2019 Oral Essays

Monday, June 10 - 8:00 am

COMPREHENSIVE GENOMIC ANALYSIS IDENTIfiES HIGH PREVALENCE OF PTCH1 MUTATIONS AND CDK4 AMPLIfiCATION IN AGGRESSIVE KERATOCYSTIC ODONTOGENIC TUMORS

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Introduction: *PTCH1* gene mutation is the etiology of KCOT, Gorlin syndrome and basal cell carcinoma. Clinically, KCOT demonstrates a range of biological behavior. Some KCOTs are small, and indolent; while others exhibit aggres-sive behavior with extensive bone destruction. The intent of this study is to report on DNA sequencing of 8 cases of KCOTs, and demonstrate the genomic profiles that distinguish between aggressive and indolent lesions. **Method & Materials:** 8 KCOTs were selected from the archives of institutional surgical pathology, and were further classified into 2 groups. Group 1) 4 cases, all males, 3 mandible, 1 maxilla that were ≤2cm radiolucencies, asymptomatic, or discovered on routine radiographic examination. Group 2) 4 cases, 3 males, 1 female, all in mandible, and radio-graphically ≥5cm radiolucencies. Next-generation sequencing (NGS) targeted 170 cancer genes including *PTCH1* & others was performed on sporadic KCOTs. DNA/RNA [T1] samples were retrieved from KCOTs for sequencing on a NGS platform. The variant and fusion calls were recorded and interpreted on software provided by Illumina & IBM. **Results:** The 4 KCOTs of group 1 exhibited *PTCH1* alterations, including missense, nonsense, frameshift, and splice site mutations. The 4 KCOTs of group 2 also exhibited *PTCH1* alterations as seen in group 1. Additionally, *CDK4* amplification was found in 3 of the 4 cases in this group. **Conclusions:** All KCOTs showed *PTCH1* mutations in contrast to previously reported 30% in sporadic KCOTs. Genetic profiling in this study seems to identify two biologically distinct groups of KCOTs, aggressive and indolent. Amplification of *CDK4* was noted in 75% of KCOTs in group 2 which demonstrated clinically aggressive behavior. The coexistence of *PTCH1* mutation and *CDK4* amplification may exert a synergistic action to promote aggressive behavior of some KCOTs. *PTCH1* mutation and *CDK4* amplification may provide a rational target for bioavailable antiproliferative drugs.

Monday, June 10 - 8:12 am

EVALUATING NOD2 IN ORAL CROHN DISEASE USING FLUORESCENCE IN SITU HYBRIDIZATION

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Introduction: Crohn disease (CD) is an inflammatory bowel disease that can affect any part of the gastrointestinal (GI) tract. Familial clustering of cases and twin studies have suggested a genetic contribution to the etiology of CD. *NOD2* on chromosome 16 is the first gene to be associated with CD susceptibility. Oral manifestations of CD are well- documented and may precede GI lesions in up to 40% of cases, especially in children. The purpose of this study is to investigate if an amplification or deletion of the *NOD2* gene can be detected using fluorescence *in situ* hybridization (FISH) analysis in oral biopsies of patients with a confirmed diagnosis of CD.

Materials and Methods: An IRB-approved retrospective search for CD, pyostomatitis vegetans, and chronic granulomatous stomatitis was performed within the archives of the University of Florida (UF), Texas A&M University (TAMU), and the University of Pittsburgh (Pitt) oral pathology biopsy services between the years 1994-2018. Cases of patients who did not have a confirmed diagnosis of CD were excluded. Tissue blocks and slides from 17 patients were retrieved. Unstained sections of each case were sent to the University of Pittsburgh Medical Center for FISH analysis. The ratio of *NOD2* and chromosome 16 control (CEP16) signals was calculated for each case.

Results: The ratio of the *NOD2* and CEP16 signals ranged from 0.95 to 1.57, with an average ratio of 1.08.

Conclusion: Neither amplification nor deletion of *NOD2* was identified in oral lesions of patients who have a history of CD. Evaluation of additional genetic markers that have been implicated in CD and employing further research to identify a reliable ancillary test that distinguishes oral granulomatous inflammation due to CD is warranted.

Monday, June 10 - 8:24 am

MECHANISM OF INTERLEUKIN 4-INDUCED MULTINUCLEATED GIANTCELL FORMATION AND ITS ROLE IN GIANT CELL-CONTAINING LESIONS

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Introduction: Multinucleated giant cells (MGCs) are found in a number of pathological lesions including reactive (foreign body granulomas), neoplastic (giant cell tumour and osteosarcoma), and genetic processes (cherubism). MGCs derive from fusion of monocytic precursors, however the molecular determinants that specify the formation and function of these cells in specific MGC-containing lesions are unknown. Notably, the morphology of MGCs in different MGC-rich lesions is strikingly similar. Accordingly, identification of a fusion-associated protein could improve characterization of MGCs and facilitate the diagnosis of MGC-containing lesions. We propose that C-type lectin domain family 10 member (CD301) is required for interleukin-4-induced MGC formation and is a marker for specific giant cell lesions. **Materials & Methods:** Tandem Mass Tag (TMT) Spectrometry was used to identify differentially expressed proteins in monocytes cultured under various pro-fusogenic conditions. We identified CD301 as a candidate protein and used immunolocalization, fusion assays with CD301 inhibition, and lectin bead-binding experiments. Three CRISPR/Cas9- knockout monocyte cell lines were generated in which isoforms a, b, or both isoforms were deleted. Statistical analysis was performed using Student's t-test. **Results:** TMT revealed that CD301 was differentially expressed by monocytic cells exposed to the pro-fusogenic cytokine interleukin-4. CD301 expression was confirmed by qRT-PCR, immunoblotting and immunolocalization. Function-inhibiting antibodies to CD301 and deletion of CD301 isoforms a and b inhibited interleukin-4-induced MGC formation by -2.3 fold and binding of lectin-bound beads by -4 fold (both tests, $r < 0.05$). **Conclusions:** In cultured monocytes, interleukin-4 strongly increases CD301 expression, which when inhibited, decreases MGC formation. Therefore, CD301 is required for the formation of MGCs involving lectin-CD301-mediated intercellular adhesion. CD301 holds the potential to act as a diagnostic marker for specific giant cell-containing lesions in human biopsy specimens.

