Tunicamycin-induced endoplasmic reticulum stress up-regulates tumour-promoting cytokines in oral squamous cell carcinoma

Tuesday, 26th June @ 15:30: (Stanley Park Ballroom – Salon 1) - Oral - Abstract ID: 268

Dr. Muhammed Yakin (Charles Sturt University), Dr. Benedict Seo (University of Otago), Prof. Alison Rich (University of Otago)

Objectives

Signal transducer and activator of transcription (STAT)-3 lies at the convergence point of key pathways involved in many malignancies including oral squamous cell carcinoma (OSCC). Endoplasmic reticulum stress (ERS) and the unfolded protein response promote either survival or apoptosis in different cancers. We investigated the expression of STAT3 pathway-related genes and proteins under ERS in OSCC.

Three normal oral keratinocyte (NOK) and three OSCC cell lines were subjected to tunicamycin to induce ERS for 24 hours or to the vehicle medium as control. A pathway-focussed array was used to analyse the modulation of STAT3 pathway gene expression under ERS using qPCR. The expression of key regulated proteins was investigated in the cell lines using immunocytochemistry and in 76 OSCC and 9 normal oral mucosa (NOM) tissue samples using tissue microarray technology and immunohistochemistry.

Findings

ERS resulted in up-regulation of interleukin-6 receptor (IL6R) gene in NOK cell lines ($p = 0.001$) and IL5 ($p = 0.005$) and IL22 ($p = 0.024$) in OSCC cell lines. Greater STAT3 ($p = 0.019$) and leukaemia inhibitory factor receptor ($p = 0.042$) protein expression was observed in treated than untreated NOK cell lines.

Conclusions

The gene and protein regulation patterns show that ERS plays a role in modifying the tumour microenvironment in OSCC by up-regulating tumour-promoting cytokines.

Clinico-pathological significance of B-catenin and E-Cadherin expression in salivary gland tumor at UCH Ibadan

Tuesday, 26th June @ 15:42: (Stanley Park Ballroom – Salon 1) - Oral - Abstract ID: 286

Dr. Bamidele Kolude (University College Hospital/University of Ibadan), Dr. Bukola Adeyemi (University College Hospital/University of Ibadan), Dr. Oluwatoyin Lawal (University College Hospital/University of Ibadan), Dr. Akinyele Adisa (University of Ibadan), Dr. Akindayo Akinyamoju (university co), Dr. Olabiyi Ogun (University College Hospital/University of Ibadan)

Objectives: β-catenin (B-Cat) is a cell adhesion molecule associated with the invasion and metastasis of carcinomas of the head and neck, esophagus while reduced expression of E-cadherin (E-cad), a transmembrane glycoprotein, is associated with loss of differentiation, acquisition of an invasive phenotype, and an unfavorable prognosis in carcinomas from several sites. B-Cat & E-Cad complex are involved in cell adhesion, signal transduction & motility. Aim is to identify the clinical / pathological significance of B- Cat & E-Cad expressions in salivary gland tumors (SGTs) presenting at the University College Hospital, Ibadan.

Findings: The expressions of β-cat & E-Cad were analyzed in 46 SGTs (10 pleomorphic adenomas PSA, 3 basal cell adenoma, 12 adenoid cystic carcinoma ADCC, 10 mucoepidermoid carcinoma MEC, 5 acinic cell carcinoma ACC, 4 polymorphous low grade adenocarcinoma PLGA & 2 papillary cystadenocarcinoma PCADCA) by immunohisto-chemistry in formalin-fixed, paraffin embedded specimens (Rabbit monoclonal; Satacruz biotechnology). Result shows immunostaining of B-cat & E-Cad were membranous & cytoplasmic without nuclear involvement, staining was more severe in the ductal areas especially in PSA, there was significant loss of membranous stain in ACC on multivariate analysis. E-Cad staining loss was significantly associated with tumour stage in ACC & MEC.

Conclusion: loss of β-catenin adhesion molecule may be involved in the development of ACC. E-cad expression is an independent indicator of clinical aggressiveness in patients with ACC.& MEC.

KRAS mutations drive adenomatoid odontogenic tumor

Tuesday, 26th June @ 15:54: (Stanley Park Ballroom – Salon 1) - Oral - Abstract ID: 213

Ms. Bruna Coura (Universidade Federal de Minas Gerais), Dr. Silvia Sousa (Universidade Federal de Sergipe), Dr. Vanessa Bernardes (Universidade Federal de Minas Gerais), Dr. Josiane Franca (Universidade Federal de Minas Gerais), Prof. Hélder Antônio Rebelo Pontes (Joao de Barros Barreto University Hospital, Federal University of Pará, Belém), Dr. Danyel Perez (Universidade Federal de Pernambuco), Dr. Ricardo Albuquerque Junior (Universidade Tiradentes), Dr. Manoela Martins (Universidade Federal do Rio Grande do Sul), Prof. Aline Batista (Federal University of Goiás), Dr. Marina Diniz (Universidade Federal de Minas Gerais),

Dr. Carolina Gomes (Universidade Federal de Minas Gerais), Dr. Ricardo Gomez (Universidade Federal de Minas Gerais)

Objective

KRAS is the most frequently mutated oncogene in human neoplasms and we have previously reported KRAS p.G12V mutations in adenomatoid odontogenic tumors (AOT). We aimed to expand this cohort of samples and to test the association of KRAS mutations with clinical and histopathological parameters. A convenience sample of 30 AOT cases was included in the study. The hotpot KRAS p.G12V mutation was assessed by TaqMan allele-specific qPCR and codon 12 was direct sequenced. Clinical information obtained included patients age, tumor site, association of the lesion with impacted teeth and clinical tumor size. In addition, tumor capsule thickness was evaluated by morphometric analysis. Statistical analysis was carried out to test the association of KRAS codon 12 mutations with clinico-pathological parameters.

Findings

Molecular results confirmed KRAS p.G12V mutation in 14/23 cases, and p.G12R in 1/23. Eight cases were wild-type and samples from 7 cases failed amplification. Codon 12 mutations were not associated with any of the clinico- pathological parameters tested ($p>0.05$).

Conclusion

AOT show high frequency of KRAS codon 12 mutations (15/23, 65%), which occur irrespectively of patients' age, tumor location, association with impacted teeth, tumor clinical size or histopathological capsule thickness. Supported by FAPEMIG, CAPES and CNPq/Brazil.

CORRELATION OF HPV16 DETECTION AND P16 EXPRESSION IN ORAL SQUAMOUS CELL CARCINOMA

Tuesday, 26th June @ 16:06: (Stanley Park Ballroom – Salon 1) - Oral - Abstract ID: 253

Dr. Smitha Thammaiah (Rajiv Gandhi University of Health sciences/ Vydehi Institute of Dental sciences)

Objectives: Oral cancer etiology is multifactorial and the main risk factors are tobacco chewing and smoking along with alcohol consumption and viruses. In the current study from a South Indian population, we sought to determine the role of HPV 16 in the pathogenesis, its concordance with p16 over expression in OSCC. Preliminary examination on FFPE embedded OSCC (n = 297) sample was observed by H & E staining, and cases showing cytological changes were selected.

Findings: HPV 16 DNA prevalence were assessed by Conventional PCR method which showed 128 out of 297 samples positive for HPV16 DNA. Further, the frequency distribution of HPV 16 E6/E7 in 128 tissues was evaluated by qPCRwhich showed 97 samples were positive for HPV16 E6 qPCR and 98 samples positive for HPV16 E7 qPCR. For the same 128 samples immunohistochemistry was conducted for p16 evaluation.Out of 128 tissue samples, 19 tissue samples were found to be positive for p16 overexpression (+++). Evaluation of mRNA expression of E6 and E7 in the 19 samples was estimated by flow-cytometry, which revealed only 7 samples positive for the mRNA expression. There was no correlation between p16 expressed (+++) samples and quantification of HPV16 mRNA expressions. From these investigations the role of HPV in the etiology, pathogenesis of OSCC was not established.

Conclusion: This led to the performance of meta-analysis in which studies pertaining to HPV-related OSCC evalu- ated by application of conventional and qPCR, flow cytometry and IHC for the detection of DNA, mRNA and p16 overexpression, respectively were included. The results obtained were indicative that HPV prevalence in the oral cavity is low and unlikely to play a major significant or decisive role in the etiology, pathogenesis of OSCC. Our results were in accordance with the meta analysis results.

Wnt/β-catenin signaling pathway regulates tumor-initiating cells in head and neck squamous cell carcinoma

Tuesday, 26th June @ 16:18: (Stanley Park Ballroom – Salon 1) - Oral - Abstract ID: 321

Dr. Chia-Cheng Li (Harvard School of Dental Medicine), Dr. Cheng-chia Yu (School of Dentistry, Chung Shan Medical University), Dr. Reshma Menon (N/A), Ms. Ingrid Carvo (Harvard School of Dental Medicine), Dr. Zhe Li (Department of Medicine, Division of Genetics, Brigham and Women's Hospital)

Objectives: Head and neck squamous cell carcinoma (HNSCC) is one of the leading cancers, with a 40% 5-year survival rate in advanced cases. HNSCC is notorious for its high recurrence rate and frequent occurrence of syn- chronous/metachronous primary tumors. Tumor initiating cells (TICs) model was proposed to explain its tumor heterogeneity and frequent recurrences. Lineage tracing is a genetic approach that allows identification of TICs in their native habitats and characterization of their in vivo behavior.

Findings: Our re-analysis of TCGA data revealed that high expression of AXIN2, a downstream target of Wnt signaling, was significantly correlated with low survival rate of HNSCC. To characterize the Wnt-responsive cell population, we established a carcinogen-induced model using Axin2-CreER reporter mice. After tamoxifen induction, clonal expansion of fluorescent reporter-positive cells (Wnt-responsive tumor cells) was visualized in the basal cell layer of epithelial dysplasia and HNSCC, suggesting the critical role of Wnt in regulating TICs in early stages of carcinogenesis. β-catenin and LEF1 immunofluorescent staining were performed to confirm Wnt activation. Lineage tracing was also accomplished in 3D organoid culture. The fluorescent reporter-positive cells were capable of forming organoids. Furthermore, application of cisplatin enriched AIXN2 cell population.

Conclusions: Activation of Wnt/β-catenin signaling pathway is an early event in HNSCC carcinogenesis. Lineage tracing using the Axin2-CreER reporter may link TICs properties with a fundamental signaling pathway in normal development. Further research is required to clarify the role of Wnt-responsive TICs in recurrence and therapy resistance of HNSCC.

Adenoid ameloblastoma: A series of 5 tumors

Tuesday, 26th June @ 16:30: (Stanley Park Ballroom – Salon 1) - Oral - Abstract ID: 58

Dr. Elizabeth Ann Bilodeau (University of Pittsburgh), Dr. Raja Seethala (University of Pittsburgh)

Background

First described in 1959 by Waldron and more fully characterized by Loyola et. al. in 2014, adenoid ameloblastoma (with dentinoid) is a rare odontogenic tumor variant with less than 20 reported cases in the literature.

Objectives

Adenoid ameloblastomas were identified after review of ameloblastomas from the University of Pittsburgh Medical Center Department of Pathology archives from 1990 to 2018.

Findings

A review of our archives yielded 6 cases of adenoid ameloblastoma in 5 patients. Of these, 2 tumors were obtained from the "in-house" pathology files with one tumor being a recurrent adenoid ameloblastoma, whereas the remain- ing were consultations. The 4 cases (in 3 patients) received in consultation had a differential diagnosis of a salivary neoplasm. All cases were in the maxilla (5/5, 100%), 60% were in males, with a mean age of 52.6 years (range 29- 65), and mean size of 5.8 cm (range 4.0 to 7.8 cm). Increased mitotic activity was present in all cases, (mean 7 mitoses/10hpf, range 3-11 mitoses/10hpf). All tumors exhibited pseudoglandular spaces. Other common histologic features included whorls or morules, clear cells, ghost cells, and matrix production with hard tissue formation. Immunohistochemically, the tumors were positive for p63 (3/3, 100%), beta catenin within the morules (2/3, 66.6%), AE1/3 (1/1, 100%), CK5/6 (1/1, 100%), vimentin (1/1, 100%), CEA (2/2, 100%), p40 (1/1, 100%) and negative for BRAF V600E (0/3, 0%), calretinin (0/2, 0%), CK7 (0/1, 0%), SMA (0/4, 0%), S100 (0/3, 0%), Sox-10 (0/1, 0%), TTF-1(0/1, 0%) , Pax-8 (0/1, 0%), p16 (0/1, 0%).

