Conclusions: P16 is not a useful predictor of malignant transformation or recurrent disease and does not correlate well with previously validated epithelial proliferative markers in oral dysplasia.

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P124. Dysregulation of the PI3K/AKT/MTOR pathway by mutation and copy number alteration in oral cavity squamous cell cancer

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Introduction: The phosphatidylinositol 3-kinase (PI3K) signaling pathway is integral to cell growth, proliferation and survival, and is upregulated in multiple human cancers. Several oncogenes and tumor suppressors within this pathway are altered by mutation, amplification or deletion, and are currently being investigated as therapeutic targets in ongoing clinical trials for head and neck and other solid cancers. The pattern of genetic alteration has not been characterized in oral cavity squamous cell carcinoma (OCSCC).

Objective: Our objective was to comprehensively analyze the pattern of mutational and copy number alteration in this pathway in OCSCC.

Methods: After IRB approval, DNA was extracted from 32 microdissected frozen OCSCC samples and matched normal tissues. High-throughput sequencing of 25 component genes of the PI3K pathway was performed. Three integrated sequence assemblies were screened for non-polymorphism coding mutations, which were independently validated on PCR. Array comparative genomic hybridization (aCGH) was then performed on the Agilent 1 M platform. The RAE computational framework was used for segmentation and identification of regions of statistically significant copy number alteration (CNA), which were then validated with qPCR. Immunohistochemistry of downstream proteins was used to confirm pathway activation.

Results: Among the 25 component genes in the PI3K pathway, activating mutations were identified in PIK3CA in 6.3% of samples. Copy number gain was present in 15 genes (including PIK3CA in 45.2%), and loss in 4 genes (including PTEN in 12.9%). Altogether, 74% of tumor samples contained either activating mutations or CNAs within the PI3K pathway. Tumors with PIK3CA mutation or EGFR amplification had a significantly higher rate of copy number alterations in the PI3K pathway.

Conclusions: In the first comprehensive mutational and copy number analysis of the 25 component genes of the PI3K pathway in cancer, we report a low frequency of somatic mutations, and high frequency of copy number alteration, in OCSCC. In contrast to colorectal, breast, ovarian, and brain cancers, copy number alteration, not mutation, appears to be the main source of pathway activation. These findings may have relevance to therapeutics targeting tumors in which the PI3K pathway is dysregulated, or in which PI3K pathway activation mediates resistance to EGFR inhibition.

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P125. Expression of midkine and its clinical significance in head and neck squamous cell carcinoma

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Background: Midkine is overexpressed in many carcinomas and thought to play an important role in carcinogenesis. However, few studies have been focused on the role of midkine in head and neck squamous cell carcinoma.

Methods: Midkine expression status was analyzed by semi-quantitative reverse transcriptase polymerase (RT-PCR), Western blot and immunohistochemistry. Clinical parameters were obtained from the medical records.

Result: Midkine is up-regulated in HNSCC, as evaluated by semi-quantitative RT-PCR, Western blot, and immunohistochemistry. Positive staining for midkine is significantly correlated with lymph node metastasis, and has poorer five-year overall survival rate ($p = 0.008$). Multivariate analysis revealed that midkine expression was an independent prognostic factor ($p = 0.002$).

Conclusion: This is a report of an association between midkine expression and all sites of HNSCC. Midkine may play an important role in the progression of HNSCC, and evaluation of midkine expression is a useful prognostic factor among patients with HNSCC.

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P126. Expression of GNA12 and its role in oral cancer

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Introduction: The variability of clinical outcomes in oral cancer patients and the heterogeneity of the disease are the main challenges for the improvement of current treatment modalities. Efforts in our laboratory have focused on the molecular profiling of oral cancer to understand the mechanisms underlying the disease. In a previous microarray study, we found Guanine nucleotide binding protein alpha-12 (GNA12) to be up-regulated in oral cancer.

Materials and methods: In this study, we validated the expression of GNA12 at the mRNA level in 47 oral squamous cell carcinoma (OSCC) and 18 non-malignant oral mucosa tissues, by quantitative polymerase chain reaction (qPCR). Further, GNA12 protein expression was accessed by immunohistochemistry (IHC) on 44 tumors and 23 non-malignant oral mucosa tissues. Using OSCC cell lines, we examined the effects of GNA12 signaling by in vitro functional assays.

Results: We demonstrated that GNA12 mRNA levels were significantly up-regulated in OSCC in comparison to the non-malignant oral mucosa tissues. Consistently, high levels of GNA12 protein expression were detected in 75% of OSCC tissues, while the non-malignant tissues showed negative or weak expression. We demonstrated that expression of activated GNA12 (GQ231L) promoted oral cancer cell migration in a monolayer wound healing assay and cell invasion through the matrigel barrier, but cell proliferation was not changed. Correspondingly, exogenous expression of the regulator of G-protein signaling (RGS) blocked G12 family signaling through RhoA, which resulted in the inhibition of cancer cell migration and invasion.