Monday, June 10 - 8:36 am

THE INHIBITORY EFFECT OF SEMAPHORIN 3F INNEUROPILIN 2-EXPRESSING ORAL SQUAMOUS CELL CARCINOMA: PRECLINICAL STUDIES.

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Introduction: The majority of oral malignancies (90%) are oral squamous cell carcinomas (OSCC) and (67%) of the patients present with disseminated disease. Enhanced tumor lymphangiogenesis correlates with increased lymph node metastasis. Neuropilin 2 (NRP2) is a cell surface receptor expressed in neonatal lymphatic endothelium and down-regulated after birth. The lymphangiogenic factor, VEGF-C binds both NRP2 and VEGFR3 in complex to stimulate the proliferation of lymphatic endothelial cells in developmental lymphangiogenesis. Another ligand of NRP2 is Semaphorin-3F (SEMA3F), which competes with VEGF-C for binding. **Objective:** To characterize NRP2 expression in normal oral tissue, oral dysplasia and OSCC and to explore the effect of SEMA3F as a therapeutic anti-tumor and anti-metastatic drug. **Methods and results:** Immunohistochemical studies show adult human and mouse normal tongue tissue sections lack NRP2 expression in the epithelium and the lymphatic endothelium. However, NRP2 is up-regulated in late oral dysplasia in the epithelium and the subjacent lymphatic vessels. Additionally, tissue sections of mouse tongue injected orthotopically with human OSCC xenografts in nude mice demonstrated high NRP2 expression that correlated with increased tumor-associated lymphatic vessel density. The growth of syngeneic OSCC xenografts were compared between wild-type and *Nrp2*-deficient mice. Lastly, anti-tumoral effect of SEMA3F protein was tested in *vitro* and in *vivo* using slow-release osmotic pumps. SEMA3F inhibited the migration and invasion of NRP2-expressing 4NQO-induced OSCC cells in a dose-dependent manner. **Conclusion:** NRP2 up-regulation in both epithelium and lymphatic vessels correlated with tumor progression in OSCC. Our data highlights the importance of the NRP2 axis in tumor lymphangiogenesis in OSCC. SEMA3F appears to be a promising inhibitor to target this pathway. Ongoing studies in K14-cre^{ERT}; NRP2-floxed mice using the 4NQO carcinogenesis model will further elucidate the role of NRP2 in OSCC tumorigenesis.

Monday, June 10 - 8:48 am

PD-1/PD-L1 EXPRESSION IS A PREDICTOR OF PROGRESSION IN ORAL EPITHELIAL DYSPLASIA (OED)

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Background: The immune checkpoint system is essential for immune homeostasis and is frequently activated in cancer to suppress anti-tumor immune responses. Programmed cell death protein 1 (PD-1) regulates T cell activity in the tumor microenvironment. Interaction of PD-1 with its ligands PD-L1/ PD-L2 inhibits T cell activation effectively suppressing anti-tumor immunity.

Hypothesis: Increased PD-1 and PD-L1 expression can be detected in Oral Epithelial Dysplasia (OED) before transformation into Oral Squamous Cell Carcinoma (OSCC) and can be used as predictive markers of malignant transformation.

Methods: Analysis of 50 oral biopsy samples, including 25 cases that transformed to OSCC and 25 non-transforming cases was done. Cases with a diagnosis of hyperkeratosis (HK), OED (including mild, moderate and severe dysplasia) and OSCC were selected from the archives of the Toronto Oral Pathology service. FFPE sections were stained with monoclonal antibodies for PD-1 and PD-L1 followed by conventional peroxidase reaction (IHC) and fluorescent immunohistochemistry (FIHC) method. Images were acquired using a spinning disk confocal microscope (FIHC) or a conventional light microscope (IHC) and analyzed. PD-1/PD-L1 staining was assessed in the epithelium and inflammatory cells in lamina propria.

Results: Using conventional IHC, we showed a small progressive increase in expression of PD-1/PD-L1 from dysplasia to OSCC. In contrast, FIHC showed 2-3-fold increase in PD-L1 expression in basal epithelial cells and PD-1 expression in inflammatory cells in progressing dysplasia compared to non-progressing dysplasia.

Conclusion: We developed a novel FIHC-based quantitative method to study PD-1/PD-L1 expression in FFPE oral biopsy samples and showed that PD-1/PD-L1 are highly expressed in progressing dysplasia. Our results indicates that immunomodulation via PD-1 pathway occurs prior to malignant transformation.

Significance/ Impact: The expression of PD-1 and PD-L1 may be used as predictive markers of transformation and the data may be used to develop early intervention in OED using PD-1/ PD-L1 inhibitors.