Conclusions

Adenoid ameloblastoma (with dentinoid) is a rare, aggressive odontogenic tumor variant with morphologic overlap with salivary neoplasms. Given the histologic similarities to other tumors and the high rate of recurrence, further characterization of this entity is needed

Primary Intraosseous Soft Tissue Myoepithelioma of the Mandible: Case Report and Literature Review

Tuesday, 26th June @ 16:42: (Stanley Park Ballroom – Salon 1) - Oral - Abstract ID: 247

Dr. Lama Alabdulaaly (Harvard School of Dental Medicine), Dr. Sook Bin Woo (Harvard School of Dental Medicine)

Introduction: Soft tissue myoepithelioma (STM) is a benign tumor composed of spindled and epithelioid cells arranged in various patterns within chondromyxoid stroma. While salivary gland myoepithelioma has been well-recognized as a variant of pleomorphic adenoma, the absence of normal myoepithelial cells within STM may be why STM was only recently-recognized.. The occurrence of STM within the bone is rare with only 16 cases reported in the literature, none of which have been reported in the mandible.

Case Report: A 14-year-old female presented with a lobulated gingival nodule measuring 1.2 x 0.8 cm between teeth #20 and 21. Radiographically, the lesion was a well-circumscribed, non-corticated multilocular radiolucency between the roots of #20 and #21 measuring 1.4 x 1.0. cm, extending from the alveolar crest to close to the root apices. An incisional biopsy was performed. Microscopically, the lesion consisted of a non-encapsulated, multilobular tu- mor composed of a proliferation of spindle and epithelioid cells within a delicate myxochondroid stroma. Tumor cells were positive for S-100, vimentin, neuron-specific enolase (NSE) and epithelial membrane antigen (EMA) and negative for CAM5.2, AE1/3, SMA, SOX-10, CD57, glial fibrillary acidic protein (GFAP), and p63. Ki-67 labeled less than 5% of the cells. These findings are suggestive of STM, and the bone and soft tissue consultant pathologist concurred with this diagnosis.

Discussion: The putative cell of origin for this tumor is a stem cell within the soft tissues that differentiates to- wards a cell with myoepithelial phenotype. Unlike salivary gland myoepithelioma which harbors *PLAG1* and *HMGA2*rearrangements, STM harbors the *EWSR1* rearrangement in up to 44% of the cases. Ectomesenchymal chon- dromyxoid tumor may represent the same entity since it has similar histomorphology and immunohistochemical profile but exhibits *EWSR1* rearrangement in only 25% of cases. To our knowledge, this is the first report of a STM occurring in the mandible.

Prognostic classifier for Oral Potentially Malignant Disorders: An integrated histopathological and molecular approach.

Tuesday, 26th June @ 16:54: (Stanley Park Ballroom – Salon 1) - Oral - Abstract ID: 35

Dr. Hans Prakash SATHASIVAM (Newcastle University), Prof. Philip Sloan (Newcastle University), Dr. Ralf Kist (Newcastle University), Dr. Max Robinson (Newcastle University)

Objectives: Oral squamous cell carcinoma (OSCC) is associated with a high degree of morbidity and mortality. OSCCs are often preceded by oral potentially malignant disorders (OPMD) which have a higher propensity to undergo malignant transformation (MT) compared to clinically normal oral mucosa. Currently there is no reliable method to determine which OPMD cases will undergo MT. This study was performed to construct a prognostic classifier for patients with OPMD by integrating clinical, histopathological and molecular factors and to discover a gene expression signature that characterises OPMD with a high risk of undergoing MT.

Findings: Statistical analysis of an OPMD patient cohort (23 MT vs. 25 with no MT) showed that site of initial OPMD ($p = 0.043$), binary oral epithelial dysplasia (OED) grading ($p = 0.009$) and loss of heterozygosity at 3p/9p/17p ($p = 0.026$) were statistically significant. Other demographic factors, clinical features and the WHO 3-tiered OED grading system were not statistically significant. Gene expression experiments revealed several genes that were differentially expressed between OPMD that underwent MT and those that did not [false discovery rate of < 0.05]. Statistical model building was performed, and the outputs were used to construct a prognostic classifier.

Conclusions: Our findings show that a classifier combining histopathological and molecular factors outperforms conventional methods for prognosticating clinical outcome in patients with OPMD. We have also shown that formalin-fixed paraffin-embedded tissue can be used to generate a molecular classification with clinical utility.

Somatic driver mutations in Oral and Sinonasal Mucosal Melanoma. A Referral Centre Experience in Mexico City

Tuesday, 26th June @ 17:06: (Stanley Park Ballroom – Salon 1) - Oral - Abstract ID: 56

Dr. Jessica Lissete Maldonado Mendoza (Universidad Autónoma Metropolitana Xochimilco), Dr. Velia Ramirez-Amador (Universidad Autónoma Metropolitana Xochimilco), Dr. Gabriela Anaya Saavedra (Universidad Autónoma Metropolitana Xochimilco), Dr. Erika Ruíz García (National Cancer Institute of Mexico), Dr. Héctor Maldonado Martínez (National Cancer Institute of Mexico), Dr. Edith Fernández Figueroa (Computational Genomics, National Institute of Genomic Medicine), Dr. Abelardo Meneses García (National Cancer Institute of Mexico)

Objective. Oral and sinonasal mucosal melanomas (OSNMM) are aggressive tumors with low survival and few therapeutic alternatives. The aim of this study was to describe the prevalence of mutations in $NRAS^{Q61K}$, $BRAF^{V600E}$, $CKIT^{L576P, K642E}$, $MITF^{E318K}$ and $PTEN^{R130;}$and to analyze the clinic-pathological features present.

Findings. Cross-sectional and observational study that included cases with OSNMM from the National Cancer Institute of Mexico City and the Oral Pathology Laboratory of UAM-X (January 2000-December 2016). Demographic and clinical data were obtained, and histopathological diagnosis was confirmed. Genomic DNA was obtained and molecular analysis was carried out through quantitative polymerase chain reactions (qPCR) (Customized *Biomarker somatic mutation Array®, Qiagen*). The statistical analysis was performed using the SPSS v20 software.

Forty-eight cases were included, 56.2% were sinonasal melanomas (SNM) and 43.7% oral melanomas (OM). The median age of the individuals was 60 years (Q_1-Q_3 = 51-74), 54.2% of the cases were men. Higher symptomatology percentages were found among SNM (100% *vs.* 52.9%, p<0.001). At the histopathological analysis, 97% of the tumors showed vertical and infiltrative growth, SNM showed a greater amount of necrosis (68% *vs.* 32%, p= 0.006) in comparison with OM. Eight (16.6%) OSNMM presented at least one mutation: 6/28 (21.4%) SNM cases and 2/20 (10%) OM. From 48 OSNMM, three (6.6%) showed V600E *BRAF*mutation, 3/48 (6.2%) Q61R mutation *NRAS* and 2/48 (4.1%) K624E mutation *KIT*. No mutations were found in *MITF or PTEN*.

Conclusions. A low prevalence of mutations was found. Somatic driver mutations might not be related with OSNMM development; thus, the current biological agents (vemurafenib and imatinib) may probably be ineffective against OSNMM. It is necessary to continue the search of other molecular alterations to suggest therapeutic alternatives for these tumors, such as: proteins amplification (c-KIT) or epigenetic mechanism which might regulate the genetic expression (miRNAs).

Application of Deep Learning Algorithms in Detection of Mitotic Events in Oral Squamous Cell Carcinoma Using Cellphone Images

Tuesday, 26th June @ 17:18: (Stanley Park Ballroom – Salon 1) - Oral - Abstract ID: 203

Dr. Amber Kiyani (Riphah International University), Dr. Hassan Aqeel (National University of Science and Technology), Mr. Asjid Tanveer (National University of Science and Technology), Mr. Wajahat Nawaz (National University of Science and Technology), Dr.

Syed Ali Khurram (University of Sheffield)

Identifying mitoses in tumors and metastatic deposits in lymph nodes can be laborious and time-consuming tasks. Advances in digital pathology and machine learning algorithms have demonstrated promising results by automating these assignments in breast tissue and sentinel lymph node sections. These breakthroughs have made automated histopathological diagnosis a possibility. All prior studies have used high-resolution images from expensive whole slide image (WSI) scanners for training and detection of cellular events. Our aim was to investigate the efficacy of deep learning algorithms for automated detection of mitotic events on low quality images of oral squamous cell carcinoma (OSCC) produced by cellphone cameras.

METHODOLOGY:

A FAST region-based convoluted neural network was trained on WSI from breast cancer. The mitotic events were highlighted through provision of pixel locations to the training algorithm, each patch was approximately 301 x 301 in size. The non-mitosis regions were randomly selected on the images. The final training data set comprised of 4407 image patches. Transfer learning was applied to generate results. Similar algorithms were employed on a data set of comparable size acquired through a cellphone camera from 13 different OSCCs at high-power (40x).

RESULTS:

The WSI demonstrated true positive rates of 0.46 and a false positive of 0.76 with an overall F1 precision of 0.57. The results from cellphone camera showed true positive rates of 0.46, and false positive rates of 0.54. The overall F1 score was 0.49.

CONCLUSION:

Although WSIs outperformed cellphone images in identifying mitoses, enhancing image quality through modified algorithms may improve efficacy. This will facilitate use of low-cost data sets for training future algorithms for automated detection of cellular events, and widen its impact by making it accessible to every pathologist with a cellphone camera.

Stratification of Head and Neck Squamous cell carcinoma using combined analysis of Programmed death ligand 1 and Semaphorin 4D expression by the inflammatory cells in the tumor microenvironment

Tuesday, 26th June @ 17:30: (Stanley Park Ballroom – Salon 1) - Oral - Abstract ID: 30

Dr. Rania Younis (University of Maryland, School of Dentistry), Dr. Sonia Sanadhya (University of Maryland Baltimore), Dr. Ioana Ghita (University of Maryland), Dr. Ingy H. Elkomary (University of Maryland School of Dentistry), Dr. Haiyan Chen (University of Maryland School of Dentistry)

Objective: Inhibition of the immune check point PD-1/PD-L1 has shown unprecedented improvement in overall sur- vival of platinum resistant head and neck squamous cell carcinoma (HNSCC) patients. PD-L1 immunohistochemical diagnostics showed to be more prognostic of the patient response. Yet, patients' response remains limited to 45% out of the PD-L1 positive cases, where PD-L1 can be expressed by the tumor cells or by the tumor associated inflamma- tory cells (TAIs). Semaphorin 4D (Sema4D) is an immune modulator molecule expressed by several inflammatory cells, as well as several tumor cell types including HNSCC. We have recently described a HNSCC stratification model based on combined analysis of PD-L1/ Sema4D IHC expression by the tumor cells. Here we would like to extend our analysis to further stratify HNSCC according to Sema4D/ PD-L1 expression by TAIs in the tumor micro-environment. Findings: IHC analysis of Sema4D/PD-L1 in 136 HNSCC tissue cores showed: 61% (83 cases) to be Sema4D +ve in TAIs, and 29% (39 cases) to be PD-L1 +ve TAIs. Accordingly, we were able to stratify the examined HNSCC cores into 4 subtypes using the expression of Sema4D/PD-L1 by TAIs in the tumor micro-environment: (1) Sema4D only positive (37%) (50 cases), (2) PD-L1 only positive (4%) (6 cases), (3) Sema4D/PD-L1 (+ve/+ve) (24%) (33 cases), and (4) 35% (47 cases) to be (-ve/-ve). Sema4D only +ve TAIs were significantly higher than PD-L1 only +ve TAIs.

Conclusion: HNSCC stratification according to Sema4D/PD-L1 expression by TAIs in the tumor microenviron- ment can open new avenues for personalized targeted therapy and might interpret resistance or cytotoxic effects to PD-1/PD-L1 inhibition in HNSCC.

