of Malaya, Malaysia. Samples were divided into two groups; 1) OSCC positive cervical lymph nodes with histological evidence of metastasis and 2) OSCC negative cervical lymph nodes without histological evidence of metastasis. Immunohistochemistry (IHC) was carried out to detect protein expression of IL17, IL22, IL23 and STAT3 using anti-human antibodies. Gene expression was performed using real time polymerase chain reaction to validate the results.

Findings: IHC results showed that expression of IL22, IL23 and STAT3 was significantly higher in negative nodes when compared with the positive group ($p<0.05$). Gene expression analysis showed no significant differences between the two groups.

Conclusion: The results suggest that there may be downstream evidence of PMN establishment in OSCC negative lymph nodes, modulated mainly by IL22, IL23 and STAT3.

VERRUCIFORM XANTHOMA: CASE SERIES OF AN UNUSUAL, COMMONLY MISDIAGNOSED LESION.

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Introduction: Verruciform Xanthoma (VX) is a benign condition occurring primarily in the oral cavity with some lesions also found on the genital mucosa or skin. VX occurs primarily in the fifth decade of life and shows a slight male predilection. This lesion generally presents as a papillary or rough-surfaced, painless, well-demarcated lesion, ranging from white, yellow-white, to orange in color. The etiology of VX is still largely unknown and definitive diagnosis is made based on histology. This lesion is thought to correlate with localized trauma or chronic inflammatory conditions such as lichen planus, lupus, epithelial dysplasia, pemphigus vulgaris, and mucous membrane pemphigoid. We report a series of three cases from our institution in which we examine the demographics associated with verruciform xanthoma as well as its connection to known inflammatory conditions.

Patient Cases: Our case series includes two males and one female patient ranging in age from 45-76 years old. The lesions were found in 3 different sites: gingival mucosa, ventral tongue, and buccal mucosa. Two of the patients had biopsy-proven oral lichen planus. The social and medical histories of each patient will be examined and compared for overlapping factors that may be of assistance in further clarifying demographic and etiological factors.

Conclusion: Clinicians should be familiar with verruciform xanthoma as it is often misdiagnosed as the more commonly occurring viral papilloma. Patients may be concerned they have contracted a viral disease and thus this lesion should be biopsied to rule out HPV infection.

CHARACTERIZATION OF THE INFLAMMATORY INFILTRATES IN ORAL EPITHELIAL DYSPLASIA AND ORAL SQUAMOUS CELL CARCINOMA USING A NEW MFCA METHOD.

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Oral cancer is a devastating disease and tumor associated inflammation is a key component of the tumor microenvironment. Current techniques to evaluate inflammatory infiltrate are based on a visual, operator-based quantification and may not accurately quantify specific inflammatory signatures.

Objective: To develop a method for characterizing the inflammatory infiltrate associated with oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC) using confocal microscopy and multichannel fluorescent colocalization analysis (MFCA).

Methods: We performed a retrospective analysis of 49 biopsy samples of lateral tongue lesions with a diagnosis of hyperkeratosis, ED and OSCC. The inflammatory infiltrate was identified using a combination of 2 primary antibodies for each cell type followed by staining with Alexa 488 or 555 tagged secondary antibodies for FIHC. Identification of the inflammatory cells was performed by 2-channel colocalization using a custom-made, semi-automated algorithm in Volocity 6.3.

Results: Using our novel analysis technique we identified and quantified neutrophils, TCD8, TCD4, eosinophils, plasma cells, B cells, Macrophages and NK cells in biopsy specimens. T-lymphocytes represented the main component of the inflammatory infiltrate in all specimens and there was a marked increase in inflammatory cell density from benign to OSCC lesions. Our results also showed that the CD4/CD8 ratio and neutrophils/lymphocytes ratio (NLR) had a progressive increase when moving from benign lesions to OSCC.

Conclusions: We described a new, method to quantify inflammatory infiltrates in oral biopsies. This semi-automated approach decreases operator bias and provides robust and reproducible data to study inflammation in tissue samples. Using this technique, we provide evidence that cancer progression is mirrored by progressive changes in the inflammatory infiltrate.

Significance: Understanding specific changes in cancer associated inflammation is essential to develop immune-targeted therapies. This technique and our current results will be further explored as a potential prognostic maker of oral cancer.

VISUALIZATION AND CHARACTERIZATION OF EXOSOMES IN BREAST CANCER CELLS.

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Objectives: Exosomes are extracellular vesicles of endocytic origin with a size range of 40-150 nm and a lipid bilayer membrane. Though exosomes are known as dynamic mediators of intercellular communication, its characteristics and function have not been fully studied. In this report, we used a metabolic labeling method to prepare fluorescent exosomes to investigate the characteristics and the movement of exosomes derived from various breast cancer cells.

Findings: MCF-7 and MDA-MB-231 cells were treated with three types of azido sugars, Ac4ManNAz, Ac4GalNAz, or Ac4GlcNAz (50 mM) for 3 days to produce the azide (-N3) containing exosomes through metabolic glycosylation. It is confirmed that the azido sugar decorated exosomes were able to maintain their original characteristics such as size, lipid bilayer morphology, and protein profile. The exosomes prepared with Ac4ManNAz have shown the highest labeling efficiency with ADIBO-Cy3 fluorescence dye in MCF-7, MDA-MB-231, BT-

549 and MDA-MB-468 cells at 17.2%, 14.8%, 13.2% and 14.9% respectively. To study their uptake capability, the labeled exosomes from different origins were incubated with a panel of breast cancer cells, including MCF-7, MDA-MB-231, MDA-MB-468 and BT-549, and normal NIH/3T3 fibroblast cells for 24 hours. The cells were then evaluated by fluorescence microscopic imaging and flow cytometry. It was observed that exosomes from different origins have a different uptake efficiency, suggesting that each exosome may have its unique navigation systems. Furthermore, the cells which have aggressive metastatic potential, such as MDA-MB-231 showed a better pickup of all exosomes. In contrast, the exosomes released by MDA- MB-468 showed higher loading in five kinds of cells.