Discussion: In summary, we demonstrated for the first time in OSCC that GNA12 is over-expressed in a large percentage of these patients and notably, the over-expression drives migration and invasion of oral cancer cells. Taken together, this information indicates that targeting GNA12 could benefit oral cancer patients by preventing the spread of the disease.

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P127. Expression of erbB, ERK, AKT, c-Fos, c-Jun and NF-kb in oral carcinogenesis golden Syria hamster model with 7,12-dimethylbenz(a)anthracene and alcohol

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Oral squamous cell carcinoma (OSCC) is a complex development process; alcohol and tobacco consumption are the major risk factors related. erbB receptor family is known to be involved in proliferation, apoptosis evasion, angiogenesis and lymphnode metastasis of OSCC. Other molecules involved with this cellular conducts are ERK, AKT, c-Jun, c-Fos and NF-kb. Identifying molecular biomarkers and clinical factors that could provide information regarding prognosis and treatment is important, however, these biomarkers could had different conduct depending the risk factor exposed. The aim of this study was determinate the relationship between 7,12-dimethylbenz(a)anthracene (DMBA) and 15% alcohol with the expression of erbB, ERK, AKT, c-Jun, c-Fos and NF-kb in hyperplasia, dysplasia and invasive carcinomas developed in hamster buccal pouch carcinogenesis model.

Methods: Fifty Syrian golden hamsters were equally divided in three experimental groups and two control groups. The experimental groups B and D were painted with 0.5% DMBA solution three times at week for 14 weeks in right cheek pouch. The experimental groups C and D consume alcohol at 15% for 14 weeks ad libitum. One of the control group (A1) remained untreated while another control (A2) was applied with mineral oil. Tumor frequency, volume, histological condition, immunohistochemistry, Western Blot and Spearman's correlation test for erbB, erk1/2, AKT, c-Jun, c-Fos and NF-kb were performed.

Results: Both control groups no present any clinical or histological alteration, B and D groups developed dysplasia and invasive well differentiated (WD) OSCC, since C group showed normal mucosa and hyperplasia; tumor volume was higher in D group. In similar histologic conditions observe that normal mucosa of C group present increase in erbB2–4, NF-kb, p-ERK, p-AKT than control. Severe dysplasia of B and D group showed difference in erbB3, c-jun and p-ERK; the WD OSCC of above groups showed difference in erbB2–4 and NF-kb. Significant correlation was observed to erbB4 and histologic condition ($p = 0.039$).

Discussion: The expression of erbB2–4 and NF-kb could be associated to risk factor exposure. Today many therapies are directed to erbB inhibition, however, the risk factor associated and the multiple downstream molecular changes are not considered. If we want understand the biological conduct of OSCC a more global vision is necessary.

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P128. High throughput mutational profiling of signaling molecules in oral cancer

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Introduction: It is well established that a plethora of mutations that occur in oncogenes and tumor-suppressor genes are the main drivers of tumorigenesis. The identification of these mutations provides important clues to the cellular processes of carcinogenesis and affords an opportunity for these genes to be used for diagnostic and therapeutic purposes. In comparison to cancers such as breast, lung and colorectal, there is a lack of systematic, large scale characterization of oncogenic mutations oral squamous cell carcinoma (OSCC).

Materials and methods: This study is a high-throughput analysis of 238 somatic mutations across 19 oncogenes in 23 OSCC cell lines and 90 OSCC tissues using the MassARRAY system (Sequenom). The mutations were further confirmed by direct sequencing.

Results: We identified 3 mutations (E545K, Q546R and E542K) in PIK3CA and 1 mutation in HRAS (G13S) in 3/23 cell lines. Likewise, PIK3CA mutations (H1047R, E545K, E542K) we detected in 6/90 patients whilst 3/90 patients carried a HRAS mutation (G13S or G12D). Surprisingly, we did not detect any mutations in the rest of the 17 oncogenes studied here.

Discussion: The results here suggest that PIK3CA and HRAS are important in the development in a subset of OSCC. Given that these mutations have been reported to modulate treatment response and are correlated with prognosis, this has clinical implications for our patients. Notably, mutations within the other 17 genes were not detected. Perhaps mutations in these genes are inherently not present in OSCC or that other mutations besides the ones examined here could be important for OSCC development. Results from this study emphasize the importance of profiling oncogenic mutations in a large pool of OSCC patients as this information has important clinical implications in the management of OSCC patients.

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P129. EZH2 overexpression in nasopharyngeal carcinoma: An independent poor prognosticator that enhances cell growth

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Purpose: As a key component of polycomb-repressive complex 2, EZH2 represses target genes through histone methylation and is frequently overexpressed and associated with poor prognosis in common carcinomas. For the first time, we reported EZH2 expression