Adenoid cystic carcinoma with high-grade transformation: a retrospective study focused on clinicopathological features and prognostic value in a single center

Tuesday, 26th June @ 17:42: (Stanley Park Ballroom – Salon 1) - Oral - Abstract ID: 290

Dr. Rong-Hui Xia (Department of Oral Pathology, Ninth People's Hospital , Shanghai Jiao Tong University School of Medicine; Shanghai Key Laboratory of Stomatology), Dr. Chun-ye Zhang (Department of Oral Pathology, Ninth People's Hospital , Shanghai Jiao Tong University School of Medicine; Shanghai Key Laboratory of Stomatology), Dr. Li-zhen Wang (Department of Oral Pathology, Ninth People's Hospital , Shanghai Jiao Tong University School of Medicine; Shanghai Key Laboratory of Stomatology), Dr. Yu-hua Hu (Department of Oral Pathology, Ninth People's Hospital , Shanghai Jiao Tong University School of Medicine; Shanghai Key Laboratory of Stomatology), Dr. Zhen Tian (Department of Oral Pathology, Ninth People's Hospital , Shanghai Jiao Tong University School of Medicine; Shanghai Key Laboratory of Stomatology), Mr. Ting Gu (Department of Oral Pathology, Ninth People's Hospital , Shanghai Jiao Tong University School of Medicine; Shanghai Key Laboratory of Stomatology), Mrs. Lei Li (Department of Oral Pathology, Ninth People's Hospital , Shanghai Jiao Tong University School of Medicine; Shanghai Key Laboratory of Stomatology), Mrs. Ying Zhang (Department of Oral Pathology, Ninth People's Hospital , Shanghai Jiao Tong University School of Medicine; Shanghai Key Laboratory of Stomatology), Mr. Jia-jun Qian (Department of Oral Pathology, Ninth People's Hospital , Shanghai Jiao Tong University School of Medicine; Shanghai Key Laboratory of Stomatology), Prof. Jiang Li (Department of Oral Pathology, Ninth People's Hospital , Shanghai Jiao Tong University School of Medicine; Shanghai Key Laboratory of Stomatology)

Objectives: High-grade transformation of adenoid cystic carcinoma(ACC-HGT) is an extremely rare phenomenon. We reported 18 cases of ACC-HGT and focused on the clinicopathological features and prognostic value in this study. Findings: 202 cases of ACC were included in the current study. According to the criteria for ACC-HGT had been published by Seethala et al., 18 cases were diagnosed as ACC-HGT. Compared to conventional ACC, ACC-HGT showed a slight male predominance (61.1% vs. 49.5%), higher lymph node metastasis (27.8% vs. 8.2%, p=0.021), higher recurrence rate (44.4% vs. 13.6%, p=0.003), higher vascular invasion rate (77.8% vs. 40.2%, p=0.002) and detected at an advanced stage (55.6% vs. 26.1%, p=0.008). Log Rank test was used to evaluated the prognostic value of the ACC- HGT. Patients with ACC-HGT had a much worse overall survival (OS) compared with conventional ACC (p<0.001). More importantly, compared with solid ACC, a subtype which generally accepted showing stronger invasiveness and having worse prognosis, ACC-HGT had even much worse OS (p=0.015).

Conclusions: Compared to conventional ACC, ACC-HGT showed a slight male predominance, higher lymph node metastasis, higher recurrence rate, higher vascular invasion rate and detected at an advanced stage. ACC-HGT had a much worse OS compared with conventional and solid type ACC. Those results suggesting that ACC-HGT is a highly aggressive tumor and should be considered for neck dissection and closer follow-up. Pathological distinction of this tumor has great significance for treating and predicting patients prognosis properly.

Antioxidant rich tropical herbs to combat Areca-Nut induced OPMDs

Tuesday, 26th June @ 15:30: (Stanley Park Ballroom – Salon 2) - Oral - Abstract ID: 211

Dr. Rasika Illeperuma (Department of Medical Laboratory Science, Faculty of Allied Health Sciences, University of Peradeniya, Sri Lanka), Mr. Kasun Bandara (Center for Research in Oral Cancer, Department of Oral Pathology, Faculty of Dental Sciences, University of Peradeniya, Sri Lanka), Dr. DO KYEONG KIM (Oral Cancer Research Institute, Department of Oral Pathology, Yonsei University College of Dentistry, Seoul, Korea), Prof. Samadarani Siriwardena (Center for Research in Oral Cancer, Department of Oral Pathology, Faculty of Dental Sciences, University of Peradeniya, Sri Lanka), Prof. Ruwan Jayasinghe (Center for Research in Oral Cancer, Department of Oral Pathology, Faculty of Dental Sciences, University of Peradeniya, Sri Lanka), Prof. Jin Kim (Oral Cancer Research Institute, Department of Oral Pathology, Yonsei University College of Dentistry, Seoul, Korea), Prof. Wanninayake Tilakaratne (Center for Research in Oral Cancer, Department of Oral Pathology, Faculty of Dental Sciences, University of Peradeniya, Sri Lanka)

Objectives: Areca-Nut (AN) induced Oral Premalignant Diseases (OPMDs) are a health burden in Asian countries which causes higher morbidity and mortality. Oral Submucous Fibrosis (OSMF) and Oral Leukoplakia (OL) are the most vulnerable AN induced OPMDs which have a considerable malignant transformation rate. The underline mechanism of the carcinogenesis in OPMDs is still obscure. It was found that the oxidative stress caused by the AN can induce the carcinogenesis in OPMDs. Based on our previous research, it was found that some of these OPMDs have DNA damage caused by oxidative stress. Tropical countries are rich of herbs with antioxidants. Our attempt was to test few herbs as a remedy to reverse the potential carcinogenesis in AN induced OPMDs by reducing the oxidative stress caused by the AN.

Findings: Expression of Phospho histone H2AX, DNA double-strand breaks (DNA DSBs) marker was tested immuno- histochemically in OPMDs with the history of AN consumption and compared with normal oral mucosa (NOM) and oral squamous cell carcinoma (OSCC). Phospho histone H2AX was significantly increased in OL and OSMF compared to the NOM ($p<0.05$). In-vitro studies using immortalized human oral keratinocytes (IHOK) shown that AN induced reactive oxygen species (ROS) production can be significantly reduced by the ethanol extracts of the antioxidant rich herbs *Shumacheria castaneifolia* leaves (SC-extract) and *Solanum nigrum* linn leaves (SN-extract). Antioxidant properties of the herbs were analyzed by DPPH assay. Furthermore, the amount of Phospho histone H2AX in response to 24hr AN treatment was considerably reduced in pretreated IHOK cell with SC extract. Murine model experiment also revealed that the herbal extracts can reduce the AN induced DNA DSBs in oral mucosa.

Conclusions: This study is evident that blocking ROS generation by herbal extracts as a promising approach to reverse DNA DSBs caused by AN. Especially, to prevent malignant transformation in OPMDs

microRNA-222 and microRNA-203 signatures in oral squamous cell carcinoma: potential role in progression and as therapeutic targets

Tuesday, 26th June @ 15:42: (Stanley Park Ballroom – Salon 2) - Oral - Abstract ID: 323

Dr. Madhura M G (DAPM R V Dental College and Hospital, Bengaluru, Karnataka, India)

Objectives: To discuss the proposed role of microRNA-222 (miR-222) and microRNA-203 (miR-203) in oral squamous cell carcinoma in the progression and as possible therapeutic targets.

Findings: miR-222 is colocalized as a cluster in the short arm of chromosome X. Luciferase reporter gene assays in oral tongue squamous cell carcinoma (OTSCC) have shown that hsa-miR-222 regulates the MMP1 expression through both direct cis-regulatory mechanism (targeting MMP1 mRNA) and indirect trans-regulatory mechanism (indirect controlling of MMP1 gene expression by targeting SOD2). Hence, hsa-miR-222 might serve as a novel therapeutic target for OTSCC patients at risk of metastatic disease.

miR-222 has been shown to regulate TRAIL resistance and enhancement of tumorigenicity through PTEN and TIMP3(Tissue inhibitor of metalloproteinase 3) downregulation.

miR-222 has been implicated to target *PUMA*(p53 up-regulated modulator of apoptosis) to improve sensitization of UM1 cells to Cisplatin.

miR-203 acts as a molecular switch between keratinocyte proliferation and differentiation in adult epidermis by targeting ΔNp63 mRNA. Following DNA damage, ΔNp63 downregulates and a possible activation of the apoptotic program in head and neck squamous cell carcinoma has been thought of.

miR-203 has been shown to target EIF5A2 in colorectal cancer cells. Serving as a tumor suppressor gene, miR-203 has been thought to be a useful potential therapeutic target in colorectal cancer. miR-203 as a therapeutic target in oral squamous cell carcinoma needs further validation.

Conclusion: In tumour progression, several cellular pathways may be affected by a single microRNA since it can target multiple mRNAs. Much more light is to be shed by developing as well as by tracking the identified microRNA signatures in oral squamous cell carcinoma, to pave the way for their future clinical use in the diagnosis, management, and prognosis.

Ladinin-1 is involved in cell motility and proliferation of oral squamous cell carcinoma cells

Tuesday, 26th June @ 15:54: (Stanley Park Ballroom – Salon 2) - Oral - Abstract ID: 304

Dr. Tatsuya Abe (Division of Molecular and Diagnostic Pathology, Niigata University Graduate School of Medical and Dental Sciences), Dr. Manabu Yamazaki (Divisions of Oral Pathology, Department of Tissue Regeneration and Reconstruction, Niigata University Graduate School of Medical and Dental Sciences), Dr. Satoshi Maruyama (Oral Pathology Section, Department of Surgical Pathology, Niigata University Hospital), Prof. Yoichi Ajioka (Division of Molecular and Diagnostic Pathology, Niigata University Graduate School of Medical and Dental Sciences)

[Objectives] Oral squamous cell carcinomas (SCCs) and carcinoma in-situ frequently form the interface between cancer and non-cancerous epithelium. Previously, we identified the altered expression of 7 specific proteins around the interface between cancer and non-cancerous epithelium using proteome analysis of oral SCC tissue sections. Among identified proteins, ladinin-1 (LAD1) expression was significantly increased in the cancer tissue adjacent to non-cancerous epithelium. However, the function of LAD1 in oral SCCs is totally unknown. Thus, the aim of this study was to examine the function of LAD1 in the oral SCCs by in-vitro analysis. [Findings] The gene and protein expressions of LAD1 were confirmed by quantitative PCR and western blotting in three oral SCC cell lines, HSC-2, -3, and -4. Using immunofluorescence, LAD1 was localized in the peripheral area of the cytoplasm of cancer cells. High resolution morphological analysis using structured illumination microscopy revealed that LAD1 was co-localized with actin filament forming “actin arc” in the cytoplasm. Three cell lines demonstrated lower growth potential under inhibition of the expression of LAD1 by using siRNA. Although early adhesion to the plates was not affected, cleaved-caspase-3 positive and TUNEL positive cell ratio were increased in LAD1-knockdown cells. Furthermore, cell motility of LAD1-knockdown cells was significantly suppressed in wound scratch assay. [Conclusions] LAD1 is potentially involved in modulation of actin dynamics in oral SCC cells, affecting their motility and proliferation at the interface between cancer and non-cancerous tissue.

Interleukin 1 receptor antagonist (IL-1RA) biology in oral epithelium, oral dysplasia and oral squamous cell carcinoma

Tuesday, 26th June @ 16:06: (Stanley Park Ballroom – Salon 2) - Oral - Abstract ID: 196

Mr. Sven Niklander (University of Sheffield), Ms. Hannah Crane (University of Sheffield), Dr. Dan Lambert (University of Sheffield), Prof. Keith Hunter (University of Sheffield)

Objectives: Knowledge of molecular biology of oral dysplasia (OD) and oral squamous cell carcinoma (OSCC) is essential in order to find novel biological markers that could serve as predictor markers for malignant transforma- tion. IL-1 receptor antagonist (IL-1RA), IL-1 natural inhibitor, is encoded by the IL1RN gene and has been reported to be downregulated in head and neck squamous cell carcinoma, but the effects of its downregulation in OSCC and OD are largely unknown. Thus, the aim of this research was to study the role of IL-1RA in oral carcinogenesis and oral keratinocyte senescence.

Findings: IL1RN, specifically intracellular IL-1RA type 1 (icIL-1RA1), is constitutively expressed in normal oral epithelium, but is downregulated, both *in vitro* and *in vivo,* in OD and OSCC cell lines. We also found an upregulation of IL-1R1 (IL-1 agonist receptor) in OSCC and OD cell lines. Using confocal microscopy, we have found that both proteins, IL-1RA and IL-1R1, are able to localize inside the nucleus, which suggests a new possible ways of interactions of intra-nuclear IL-1α in oral keratinocytes. Transient transfection in OSCC and OD cell lines with a plasmid encoding for icIL-1RA1, showed no or limited effects on cell migration (by cell exclusion and transwell assay), cell proliferation (by EDU incorporation) and IL-6 and IL-8 secretion (by ELISA). Preliminary data suggests an increase of IL-1 alpha and a decrease of icIL1-RA mRNA expression as primary oral keratinocytes and mortal OD cells senesce.