Monday, June 10 - 9:00 am

ASSESSMENT OF MDM2 AMPLIfiCATION BY FLUORESCENCE IN-SITU HYBRIDIZATION IN JUVENILE ACTIVE OSSIFYING FIBROMA

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Introduction: Juvenile active ossifying fibroma (JAOF) is an uncommon benign fibro-osseous lesion (FOL) of the maxillofacial bones with locally aggressive behavior and a high recurrence potential. Murine Double Minute 2 (*MDM2*) is an oncogene located at chromosome 12 (12q13-15) which inhibits tumor suppressor gene *TP53*. The presence of *MDM2* gene locus amplification is a useful molecular diagnostic adjunct in the evaluation of some sarcomas including low grade intramedullary osteosarcoma and liposarcoma. JAOF and low grade intramedullary osteosarcoma may have some overlapping clinical and histomorphological features. The aim of this study is to evaluate a series of JAOF for the presence of *MDM2* gene locus amplification using fluorescence in-situ hybridization (FISH). **Materials and Methods**: With IRB approval, a search of the institutional files of the Department of Oral Pathology and the Department of Pathology at the University of Florida Shands Hospital was performed and 9 cases of JAOF were retrieved. The diagnosis of JAOF was confirmed by a group consisting of a senior resident in oral pathology, a board certified oral and maxillofacial pathologist, and a bone and soft tissue pathologist. Testing for MDM2 protein expression by FISH testing for *MDM2* gene locus amplification was performed for all cases. **Results**: All cases were negative for MDM2 amplification via FISH testing. **Conclusion:** In our small series of cases, JAOF did not demonstrate the *MDM2* gene locus abnormality characteristic of low grade intramedullary osteosarcoma, indicating the likelihood of a separate distinct underlying pathogenesis. If confirmed in a larger series, these findings may be a useful adjunct to microscopy in distinguishing these two entities, especially in cases with confounding and overlapping features or inadequate sample submissions.

Monday, June 10 - 9:12 am

DIFFERENTIAL EXPRESSION OF PROGRAM DEATH LIGAND 1 IN PROLIFERATIVE VERRUCOUS LEUKOPLAKIA

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Introduction: Upregulation of program cell death ligand 1 (PD-L1) in some cancers allows immune surveillance evasion and proliferation of the cancer cells. Expression of PD-L1 is currently utilized as a biomarker for anti- program death 1 (PD-1) therapy in many cancers. Thus far, investigation of PD-L1 expression in precancerous conditions has been minimal. We explored the expression of PD-L1 in precancerous lesions from patients with proliferative verrucous leukoplakia (PVL).

Materials and Methods: The residual formalin-fixed, paraffin embedded tissue of oral mucosal biopsies diagnosed as hyperkeratosis, dysplasia, verrucous hyperplasia, and carcinoma in-situ from patients with PVL were searched in the University of North Carolina at Chapel Hill School of Dentistry Oral and Maxillofacial Pathology Service. Patients with at least one low-risk group lesion (hyperkeratosis and low-grade dysplasia) and at least one high-risk group specimen (high-grade dysplasia) and their specimens were retrieved. The number of high- and low-risk group specimens were matched for each patient. Amalgam tattoo specimens were selected as control. Immunohistochemistry was performed on tissue sections using PD-L1 antibody. The proportion of epithelial cells expressing PD-L1 in epithelium was scored by three observers.

Results: Seven female and one male patients were selected. The average ages at the time of biopsy were 57, 55, and 59 years for the control, low-risk, and high-risk groups, respectively. The agreement between the observers in scoring PD-L1 expression was very good (ICC=0.94). All 16 control specimens showed 0% PD-L1 expression. Of 12 low-risk specimens, 1 specimen showed ≥1% PD-L1 expression (8.3%). Of 18 high-risk specimens, 15 specimens showed ≥1% PD-L1 expression (83%). There was significant association between the risk groups and PD-L1 expression ($\chi 2=8.3$, df=1, p=0.004), and the odds of high-risk lesions having PD-L1 expression ≥1% was 54.

Conclusion: Our results suggest that anti-PD-1/PD-L1 therapy may be beneficial for patients with PVL who have developed high-risk lesions.

Monday, June 10 - 9:24 am

SJÖGREN-LIKE SYMPTOMS IN PATIENTS WITH IDIOPATHIC PULMONARY FIBROSIS.

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INTRODUCTION: Idiopathic pulmonary fibrosis (IPF) is a chronic lung condition characterized by a progressive diminution in lung function. Certain forms of interstitial lung disease have been reported secondary to Sjögren syndrome (SS). However, IPF-related oral symptoms mimicking SS have not been reported. This study is the first retrospective case series of patients diagnosed with IPF with symptoms mimicking SS.

METHODS: An IRB approved retrospective chart review encompassing years 2005 to 2018, available through the University of Florida (UF) Oral Pathology Biopsy Service and the Center for Orphaned Autoimmune Disorders, was performed. Inclusion criteria included patients referred for a labial salivary gland biopsy with a diagnosis of IPF and Sjögren-like symptoms. Demographics, clinical data, laboratory test result, and microscopic diagnoses were reviewed and analyzed.

RESULTS: A total of 12 cases were included in the study. The mean age was 67.2 years (range 55-76) with a female to male ratio of 1:3. About 25% of patients had a history of occupational exposure to asbestos or chemicals and 66.7% had a smoking history. All patients reported symptoms of dry mouth and 9/12 patients reported dryness of eyes as well. About 75% of the patients were negative for anti-SSA and 71.9% were negative for anti-SSB. Positive ANA was seen in two patients and an additional two had a positive rheumatoid factor. Only one of the biopsies demonstrated positive focal lymphocytic sialadenitis (focus score ≥1 per 4 mm^2). Overall, 91.7% of the IPF cases did not meet the 2002 American-European Consensus Criteria for SS.

CONCLUSION: IPF patients may present with SS-like symptoms with positive autoantibodies. However, the majority of the IPF patients did not fulfill the current diagnostic criteria for SS. Future investigations are essential to delineate commonality, causality, and distinctions in the pathogenesis of both conditions to clarify diagnosis and management.

Monday, June 10 - 9:36 am

SATB2 EXPRESSION IN MYOfiBROBLASTIC AND fiBROBLASTIC PROLIFERATIONS OF THE HEAD AND NECK

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Objectives: To characterize the expression of special AT-rich sequence-binding protein 2 (SATB2) in myofibroblastic and fibroblastic proliferations of the head and neck.