Conclusions: Our development has demonstrated an effective labeling method that can facilitate exosome research by providing a new way of quantification and tracking in vitro and potentially in vivo studies.

THE CYTOSKELETAL ALTERATION MODULATES CELL INVASIVENESS OF OSCC CELLS THROUGH RHOA-YAP SIGNALING IN STROMAL FIBROBLASTS.

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Objectives: Cancer-associated fibroblasts (CAFs) are most abundant stromal cells among tumor microenvironment that participate in carcinogenesis. This study aimed to investigate the mechanism of cytoskeletal alteration of CAFs and its role in carcinogenesis of oral squamous cell carcinoma (OSCC).

Findings: We first evaluated if immortalized normal fibroblasts(hTERT-hNOFs) can be substituted for CAFs. hTERT- hNOFs co-cultured with OSCC cells showedmyofibroblastic and senescent phenotypeslike CAFs.Next, we observed the cytoskeletal alteration in hTERT-hNOFs co-cultured with OSCC cells, including enlarged cellular size, distinct F-actin assembly (stress fibers). To further understand the mechanisms, we identified the expression of RhoGT- Pase gene family. Among them, RhoA was significantly increased. These results were confirmed by RhoA-ROCK inhibitor(Y27632). In spite of fibroblasts grown with OSCC cells, Y27632 reduced cell size and stress fibers. Furthermore,YAP distribution, as a downstream transcriptional factor of RhoA, was examined. YAP was mainly localized at nucleus in hTERT-hNOF co-cultured with OSCC cells, unlike hTERT-hNOFs co-cultured with HEK(human normal epidermal keratinocyte). To further verify if RhoA and cytoskeletal change modulate YAP distribution, Actin polymerization inhibitor(Lat.A) and Y27632 were used. As results, the inhibitors interrupted nuclear YAP localization, suggesting that YAP can be regulated by RhoA-induced cytoskeletal alteration. Lastly, we examined if nuclear YAP localization of fibroblasts exacerbates OSCC progression. YAPS127A mutant fibroblasts, maintained in nuclear YAP, were generated. As results, YAPS127A showed cytoskeletal rearrangement, such as increased gel contractility and matrix stiffness, and thereby enhanced the invasiveness of OSCC cells.

Conclusions: The alteration of tumor microenvironment, such as cytoskeletal change and matrix remodeling via RhoA-YAP in CAFs, modulates OSCC progression. These understandings will provide the novel approaches for CAFs-based OSCC therapy.

OPG AND BCL-2 PROMOTE AMELOBLASTOMA CELL TUMORIGENESIS AND PREDICT PROGNOSIS FOR AMELOBLASTOMA PATIENTS.

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Ameloblastoma is the most frequent odontogenic epithelial tumor in the jaw. Though ameloblastoma belongs to benign odontogenic tumors, it exhibits a locally aggressive behavior with high recurrence rate. However, molecular markers predicting the recurrence have not been reported yet. The aim of this study was to find the prognostic markers in ameloblastoma. To detect apoptosis-related genes showing difference of expression level between ameloblastomas and normal oral tissues, the public database was analyzed. As results, OPG and Bcl-2 were identified as 2 most upregulated genes in ameloblastomas. To confirm public database analysis, in vitro study was conducted by use of AM-1 cell line. AM-1 cells expressed higher level of OPG and Bcl-2, compared with normal human epidermal keratinocytes (HEK). Exposing AM-1 cells to various environmental factors during culture in the 3-dimensional collagen gels were increased level of OPG and Bcl-2 than monoculture. To evaluate tumor-forming properties of AM-1 cells, subrenal capsule assay was conducted using AM-1 cells with hTERT-hNOF. As results, tumor formation were observed in 3 weeks, in which OPG and Bcl-2 expression was identified. To evaluate whether OPG and Bcl-2 regulates cell viability and apoptosis in AM-1 cells, siRNA transfection was conducted. As results, the knockdown of OPG and Bcl-2 reduced the cell viability and promoted the apoptosis of AM-1 cells. Knockdown of OPG and Bcl-2 decreased tumorigenesis. Eighty-nine cases of ameloblastomas were used for this study. Recurrence rate was 20.2%. Then, to validate whether these genes are associated to recurrence in ameloblastomas, immuno-histochemistry were performed. Each positivity classified 2 group by appropriate scoring system, low and high expression. The OPG and Bcl-2 expression was significantly associated with recurrence in conservative treatment group. These studies indicate that OPG and Bcl-2 status were independent predictive factors for recurrence.

10 YEAR REVIEW OF CHRONIC GRANULOMATOUS INFLAMMATORY REACTIONS FOUND IN THE ORAL CAVITY: 2007-2016.

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Chronic granulomatous inflammatory reactions are uncommon in the oral cavity. These lesions are reactive in origin and are characterized by macrophages which fuse to form multinucleated giant cells or transform into epithelioid histiocytes. Multiple etiologies exist for CGIR and include foreign body reactions to endogenous and exogenous materials, allergic