and its biological and clinical significance in nasopharyngeal carcinoma (NPC).

Materials and methods: In NPC cell lines and specimens, endogenous expression of EZH2 mRNA and protein was determined by semiquantitative RT-PCR and immunoblotting, respectively. To analyze the effect on cell growth, stable silencing of EZH2 was established in EZH2-expressing TW02 NPC cells with RNA interference. EZH2 immunolabeling was assessed for 89 primary NPC biopsies and correlated with clinicopathological variables, disease-specific survival (DSS), and overall survival (OS).

Results: Growth activity of TW02 cells was significantly suppressed (p < 0.001) with stable EZH2 silencing. Compared with normal nasopharyngeal tissue, expression levels of EZH2 transcript and protein were apparently upregulated in NPC specimens. As a continuous variable, higher EZH2 expression preferentially occurred in NPCs of pT3–4 stages (p = 0.03) and significantly predicted inferior DSS (p = 0.0010) and OS (p = 0.004). The prognostic implications for DSS (p = 0.010) and OS (p = 0.006) still remained valid when using the median (60%) of EZH2 immunolabeling index to dichotomize the cohort. In the multivariate model, higher EZH2 expression was an independent adverse factor of both DSS (p = 0.012) and OS (p = 0.011), along with AJCC stages III–IV (p = 0.024 for DSS, p = 0.017 for OS).

Conclusion: At least partly through promoting cell growth, EZH2 implicate cancer progression, confers tumor aggressiveness, and represents an independent adverse prognosticator in patients with NPC.

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P130. The role of GSN expression in the modulation of oral cancer malignant phenotypes and its therapeutic implications

P.-W. Wang a,b, A.-A. Ding a, D.-B. Shieh b,c

a Institute of Basic Medical Sciences, National Cheng Kung University, Tainan, Taiwan
b Institute of Oral Medicine, National Cheng Kung University, Tainan, Taiwan
c National Cheng Kung University Hospital, and NCKU Centre for Micro Nano Science and Technology, Tainan, Taiwan

Gelsolin is a multifunctional actin binding protein responsible for actin polymerization and depolymerization by sequestering actin polymer and nucleate it monomers as well as capping actin filaments. In later study, gelsolin was found to play multiple important cellular functions including cell signaling, motility, apoptosis, proliferation, differentiation, and epithelial mesenchymal transformation (EMT). The association of gelsolin expression present prognostic significance in a wide range of cancer types such as lung, breast, colorectal, prostate, gastric and oral. Previous studies revealed gelsolin and its C-terminal half as a common effector of apoptosis, that forms complex with phosphatidylinositol 4,5-bisphosphate and prevent mitochondrial from losing of membrane potential and releasing of cytochrome C. Our previous study showed the significant influence of gelsolin in apoptotic activity, it is conceivable thus to investigate the possibility of cancer drug resistance and gelsolin activity and the potential disease molecular staging and therapeutic implications. In vitro enrichment of cisplatin resistant head and neck cancer cell line revealed that gelsolin expression is significantly increased in the chemoresistant lines (HONE1-DR) than their parental cells (HONE1). Overexpression of full length gelsolin enhanced survival of oral cancer cell SCC9 and SCC15 also presence the resistant to cisplatin treatment. As n-terminal and c-terminal gelsolin fragments are responsible for caspase-3 cleavage, we constructed DQTN352S mutant of gelsolin that resist caspase-3 cleavage and perform a transfection to the oral cancer cells (SCC15). While overexpression of intact gelsolin (I-GSN) and N-terminus GSN fragment (N-GSN) result in cisplatin resistance in oral cancer cells SCC15, overexpression of the C-terminus GSN fragment (C-GSN), and caspase3 resistance mutant gelsolin (M-GSN) failed to promote cisplatin resistance in the transfected oral cancer cells. It shows that I-GSN resulting cisplatin-resistant was associated with the caspase3 activity by measuring the caspase3/7 activity through Caspase-Glo® 3/7 luminescent assay. These findings support that gelsolin plays important functions in the regulation of chemoresistance in head and neck cancers and suggest the potential targeting of gelsolin as an effective way to overcome chemoresistant cancers.

