O54. Overexpression of macrophage inflammatory protein-3alpha in oral cavity squamous cell carcinoma is associated with nodal metastasis

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Introduction: We examined the role of macrophage inflammatory protein (MIP)-3alpha on oral cavity squamous cell carcinoma (OSCC) and whether it was involved in modulating OSCC cell functions.

Methods: The study population was comprised of 102 patients with OSCC. MIP-3alpha levels in tissues were examined by immunohistochemistry and quantitative real-time RT-PCR. Effects of MIP-3alpha on OSCC cell function were investigated by cell proliferation assays, trans-well migration/invasion assays, and RNA interference.

Results: We found that MIP-3alpha was overexpressed in OSCC tumor cells. MIP-3alpha expression was significantly higher in tumor cells *versus* normal epithelial cells, as determined by both quantitative real-time RT-PCR and immunohistochemistry. Overexpression of MIP-3alpha was significantly correlated with positive pN status ($P = 0.036$). Nevertheless, there were no correlations related to patient age, pT status, overall pathological stage, cell differentiation, or perineural invasion. The long-term disease-specific survival for patient subgroups stratified by the absence or presence of MIP-3alpha overexpression was 70.9% vs. 54.7% ($P = 0.041$). Multivariate analysis indicated that MIP-3alpha overexpression had a significantly lower disease-specific survival (hazard ratio: 2.158; $P = 0.037$). Additionally, *in vitro* suppression of MIP-3alpha expression in OECM-1 cells using specific interfering RNAs attenuated cell migration and invasiveness.

Discussion: These findings suggest that MIP-3alpha overexpression in OSCC is associated with a poorer prognosis for patient survival and contributes to tumor metastasis.

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O55. MDM2 splice variants in oral cancer

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Introduction: MDM2 is a negative regulator of the p53 tumor suppressor protein. The presence of a variety of MDM2 splice variants have been reported in many different types of tumor and some have been shown to be associated with patient prognosis.

Materials and methods: In this study, we demonstrated the occurrence of MDM2 splice variants in OSCC tissues. RNA was extracted from 45 OSCC tissues and 17 normal oral mucosa tissues and reverse transcribed into cDNA. Nested PCR was performed to amplify the MDM2 transcripts and the PCR amplicons were cloned into cloning vector pTZ57R/T and sequenced.

Results: We demonstrated that 37/45 OSCC and 14/17 normal oral mucosa tissues contained MDM2 splice variants and a total of 34 transcript variants were found. Comparing with the reported splice variants in GenBank, four known variants, MDM2B, MDM2C, MDM2-EU2 and MDM2-PM2 were found in tumor samples with MDM2B being the most common variants found in our samples. None of these known variants were found in normal oral mucosa tissues. The other 30 transcript variants were novel, where 3 of these were present in both tumor and normal tissues, 23 were found only in tumor tissues and 4 were exclusive to normal oral mucosa tissue.

Discussion: The common loss among the variants was sequences coding for the p53 binding domain, acidic domain, nuclear localization and nuclear export domains. In general, all 30 novel transcript variants were aberrantly spliced, however, interestingly, 9/23 aberrantly spliced variants in OSCC codes for an in-frame putative protein, suggesting that despite the aberrant splicing, these transcripts may still result in a functional protein and therefore further work to study how these splice variants affect MDM2 function directly or how they may affect other binding partners is needed.

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O56. Genome wide profiling of tongue and cheek cancer using high resolution array based CGH

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Introduction: Tongue and cheek cancer have different behaviors. In order to understand these behaviors, there is a need to look into the chromosomal alterations and gene pathways that maybe associated with oral cancer at these sites. Therefore, the objective of this study is to determine the differences in chromosomal aberrations and gene pathways involved in tongue and cheek cancer using high resolution array CGH.

Methods: A genome wide screening with array CGH (SurePrint G3 CGH 1x1M microarray) was performed using gDNA from 20 snap frozen fresh tissues consisting of 12 tongue and 8 cheek oral squamous cell carcinomas (samples from the Malaysian Oral Cancer Data and Tumour Bank System [MOCDTBS] coordinated by OCRCC-UM). Cytosure Software was used to detect the chromosomal aberrations and candidate genes related to the selected regions. Pathway analysis was done using MetaCore™software for selected genes.

Results: The mean number of chromosomal aberrations per tumour for tongue cancer (22 ± 24.97) was higher than cheek cancer (8.38 ± 11.98). The most common amplified regions in tongue cancers were 8q24.22 (33.33%), 8q24.3 (33.33%), 11q13.1 (33.33%), 11q13.2 (33.33%), 12q13.13 (33.33%), 14q32.33 (33.33%) and for cheek cancer the most common amplified region was 22q12.3 (25%). For the deleted regions, the most common for tongue cancer were 2q21.1 (16.67%), 6q21 (16.67%) and for cheek cancer were 2q22.1 (25%), 7q35 (25%), 19q13.33 (25%). The most significant pathway based on $p < 0.001$ involved in tongue cancer is cell adhesion ECM remodeling pathway Among the genes involved in amplified region, were EGFR, MMP1, MMP10, MMP12, MMP3, MMP7 and MMP9. For cheek cancer, the cancer associated significant pathway was apoptosis and survival (the role of CDK5 in neuronal death and sur-

vival). An oncogene located at 8p12 was identified in this pathway is NRG1.

