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

cellular proliferation markers in ameloblastomas (AMs) and unicystic ameloblastomas (UAMs).

Findings: Immunohistochemical and western blotting results determine that for both variants, AM and UAM, the Label index (Li) showed a major value for MCM6 protein, followed by MCM5, MCM4 and lastly by Ki67 expression (p value <0.05). The immunoexpression of Ki67 and MCM5 was exclusively nuclear in basal tumoral cells of both variants. On the other hand, MCM4 and 6 were located in the nucleus and cytoplasm of basal and columnar epithelial cells and those that resemble the stellar reticulum. There were no significant differences in the results between the AM and UAM.

Conclusions: Results suggest that MCM5 protein could be a good proliferation marker, with greater sensitivity in comparison with Ki67. Moreover, MCM markers could be used to predict AM and UAM cell proliferation. Further studies with the inclusion of others odontogenic tumors are necessary to confirm the real potential of MCM proteins, more specifically MCM5.

ASSOCIATION OF MAPK/ERK PATHWAY ACTIVATION WITH KRAS MUTATIONS IN ADENOMATOID ODONTOGENIC TUMORS.

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Objectives: Adenomatoid odontogenic tumors (AOT) are benign tumors derived from odontogenic epithelium, and they account for 2-7% of all odontogenic tumors. Intraosseous AOTs are thought to be associated with unerupted permanent teeth, although their pathogenesis is still unclear.KRAS mutation, which is involved in the pathogenesis of some malignant tumors as driver mutation, was recently detected in AOT suggesting its association with tumorigenesis.The aim of this study was to assess the frequency of KRAS mutation and his association with the presence of the MAPK / ERK signaling pathway proteins.

Finding: Paraffin-embedded tissue samples from 9 AOT patients (3-47 years old, mean 24.7 years) were obtained for this study. Genomic DNA was extracted from each sample, and in one case, genetic mutations in 50 cancer-associated genes were examined by next-generation sequencing. A KRAS G12D missense mutation was detected in the DNA sequence of the tumor cells, but it was not detected in that of the stroma tissue. Based on this result, hotspot mutations in the RAS family were analyzed by PCR-rSSO using the remaining 8 cases. KRAS G12V and KRAS G12R mutations were detected in 2 and 4 cases, respectively. Subsequently, in the paraffin blocks, immunohistochemistry was performed to visualize the presence of the proteins involved in the MAPK / ERK signaling pathway. All the cases were EGFR, KRAS,CRAF, BRAF positive, one case was ERK negative, and one case was MEK and ERK negative, all the other remaining cases were MEK and ERK positive.

Conclusions: In conclusion, KRAS mutation was frequently detected in AOT, suggesting its association with tumorigenesis of AOT. However, since EGFR was positive, how the mutation affects the tumor development is still unclear.

CLINICOPATHOLOGICAL SIGNIFICANCE OF EXPRESSION OF MIR-26A, MIR-107, MIR-125B AND MIR-203 IN HEAD AND NECK SQUAMOUS CELL CARCINOMAS.

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Objectives: MicroRNAs play an important role in the development and progression of head and neck squamous cell carcinomas (HNSCC). In the current study, we compared the expression levels of microRNAs in primary HNSCC with and without cervical lymph node metastasis and determined their clinicopathological significance. The expression levels of miR-26a, miR-107, miR-125b and miR-203 in primary HNSCC with cervical lymph node metastasis (n=16), and their matched lymph node metastasis, and primary tumors without metastasis (n=16) were determined by quantitative RT-PCR. Furthermore, we evaluated the association of those microRNAs with clinicopathological features and survival of patients with HNSCC.

Findings: miR-26a ($p<0.05$) and miR-125b ($p<0.01$) expression levels were significantly higher in primary HNSCC with lymph node metastasis than in tumors without metastasis, while that the levels of miR-203 ($p<0.01$) were significantly lower in the metastatic tumors. Compared with matched metastatic lymph node tissues, miR-125b ($p<0.01$) exhibited a significantly lower expression and miR-203 ($p<0.01$) demonstrated higher expression in the primary tumors. The expression of the microRNAs was associated with various HNSCC clinicopathological risk features, including miR-26a high expression and N stage (p=0.04), poor histological differentiation of tumors (p=0.005) and recurrence (p=0.007), miR-125b high expression and N stage (p=0.0005) and death (p=0.02), and low levels of miR-203 and N stage (p=0.04). Importantly, high expression of miR-26a was significantly associated with shortened disease-free survival (disease relapse) and high miR-125b levels was an independent risk factor for poor disease-specific survival patients with HNSCC.

Conclusion: These findings suggest that miR-26a and miR-125b may be associated with progression and metastasis of HNSCC.

INFLUENCE OF RADIATION DOSE IN COLLAGEN IV AND MMP20 IMMUNOEXPRESSION AND THE TOOTH IMMEDIATE ADHESIVE PROPERTIES.

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Objectives: Radiation-related caries is an important collateral effect in patients with head and neck cancer subjected to radiotherapy, with rapidly progressive, asymptomatic and ample lesions, associated to direct and indirect effects of radiation. The present study has the aim of determine the alterations of the immunoexpression of collagen IV and MMP20 in the amelodentin junction and its relationships with odontoblasts according to the radiation dose (0, 20, 40, and 70Gy) and its influence on the