Methods: Following IRB approval, 14 cases diagnosed as myofibroma (MF), 5 cases of myofibroblastic proliferation (MFP), 4 cases of inflammatory myofibroblastic tumor (IMT), 12 cases of nodular fasciitis (NF), 9 cases of solitary fibrous tumor (SFT), and 12 cases of leiomyoma (LM) were retrieved from archives of the Oral Pathology Consultants at The Ohio State University College of Dentistry between the years of 1998 and 2017. SATB2 immunohistochemical probe was used to analyze all samples. Staining was initially evaluated by manual scoring followed by image software analysis. SATB2 expression was also examined in twentyve additional cases from the University of Texas Southwestern Medical Center (UTSMC) representing both benign and malignant myofibroblastic/fibroblastic entities, as well as their histologic mimics.

Results: Moderate to strong SATB2 nuclear expression was seen at least focally in 13/14 MF, 4/4 IMT, 4/5 MFP, 6/12 LM, and 3/12 NF. Weak, focal positivity was noted in 1/14 MF and 3/12 NF. Lack of SATB2 expression was observed in 1/14 MF, 6/12 LM, 6/12 NF, and 8/9 SFT. The UTSMC cases showed positivity for SATB2 in 3/3 MF, 5/5 NF, 1/2 IMT, 2/3 radiation fibroblastic proliferations, 1/2 low-grade fibromyxoid sarcomas, 2/3 low-grade myofibroblastic sarcomas, 1/2 biphenotypic sinonasal sarcomas, 0/2 leiomyosarcomas, and 0/3 spindle cell squamous carcinomas.

Conclusions: Although originally described as a marker of osteoblastic differentiation, SATB2 appears to be expressed with high sensitivity in myofibroma and other myofibroblastic proliferations. Given its lack of specificity across a broad range of both benign and malignant spindle cell lesions, the use and interpretation of SATB2 for diagnostic purposes may warrant caution.

Monday, June 10 - 9:48 am

T CELL LYMPHOMA OF THE ORAL CAVITY: CASE SERIES AND REVIEW OF THE LITERATURE

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Introduction: T cell lymphomas (TCL) are rare neoplasms within the oral cavity. We present a series of intraoral TCLs and describe their clinical presentations, histologic and immunohistochemical features.

Materials and Method:

Our archives were searched from 2007-2019 yielding 22 cases diagnosed as TCL. Immunohistochemical analysis was performed on all cases. 11 cases underwent PCR analysis for T-cell receptor gene rearrangements.

Results: The 22 cases were noted in 20 patients, 2 patients had 2 lesions. Clinical presentations varied and included leukoplakias, ulcerations, and masses. One patient was HIV +, one had a history of TCL of the skin, another had a history of liposarcoma. A male predilection and a mean age of 61.9 years were identified. The tongue was the most common site. The histologic findings varied, however, an infiltrate of histiocytes, eosinophils and occasionally neutrophils was frequently admixed with the atypical T lymphocytes.

IHC analysis revealed that CD 8 expression was noted in the majority of cases, as was CD4 and CD3. Down regulation or loss of CD7 was common. CD30 positivity of large atypical cells was often seen.

PCR analysis for T-cell receptor gene rearrangement was performed on 11 cases. 7 demonstrated T cell gene rear- rangement.

Conclusion: Oral TCL are rare and can show a wide range of clinical presentations. TCL should be included in the differential diagnosis particularly when a paucity of B lymphocytes is identified in an atypical and mixed inflammatory infiltrate.

An immunohistochemical profile demonstrating loss of CD 7 expression in conjunction with CD8, CD4 and CD 3 positivity with or without CD 30+ cells is helpful. Immunohistochemistry should be used to confirm a diagnosis of TCL. T-cell receptor gene rearrangement can be helpful but not definitive in establishing the diagnosis. The results must be interpreted in the setting of appropriate clinical, histologic and immunologic findings.

Monday, June 10 - 10:00 am

A RETROSPECTIVE STUDY OF BUCCAL MUCOSAL SALIVARY NEOPLASMS

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Introduction: Salivary gland neoplasms of the buccal mucosa are relatively rare and often present with an unusual profile when compared with the same neoplasms seen in more common locations such as the hard palate and upper lip. We present a series of minor salivary gland neoplasms of the buccal mucosa and discuss demographics, clinical presentation, and histologic findings. **Materials and Methods:** With IRB approval, the archives of the University of Florida Oral Pathology Biopsy Service was retrospectively searched from 1994-2018 and all benign and malignant salivary gland neoplasms of the buccal mucosa were identified. Age, gender, clinical presentation, diagnosis, and category of neoplasm were recorded. **Results:** 68 cases were included. Most patients were female (71.6%). Patient age at presentation ranged from 11 to 93 years with a mean of 62.8 years. Clinical impression in descending order of frequency included: mucocele, papilloma, salivary tumor, sialolith, lipoma, fibroma, and sebaceous cyst. Benign neoplasms represented 57.4% of overall cases, while malignant lesions comprised 42.6%. Mucoepidermoid carcinoma was the most common neoplasm (26/68, 38.2%), followed by monomorphic adenoma) (18/68, 26.5 %), ductal papilloma (10/68, 14.7%), cystadenoma (9/68, 13.2%), pleomorphic adenoma (2/68, 2.9%), and 1(1.5%) each for mammary analogue secretory carcinoma, adenoid cystic carcinoma and adenocarcinoma NOS. **Conclusion:** Our patient demographics and percentage of benign and malignant buccal mucosal salivary gland neoplasms aligned with previously published studies. However, benign neoplasms occurring in the buccal mucosa were more diverse than those found in other minor salivary gland locations. In addition, lesions in this location are less likely to be recognized as possible salivary gland neoplasms. Both benign and malignant salivary gland neoplasms should be included in the differential diagnosis of submucosal buccal masses. Additional large studies with more detailed treatment and outcome data would assist in further understanding the behavior of these neoplasms.

