at the leading edge of invasion. In keratinized tumor nests, staining was strongest peripherally and became diminished in central keratinized zones.
Conclusions: New parameters of tumor aggressiveness are needed for more effective triaging of patients to appropriately aggressive therapies. The present findings suggest that the potent apoptotic inhibitor XIAP may be such a biomarker in head and neck squamous carcinomas, either of resistance to apoptosis-inducing therapies, or of responsiveness to a new class of XIAP-suppressive drugs already in clinical trials.



993 Primary Mucin Producing Tumors of the Salivary Glands: A Clinicopathologic and Morphometric Study

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Background: Mucin producing adenocarcinomas of the salivary glands, including mucinous (colloid) carcinoma (MC), mucinous cystadenocarcinoma (MCAC), and mucin-rich salivary duct carcinoma (SDC), are rare neoplasms histologically characterized by large pools of extracellular mucin. The morphological features of these tumors often overlap creating difficulties in differential diagnosis. The aim of this study was to determine clinicopathologic and morphometric features that discriminate between these rare entities.
Design: Mucin producing carcinomas were reclassified using a strict definition of mucinous carcinoma with ≥90% of tumor cells being surrounded by pools of mucin material with <10% of the tumor cells touching peripheral fibrous stroma. Eleven mucin producing tumors were stratified into 4 MC, 4 MCAC, and 3 mucin-rich SDC. Computerized morphometry was performed using Image-Pro Plus software. Sixty microscopic images were obtained. A total of 6,441 nuclei were analyzed for size (area, diameter), shape (ellipticy, elongation), and chromatin texture (margination, density).
Results: The median patient age was 73, 67, and 43 years for MC, MCAC, and SDC, respectively. Eight of 11 patients were females. Tumor size did not differ significantly between these groups. Two cases of MC originated from parotid gland, and 2 from minor salivary glands; MCAC and SDC showed predilection to the major salivary glands. Clinical follow-up was available for 10 cases; mean follow-up period was 51 months. One patient died from disease in the MC group, and one in the SDC group; no patients died from disease in the MCAC group. Histologically, MC and MCAC showed bland nuclear features, whereas SDC nuclei were more atypical. Mitotic figures were more frequent in SDC group, as compared to MC and MCAC (5, 2.5, and 3.6/10 hpf). Histomorphometric measurements revealed that nuclear area and diameter in MC and MCAC groups were significantly smaller as opposed to the SDC group (area = 18, 17,

and 27, $P<0.001$). Moreover, in MCAC group, nuclei were significantly more elongated, more irregular with more heterogenous chromatin as compared to the MC.
Conclusions: Strict morphologic criteria of MC coupled with the nuclear features (size, shape, and chromatin texture) facilitate discrimination between rare mucin producing tumors of the salivary glands. Different clinical behavior emphasizes the importance of differentiating MC from MCAC and mucin-rich SDC.

Hematopathology

994 Megakaryocytic Nuclear Phospho-STAT5 Staining in Non-CML Myeloproliferative Disorders Correlates with JAK2 V617F

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Background: The diagnosis and classification of chronic myeloproliferative disorders (MPDs) is often challenging. Although attempts at finding reliable diagnostic immunophenotypic markers have been made, no such markers exist. The novel activating JAK2 V617F is found in many non-CML MPDs and in only rare cases of other myeloid disorders such as myelodysplastic syndrome (MDS) or overlap MDS/MPD. Activated JAK2 phosphorylates STAT5 (p-STAT5), resulting in translocation to the nucleus, where it promotes cell survival and proliferation. We used immunohistochemistery (IHC) to examine the diagnostic utility of activated p-STAT5 (nuclear p-STAT5) in non-CML MPDs.
Design: We examined bone marrow trephine biopsies (BM) from 44 patients with non-CML MPD (16 polycythemia vera [PV], 15 essential thrombocythemia [ET], and 13 chronic idiopathic myelofibrosis [CIMF]) for nuclear p-STAT5 expression by IHC. We similarly examined 6 cases of MDS and 11 cases of MDS/MPD overlap syndrome. Our control group included 20 BMs submitted for lymphoma staging and other non-myeloid disorders. Positive p-STAT5 staining was defined as nuclear staining in > 10% of megakaryocytes (nMEG p-STAT5). JAK V617F status was determined by allele specific PCR or LightTyper assay.
Results: The results are summarized in Table 1. In the control group nMEG p-STAT5 was observed in 2/20 patients; interestingly, both were receiving growth factor support (EPO and G-CSF), which is known to activate STAT5. When excluding growth factor therapy, IHC for nMEG pSTAT5 was 100% sensitive and 90% specific for JAK2 V617F. Similarly, when growth factor therapy is excluded, nMEG pSTAT5 was 86% sensitive and 100% specific for the diagnosis of non-CML MPD in our patient population (PV, ET and CIMF) compared to the control group.

