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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 immunohistochemically 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.

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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.

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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.

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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 transformation. 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.

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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 $\geq$10% were related to poor recurrence-free survival (RFS), distant disease-free survival (DDFS) and disease-free survival (DFS) in conventional SC. Significantly decreased RFS and DFS were seen in patients with extraparenchymal extension. T3/T4 stage, age greater than 44 years and markedly hyalinized fibrous septa were associated with worse DDFS. By using multivariate analysis, the Ki67 index was found to be an independent prognostic factor for RFS ($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.

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Objectives: MAML2 expression has been demonstrated in the majority of mucoepidermoid carcinomas (MEC) arising 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.