Monday, June 10 - 10:12 am:

CAMTA 1 AND TFE 3 CONfiRMATION OF ORAL CAVITY EPITHELIOID HEMANGIOENDOTHELIOMA

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Introduction: Epithelioid hemangioendothelioma (EHE) is an unusual vascular neoplasm of indeterminate biologic behavior, classified as intermediate between benign and malignant. It may microscopically mimic other vascular and spindle cell lesions, and definitive diagnosis is paramount owing to its potential for local recurrence and infiltrative nature. Recently, WWTR1-CAMTA1 (CAMTA1) fusion gene has been described in EHE allowing for a more definitive diagnosis. Moreover, a subset of cases have also been found to be positive for YAP1-TFE3 translocation (TFE3) which may be associated with more aggressive behavior. The purpose of this study is to evaluate archived oral cavity cases of EHE for immunohistochemical expression of CAMTA1 and TFE3, and to confirm the diagnosis and evaluate utility of these markers in diagnosing oral EHE. Materials and methods: With IRB approval cases diagnosed as EHE were retrieved from the archives of the oral pathology biopsy services at University of Florida and Kentucky Colleges of Dentistry from 1994-2018. The slides were reviewed in order to assure diagnostic agreement, and case demographic and diagnostic information was aggregated. All included cases were submitted for immuno- histochemical (IHC) testing for both CAMTA1 and TFE3 antibodies. Results: A total of 6 cases were included. The mean age was 35.8 years (range 14-69). Three of 6 cases were found to affect the gingiva. Other affected sites were mandible (2) and buccal mucosa (1). All cases had previous IHC demonstrating positive vascular markers. CAMTA1 expression in all the six cases exhibited diffuse positive nuclear staining. However, positive TFE3 expression was found in only one case. Conclusion: CAMTA1 appears to be of diagnostic value in confirmation of diagnosis of oral EHE. However, expression of TFE3 in oral EHE appears to be rare, and since TFE3 positivity may affect prognosis, additional studies are warranted.

Monday, June 10 - 10:24 am

GINGIVAL LEUKOPLAKIA: PREVALENCE, HISTOLOGIC FEATURES AND COMPARISON WITH OTHER KERATINIZED TISSUE SITES

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Introduction: The gingiva is the third most common location for leukoplakia, after the tongue and the buccal mucosa, but with a high recurrence rate after treatment. The aim of this study is to review the histopathology of keratotic lesions on the gingiva and determine the proportion of reactive versus nonreactive keratoses.

Materials and methods: Cases that were submitted with a clinical diagnosis of hyperkeratosis or leukoplakia located on the gingiva or alveolar mucosa from January 2018 to December 2018 were evaluated, demographic and clinical data were abstracted, and histopathology reviewed. Cases of lichen planus were excluded.

Results: There were 110 biopsies from 99 patients with 67 males (61%); 5 patients had proliferative leukoplakia (PL). The median age was 66 years (range 30-88). The most common location was the gingiva (43%) followed by the hard palatal mucosa (24%), retromolar pad (16%) and alveolar ridge mucosa (15%). Reactive keratoses constituted 58 (53%) cases. Of these, 66% were nonspecific and 34% were benign alveolar ridge keratosis. Non-reactive keratoses constituted 47% of cases. Of these, 33% were epithelial dysplasia (ED), 10% were atypical verrucous hyperplasia (AVH), and 56% were keratosis of uncertain significance (KUS), without obvious dysplasia. The highest prevalence of non-reactive keratoses occurred on the gingiva (60%) followed by hard palatal mucosa (24%), alveolar ridge mucosa (10%), and retromolar pad (6%); 80% of VH cases occurred on the gingiva. The 5 patients with PL had a total of 11 biopsies, of which 80% were from the gingiva. The most common diagnosis was KUS (64%), and 36% were ED and 36% had lichenoid features.

Conclusions: Reactive and nonreactive keratoses constituted 33% and 67% respectively of keratotic lesions on the gingiva. Of the nonreactive keratoses, 47% were ED or AVH, while the remaining were KUS.

Monday, June 10 - 10:36 am

PROGRAMMED CELL DEATH LIGAND 1 IN MORPHOLOGICALLY NORMAL EPITHELIAL MARGINS OF HEAD AND NECK SQUAMOUS CELL CARCINOMA RESECTION TUMOR TISSUE

Dr. Manar Elnaggar (Department of Oral Oncology and Diagnostic Sciences at University of Maryland Baltimore and Oral pathology Department, Faculty of Dentistry, Alexandria University, Egypt), Prof. Risa Chaisuparat (Chulalongkorn University), Dr. Ioana Ghita (University of Maryland Baltimore), Prof. Joshua Lubek (University of Maryland Baltimore), Prof. Rania Younis (University of Maryland Baltimore)

INTRODUCTION: Programmed cell death ligand 1 (PD-L1) is an immune-checkpoint regulator. Expression of PD- L1 in a subset of the head and neck squamous cell carcinoma (HNSCC) tumor islands has been shown to have an increasing survival benefit to patients undergoing immunotherapy. The objective of this study was to investigate the potential role of PD-L1 as a biomarker for tumor progression in HNSCC.

MATERIALS AND METHODS: We investigated the expression of PD-L1 in the morphologically normal epithelial margins (MNEM) of HNSCC resection tumor tissue. Thirty three HNSCC cases were obtained prospectively under institutional review board approval. PD-L1 [clone 28-8] immunohistochemical staining was carried out and analysed both subjectively and digitally using aperio image scope. Membranous PD-L1 expression was considered positive. RESULTS: Twenty seven cases proved to have MNEM (0.5 cm to 1.5 cm margin). All cases were of the oral and mobile tongue. Two cases were form the base of the tongue and one case was of tonsillar origin. One case was HPV-associated. Our data showed that the MNEM were negative for PD-L1 in 12 out of the 27 cases (44%). 11 (40%) showed PD-L1 positivity. The MNEM of the base of the tongue lesions showed basal and parabasal PD-L1 positivity. MNEM of the dorsum tongue showed PD-L1 focal positivity especially where foci of inflammation were observed in the stroma. PD-L1 expression in only prickle layers as it approached the tumor was observed in 2 cases. 4 cases showed transition from MNEM to dysplasia to malignancy, the PD-L1 expression was remarkably increased in dysplastic areas.