Disease Type	JAK2 V617F		Wild Type JAK2	
	p-STAT5 (+)	p-STAT5 (-)	p-STAT5 (+)	p-STAT5 (-)
Control (20)	0	0	2	18
MDS (6)	0	0	0	6
MDS/MPD (11)	1	0	2	8
PV (16)	14	0	0	2
ET (15)	11	0	2	2
CIMF (13)	11	0	0	2

Table 1

Conclusions: nMEG pSTAT5 expression in V617F MPDs appears to reflect JAK2 activation. In the absence of growth factor therapy, it strongly suggests the diagnosis of a myeloid malignancy and should prompt confirmatory molecular testing for JAK2 V617F. Finally, the presence of JAK2 V617F and nMEG pSTAT5 in some cases of MDS/MPD overlap syndrome suggests closer biologic similarity of such cases to MPDs than to MDS.

995 Extranodal Diffuse Large B Cell Lymphoma of Cutaneous Follicular Lymphoma Type

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Background: Cutaneous follicular lymphoma (cFL) and marginal zone lymphoma (MZL) are distinct WHO/EORTC clinico-pathological entities of cutaneous lymphomas carrying an excellent prognosis. MZL is well recognized to occur in other extranodal locations. The aim of the study was to investigate, whether diffuse large B cell lymphomas (DLBCL) of cFL type could also be identified in other extranodal localizations and whether it could also be associated with a similar distinct clinical course.
Design: Among 1500 consecutive lymphoma diagnoses on file in the Department of Pathology of the University Hospital Zurich (from 1992 to 2004) 111 cases of non cutaneous non gastrointestinal DLBCL were retrieved and reviewed. 10 cases were associated with HIV positivity, 1 case with immunosuppression after organ transplantation, 55 cases had documented previous nodal disease or documented other nodal disease at presentation, 5 cases represented Burkitt lymphoma or blastoid mantle cell lymphoma, and 4 cases had no paraffin material left for analysis. The 36 remaining cases were analyzed for nuclear morphology and immunohistochemical profile and classified according to WHO/EORTC criteria into DLBCL of cFL type (centrocytoid, BCL-6+ CD10-/+ BCL-2- IRF-4-) or in DLBCL of non cFL type (centroblastic or immunoblastic, BCL-6+/- CD10+/- BCL-2+ IRF-4+).
Results: Lymphomas that matched the WHO/EORTC criteria for DLBCL of cFL type by morphology, immunohistochemical profile and clinical course could be identified. One case showed a localized extranodal extracutaneous recurrence with a follicular growth pattern 9 years after primary diagnosis and local radiation therapy, 1 patient was free of disease 8 years after surgical excision alone. 8/36 cases were classified as DLBCL of cFL type, 28/36 cases were classified in analogy to DLBCL of non cFL type. 1/8 patients (13%) died of lymphoma in the former group, 4/28 patients (14%) died of lymphoma in the latter group (median follow up 60 months).
Conclusions: In analogy to extranodal MZL the data suggest that DLBCL of cFL type as defined by WHO/EORTC criteria does occur in other extranodal extracutaneous localizations. Limited stage extranodal lymphoma can be associated with an excellent disease specific survival. No difference in survival was found between cases that matched the WHO/EORTC criteria for cFL and DLBCL cases of non cFL type.