Conclusions: IL1RN is downregulated in oral dysplasia and oral cancer. How this downregulation favours oral carcinogenesis it is not yet known, but might be related with the oral senescence program.

Extraparenchymal extension, lymph node involvement, and a higher Ki67 index were high risk factors for worse prognosis in conventional mammary analogue secretory carcinoma

Tuesday, 26th June @ 16:18: (Stanley Park Ballroom – Salon 2) - Oral - Abstract ID: 252

Dr. Jingjing Sun (Department of Oral Pathology, Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine; Shanghai Key Laboratory of Stomatology, National Center for Clinical Medicine of Oral Diseases.), Dr. Zhen Tian (Department of Oral Pathology, Ninth People's Hospital , Shanghai Jiao Tong University School of Medicine; Shanghai Key Laboratory of Stomatology, National Center for Clinical Medicine of Oral Diseases.), Dr. Ronghui Xia (Department of Oral Pathology, Ninth People's Hospital , Shanghai Jiao Tong University School of Medicine; Shanghai Key Laboratory of Stomatology, National Center for Clinical Medicine of Oral Diseases.), Dr. Li-zhen Wang (Department of Oral Pathology, Ninth People's Hospital , Shanghai Jiao Tong University School of Medicine; Shanghai Key Laboratory of Stomatology, National Center for Clinical Medicine of Oral Diseases.), Dr. Chun-ye Zhang (Department of Oral Pathology, Ninth People's Hospital , Shanghai Jiao Tong University School of Medicine; Shanghai Key Laboratory of Stomatology, National Center for Clinical Medicine of Oral Diseases.), Dr. Yu-hua Hu (Department of Oral Pathology, Ninth People's Hospital , Shanghai Jiao Tong University School of Medicine; Shanghai Key Laboratory of Stomatology, National Center for Clinical Medicine of Oral Diseases.), Prof. Jiang Li (Department of Oral Pathology, Ninth People's Hospital , Shanghai Jiao Tong University School of Medicine; Shanghai Key Laboratory of Stomatology, National Center for Clinical Medicine of Oral Diseases)

Objective: The prognostic factors of salivary gland (mammary analogue) secretory carcinoma (SC) are unclear because of the rarity of the tumors. Here we presented the largest case series to investigate the prognosis related clinicopathological factors in salivary conventional SC.

Findings: The study was based on a retrospective cohort of patients whose sections were reviewed and newly diagnosed as SC by the detection of *ETV6* rearrangement from 1993 to 2015. The clinicopathological features were analysed as the primary predictors and patients' final outcome was collected. Survival analysis was performed in conventional SC by using Kaplan-Meier method and Cox proportional hazards regression model. In our study, totally sixty-two cases of SC were confirmed. Fifty-nine out of 62 cases were conventional SC with a mean age of 43.2 years, showing significant male predilection (49/59, 83.1%) and mostly occurred in parotid glands (49/59, 83.1%). Additional 3 cases were identified as SC with high-grade transformation (HG-SC), with a mean age of 20 years older than that of patients with conventional SC. Lymph node metastasis and Ki67 expression ≥10% were related to poor recurrence-free survival (RFS), distant disease-free survival (DDFS) and disease-free survival (DFS) in conventional SC. Significantly decreased RFS and DFS were seen in patients with extraparenchymal extension. T3/T4 stage, age greater than 44 years and markedly hyalinized fibrous septa were associated with worse DDFS. By using multivariate analysis, the Ki67 index was found to be an independent prognostic factor for RFS ($p = 0.008$) and DFS ($p = 0.003$) in conventional SC. Much more worse RFS and DFS were presented in HG-SC due to its aggressive behaviour.

Conclusion: In conventional SC, patients with extraparenchymal extension, lymph node involvement, and higher Ki67 index exhibited poor clinical outcome. Moreover, Ki67 was a potential predictor of RFS and DFS of conventional SC.

THE EXPRESSION OF MAML2 GENE REARRANGMENT IN CASES OF GLANDULAR ODONTOGENIC CYSTS AND MUCOEPIDERMOID CARCINOMAS WITH OVERLAPPING HISTOLOGIC FEATURES

Tuesday, 26th June @ 16:30: (Stanley Park Ballroom – Salon 2) - Oral - Abstract ID: 75

Dr. Rekha Reddy (University of Flo), Dr. Liya Davidova (University of Florida), Dr. Mohammed Islam (University of Florida), Dr. Indraneel Bhattacharyya (University of Florida), Dr. Donald Cohen (University of Florida), Dr. Sarah Fitzpatrick (University of Florida)

Objectives: MAML2 expression has been demonstrated in the majority of mucoepidermoid carcinomas (MEC) aris- ing in the salivary glands. MEC may also arise intraosseously in the jawbone (IMEC). Glandular odontogenic cyst (GOC) is an odontogenic cyst with some histologic overlap with IMEC. MAML2 expression has not been extensively studied in IMEC or in GOC. This study will test the reliability of MAML2 in distinguishing cases of IMEC from GOC that share similar histologic features.

Methods: An IRB-approved retrospective search of IMEC, GOC, and IMEC with prior history of GOC was performed within the archives of the UF Oral Pathology Biopsy Service from 1994-2017. Eight cases from four patients were selected with diagnoses of either IMEC with earlier GOC, GOC with IMEC features, or IMEC with GOC features. Tissue was available for six out of the eight cases, on which break apart fluorescent in situ hybridization (FISH) analysis was performed for the presence of MAML2 rearrangement.

Findings: Lesions from two of the patients were negative for the MAML2 gene rearrangement while lesions from the other two patients were positive for the MAML2 gene rearrangement.

Conclusion: Although it can be concluded that the two patients with positive translocation for MAML2 had a diag- nosis of IMEC, the same conclusion could not be drawn for the two patients with negative translocation. Whether the cases that were negative for the translocation are GOCs with MEC-like islands or MAML2 negative IMEC could not be ascertained. Therefore, MAML2 gene rearrangement is not always dependable in differentiating IMECs and GOCs that share similar histologic overlap. The limited nature of the study due to small sample size precludes a more definitive conclusion. Collaboration vis-à-vis cases and data exchanges between oral and maxillofacial pathology centers may help achieve a better understanding of two uncommon, but clinically impactful, entities.

TUMOR ASSOCIATED MACROPHAGES: TAGGING AGGRESSIVENESS IN ORAL SQUAMOUS CELL CARCINOMA.

Tuesday, 26th June @ 16:42: (Stanley Park Ballroom – Salon 2) - Oral - Abstract ID: 162

Dr. Anupama Mukherjee (Goa Dental College and Hospital- Goa University), Dr. Anita Spadigam (Goa Dental College and Hospital- Goa University), Dr. Anita Dhupar (Goa Dental College and Hospital- Goa University), Dr. Karla Carvalho (Goa Dental College and Hospital- Goa University), Dr. Shaheen Syed (Goa Dental College and Hospital- Goa University)

INTRODUCTION:

A tumor cannot progress independently of its micro-environment- the stromal cells, tumor associated inflamma- tion, metabolic alterations and extracellular matrix remodeling is significant in disease progression, evolution and metastasis. Bi-directional interactions between the tumor cells and stromal elements determine individual tumor behavior as reflected in the prognostic variability of cases within the same histological grade. However, pivotal findings relating to the tumor micro-environment (TME) in Oral Squamous Cell Carcinoma (OSCC) still remain unaccounted for in the standard grading and staging systems. Thus evaluation of the TME could provide a more robust and accurate predictive assessment of OSCC.

Tumor Associated Macrophages (TAM) constitute the major inflammatory cell population of the TME, with a promi- nent role in stromal modulation and tumor progression. TAMs have also been regarded as suitable demonstrators of the "seed and soil theory" of metastasis.

OBJECTIVE:

To correlate the presence and role of TAMs in OSCC with the STNMP staging system.

FINDINGS:

Immunohistochemical evaluation revealed a definitive presence of TAMs at the advancing front of the tumor. The density of cells escalated from STNMP stage-1 to stage-4. A statistically significant, strong positive correlation was noted between- TAMs, tumor stage, tumor size and nodal status. A poor correlation between TAMs and tumor grade was noted.

CONCLUSION:

In India, of the 77,003 new cases of OSCC registered annually, 67.7% of the patients are lost to the disease. While tumor grade is indicative of the degree of differentiation of OSCC, it is inadequate as a sole predictor of tumor behavior and prognosis. A holistic evaluation of tumors and their TME may be the remedy. Thus, it has emerged that TAMs being dynamic cells of the TME, could be utilized as indicators of tumor behavior and aggressiveness.

Plaque-type lichen planus or leukoplakia with lymphocytic host response?

Tuesday, 26th June @ 16:54: (Stanley Park Ballroom – Salon 2) - Oral - Abstract ID: 332

Dr. Ibrahim Akeel (Harvard School of Dental Medicine), Dr. Sook Bin Woo (Harvard School of Dental Medicine)

Introduction: Oral epithelial dysplasia (OED) and oral squamous cell carcinomas (SCCAs) often exhibit a lympho- cytic host response (LHR) present as a band at the epithelium-connective tissue interface. Because these are often diagnosed as dysplasia with lichenoid mucositis or lichenoid dysplasia, clinicians assume that such lesions repre- sent dysplasia or SCCA arising within lesions of oral lichen planus (OLP). If the clinical lesion is a solitary plaque, the diagnosis of plaque-type OLP may be made. Lichenoid lymphocytic reactions are not specific to OLP and may be seen in drug-induced, contact hypersensitivity reactions and other conditions. The objective of this study is to review cases of leukoplakia with a lichenoid LHR.

Materials and Methods: Cases diagnosed as OED with lichenoid features or lichenoid mucositis that represented biopsies from solitary white lesions were identified from the files of one laboratory from January 2013 to December 2018.

Results: There were 13 males and 11 females (1.2:1 male to female ratio), and the median age was 61 (range 37 – 90). All lesions were unilateral and the two most common locations were the tongue (12 cases, 50.0%) and the gingiva (5 cases, 20.8%). Hyperkeratosis and/or parakeratosis and epithelial atrophy was present in 23 (95.8 %) and 10 cases (41.6%) respectively while degeneration of the basal cells was present in 7 cases (29.1%) only. OED was present in 13 (54.1%) of the cases (5 mild, 5 moderate, 2 severe, and 1 carcinoma-in-situ); 36.3% of the cases that showed epithelial atrophy also showed OED. A lymphocytic band was present in 24 cases (100%).

Conclusion: These lichenoid lesions were solitary plaques located most commonly on the tongue and gingiva, common sites for leukoplakia with 54.1% exhibiting OED. As such, these lesions more likely represent leukoplakia with a LHR rather than OLP. Clinicopathologic correlation is essential for accurate diagnosis.

Comparative Assessment of p16 Protein Expression in Normal and Dysplastic Oral Mucosal Epithelium

Tuesday, 26th June @ 17:06: (Stanley Park Ballroom – Salon 2) - Oral - Abstract ID: 226

Dr. Vimi Mutalik (The Ohio State University), Dr. Kristin McNamara (Ohio State University), Dr. John Draper (The Ohio State University), Dr. John Kalmar (The Ohio State University)

Objective: Expression of the protein marker p 16^{INK4a} is used as a surrogate for human papillomavirus (HPV) infection in biopsies of oral and tonsillar mucosa. While HPV infection accounts for <5% of oral cavity cancers, its association with oral epithelial dysplasia (OED) is unclear, with prevalence estimates ranging from zero to greater than 90%. In this study, the expression of p 16^{INK4a} was examined in archived biopsy specimens by immunohistochemistry within three groups: control mucosa (CM), low-grade dysplasia (LGD) and high-grade dysplasia (HGD). Tissue samples were age-, sex- and site-matched with 24 cases in each group. Grading of p16 expression was performed according to the criteria of intensity and proportion of cells as described by Grobe *et al*.