CONCLUSIONS: To our knowledge, this is the first study to investigate the expression of PD-L1 in MNEM of HNSCC resection specimens. We conclude that PD-L1 expression can vary according to the location of the MNEM, with increased expression in dorsum and base of tongue.

Monday, June 10 - 10:48 am

ACTIVATION OF MULTIPLE MYD88-DEPENDENT TLR SIGNALING PATHWAYS MEDIATES LOCAL AND SYSTEMIC INflAMMATION IN A MOUSE MODEL OF PRIMARY SJÖGREN'S SYNDROME

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Introduction: Primary Sjögren's syndrome (pSS) is an autoimmune disease characterized by exocrine gland dysfunction and immune hyperactivity. The adaptor Myd88 is critical for immune function, as most TLRs utilize Myd88 for signal transduction. Previous work by our group found Myd88 is required for pSS disease. Our objective was to identify the specific Myd88-dependent TLR pathways that mediate salivary and systemic inflammation in pSS. **Materials and Methods:** We used the pSS mouse model NOD.B10Sn-$H2^b$/J (NOD.B10), *Myd88*-deficient NOD.B10 mice (NOD.B10$^{Myd88-/-}$), and *Myd88*-deficient or sufficient C57BL/10 controls. We isolated spleens from NOD.B10 mice and age and gender-matched C57BL/10 controls and performed RNA-sequencing. We then harvested salivary tissue and spleens from NOD.B10, NOD.B10$^{Myd88-/-}$, C57BL/10, and C57BL/10$^{Myd88-/-}$mice. We performed quantitative PCR and flow cytometry to assess expression of Myd88-dependent TLRs in B cells derived from splenic tissue and salivary glands. Next, we cultured splenocytes with TLR2 and TLR4 agonists, harvested the supernatants, and performed IL-6 ELISAs to determine whether cells derived from NOD.B10 animals were hyper-responsive to TLR ligation. Finally, we cultured salivary tissue from NOD.B10 and NOD.B10$^{Myd88-/-}$ mice and performed cytokine multiplex arrays on the supernatants. **Results:** We identified dysregulation of numerous TLR-related networks in pSS splenocytes, particularly those employed by TLR2 and TLR4. We found altered expression of TLR1, TLR2, TLR6 and TLR4 in both the spleens and salivary tissue from NOD.B10 mice. Moreover, splenocytes from NOD.B10 females with clinical disease were hyper-responsive to TLR2 ligation as compared to C57BL/10 controls. Finally, salivary tissue from *Myd88*-sufficient NOD.B10 females exhibited spontaneous inflammatory cytokine secretion and this was diminished in that derived from NOD.B10$^{Myd88-/-}$animals. **Conclusion**: Our data demonstrate that Myd88-dependent pathways contribute to the inflammatory landscape in pSS, and inhibition of such will likely have therapeutic utility.

Monday, June 10 - 11:00 am

IMMUNE CHECKPOINT INHIBITOR SICCA (ICIS): A NEW, POTENTIALLY SEVERE IMMUNE RELATED ADVERSE EVENT (IRAE).

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Introduction Immune checkpoint inhibitors (ICI), biologic agents that augment the immune system to establish tumor immunity, are breakthrough cancer therapeutics. However, adverse effects from an augmented immune response, termed "immune-related adverse events" (irAEs), have been reported in up to 60% of patients. We published the first comprehensive description of ICI-induced sicca (ICIS) however the mechanism remains unknown. Presently, we sought to gain mechanistic insight into ICIS pathogenesis focusing on immune dysregulation in the salivary complex. Methods: Patients (N=26) with ICIS and healthy volunteers (N=9) underwent comprehensive evaluations including salivary assessments (sialometry, ultrasonography) and minor salivary gland (MSG) biopsies. MSG were used for histopathology, immunohistochemistry, RNA sequencing, and ex vivo functional and immunological assays.
Results: 'Dry mouth' was reported by all subjects; 97% had objective salivary hypofunction. Microscopically, MSG demonstrated mild-to-severe chronic sialadenitis with fibrosis and atrophy; a third of cases exhibited lymphocytic aggregates (focus score ≥1) and three exhibited severe sialadenitis (focus score >8). Immunohistochemical immunophenotyping exhibited a nearly exclusive $CD3^+$T-lymphocytic infiltrate with a predominance of $CD4^+$cells and a paucity of B cells. T cell infiltrates were PD-1^+; epithelial PD-$L1^+$was present in severe sialadenitis cases. Ex vivo MSG functional studies demonstrated deficits in agonist-stimulated fluid secretion and impaired calcium release and influx. Flow cytometry on MSG exhibited increased cytotoxicity of infiltrating T lymphocytes. MSG RNAseq confirmed profound immune dysregulation in a subset of ICIS patients with severe sialadenitis with enrichment of immunoregulatory interactions between lymphoid and non−lymphoid cells, interferon (gamma and alpha/beta), the PD-1 pathways.
Conclusions: ICI therapies can elicit severe and long-lasting effects on salivary secretion. We illustrate that the immune activation and resultant salivary gland hypofunction is distinct from other immune-related conditions affecting the salivary complex (e.g., Sjögrens syndrome). These data provide insight into targetable pathways for prevention and treatment of this potentially severe irAE.

