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IMMUNE CHECKPOINTS INDOLEAMINE 2,3-DIOXYGENASE 1 AND PROGRAMMED DEATH-LIGAND 1 IN ORAL MUCOSAL DYSPLASIA. *MS. MERI SIEVILÄINEN[A], DR. FABRICIO PASSADOR-SANTOS[B], MS. RABEIA ALMAHMOUDI[A], MR. SOLOMON CHRISTOPHER[A], DR. MARIA SIPONEN[C], DR. SANNA TOPPILA-SALMI[A], PROF. TUULA SALO[A], DR. AHMED AL-SAMADI[A]. [A] UNIVERSITY OF HELSINKI, [B] SÃO LEOPOLDO MANDIC RESEARCH CENTRE, [C] KUOPIO UNIVERSITY HOSPITAL*

Objectives: Oral mucosal dysplasia is a potentially malignant disorder that is associated with risk of transformation to carcinoma. During malignant transformation, dysplastic cells escape from immune-mediated destruction. We hypothesized that adaptive immunity is inhibited by activation of distinct immune checkpoint molecules, such as indoleamine 2,3-dioxygenase 1 (IDO1) and programmed death-ligand 1 (PD-L1). We collected 64 oral dysplasia samples from 47 patients. Nine biopsies from alveolar mucosa during wisdom teeth extractions were used as healthy controls. Tissue samples were stained and scored for IDO1 and PD-L1. Additionally, dysplasia grades and inflammatory cell infiltration were evaluated. Nine patients were followed up to 36 months to evaluate dysplasia progression, inflammation, and immune checkpoint molecule expression.

Findings: Dysplastic epithelium had significantly lower IDO1 expression than that of healthy controls. Cells positive for PD-L1 in the lamina propria were mainly in dysplastic samples and seldom in healthy controls. Dysplasia grade associated negatively with epithelium IDO1 and positively with IDO1 and PD-L1 expression in the lamina propria. There was a positive association between dysplasia grade and level of inflammatory cell infiltration. During follow-up, dysplasia grade, inflammatory cell infiltration, and the immune checkpoint expression fluctuated over time.

Conclusions: The immune checkpoint molecules IDO1 and PD-L1 are modulated during oral epithelial dysplastic changes and their expression is associated with inflammatory cell infiltration in the lamina propria. As immune checkpoint molecule expression fluctuates over time, these molecules are not useful as biomarkers for oral mucosal dysplasia progression.

KNIEST SYNDROME: CASE REPORT AND REVIEW OF LITERATURE. *DR. TANIA JHAMB, DR. HAYAT MASOOD, DR. EMILE ROSSOUW. EASTMAN INSTITUTE FOR ORAL HEALTH, UNIVERSITY OF ROCHESTER*

Background: Kniest syndrome (dysplasia) is a rare autosomal dominant chondrodysplasia that is characterized by distinct musculoskeletal and craniofacial irregularities. These abnormalities result from a mutation of the collagen type II gene (COL2A1) resulting in an abnormal type II collagen product. Craniofacial abnormalities seen in this syndrome include prominent eyes, flat nasal bridge, cleft palate, midface anomalies, tracheomalacia, and hearing loss. This report illustrates a case of Kniest syndrome with severe dentoskeletal malformation with cleft palate treated at Eastman Institute for Oral Health. In addition, the report also outlines clinical, histopathological and radiographic findings of the condition with a review of literature of Kniest syndrome.

Method: Case study of a 16 year old male with a history of Kniest syndrome presented to the Orthodontic clinic seeking treatment for misaligned teeth. The patient showed clinical features of this syndrome which included dwarfism, severe midface hypoplasia, flattened and rounded face with prominent eyes and nasal atresia. Patient had a history of cleft palate repair. Intraoral findings included severe gingival hyperplasia, high arched palate and abnormal dentoalveolar development.

Conclusion: Kniest syndrome (dysplasia) is a rare chondrodysplasia with differential diagnosis that can include Spondyloepiphyseal dysplasia, Spondyloepimetaphyseal and Metatropic dwarfism. In addition to genetic testing, distinct radiographic features and histopathological studies are crucial in determining the proper diagnosis of the condition.

VALIDATION OF A FOUR PROTEIN SIGNATURE FOR DETERMINING LYMPH NODE METASTASIS AND SURVIVAL IN ORAL SQUAMOUS CELL CARCINOMA IN 3 MAIN DIAGNOSTIC CENTERS IN MALAYSIA. *DR. FAIRUZ ABDUL RAHMAN[A], DR. ANAND RAMANATHAN[A], DR. LAU SHIN HIN[B], DR. GEORGE BOEY TEIK FOO[C], PROF. SOK CHING CHEONG[D], DR. NOOR AKMAR NAM[E], PROF. ROSNAH MOHD ZAIN[F]. [A] UNIVERSITY OF MALAYA, [B] INSTITUTE FOR MEDICAL RESEARCH, MALAYSIA, [C] QUEEN ELIZABETH HOSPITAL, KOTA KINABALU, SABAH, [D] CANCER RESEARCH MALAYSIA, [E] UNIVERSITI SAINS ISLAM MALAYSIA, [F] MAHSA UNIVERSITY; ORAL CANCER RESEARCH AND COORDINATING CENTRE, UNIVERSITY OF MALAYA*

Introduction: Despite advances in screening and detection tools, the overall accuracy for current pre-operative assessment of regional lymph node (LN) metastasis is still limited with low sensitivity (70%) having a false negative rate of 30%.

Objectives: To validate the previous study (Zanaruddin et al. 2013) that has identified 4-protein signature (EGFR, HER2/neu, LAMC2 and RHOC) in primary oral squamous cell carcinoma (OSCC) that could reliably distinguishes patients with and without LN metastasis.