Findings: Fifteen of the 24 HGDs (62.5%), fourteen of the 24 LGDs (58.3%) and four of the 24 CMs (16.6%) were positive for p 16^{INK4a} expression. The difference in p16 expression between HGD versus CM and LGD versus CM were analyzed by Wilcoxon signed rank test and statistically significantly different at *level with p-values of 0.0001 and 0.0009, respectively. Greater p16 expression was noted in HGDs compared to LGDs (p-value = 0.01960, which was significant at level). A step-down Holm-Bonferroni method to account for multiple comparisons showed adjusted p- values of 0.0003 (HG versus CM), 0.0018 (LG versus CM) and 0.0196 (HG versus low LG) among three groups.*

Conclusion: *p16 expression was statistically-significantly greater in LGD and HGD lesions compared to CM, with a trend of greater expression being associated with higher grade of dysplasia.*

Determining the inflammatory response in oral squamous cell carcinoma by saliva analysis

Tuesday, 26th June @ 17:18: (Stanley Park Ballroom – Salon 2) - Oral - Abstract ID: 301

Dr. Catherine Laliberte (University of Toronto, Faculty of Dentistry), Ms. Denise Lopez Eymael (Faculty of Dentistry, University of Toronto), Dr. Grace Bradley (University of Toronto, Faculty of Dentistry), Dr. Marco Magalhaes (University of Toronto, Faculty of Dentistry)

Objectives: Oral squamous cell carcinoma (OSCC) often shows a pronounced inflammatory infiltrate and there is accumulating evidence that this inflammatory infiltrate plays an active role in tumor development and progression. Analyses of saliva may provide a non-invasive approach to study the inflammatory response in OSCC. Our aim is to develop a protocol for collection and analysis of saliva in OSCC patients and to use it to characterize both the inflammatory cell profile and cytokine profile in the saliva of patients across all stages of OSCC. **Methods:** 42 patients undergoing treatment for OSCC at the Toronto Odette Cancer Centre (stages I to IV), 25 healthy patients, and 9 patients with periodontitis were enrolled. Saliva samples were obtained by rinsing with 3 ml of saline for 30 sec. The samples were kept on ice and stabilized with protease inhibitor until filtration using a 20 μm membrane filter. Cell pellets were separated by centrifugation and supernatants were analyzed using a BioFLex 30-Plex inflammatory panel (BioRad) and Luminex® detection technology. Cell pellets were fixed in 4% PFA and analyzed using multichannel flow cytometry. The fluorescent markers included CD45, CD66b, CD3, CD4, CD8, CD25, CD56, CD68, CD138, Siglec8, PD1 and PDL1. **Findings**: Distinctive, reproducible changes were observed in salivary cytokines and inflammatory cell profile of patients with OSCC compared to control and periodontal disease patients. Using our protocol, we were able to describe specific patterns of inflammation for oral cancer, including altered CD4/CD8 ratio and marked increase in IL-1b, IL-6 and TNF-a. **Conclusion**: We created a reproducible protocol to collect saliva and perform high-throughput analysis of inflammatory profile of saliva. This technology can be used to develop non-invasive, early detection/prognostic tests for OSCC, new adjuvant therapies and new techniques to monitor response to treatment.

CHRONIC ULCERATIVE STOMATITIS: A LICHENOID OR VESICULOBULLOUS DISEASE?

Tuesday, 26th June @ 17:30: (Stanley Park Ballroom – Salon 2) - Oral - Abstract ID: 74

Dr. Rekha Reddy (University of Florida), Dr. Sarah Fitzpatrick (University of Florida), Dr. Liya Davidova (University of Florida), Dr. Indraneel Bhattacharyya (University of Florida), Dr. Donald Cohen (University of Florida), Dr. Mohammed Islam (University of Florida)

Objectives: Chronic ulcerative stomatitis (CUS) is a rare disease of unknown etiology. The histopathologic features are similar to lichen planus, but direct immunofluorescence (DIF) studies show characteristic presence of IgG in basal and parabasal epithelial nuclei.This study will review a case series of CUS and assess if the entity is more similar to lichen planus or vesiculobullous diseases. ***Methods:*** An IRB-approved retrospective search of CUS was performed within the archives of the UF Oral Pathology Biopsy Service between 2007 and 2017. ***Findings:*** Sev- enteen cases, all female, were included. The median age was 64 years (range 47-83 years). Eleven patients were Caucasian, one was Asian, and one was African-American. Race was not specified in four cases. Buccal mucosa (8/17) was the most common location, followed by gingiva (7/17), buccal vestibule (1/17), and gingiva/buccal mucosa (1/17). The most common clinical presentations were pain/burning (13/17), erythema (13/17), whiteness (11/17), ul- cerations/erosions (5/17), blisters/positive Nikolsky's sign (5/17), sloughing (2/17), striae (2/17), and recession (1/17). The clinical impression was lichen planus in 12 cases. Of these twelve cases, 4 included vesiculobullous disease as a differential. Four cases did not include a clinical impression and one listed erythema multiforme as the clinical impression. All cases were confirmed with DIF testing that showed a characteristic speckled pattern of IgG in basal and parabasal cells. Eleven of these cases were also positive for fibrinogen and two cases were faintly positive for C3. None of the cases were positive for IgA or IgM. ***Conclusion:*** Since CUS has overlapping clinical, histological, and immunofluorescence features with lichen planus and vesiculobullous diseases, clinicians and pathologists should consider this unusual, but significant, entity whenever oral ulcerative diseases with mixed features are encoun- tered.

IMMUNOHISTOCHEMICAL ANALYSIS OF INFLAMMATORY RESPONSE IN VERRUCOUS CARCINOMA COMPARED TO CONVENTIONAL ORAL SQUAMOUS CELL CARCINOMA

Tuesday, 26th June @ 15:30: (Stanley Park Ballroom – Salon 3) - Oral - Abstract ID: 232

Dr. Liya Davidova (University of Florida), Dr. Rekha Reddy (University of Flo), Dr. Sarah Fitzpatrick (University of Florida), Dr. Indraneel Bhattacharyya (University of Florida), Dr. Donald Cohen (University of Florida), Dr. Mohammed Islam (University of Florida)

Introduction: Studies on inflammatory response to oral squamous cell carcinoma (OSCC) generally do not include verrucous carcinoma (VC), which typically carries a far better prognosis. While high CD8 expression is associated with favorable outcome in head and neck cancers, the role of CD4+ lymphocytes remains controversial. B cell involvement has been suggested to enhance T cell response. The aim of this study is to evaluate differences in inflammatory infiltrate immunohistochemistry (IHC) between OSCC and VC.

Materials and Methods: The archives of the UF College of Dentistry oral pathology biopsy service were retrospectively searched for OSCC and VC. Slides were reviewed and 10 cases of VC, 10 cases of well differentiated SCC (SCC- WD), and 10 cases of poorly differentiated SCC (SCC-PD) were selected for testing. IHC staining for CD4, CD8, and CD20 was performed for 30 selected cases. The results were assessed via Aperio Image Scope positive pixel count assessment and analyzed statistically using ANOVA comparison of means with significance measured at $p<0.05$.

Results: A total of 90 scanned slides were evaluated. Analysis of the results showed no significant difference in mean scores of CD8 or CD20 across groups; however, there was significant difference in CD4 mean scores, with increasing scores noted from VC to SCC-WD to SCC-PD ($p=0.002$). The CD8:CD4 ratio was highest in VC followed by SCC-WD then SCC-PD, but the difference was not statistically significant. No significant difference in B: T cell ratio was observed between diagnostic groups.

Conclusions: This study demonstrated a lower level of CD4 positive lymphocytes and a slightly increased CD8:CD4 ratio within the VC infiltrate as compared to SCC. B lymphocyte involvement did not appear to differ between VC and SCC in this sample. Further studies may help elucidate the clinical implications of these differences.

Oral Syphilis: A Report of Two Cases and a Literature Review of This Re-Emerging Entity

Tuesday, 26th June @ 15:42: (Stanley Park Ballroom – Salon 3) - Oral - Abstract ID: 86

Dr. Richard J. Vargo (University of Pittsburgh), Dr. Elizabeth Ann Bilodeau (University of Pittsburgh)

OBJECTIVE: Syphilis is a sexually transmitted, infectious disease caused by *Treponema pallidum*. It can manifest clinically in three stages: primary, secondary, and tertiary. While rare, oral syphilis cases are starting to re-emerge. Our objective is to report 2 additional cases of oral syphilis—one case of primary syphilis and 1 case of secondary syphilis—to highlight the need to consider this entity in the histopathologic differential diagnosis of nonspecific mucositis.

FINDINGS: Both cases presented to outside oral surgeons in separate geographic regions. In case 1, a 25-year-old male presented with a six-week history of a 1.0 cm non-healing ulcer of the right lateral tongue. In case 2, a 28- year-old male presented with welt-like, slightly raised, red/white lesions of the lateral tongue, buccal mucosa, and hard and soft palates. The clinician reported no other lesions on the body and a negative STD test. An excisional biopsy was performed for case 1 and an incisional biopsy for case 2. Histopathologically, case 1 showed an ulcer with an underlying lichenoid lymphoplasmacytic infiltrate with perivascular plasma cells. Case 2 showed epithelial spongiosis and prominent neutrophilic microabscesses with an underlying lichenoid lymphoplasmacytic infiltrate. Perivascular and perineural plasma cells were also present. Because of the perivascular plasma cells in both cases, Treponema immunohistochemistry was ordered, and it highlighted many spirochetal organisms in the epithelium and superficial lamina propria in both cases. Given their respective clinical presentations, case 1 was diagnosed as a chancre of primary syphilis, while case 2 was diagnosed as a mucous patch of secondary syphilis.

CONCLUSION: Due to the resurgence of oral syphilis cases, clinicians should be aware of the histopathologic features and order the appropriate ancillary studies in suspected cases. Proper histopathologic diagnosis is important to prevent the spread and further re-emergence of this treatable infection and avoid misdiagnosis as nonspecific mucositis.

ODONTOGENIC MYXOMA: A 23-YEAR RETROSPECTIVE SERIES OF 38 CASES

Tuesday, 26th June @ 15:54: (Stanley Park Ballroom – Salon 3) - Oral - Abstract ID: 106

Dr. Abdulaziz Banasser (University of Florida College of Dentistry), Dr. Indraneel Bhattacharyya (University of Florida), Dr. Sarah Fitzpatrick (University of Florida College of Dentistry), Dr. Donald Cohen (University of Florida), Dr. Mohammed Islam (University of Florida)

Introduction: Odontogenic myxoma (OMX) is an uncommon benign tumor arising in the jaw. Though it has some histologic overlap with other entities, correct diagnosis is imperative considering the aggressive nature, high re- currence rate, and necessity of radical surgical intervention in large sized lesions. **Materials and Methods:** With IRB approval, a retrospective search of the University of Florida College of Dentistry Oral Pathology Biopsy Ser- vice archives from 1994-2017 for diagnosis of OMX of the mandible or maxilla was performed. Biopsy reports and original slides for each case were assessed and reviewed along with any accompanying radiographs to confirm the diagnosis. Immunohistochemical (IHC) staining was utilized to exclude entities with histologic overlap such as in- traosseous myxoid neurofibroma. **Results:** A total of 38 cases were included. The patients' ages ranged between 13 to 82 years, with a mean age of 38.5 years. Females comprised two-thirds of the cases (n=25) versus males (n=13). The mandible was the most affected at 56% (n=21), followed by maxilla 34% (n=13) with 10% (n=4) not specified. Posterior jaw involvement was higher than anterior in both the mandible (n=17 versus n=1) and the maxilla (n=8 versus n=4). The right side was more commonly affected than the left side in both arches. Most lesions presented clinically as expansile masses with variable radiographic appearance, and the submitting providers' clinical im- pressions included gelatinous masses, reactive gingival lesions, abscess, odontogenic lesions, fibro-osseous lesions, and soft tissue or bone neoplasms. In 30 cases (79%) the histologic diagnosis was compatible with OMX, while in 8 cases (21%) a more fibrous stroma was identified with diagnoses of fibromyxoma. **Conclusion:** OMX may ex- hibit varied demographic and clinical profile and wide spectrum of histologic presentation. Oral and maxillofacial pathologists and surgical pathologists should be sentient of this variability of presentation for accurate diagnosis and management.