Monday, June 10 - 11:12 am

NANOPHOTONICS FOR IN VIVO TARGETING AND DETECTING ORAL CANCER

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Introduction: Nanophotonics has emerged as a revolutionized way in the field of medicine to detect and treat cancer. We present a novel non-invasive cancer-detection technique that utilizes the unique absorption properties of gold-nanorods (GNRs) in the near-infrared region. The method is based on diffusion reflection (DR) measurement of gold-nanorods bio-conjugated (C-gold-nanorods) to anti-epidermal growth factor receptor (EGFR) monoclonal antibodies exclusively attached to OSCC cells. The ability to specifically deliver systemically and target high concentration of GNRs exclusively to the tumor, significantly change its optical properties, enabling the discrimination between cancerous and non-cancerous tissues.

Objective: To investigate the targeting potential of systemically injected C-gold-nanorods to OSCC cells in a rat model of oral carcinogenesis and to develop a methodology for in-vivo detection of oral cancer by using DR optical method.

Methods: DR measurements of C-gold-nanorods injected systemically were recorded from the surface of the rat tongue where OSCC has been induced by the carcinogen 4-nitroquinoline-N-oxide (4NQO). 26 Wistar-derived male rats were used, divided into experimental (20 rats) and control (6 rats) groups. C-gold-nanorods were injected systemically to the tail vein. DR measurements were taken from the surface mucosa of the tongue following washout time of 96 hours. The results of the DR measurements were compared with the histologic diagnosis.

Results: OSCC was detected in the posterior dorsum of the tongue in all experimental rats after week 22. Following systemical injection of C-gold-nanorods, significant high DR values were recorded in all rats in the area corresponding to carcinoma compare with the unaffected tip of the tongue and with the control healthy rats.

Conclusion: the present study clearly demonstrates the specific property of systemically injected C-gold-nanorods to target cancer cells in the oral cavity and the detection sensitivity using Nanophotonic optical method.

Monday, June 10 - 11:24 am

BIALLELIC PTCH1 INACTIVATION IS A DOMINANT GENOMIC CHANGE IN SPORADIC KERATOCYSTIC ODONTOGENIC TUMORS

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Introduction: Keratocystic odontogenic tumors (KCOTs) are locally aggressive odontogenic neoplasms with recurrence rates up to 60%. Less than 10% of KCOTs are associated with nevoid basal cell carcinoma (Gorlin) syndrome and up to 85% of these show *PTCH1* inactivation. Sporadic KCOTs show *PTCH1* mutations in ~30% of cases but previous studies have been limited by low DNA yield. The absence of consistent genomic alterations prompted reclassification to odontogenic keratocyst in 2017 by the WHO. The aim of this study was to analyze sporadic KCOT for recurrent genomic aberrations, specifically those impacting the SHH signaling pathway.

Materials and Methods: 44 KCOTs diagnosed between 2013-2018 and containing at least 30% neoplastic cells were retrieved from institutional archives. DNA extracted from FFPE tissue was subjected to targeted next-generation sequencing (NGS) interrogating the exonic sequences of 447 cancer-associated genes for mutations and copy number variations, and 191 introns across 60 genes for gene rearrangements.

Results: Sporadic KCOTs occurred in 23 female and 21 male patients with a median age of 50 (range, 10-82) years. 33 cases were located in the mandible, 11 in the maxilla. NGS identified loss of function *PTCH1* mutations in 41/44 (93%) cases; 21 cases harbored 2 concurrent *PTCH1* mutations and 14 cases showed 9q copy neutral loss of heterozygosity involving the *PTCH1* locus, for a total of 35 (80%) cases with evidence for *PTCH1* biallelic inactivation. 1 case showed a pathogenic *SMO* missense mutation and 1 case showed *GLI1/2* missense mutations; no mutations were detected in *SUFU*.

Conclusion: We identify inactivating *PTCH1* mutations in 93% of sporadic KCOTs, indicating that SHH pathway alterations are a near-universal event in these benign but locally aggressive neoplasms. The high frequency of biallelic *PTCH1* loss of function may provide a rational target for SHH pathway inhibitors to be explored in future studies.

Monday, June 10 - 11:36 am

DMBT1 SUPPRESSES TUMOR PROGRESSION IN HEAD AND NECK CANCER.

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Introduction: Invasion is a critical phenotype in progression of head and neck squamous cell carcinoma (HNC). Deleted in malignant brain tumors 1 (DMBT1) is a tumor suppressor that is downregulated in brain and lung cancers. In previous in vitro studies, we showed that DMBT1 expression is suppressed in HNC and it inhibits invasion in vitro. **Goals**: We investigated the in vivo effects of modulation of DMBT1 on tumor progression in HNC and the mechanistic basis of its effects. **Materials and Methods**: Clinical outcome of low DMBT1 was investigated in a HNC dataset. HNC cell lines with stable overexpression and downregulation of DMBT1 were generated for gain-of-function and loss- of-function studies. The impact of DMBT1 expression on tumor growth and invasion were investigated in vivo using two different models. **Results**: Low DMBT1 is correlated with reduced metastasis-free survival of patients. Overexpression of DMBT1 reduced tumor growth in a murine model of HNC. The histopathologic features were less aggressive when DMBT1 was overexpressed whereas control tumors with low DMBT1 exhibited an aggressive, invasive phenotype. In the chick chorioallantoic membrane in vivo model of HNC, overexpression of DMBT1 in HNC cells suppressed invasion and metastasis, and downregulation of DMBT1 had the reverse effect. Suppression of DMBT1 leads to a mesenchymal phenotype. **Conclusion**: Elucidation of the mechanism of suppression of DMBT1 in HNC could provide a treatment target to suppress invasion in HNC. (This work was supported by NIH/NIDCR grant DE027551).

Monday, June 10 - 11:48 am

VALIDATION OF AN ORAL CANCER AND PAIN MOUSE MODEL

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Introduction: Oral cancer patients report severe function related pain. Not all oral cancers are painful. Patients with oral dysplasia do not experience pain. We use the 4-nitroquinoline-1-oxide (4NQO) rodent carcinogenesis model that recapitulates oral cancer progression to study nociception (the animal equivalent of pain). Carcinogen treated animals develop multiple tongue lesions, including field changes, dysplasias, papillomas and invasive cancers. Cancers are present in 30-40% of animals. Dysplasia, without cancer is seen in the remaining mice. The 4NQO-treated animals display quantifiable nociceptive behavior. We are examining the association of histopathology with nociceptive behavior.