Discussion: This study showed a different pattern of chromosomal aberrations in tongue and cheek cancer with different significant gene pathways. Tongue cancer behaves more aggressively than cheek cancer which may be due to the involvement of ECM remodeling pathway, where MMP family which are the proteolytic enzyme that degrade various component in the extracellular matrix would favor invasion and metastasis.

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O57. TSG101 downregulates FLJ10540 and modulates migration and invasion of OCSCC via AKT pathway

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Purpose: OCSCC is associated with high rates of recurrence and mortality. Although several well-known markers correlated with poor/metastasis prognosis in OCSCC patients was reported, the molecular mechanisms of OCSCC development are still not clear. Here, we explored TSG101 and its-elicited signaling pathway participates in the metastatic progression of OCSCC.

Methods: The semi-quantitative-RT-PCR/Q-RT-PCR/Western blot/IHC approaches were used to evaluate the mRNA/protein expressions of TSG101 in paired OCSCC specimens. Immunohistochemical staining of TSG101 expression with clinic-patholgic characteristics was examined using univariate and multivariate analyses. Human oral cancer cell lines with overexpressing-TSG101 or TSG101-mediated siRNAs were generated by transfection. Transwell chamber-, Western blot-, pharmacological-inhibitor-, and immunohistochemical- assays were done to evaluate the signaling pathways that were involved.

Results: We created a bioinformatics scheme consisting of integrating two gene expression profile datasets, including un-pairwise OCSCC, and secondary metastatic tumors vs. benign tumors. Among the novel targets identified, TSG101 was down-regulated in OCSCC tissues and is associated with cancer metastasis. Furthermore, we employed two co-expression strategies to identify in which pathway TSG101 was involved. By semi-quantitative-RT-PCR/Q-RT-PCR/Western blot/IHC approaches, we found that TSG101 is not only an indicator of poor survival, but also exhibits negative correlations with FLJ10540 expression in OCSCC specimens. In vitro study, TSG101-overexpressing transfectants could inhibit the motility of cells via suppression FLJ10540/p-AKT expression. Conversely, the motility of oral cancer cells could be increased by knock-down endogenous TSG101. These data indicated that TSG101 prevents oral cancer metastasis via FLJ10540/AKT pathway.

Conclusions: These finding suggested that TSG101 is not only an important prognostic factor but also a new therapeutic target in the FLJ10540/AKT pathway for OCSCC treatment.

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O58. Effects of smoking on oral cancer transcriptome

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Introduction: Oral cancer is a debilitating disease and the survival rates for these patients have not improved over the past decades. Tobacco smoking is one of the most common risk factor associated with oral cancer.

Objectives: To explore and identify differential genes expression associated with tobacco smoking.

Methodology: Next generation sequencing using the Illumina Genome Analyzer was done to sequence five fresh frozen oral cancer tissue samples from smoking patients, two of which were former smoker and an additional 8 samples consisting of normal oral mucosal tissues from the alveolar mucosa of non-cancer patients where 4 were smokers and another 4 were non-smokers. The tissues and socio-demographic information were obtained from the Malaysian Oral Cancer Data and Tumour Bank System (MOCDTBS) at the Oral Cancer Research and Coordinating Centre (OCRCC). High quality poly A+ RNA was extracted from macrodissected tumour and normal epithelial tissue to obtain >70%. Validation of second generation sequencing was done using commercial and custom microarrays. Principle component analysis (PCA) was applied to the sequenced generated data.

Results and discussion: PCA showed distinct clusters separating groups of current and former smokers. The former smoker who ceased smoking >25 years clustered closely with normal non-smoker suggesting expression levels of some of the genes related to tobacco smoking could return to levels similar to never smokers upon cessation of smoking. When comparing differential gene expression between tumor in former and current smokers, ACTC1, MYH2, DES, MYBPH, MYLPF were the top 5 most down-regulated genes. Meanwhile, CYP2W1, FAIM2, OLFML1, KRT13 were found to be up-regulated when comparing differential gene expression between these groups (fold change > 8). Despite prolonged smoking cessation, some gene expression could appear to be permanently altered and these irreversible changes may account for oral cancer risk despite smoking cessation.

Conclusion: These findings illustrate the potential for next generation sequencing to provide insights into the unique gene expression profiles associated with risk habits which will be useful in developing biomarkers for prognostic and therapeutic applications in the future.

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