IMMUNOHISTOCHEMICAL EXPRESSION OF EZH2 IN ATYPICAL PAPILLARY EPITHELIAL PROLIFERATIONS OF THE ORAL CAVITY: A POTENTIAL MARKER FOR MALIGNANT TRANSFORMATION

Tuesday, 26th June @ 16:06: (Stanley Park Ballroom – Salon 3) - Oral - Abstract ID: 94

Dr. Faraj Alotaiby (University of Florida College of Dentistry), Dr. Sarah Fitzpatrick (University of Florida), Dr. Mohammed Islam (University of Florida), Dr. Indraneel Bhattacharyya (University of Florida), Dr. Donald Cohen (University of Florida)

Background: Enhancer of zeste homolog-2 (EZH2) is a member of the polycomb group PcG of proteins; the genes that are involved in transcriptional repression. Cell cycle regulation and cell proliferation is associated with EZH2 expression and EZH2 overexpression stimulates cell proliferation and invasiveness. Conversely, inhibition of EZH2 precludes cancer cell invasiveness through inhibition of cell proliferation. Atypical papillary epithelial prolifera- tion (AEP) is a histologic diagnosis rendered for oral lesions with confounding microscopic features, neither overtly benign nor unequivocally malignant. **Aim:** To evaluate EZH2 antibody expression through immunohistochemical testing to delineate the potential of malignant transformation in AEP by comparing and contrasting with unequiv- ocally benign papillary lesions represented by inflammatory papillary hyperplasia (IPH) and malignant papillary lesions represented by papillary well differentiated squamous cell carcinoma (PSCC). **Materials and Methods:** 10 cases each of AEP, IPH and PSCC were retrieved from the University of Florida, Oral Pathology Biopsy Service archive and stained with Anti-KMT6/EZH2 antibody. The cases were reviewed and the extent and pattern of EZH2 expres- sion were assessed. The results were analyzed for statistical significance using Fischer's exact test. **Results:** The pattern and intensity of EZH2 expression in AEP and PSCC demonstrated statistically significant differences when compared to IPH ($p=0.002$). In addition, the basal cell layer showed EZH2 expression in all the cases of AEP (100%) and PSCC (100%) but only 3 out of 10 (30%) in IPH ($p=0.000$), comparable to normal oral epithelial control tissue. **Con- clusion:** EZH2 expression in AEP is more similar to malignant processes than benign lesions. The pattern of basal cell layer expression of EZH2 could be a potential prognostic indicator of malignant transformation risk in oral AEP lesions. A subsequent study by our group to assess EZH2 expression with respect to clinical outcome in AEP lesions is ongoing.

Inter-observer Variability among Pathologists in the Interpretation of Lesions of Proliferative Verrucous Leukoplakia Spectrum: A Collaborative Pilot Study

Tuesday, 26th June @ 16:18: (Stanley Park Ballroom – Salon 3) - Oral - Abstract ID: 228

Dr. Jasbir Upadhyaya (University of Florida College of Dentistry), Dr. Donald Cohen (University of Florida), Dr. Indraneel Bhattacharyya (University of Florida), Dr. Mohammed Islam (University of Florida), Dr. James Lewis (Vanderbilt University Medical Center), Dr. John Wright (Texas A&M College of Dentistry), Dr. Lester Thompson (Woodland Hills Medical Center), Dr. Susan Muller (Atlanta Oral Pathology), Dr. Elizabeth Ann Bilodeau (University of Pittsburgh), Dr. Jinping Lai (University of Florida College of Medicine), Dr. Marino Leon (University of Florida College of Medicine), Dr. Ricardo Padilla (University of North Carolina), Dr. Justin Bishop (University of Texas Southwestern Medical Center), Dr. Raja Seethala (University of Pittsburgh), Dr. Roman Carlos (Centro Clínico de Cabeza y Cuello / Herrera Llerandi Hospital), Dr. Sarah Fitzpatrick (University of Florida)

Objective: The use of diverse terminology may lead to inconsistency in the diagnosis and subsequent treatment of lesions within the proliferative verrucous leukoplakia (PVL) spectrum. The objective of this study was to determine inter-observer variability between pathologists in the diagnosis of PVL spectrum lesions.

Methods: Digitally scanned slides of 40 PVL lesions of varying stages were diagnosed by six oral pathologists (OP) and six head and neck pathologists (HNP) at multiple institutions. Inter-observer agreement on diagnoses was evaluated by Fleiss' kappa analysis using Microsoft Excel 2013 and IBM SPSS version 25 software.

Results: The responses provided were grouped into five broad categories. Category 1, simple hyperkeratosis with/without low-grade dysplasia; category 2, verrucous hyperplasia/keratosis with/without low-grade dysplasia; category 3, high-grade dysplasia or carcinoma-in-situ with/without verrucous surface change; category 4, verrucous carcinoma (VC) or atypical epithelial proliferation suggestive of but not fulfilling criteria of VC or squamous cell carcinoma (SCC) and; category 5, papillary or conventional SCC. The overall level of agreement between all pathologists for all cases as measured by Fleiss' kappa (K_F) was 0.270, considered fair agreement. Amongst OP the K_F was 0.225, whereas amongst HNP the K_F was 0.344. The best agreement between pathologists was on category 5 lesions (K_F=0.650) followed by category 1 (K_F=0.312). The least agreement was within categories 2 (K_F=0.150), 3 (K_F=0.192) and 4 (K_F=0.156).

Conclusion: This study reflects the lack of standardized diagnostic criteria and terminology for lesions in the PVL spectrum. We recommend that standardized criteria and terminology be proposed and established by an expert panel position paper, which would assist pathologists and clinicians to uniformly diagnose and manage PVL spectrum lesions more effectively.

Salivary Gland Anlage Tumor: Molecular Profiling Sheds Light on a Morphologic Question

Tuesday, 26th June @ 16:30: (Stanley Park Ballroom – Salon 3) - Oral - Abstract ID: 193

Dr. Scott Peters (Columbia University), Dr. Andrew Turk (Columbia University)

Objectives: The salivary gland anlage tumor (SGAT), previously referred to as a "congenital pleomorphic adenoma" or a "squamous proliferative lesion," is a rare, benign entity which presents within the first few months of life. It occurs almost exclusively in the nasopharynx or the posterior nasal cavity, and affected neonates typically present with respiratory distress and difficulty feeding. Despite this ominous clinical picture, the SGAT can be easily treated by surgical excision, with no recurrence reported in the limited cases available in the literature. Histologic exami- nation of this lesion reveals a distinct biphasic composition containing both epithelial and mesenchymal elements. Although the clinical and histologic features of the SGAT are well-described, the etiology of this entity is still poorly understood. The SGAT is currently believed to be a hamartoma rather than a true neoplasm due to its benign nature and lack of reported recurrence following treatment, however molecular studies have yet to be performed to verify this claim.

Findings: We present three new cases of SGAT on which whole exome sequencing has been performed. Specific attention was given to variants affecting 964 cancer-related genes compiled from five sources: the Cancer Gene Census, Oncomine, and the targets of the cancer panels designed by the Columbia Combined Cancer Panel, Memorial Hospital for Cancer and Allied Diseases, and Foundation Medicine. In the current study, examination of the entire exome from the three cases shows no plausible sequence-level driver mutations.

Conclusions: Our demonstration of apparently normal exome sequences from the three cases provides molecular support for the concept of SGAT as a non-neoplastic process. These results enhance the characterization and un- derstanding of this tumor, and illustrate the manner in which molecular studies may contribute to resolution of morphologic debates and impasses.

Ameloblastoma Arising in Odontogenic Keratocyst: Report of Four Rare Cases, Immunohistochemical Analysis and Review of Literature

Tuesday, 26th June @ 16:42: (Stanley Park Ballroom – Salon 3) - Oral - Abstract ID: 250

Dr. Moni Ahmadian (New York Presbyterian Queens), Dr. Paul Freedman (New York Presbyterian Queens), Dr. Renee Reich (New York Presbyterian Queens)

Odontogenic keratocyst (OKC) is a developmental cyst of the gnathic bones arising from the rests of dental lamina. This cyst demonstrates propensity for aggressive behavior and a relative high rate of recurrence compared to the other odontogenic cysts. Ameloblastoma is a benign neoplasm of odontogenic epithelium. It is the most common clinically significant odontogenic tumor that may demonstrate a locally aggressive clinical behavior. Ameloblas- toma may theoretically arise *de novo* from the rests of dental lamina as well as a developing enamel organ or from the epithelial lining of a pre-existing odontogenic cyst. Rare cases of ameloblastoma arising in the wall of dentiger- ous cyst, calcifying odontogenic cyst, glandular odontogenic cyst, radicular cyst, and residual cyst have been pre- viously documented in the existing literature. Furthermore, ameloblastomatous changes of cysts in nevoid basal cell carcinoma syndrome (NBCCS) have been previously reported. To our knowledge, only one case of ameloblas- toma combined with an OKC in a non-syndromic patient has been reported in the English language literature so far. Here we report four additional and extremely rare instances of ameloblastoma arising in combination with an OKC. Microscopically, all the cases exhibit the distinctive histopathologic features of OKC and ameloblastoma. Immunohistochemical staining for CD56, which has been reported to stain the peripheral layer of ameloblastomas and calretinin was performed on all cases. Additionally, two OKCs were stained with both markers as controls. No case demonstrated calretinin positivity, including in the obvious ameloblastic islands. CD56 highlighted only the ameloblastic areas while the areas of OKC were negative. The lack of staining in the areas typical of OKC help high- light the combined nature of the lesions. These findings suggest that much may still be unknown about the biologic potentials of OKC and that the pluripotentiality of the odontogenic epithelium may be the driving force behind such rare findings.

CLINICAL ORAL PATHOLOGY CONSULTS IN A US DENTAL SCHOOL: A RETROSPECTIVE ANALYSIS OF UTILIZATION AND EFFICACY

Tuesday, 26th June @ 16:54: (Stanley Park Ballroom – Salon 3) - Oral - Abstract ID: 231

Dr. Liya Davidova (University of Florida), Dr. Rekha Reddy (University of Florida), Dr. Sarah Fitzpatrick (University of Florida), Dr. Indraneel Bhattacharyya (University of Florida), Dr. Donald Cohen (University of Florida), Dr. Mohammed Islam (University of Florida)

Introduction. Chairside clinical oral pathology consultations are frequently provided in most dental schools; however, the outcome and efficacy of those consults remains largely unanalyzed. We designed a retrospective study to assess the utilization of consults by Oral and Maxillofacial Pathology (OMP) providers at the UF College of Den- tistry (UFCOD). ***Materials and Methods***. With IRB approval, the clinical record system (AxiUm) at the UFCOD was searched from January 1, 2011 until July 1, 2017 for oral pathology consultations. The following information was collected for these consults: year of consult, requesting clinic, reason/clinical impression, presumptive diag- nosis, recommended plan of action, and outcome (follow up). ***Results.*** A total of 418 consults were included in this study, of which 11 were repeat consults on the same lesion on different occasions. The most frequent clinics requesting consults were in decreasing order: undergraduate DMD clinics, followed by faculty practice, graduate prosthodontics, graduate periodontics clinic, with other clinics requesting consults infrequently. The most com- mon reasons consults were requested in descending order were: white lesions, ulcerations, nodules, pigmented lesions, swellings/enlargements, and erythematous lesions. Radiographic consults were uncommon in our study as at UFCOD, these are usually assigned or re-assigned to oral radiology. The disposition of the consults resulted in the following recommendations: 35% for observation/re-evaluation (ORE) in original clinic, 24% referred for biopsy, 19% treatment in original clinic followed by ORE, 12% referral to specialty clinic for treatment, and 10% multiple recommendations/lesions. In terms of outcome, biopsy and referral compliance was relatively reasonable, however ORE remained problematic. ***Conclusions***. This study illustrates the scope and difficulties associated with clinical consults in dental school and identifies areas of potential improvement.

HPV DOWN-REGULATES THE STEM CELL MARKER CD44 IN VIRAL-RELATED ORAL EPITHELIAL DYSPLASIA AND HNSCC

Tuesday, 26th June @ 17:06: (Stanley Park Ballroom – Salon 3) - Oral - Abstract ID: 324

Mr. Jordan Bolger (University of Minnesota School of Dentistry), Dr. Prokopios Argyris (University of Minnesota School of Dentistry), Ms. Christine Goergen (University of Minnesota School of Dentistry), Dr. Ali Khammanivong (Veterinary Clinical Sciences, University of Minnesota), Dr. Mark Herzberg (University of Minnesota School of Dentistry), Dr. Erin Dickerson (Veterinary Clinical Sciences, University of Minnesota), Dr. Raj Gopalakrishnan (University of Minnesota School of Dentistry)

Objective: Head and Neck Squamous Cell Carcinoma (HNSCC) represents the sixth most common malignancy worldwide and is characterized by dismal prognosis and poor patient survival. More than 75% of HNSCCs arise on a precancerous lesion. CD44 is a membrane bound glycoprotein stem-cell marker strongly expressed in normal oral mucosal epithelium. Upregulated in HNSCC, CD44 participates in key cell functions including cell division, migration and adhesion, and is recognized as a negative prognosticator for the disease. In addition, HPV(+) tumors show decreased CD44 levels when compared to HPV(-) neoplasms. We aimed to investigate the role of HPV infection in the regulation of CD44 expression in oral epithelial dysplasia (OED) and invasive HNSCC.