Methods: Mice (C57BL/6 female mice, n=40) were offered 4NQO (100μg/ml) in the drinking water for 16 weeks. Nociceptive behavior is being measured with the dolognawmeter device. The assay measures time to escape from confinement in a tube by gnawing through a dowel blocking exit from the tube (gnaw time). At 28 weeks after initial exposure to 4NQO, tongues and lymph nodes will be harvested for histologic review. The nociception score (average percent change in gnaw time above baseline) will be compared with histopathology. Analysis of variance (ANOVA) will be used to test for differences between groups (nociception score, pathologic lesion type).

Results: Mice have been trained on the device for 21 weeks. All experimental animals display stable gnaw times. Behavior testing continues twice weekly. In a pilot study of eight mice (invasive squamous cell carcinoma (SCC), n=4; microinvasive SCC (n=2); papillomas only, n=2), nociception scores ranged from -65 to 259% of baseline.

Conclusions: Pilot data indicate that mice with oral lesions display a range of nociception scores. The larger on- going study will determine whether the 4NQO model recapitulates variation in pain experienced by oral cancer patients. A validated oral cancer pain mouse model has potential for evaluation of therapies and improved understanding of oral-cancer pain.

Monday, June 10 - 12:00 pm

POTENTIAL UTILITY OF HISTOLOGY-GUIDED MASS SPECTROMETRY FOR THE DIAGNOSIS OF PROLIFERATIVE VERRUCOUS LEUKOPLAKIA

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Proliferative verrucous leukoplakia (PVL) is a relentless, slowly-progressive oral mucosal potentially malignant dis- order with a very high rate of malignant transformation. Timely diagnosis is difficult in part because early lesions are clinically and pathologically similar to other white or white/red lesions. The **objective** of this pilot study was to determine if MALDI mass spectrometry could identify a proteomic profile in early-intermediate stages of PVL that separates it from non-PVL hyperkeratosis and lichen planus (OLP). **Methods and Results:** We performed histology- guided mass spectrometry (HGMS) profiling on 149 samples derived from early (24) and intermediate PVL (25), hyperkeratosis with minimal to no atypia (25), OLP (25), normal epithelium (24), and squamous cell carcinoma (SCC, 26). Representative areas of the epithelium and connective tissue were evaluated using a Bruker MALDI-TOF mass spectrometer, followed by statistical analysis and classification algorithm generation using SCiLS Lab software. We show that early-intermediate PVL can be differentiated from non-PVL hyperkeratosis and OLP with an accuracy of 92% when performing a leave-10%-out repeated subsampling internal cross validation. A total of 508 peaks were used to build the model, of which, 369 peaks were found to be statistically significant between the two groups after applying a Bonferroni correction (p-value $<9.84 \times 10^{-5}$). A total of 16/508 peaks corresponding to 10 unique peptides had areas under Receiver Operating Characteristic (ROC) curves greater than 0.8 indicating these peptides were significantly more abundant in PVL. Five of these peptides were also more highly expressed in PVL than in SCC. Comparisons of PVL and normal epithelium resulted in a total of 152/508 peaks with areas under ROC curves >0.8 indicating significantly higher expression in PVL. **Conclusion:** HGMS technology may be a powerful tool for differentiating PVL from selected non-PVL lesions. Validation studies are underway.

Acknowledgements: We thank histotechnologist Regina Hand for the preparation of tissue sections.

Monday, June 10 - 12:12 pm

ENDOGENOUS EXPRESSION OF DNA CYTOSINE DEAMINASE APOBEC3B IS UPREGULATED IN ORAL MUCOSAL MELANOMA

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Introduction: Oral mucosal melanoma (OMM) exhibits aggressive behavior, dismal prognosis (5-year survival rate 10-25%) and is characterized by elevated mutation loads. The molecular mechanisms responsible for the high genomic instability observed in OMM remain elusive. DNA cytosine deaminase APOBEC3B (A3B) is a major endogenous source of mutation in a wide variety of cancers. A3B-driven signature mutations in tumors are identified through C-to-T and C-to-G base substitutions in 5'-TCA/T trinucleotide motifs. We *hypothesized* that A3B is overexpressed in OMM inflicting a heightened state of somatic mutations.

Materials and Methods: A3B levels were assessed in OMM (N=10) by immunohistochemistry using a custom rabbit monoclonal antibody (5210-87-13) developed by our group. Benign oral melanocytic nevi (N=13) served as a control group. Nuclear A3B immunoreactivity in all lesions was visualized with the Aperio ScanScope XT and quantified using the Aperio Nuclear Algorithm. Non-parametric Mann-Whitney U test was utilized for statistical analysis. Additionally, published molecular data sets focusing on whole genome landscapes of major melanoma subtypes were further analyzed to assess the impact of A3B in mucosal melanomas.

Results: Among OMMs, 6 cases involved the palate and 1 case each the maxillary gingiva, floor of the mouth and upper lip (mean age: 67.4 years, range 54-84, M:F ratio=5:4). One case represented locoregional recurrence 2 years after initial diagnosis. Strong, focal to diffuse, nuclear A3B immunopositivity was observed in 9/10 OMMs (median H-Score=38.9), whereas oral nevi were mostly A3B negative (median H-Score=5.6). Overall, A3B protein levels were significantly higher in OMMs ($p<0.0001$). Genomic analysis showed that the overall mutation load, number of C-to- T transitions and proportion of A3B-related mutation signatures 2 and 13 were higher in head and neck mucosal melanomas than melanomas developing in other mucosal sites.

Conclusions: These preliminary immunohistochemical and genomic data implicate the mutagenic factor A3B in the pathogenesis of OMM.