Method: A total of 83 cases of OSCC samples, their socio-demographic and clinic-pathologic data were collected from three centers. Four proteins (EGFR, HER2/neu, LAMC2 and RHOC) expression were evaluated using immunohistochemistry based on the intensity and percentage of staining.

Results: All four proteins evaluated, were found to be significantly associated with the presence of LN metastasis.

EGFR, HER2/neu, LAMC2 showed high expression whereas RHOC showed low expression with LN metastasis. The cutoff point of at least four proteins with cumulative score of 3 would reflect the best sensitivity and specificity. The 4-protein signature showed sensitivity of 90.1% and specificity of 64.1% and prognostic accuracy of 77.5% in correlation with LN metastasis in general for an overall 83 OSCC samples and in particular for each set of OSCC samples from each center demonstrates the robustness and accurateness of this 4-protein signature in predicting LN metastasis. Kaplan-Meier survival curves showed significant survival probability difference between two groups in 4-protein signature for overall 83 samples.

Conclusions: Four protein signature have been shown to have potential to be used as prognostic indicators of LN metastasis in OSCC. It can be an useful prognostic tool in the clinical setting to facilitate the prediction of LN metastasis. This study also concluded that the survival probability is inconclusive. However, it is found that the 4-protein signature has shown a trend for prediction of overall survival.

DOWN-EXPRESSION OF TETRASPANIN CD9 IS A SENSITIVE MARKER FOR IDENTIFYING PRE-MALIGNANT CHANGES IN THE ORAL EPITHELIUM.

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Objectives: Tetraspanins, cell surface proteins that mediate cell-cell and cell-extracellular matrix interactions, are capable to modify cell motility, thus being potential diagnostic markers in pre-malignant conditions. We examined the immunohistochemical expression of tetraspanins CD9, CD81 and CD63 in normal oral mucosa as well as in inflamed, dysplastic and neoplastic epithelial lesions.

Findings: Included were cases of normal oral mucosa (NOR, N=15), oral lichen planus (OLP, N=51), hyperkeratosismild dysplasia (HK, N=29), moderate-severe dysplasia (DYSP, N=22), oral squamous cell carcinoma (OSCC, N=31), and normal-looking mucosa nearby OSCC (N-OSCC, N=18). Staining, assessed as percent of stained cells multiplied by staining intensity (1=weak, 2=strong), was evaluated per epithelial thirds (basal, middle and upper) and then as total staining score (sum of all thirds). Statistical analysis was performed using One-way ANOVA. Receiver operating characteristic (ROC) curve was used for diagnostic sensitivity. Statistical significance was set at $P<0.05$. Expression of CD9 was highest in NOR compared to all other lesion types and higher in OLP, HK and DYSP than in N-OSCC and OSCC ($p<0.001$). A higher expression of CD81 in NOR, OLP and HK differentiated these lesions from DYSP, OSCC and N-OSCC ($p<0.001$). CD63 was usually inconclusive. CD9 was the only tetraspanin to significantly distinguish NOR from all other lesion types (area under ROC, 0.9; $P < 0.001$) with high sensitivity and specificity (80% for both, at a total staining score of 12.5).

Conclusions: CD9 could accurately discriminate between normal (high expression) and all other types of pathologies (lower expression) with high diagnostic sensitivity. In addition, expression of CD9 in neoplasia and the nearby histologically "normal-looking" epithelium was similar but significantly lower than in dysplasia and OLP. Therefore, the expression of CD9 could aid in defining the nature of equivocal histopathological changes in oral epithelial lesions.

HIGH THROUGHPUT SEQUENCING REVEALS CIRCADIAN RHYTHM GENE RORA IS COOPERATIVELY SUPPRESSED BY MULTIPLE MICRORNAS IN ORAL SQUAMOUS CELL CARCINOMA.

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Objectives: To explore the differentially expressed mRNAs and miRNAs in OSCC tissues, and identify the interaction network between miRNAs and trascription factors (TFs). Among them, the regulatory network of miRNAs - circadian gene RORα on proliferation in OSCC was further elucidated.

Findings: RNA-seq and microRNA-seq analyses show that upregulation of microRNA in OSCC samples significantly contribute to the globally down-regulated transcription factors (TFs) in OSCC. Circadian rhythms genes including three members of retinoic acid receptor-related orphan receptor family (RORα, RORβ and RORγ) and CLOCK were among the down-regulated TFs. RORα was predicted to be targeted by 25 co-upregulated miRNAs, of which, miR-503- 5p, miR-450b-5p, miR-27a-3p, miR-181a-5p and miR-183-5p were further testified to directly target RORα, resulting in a more stronger effect on RORα suppression by mixing together. In addition, we showed that RORα was significantly decreased in most OSCC samples (37 of 44, 84%), and significantly suppressed the proliferation of OSCC cells in vitro and in vivo. Attenuated RORα decreased p53 protein expression and suppressed p53 phosphorylation activity.

Conclusions: The abnormal miRNAs-mediated TFs network could play important role in OSCC tumorigenesis. Among those TFs, circadian gene RORα acted as a tumor suppressor in OSCC by inhibiting tumor proliferation and could be negatively regulated by miR-503-5p, miR-450b-5p, miR-27a-3p, miR-181a-5p and miR-183-5p cooperatively, which provides clues to understand the clinical link between circadian rhythms and cancer therapy.

COMPARATIVE STUDY OF KI67 AND MCM4-6 COMPLEX IN AMELOBLASTOMA AND UNICYSTIC AMELOBLASTOMA.

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Objectives: The aim of the present study was to determine the patterns of immunoexpression of minichromosomal maintenance proteins (MCM) 4, 5, 6 and corelate them with the presence of Ki67, in order to evaluate their utility as possible