Methods: Formalin fixed, paraffin embedded specimens of HPV(+) OED (N=16), HPV(-) OED (N=15) and HNSCC (N=29) were evaluated by immunohistochemistry for CD44. Among the carcinoma specimens, five were HPV(+) and 24 HPV(-); 13 well-differentiated (WD), 5 moderately-differentiated (MD) and 6 poorly-differentiated (PD). HPV positivity was confirmed by immunohistochemistry for the surrogate marker p16. CD44 immunoreactivity was semi-quantitatively evaluated. Statistical analysis was performed using one-way ANOVA. **Results:** HPV(+) OEDs (mean expression=1.74) showed significantly lower CD44 membranous immunoexpression than HPV(-) OEDs (mean expression=2.42, $p<0.01$). Similarly, HPV(+) HNSCCs (mean expression=0.98) exhibit prominent loss of CD44 expression when compared to HPV(-) cancers (mean expression=2.99, $p<0.01$). Interestingly, CD44 expression appeared to associate with tumor differentiation since WD and MD specimens collectively (mean expression=3.18) display higher CD44 positivity than PD (mean expression=2.10, $p<0.05$). **Conclusions**: Lower CD44 expression in HPV(+) OEDs and HNSCCs may reflect decreased numbers of stem cells in HPV-driven lesions. The latter, can explain the limited frequency of malignant transformation in HPV(+) OEDs and better survival rates for patients with HPV(+) tumors.

USP6 gene rearrangement testing of gnathic aneurysmal bone cysts: a multicenter analysis of ten cases

Tuesday, 26th June @ 15:30: (Cypress Room 1 & 2) - Oral - Abstract ID: 38

Dr. Mark Mintline (University of Florida), Dr. Molly Smith (University of Kentucky), Dr. Sarah Fitzpatrick (University of Florida), Dr. Paras Patel (Texas A&M College of Dentistry), Dr. Harvey Kessler (Texas A&M College of Dentistry), Dr. Kristin McNamara (Ohio State University), Dr. Elizabeth Ann Bilodeau (University of Pittsburgh), Dr. Raja Seethala (University of Pittsburgh), Dr. Donald Cohen (University of Florida), Dr. Julia Bridge (University of Nebraska Medical Center), Dr. John Reith (Cleveland Clinic)

BACKGROUND: The jaws are an uncommon location for primary aneurysmal bone cysts (ABCs), and few gnathic cases have been tested for USP6 rearrangement. Rearrangements of CDH11 and/or USP6 are identified in approxi- mately 70% of primary extragnathic ABCs.

MATERIALS/METHODS: Herein, this multi-institutional, IRB-approved study investigates the USP6 status and clinicopathologic characteristics of cases histopathologically diagnosed as primary gnathic ABC. This study retrospectively identified 11 cases from four Oral & Maxillofacial Pathology Services and submitted them for USP6 analysis. Evaluation of one case failed, but the results of FISH testing, histomorphology, patient age and sex, lesion location, lesion duration, and clinical impression were abstracted for the remaining 10 cases.

RESULTS: The patients ranged in age from 10 to 43 years (mean: 25.4 years), with a male-to-female ratio of 1:1 (5:5). Nine cases occurred in the mandible and one case in the maxilla. The majority of lesions were present for an unknown duration or more than one month. Morphologically, five cases exhibited classic ABC features while five exhibited few cystic spaces with "solid" morphology. None of the tested cases demonstrated rearrangement of the USP6 locus by FISH.

CONCLUSION: In the jaws, lesions that morphologically mimic primary extragnathic ABC rarely show USP6 abnormalities, and may be genetically distinct lesions. Lesions with USP6 rearrangement are neoplastic; however, the etiology of lesions lacking rearrangement is less certain and may represent primary ABC without USP6 rearrangement, secondary ABC that have effaced the lesion of origin, or ABC-like degenerative lesions.

Genetic Polymorphism of tumor necrosis factor alpha (TNF-α) and tumor necrosis factor beta (TNF-β) genes and risk of oral pre cancer and cancer.

Tuesday, 26th June @ 15:42: (Cypress Room 1 & 2) - Oral - Abstract ID: 92

Dr. Shalini Gupta (King George's Medical University), Dr. Omprakash Gupta (Prasad Medical University,Lucknow), Dr. Shaleen Chandra (King George's Medical University)

Abstract

Background: Inflammatory cytokines such as TNF-α andTNF-β may play a pathogenic role in the development of oral precancerous lesions and oral cancer. Genetic polymorphisms of in these genes are known to predispose to malignant disease. We hypothesized that the risk of oral precancerous lesions and oral cancer might be associated with polymorphisms in these two inflammatory genes.

Methodology: A total 500 patients with oral pre cancer &cancer and 500 healthy volunteers were genotypes for the TNF-α (-238) G/A and TNF-β (252) A/G gene polymorphism. Genotypes were identified by polymerase chain reaction (PCR) restriction fragment length polymorphism(RFLP). Genotype frequencies were evaluated by Chi-square test.

Results: Compared to the GG genotype the GA genotype of TNF-α (G238A) polymorphism has been found to sig- nificantly increase the risk of oral disease (OR= 1.99) and especially the risk of Lichenplanus and oral malignancy (OR= 2.805 and 5.790 respectively). The risk of overall oral disease, Lichenplanus and oral malignancy were also high with allele A compared to allele G of TNF-α (G238A) polymorphism (OR =1.88, 2.34, 4.42) and respectively). Similarly, the risk of oral disease was also more in the heterzygote (AG) than the common allele homozygote (AA) of TNF-β (A252G) polymorphism (OR= 1.483) .

Conclusions: We conclude that the TNF-α (-238) G/A, TNF-β(252) A/G polymorphism were significantly associated with Oral pre cancer & cancer.

Keywords: Oral pre cancer & cancer, cytokines, tumor necrosis factor-α,β, inflammation, PCR-RFLP, Gene Polymor- phism.

The “old sailors “ illness makes a return

Tuesday, 26th June @ 15:54: (Cypress Room 1 & 2) - Oral - Abstract ID: 254

Dr. Suma Sukumar (University of Sydney), Prof. Hedley Coleman (Institute of Clinical Pathology and Medical Research, Westmead Hospital)

Vitamin C, also known as ascorbic acid, is a co-factor in multiple enzymatic reactions including that of collagen synthesis. Due to the absence of the enzyme L-gulonolactone oxidase, humans are unable to synthesise ascorbic acid; hence it is recognised as an essential nutrient. Scurvy refers to the clinical presentation of a deficiency of ascorbic acid, which occurs as a result of inadequate dietary intake. It has long been considered an illness of historical rather than contemporary significance. However, a tendency towards Western diets rich in processed foods and lacking in fresh produce has given rise to the re-emergence of the condition in the developed world. Current evidence suggests that there is a resurgence of scurvy in Sydney. A case is highlighted of an otherwise healthy 35year old male who presented to a hospital emergency dental clinic with generalised red, boggy gingivae. Clinical examination revealed bilateral involvement of the buccal and lingual/palatal gingivae in both the mandibular and maxillary arches, predominantly affecting the interdental papillae. Radiographic examination confirmed there was no associated bone loss or bony pathology. The clinical differential diagnoses included leukaemia, Kaposi sarcoma or possible scurvy. A battery of serological investigations yielded the eventual diagnosis of severe ascorbic acid deficiency (vitamin C level <5μmol/L, HIV negative and blood films and blood counts that did not show features of leukaemia). Dietary intake was immediately instituted in addition to 250mg daily vitamin C supplementation. The condition improved within weeks and completely resolved within 3 months (progress vitamin C level 55 μmol/L) and the patient was educated to henceforth maintain an adequate nutritional intake of ascorbic acid. Though these cases will be infre- quent, prudent clinicians need to re-familiarise themselves with the signs and symptoms of scurvy and maintain a wide diagnostic radar in order to ensure a speedy and accurate diagnosis.

The Role of Epigenetic and Epistatic Interactions in the Pathogenesis of Oral Submucous Fibrosis

Tuesday, 26th June @ 16:06: (Cypress Room 1 & 2) - Oral - Abstract ID: 264

Prof. Raghu Radhakrishnan (Manipal Academy of Higher Education), Dr. Mohit Sharma (Reader, Department of Oral Pathology, ITS Dental College, Hospital and Research Center, Greater Noida - 201308)

Objectives:

Epigenetic factors have shown to play an important role in the development of fibrosis. Persistent injury to oral mucosa because of habitual quid chewing resulting in the upregulation of inflammatory cytokines, leading to my- ofibroblastic persistence underlies an epigenetic aberration in oral submucous fibrosis (OSF). There is however, a paucity of literature showing the role of epistasis in the pathogenesis of OSF.

Findings:

Epistasis of IGF-1, TGF-1, COX and Lipoxygenase (LIOX) on PTEN are some of the relevant epistatic interaction relevant to the pathogenesis of OSF. Additionally, NF-κB is epistatic to PTEN, which is specifically arbitrated via p65 subunit of NF-κB.

Conclusions:

Given the importance of epigenetic modification in the pathogenesis of OSF the potential role of DNMT and HDAC inhibitors as a therapeutic option holds promise in OSF. Inhibitory microRNAs against profibrotic genes and/or stimulatory microRNAs against antifibrotic genes could be another viable in-vivo therapeutic alternative for the treatment of OSF.

Ameloblastic fibro-odontoma: A distinct entity

Tuesday, 26th June @ 16:18: (Cypress Room 1 & 2) - Oral - Abstract ID: 334

Dr. Molly Smith (University of Kentucky), Dr. Craig Fowler (University of Kentucky), Dr. Indraneel Bhattacharyya (University of Florida), Dr. Rekha Reddy (University of Florida), Dr. Douglas Damm (University of Kentucky)

Background: Ameloblastic fibro-odontoma (AFO) is a benign odontogenic tumor first described by Hooker in 1967. Its etiology and behavior have long been debated, as some investigators have proposed that AFO may represent a stage in development of an odontoma. For this reason, AFO was eliminated from the most recent Odontogenic and Maxillofacial Bone Tumor section of the World Health Organization (WHO) Head and Neck classification system. Occasional AFOs, however, have been found in patients older than the proposed age for odontoma completion (22 years) or present as large radiolucent lesions consisting mainly of the ameloblastic fibroma (AF) pattern with only foci of mineralized product formation. Herein, we present seven cases of AFO, all of which demonstrate particularly aggressive radiographic and/or histopathologic features and do not support the contention that all AFOs represent maturing odontomas. **Materials and Methods:** An IRB-approved retrospective search of the oral pathology biopsy services at the Universities of Kentucky and Florida between January 1, 1975 and January 1, 2018 was completed. Cases with appropriate histopathological and radiographic documentation were selected. **Results:** Seven patient cases were identified with ages 8, 8, 12, 16, 17, 27, and 29 years. Six cases were from the posterior mandible, and one was located in the posterior maxilla extending into the maxillary sinus close to the floor of the orbit. Only two of the cases have follow-up information, both of which demonstrate no evidence of tumor following conservative treatment. **Conclusion:** Although the majority of cases diagnosed as AFO likely represent developing odontomas, we present seven cases in which the clinical, histopathologic, and/or radiographic features suggest that AFO should exist as a distinct entity and be treated similarly to an AF.

GENETIC POLYMORPHISM AND GENE EXPRESSION OF PI3K GENE IN AMELOBLASTOMA

Tuesday, 26th June @ 16:30: (Cypress Room 1 & 2) - Oral - Abstract ID: 188

Prof. Aadithya B Urs (Maulana Azad Institute of Dental Sciences, New Delhi-02), Dr. Hanspal Singh (Maulana Azad Institute of Dental Sciences, New Delhi-02), Prof. Mahesh Verma (Maulana Azad Institute of Dental Sciences, New Delhi-02)

Objective:

Ameloblastoma is a benign and local aggressive odontogenic tumor. Many genes and their respective signalling pathways are involved in the pathogenesis i.e. Patch, SHH, SMO, PI3K, AKT, mTOR etc. PI3K has an important role i.e. cellular quiescence, proliferation, cancer, and longevity in the pathogenesis of Ameloblastoma through PI3K/AKT/mTOR signalling pathway. The study was designed to evaluate the gene expression and gene polymorphism of PI3K gene.

The present study was a prospective preliminary study, which was carried out in 20 patients of confirmed ameloblastoma cases. 5 tooth germs were taken as control to compare. Biopsy was taken with patient's consent. Genomic DNA was extracted to assess the polymorphism of PI3K gene gene sequencing method in exon 9 and exon 20 in association with immunohistochemical analysis respectively.