996 Differential Expression of Cyclin Dependent Kinase 1 (CKS-1) in Small Cell and Blastoid Variant Mantle Cell Lymphoma

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Background: Mantle cell lymphoma (MCL) is a distinct type of B-cell lymphoma characterized by the t(11;14)(q13;q32). Most cases of MCL are composed of small lymphocytes, but a subset of cases is composed of larger or more immature cells, known as blastoid variant. Blastoid MCL is associated with a higher proliferation rate, more aggressive clinical course, and commonly has genetic alterations affecting the cell cycle in addition to the t(11;14). Cyclin dependent kinase (CDK) subunit-1 (CKS-1) is essential for the ubiquitination and subsequent degradation of p27, a universal CDK inhibitor.
Design: We analyzed CKS-1 and p27 expression in four MCL cell lines including Mino, SP53, Jeko and Z-138 using Western blot analysis after subcellular fractionation. CKS1 and p27 expression and localization was further assessed in two of these cell lines (Mino, SP53) by immunohistochemistry in paraffin-embedded cell blocks. CKS1 and p27 expression was also assessed in 51 MCL tumors (35 small cell, 16 blastoid) using immunohistochemical methods and correlated with proliferation index (MIB1) and apoptotic rate (TUNEL) of these neoplasms.
Results: High levels of CKS-1 were detected in the Mino, SP-53 and Jeko cells. CKS1 was predominantly cytoplasmic. In MCL tumors, using a 10% cutoff, 10 of 35 (28.6%) small cell tumors versus 14 of 16 (87.5%) blastoid tumors were positive for CKS-1 (p=0.0002). Analyzed as a continuous variable, the percentage of CKS-1 positive tumor cells also significantly correlated with blastoid variant (p=0.001). p27 levels were low in the Mino and SP53 cell lines. Using a 25% cutoff, p27 was expressed in 12 of 51 (23.5%) mantle cell lymphoma tumors. However, there was no correlation between p27 expression levels and histologic type (small cell vs. blastoid) of mantle cell lymphoma. Proliferation rate, as assessed by Ki-67 expression, was higher in blastoid variant than in small cell tumors and was inversely associated with p27 levels in the small cell but not in the blastoid MCL group. In the small cell MCL group, progression-free survival did not differ significantly in patients with CKS1+ versus CKS1- tumors. However, in the blastoid MCL group, 2 patients with CKS1- tumors did not relapse or progress.
Conclusions: We conclude that CKS-1 is commonly over-expressed in mantle cell lymphoma cell lines and tumors, particularly in the blastoid variant. CKS-1 overexpression appears to be a manifestation of cell cycle dysregulation in mantle cell lymphoma.

997 Differential Expression of Smac/DIABLO in B-Cell Non-Hodgkin Lymphomas (NHL)

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Background: The pathogenesis of B-cell NHL may be regulated by apoptotic mechanisms. Low-grade lymphomas (e.g. CLL/SLL) are characterized by slow accumulation of mature B cells, suggesting that these diseases have defects in the regulation of apoptosis. By contrast, high-grade lymphomas are highly proliferative and can be associated with either low (e.g. pre-B LBL) or high levels of apoptosis (e.g. Burkitt lymphoma). Caspases, the central component of the apoptotic machinery, can be inhibited by members of the inhibitor of apoptosis protein (IAP) family. Other proteins can negatively regulate IAPs, one of which is Smac/DIABLO. In this study, we assessed for expression of Smac/DIABLO in B-cell lymphoma cell lines and primary B-NHL tumors.
Design: The study group included 187 B-NHL tumors and 10 B-NHL cell lines: 3 MCL, 2 DLBCL, 2 Burkitt, 2 pre-B LBL, and 1 primary effusion lymphoma (PEL). Expression of Smac/DIABLO was determined by immunohistochemistry and Western blot analysis using a polyclonal antibody (Imgenex, San Diego, CA). For immunostaining, tumors were analyzed using either tissue microarrays or full tissue sections.
Results: Smac/DIABLO was positive in 7 of 10 cell lines (negative in 1 DLBCL, 1 Burkitt, and 1 PEL). Immunostaining in tumors showed a variable pattern of expression in different NHL types (see table). In particular, both CLL/SLL and Burkitt showed no staining of Smac/DIABLO. By contrast, over half of follicular lymphomas (mostly high-grade), MCL, DLBCL, and pre-B-LBL showed moderate to strong expression, with the strongest intensity and highest frequency of expression being in pre-B LBL (68%).
Conclusions: We conclude that Smac/DIABLO is differentially expressed in B-NHLs, suggesting that different apoptotic mechanisms are involved in the pathogenesis of these diseases. The lack of Smac/DIABLO immunostaining in CLL/SLL supports the notion that this neoplasm is characterized by defective apoptosis. By contrast, the high apoptotic rate in Burkitt lymphoma is most likely regulated by mechanisms different from the IAP-Smac/DIABLO pathway. Smac/DIABLO may be a potential target for therapy in tumors that are most often positive, such as pre-B LBL.

Expression of Smac/DIABLO Proteins in B-cell NHL

Lymphoma Type	No.	Smac/DIABLO
Follicular lymphoma	30	19 (63%)
Mantle cell lymphoma (MCL)	38	20 (53%)
CLL/SLL	9	0
Lymphoplasmacytic lymphoma (LPL)	7	2 (29%)
N-marginal zone lymphoma (MZL)	7	3 (43%)
E-MZL	10	1 (10%)
SMZL	8	3 (38%)
Diffuse large B-cell lymphoma (DLBCL)	38	20 (53%)
Burkitt	12	0
Pre-B lymphoblastic lymphoma (LBL)	28	19 (68%)