Findings: Insertion of AA is noticed as the most common variation among 12 samples out of 20 identified at Exon 9 near to the splice site of PIk3CA (g.24751_24752insAA) (chr3:178890652_178890653insAA). However, no variation at Exon 20 was observed. Variant was neither found in ExAC nor 1000G. No differences were noted in the frequency and type of mutations analyzed by sex, age, or histologic features. The gene expression of PIK3CA was significantly higher in tumor epithelial cells. Such genetic polymorphisms are vital because they can be used as biomarkers that indicate for prognosis of tumor and its biological behavior.

Conclusion: These results suggest that common genetic variations in these pathways may modulate risk and clinical outcomes of ameloblastoma. Further replication and functional studies are needed to confirm these findings. It will be of benefit to the patient, if we target the mutation or aberrant protein products at the appropriate time by intervention of précised therapy.

Keyword- Ameloblastoma, Immunoexpression, mutation, PI3KCA.

Plasma cell gingivitis due to cosmetics related Iodopropynyl Butylcarbamate (IPBC) allergy in a teenage female patient masking as desquamative gingivitis.

Tuesday, 26th June @ 16:42: (Cypress Room 1 & 2) - Oral - Abstract ID: 209

Dr. Sonia Sanadhya (University of Maryland, School of Dentistry), Dr. Ronald Brown (Howard University College of Dentistry), Dr. John Basile (University of Maryland, School of Dentistry), Dr. Rania Younis (University of Maryland, School of Dentistry), Dr. Roy Eskow (Bethesda Dental Implant Center)

Plasma cell gingivitis (PCG) is a rare lesion found on the attached and free gingiva, often extending to the mucogin- gival junction. Clinically, PCG can appear as sharply delineated erythematous lesions which can be accompanied by edema. We present a case of PCG in a 13 year old female patient which clinically presented as generalized desqua- mative gingivitis involving the facial aspects of the attached and marginal gingiva that persisted despite substituting to a non flavored dentifrice and failure to elicit any suspected drug or food allergies. Dermatologic patch testing proved positive for Iodopropynyl Butylcarbamate (IPBC), a water based preservative or biocide used in personal care products. Microscopically, there was an intense inflammatory infiltrate in the lamina propria composed pre- dominantly of mature plasma cells. Immunohistochemistry and in situ hybridization showed marked unrestricted cytoplasmic positivity for kappa and lambda light chains. IPBC is used in personal care products comprising lip balms, moisturizers, sunscreens, concealers and body washes due to its effectiveness at preventing fungal growth in topical products. The maximum level for safe use in leave on products is 0.1% but cosmetic products continue to use 10 times more than the safe levels. In the differential spectrum it is pertinent to discriminate erosive lichen planus, cicatricial pemphigoid, acute leukemia, HIV infection clinically; multiple myeloma and plasmacytoma his- tologically. Our case highlights the importance of patch testing for IPBC allergies in the oral mucosa. IPBC can lead to sensitization and contact dermatitis due to prolonged exposure; as its use in cosmetics continues to rise, and it is difficult to completely eliminate exposure to products containing IPBC especially in the context of teenaged girls and adult female patients.

RAMAN SPECTRAL STUDY OF SALIVA: A NEW TOOL FOR DETECTION OF MALIGNANT AND PREMALIGNANT ORAL LESIONS

Tuesday, 26th June @ 16:54: (Cypress Room 1 & 2) - Oral - Abstract ID: 68

Dr. Genecy Calado (Dublin Institute of Technology), Ms. Isha Behi (Dublin Institute of Technology), Dr. Marina Leite Pimentel (Dublin Dental University Hospital), Dr. Sheila Galvin (Dublin Dental University Hospital), Dr. Stephen Flint (Dublin Dental University Hospital), Prof. Hugh J Byrne (Dublin Institute of Technology), Prof. Fiona Lyng (Dublin Institute of Technology)

The overall aim of this study is to develop methodologies for analysis of human saliva using Raman spectroscopy with a future applicability for oral cancer diagnosis.Artificial saliva was prepared in different concentrations, aim- ing to optimise the spectroscopic acquisition protocol. Furthermore, saliva samples were collected from 10 healthy volunteers by a non-stimulated collection method and from 10 healthy volunteers by a stimulated collection method and frozen for further analysis. Also, saliva samples from 20 patients with oral cancer and oral dysplasia were collected for initial characterization and analysis. Centrifugal filtration was performed to concentrate the saliva samples. The optimization of the different parameters required for Raman spectral acquisition using a HORIBA Jobin-Yvon HR-800 confocal Raman microspectrometer was carried out. Raman spectra were recorded using dif- ferent wavelengths (532nm and 785nm), various objectives (x10, x50 and x60) and a diffraction grating of 600g/mm using both upright and inverted geometries and different substrates. Following pre-processing, spectra were sub- jected to principal component analysis (PCA) and principal component-linear discriminant analysis (PC-LDA).The 532nm source, inverted geometry, 10x objective and 96 well plate produced the best spectral quality and may be considered readily adaptable for clinical applications. Centrifugal filtration using a 3K device improved the spec- tra of the concentrate. PCA-LDA could discriminate between the healthy volunteer samples collected by stimulated or non-stimulated methods with resonable accurancy (83%). Furthermore, a specificity and a sensitivity as high as 91% and 94%, respectively, could be achieved when diferentiating healthy volunteer samples from patient samples. In this study, methodologies for the analysis of saliva by Raman spectroscopy have been developed to demonstrate the applicability of Raman microspectroscopy for providing molecular level insights from human saliva samples. The study also indicates the future potential for screening of saliva samples for oral pre-cancer and cancer.

Non-habit related oral squamous cell carcinoma: possible etiologic factors and probable prevention in Indian scenario

Tuesday, 26th June @ 17:06: (Cypress Room 1 & 2) - Oral - Abstract ID: 48

Prof. Susmita Saxena (Oral Pathology, ESIC Dental College, Rohini, New Delhi), Prof. Sanjeev Kumar (Oral Surgery, ITS Dental College and Research Centre, Muradnagar, Uttar Pradesh)

Introduction- India has the highest number of oral cancer cases in the world. Approximately 130,000 people succumb to oral cancer in India annually. Habits such as tobacco and alcohol consumption are well established etiologic factors in causing oral cancer. However, in recent years oral cancer cases are in the rise which do not have any known causative factors and studies have associated nutritional status, Human Papilloma Virus or poor oral hygiene as the probable cause.

Objectives- This paper aims at finding the possible etiologic factors in non-habit associated oral cancer through an extensive literature search keeping in view the incidence of reported cases in the Indian sub-continent. Studies reveal that 4-6% of oral cancer cases are not associated with any oral habits. It is important to be aware of the possibility of other factors contributing to the occurrence of oral cancer and aim at its prevention.

Findings- Significant number of studies and reported cases in India have shown that incidence of oral cancer in women without the exposure of any potential risk factors is alarmingly on the rise. The age range is lower as compared to habit associated cases where middle aged men are predominant. Other etiologic factors correlated with OSCC are viral infections like EBV, HPV, immunosuppression, familial factors, genetic predisposition, chronic mechanical irritation, dietary factors and hormonal factors.

Conclusions- OSCC is more prevalent amongst the lower socio-economic strata of the society in India where oral deleterious habits are common. The rising trend of oral cancer affecting people, especially women, without exposure to potentially harmful irritants should motivate researchers in identifying the possible etiologic factors. HPV virus association, genetic counselling, hormonal and dietary factors are to be considered to correlate cause and effect of such non-habit associated OSCC and adequate measures taken towards its prevention.

Investigation of foreign materials in gingival biopsies: a clinicopathologic, energy-dispersive x-ray microanalysis, and in vitro study

Tuesday, 26th June @ 17:18: (Cypress Room 1 & 2) - Oral - Abstract ID: 227

Dr. Leticia Ferreira (University of the Pacific Arthur A. Dugoni School of Dentistry), Dr. Hsin-Hsin Peng (Center for Molecular and Clinical Immunology - Chang Gung University), Dr. Darren Cox (University of the Pacific Arthur A. Dugoni School of Dentistry), Dr. David W. Chambers (University of the Pacific Arthur A. Dugoni School of Dentistry), Mrs. Avni Bhula (University of the Pacific Arthur A. Dugoni School of Dentistry), Dr. David Ojcius (University of the Pacific Arthur A. Dugoni School of Dentistry), Dr. John D. Young (Center for Molecular and Clinical Immunology - Chang Gung University), Dr. Erivan Ramos-Junior (University of the Pacific Arthur A. Dugoni School of Dentistry), Dr. Ana Morandini (University of the Pacific Arthur A. Dugoni School of Dentistry)

Foreign body gingivitis (FBG) has been previously described as a localized inflammatory reaction associated with the presence of foreign material in gingival tissues. However, among the gingival biopsies submitted to the Pacific Oral Pathology Laboratory (POPL) for diagnosis, we have identified foreign material in lesions that are markedly keratinized and described clinically as white plaques rather than inflamed lesions.

Objectives: To evaluate the clinical and histopathological features of 86 gingival biopsies containing foreign mate- rial retrieved from the POPL archives and to identify the composition of these particles by energy-dispersive x-ray spectroscopy (EDX). Further, primary human gingival fibroblasts (HGF) were stimulated with silica (SiO_2) microparticles to investigate the production of COL-1, MMP2 and inflammatory cytokines.

Findings: Foreign material was most commonly found in women (61%), in the 6^{th} or 7^{th} decade of life, and the clinical lesions were most frequently described as white plaques involving posterior mandibular gingiva. Interestingly, histopathological examination identified verrucous hyperplasia in 60.5% of the cases and epithelial dysplasia in 28.5% of the cases. EDX microanalysis revealed that Si (94%) followed by Ca (85%) and Al (66%) were the most frequently detected elements in the foreign particles. Silica microparticles induced higher COL-1 expression and increased MMP-2 activity in HGF, and higher levels of pro-inflammatory cytokines such as IL-6, IL-8 and TGF-ß in a microparticle-concentration-dependent manner.

Conclusions: Our study demonstrates that there is a strong association between the presence of foreign material in gingiva and clinically and microscopically demonstrable hyperkeratotic verrucous plaques. Moreover, we found that the most common element in the foreign material is Si which is usually found in the Earth's crust as silica. Our in vitro findings demonstrate the importance of silica-mediated effects on gingival fibroblasts, suggesting that the presence of silica in gingival biopsies could modulate the host inflammatory response and should be further investigated.

Odontogenic keratocyst - The role of PTCH1 and Hedgehog signaling in its pathogenesis

Tuesday, 26th June @ 17:30: (Cypress Room 1 & 2) - Oral - Abstract ID: 60

Prof. Tiejun Li (Peking University School of Stomatology), Mr. Jianyun Zhang (Peking University School of Stomatology)

Objectives: Mutations in *PTCH1*gene, a receptor in the Hedgehog (Hh) signaling, are responsible for Gorlin syn- drome (GS) and are related in tumors associated with this syndrome. The aims of this series of studies were to determine the role of *PTCH1* mutation and misregulation of the Hh signaling in the pathogenesis of GS-related and sporadic odontogenic keratocysts (OKCs).

Findings: Based on screening of 73 sporadic and 30 GS-related OKCs, we identified *PTCH1* mutations in 35.6% (so- matic, 26/73) of sporadic cases and 83.3% (germ-line, 25/30) of GS-related OKCs. However, a much higher mutation rate (79%, 30/38) in sporadic OKCs was detected by analyzing epithelial samples separated from the fibrous capsules. The previously underestimated mutation rate in sporadic cases might be due to the masking effect of the attached stromal tissues. Mutations in other genes of the Hh signaling such as *PTCH2*, *SUFU*, and *SMO* were rare and their pathologic roles in OKC were uncertain. Using whole-exome sequencing (WES), we further characterized the mu- tational landscape of 5 OKC samples lacking *PTCH1* mutation and revealed 22 novel mutations, among which two significantly altered genes (CDON and MAPK1) were predicted to affect Hh signaling activity in two cases. However no recurrent mutations were identified in the WES samples and validation cohort of 10 OKCs. Functional analysis revealed that *PTCH1* mutations activated Hh signaling and resulted in aberrant cell proliferation via both classical and non-canonical Hh pathways. Conclusions: Our data confirmed the high *PTCH1*mutation rate in both GS-related and sporadic OKCs. In *PTCH1*-negative cases, other genetic alterations were rare, but could also be related to Hh signaling. These results suggested that an inhibitor of the Hh pathway may be effective for the treatment of OKCs.