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P131. Development of an ELISA method for the detection of HPV 16 in oral cancer patients

G.R. Wong a,b, K.O. Ha a,b, W.H. Himratul-Aznita c, S.C. Cheong b,d, R. Saini e, W.M. Wan Mustaffa f, N. Jalil f, L.P. Karen- Ng g, R.B. Zain h

a Oral Cancer Research & Coordinating Centre (OCRCC), Faculty of Dentistry, University of Malaya, Malaysia
b Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, University of Malaya, Malaysia
c Department of Oral Biology, Faculty of Dentistry, University of Malaya, Malaysia
d Oral Cancer Research Team, Cancer Research Initiative Foundation (CARIF), Malaysia
e School of Dental Sciences, Universiti Sains Malaysia, Malaysia
f Oral Health Division, Ministry of Health, Malaysia

Introduction: HPV infection has been associated with a subset of head and neck cancers and current evidence suggest that it may be an important risk factor for oral cancer. Using polymerase chain reaction (PCR) and sequencing, we recently demonstrated the presence of HPV in more than 50% of oral squamous cell carcinoma (OSCC) patients and that high-risk HPV is significantly associated with OSCC. Serological detection of HPV has been reported the most convenient method for detecting HPV. However, currently there is a lack of serological assays for the detection of the HPV. The HPV E6 viral oncprotein is known to play crucial role in tumorigenesis, therefore detecting the presence of the E6 protein could be a useful biomarker for HPV detection.

Methods: A pGEX plasmid containing HPV 16 E6 gene was ligated with KT3 oligonucleotide. Constructed plasmid was then transformed into Escherichia coli for production of the recombinant protein which was used as antigen in ELISA assay. ELISA was optimized using anti KT3 antibody to detect the recombinant antigen. HPV ELISA was performed on sera from 18 healthy and 15 OSCC patients obtained from the Malaysian Oral Cancer Database & Tissue Bank System (MOCDBTS) which is coordinated by Oral Cancer Research & Coordinating Centre (OCRCC). Sera that have net OD above calculated cutoff value were determined as HPV seropositive. Fisher’s Exact test was used for statistical evaluation.

Results: An ELISA method to detect the presence of HPV16 E6 protein was successfully developed. Using this method, 33.3% (5/15) of OSCC and 16.7% (3/18) of healthy patients were found to be HPV 16 seropositive. No significant association was found between HPV 16 seropositivity and OSCC occurrence (P value = 0.428).

Discussion: Although there is a trend to support our previous findings using PCR where a larger proportion of OSCC patients were
P132. MicroRNA profiles of oral squamous cell carcinoma (OSCC) using formalin-fixed, paraffin embedded (FFPE) tissue

M.Y. Siow **a,b, L.P. Karen Ng c, M.T. Abraham c, Z.M. Zain a,b, S.C. Cheong a,b, R.B. Zain a,b

a Oral Cancer Research & Coordinating Center (OCRCC). Faculty of Dentistry. University of Malaya. Malaysia
b Dept. of Oral Pathology, Oral Medicine & Periodontology. Faculty of Dentistry. University of Malaya. Malaysia
c Oral Health Division, Ministry of Health, Malaysia. Malaysia

Introduction: MicroRNAs (miRNAs) have previously been found to be highly tissue- or disease-specific biomarkers with clinical applicability. However, the role of miRNAs in OSCC progression has yet to be well-studied. Here, we demonstrated the use of FFPE specimens in microarray profiling and quantitative real-time PCR (qPCR) approaches to identify miRNAs that are differentially expressed in OSCC compared with non-tumour tissues.

Materials and methods: Four OSCC and four non-tumour FFPE tissues were obtained from the Malaysian Oral Cancer Database and Tumour System (MOCDTS), coordinated by OCRCC-UM and profiled using Agilent 8 × 15 k mammalian miRNA microarray. Based on microarray results, TaqMan miRNA Assay (qPCR) is underway to validate the selected differentially expressed miR-31, miR-7, miR-375 and miR-151-3p with an endogenous control RNU-44 using an independent set of FFPE tissue (seven OSCC and three non-tumour tissues). Biological functions of targeted miRNAs were analyzed using GeneGo Pathway software.

Results: Analysis of profiling identified 19 significantly up-regulated miRNAs (2-fold change) and four significantly down-regulated miRNAs (2-fold change). The targeted miR-31, miR-7 and miR-151-3p were up-regulated whilst miR-375 was down-regulated in OSCC compared with non-tumour tissues. These initial microarray profiling results had been subsequently confirmed in qPCR validation except miR-151-3p, which was validated as down-regulated miRNA.

Discussion: Interestingly, we have identified miRNA targets that can potentially be exploited to differentiate between betel-quid exposure OSCC with no risk habit related OSCC. These miRNA targets were found to interact with protein BuBRI in regulation of cellular component organization (p0.05, GeneGo Pathway software). BuBRI can be modified by arecoline in betel nut leads to abnormal cell cycle progression. Further analyses with larger sample set are on-going to validate the preliminary result and examine if the betel-quid exposures related with OSCC carcinogenesis may be acting through a specific miRNA alterations to lead to a malignant phenotypes. This better understanding of the contribution of miRNA to OSCC may aid in earlier diagnosis, improved prognosis, and novel targets for therapeutic intervention.

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P133. Tissue microarray analysis – A new revolution in diagnosis of oral cancers

K. Gigi Samuel*, S.C. Selvamuthukumar

Sree Balaji Dental College & Hospital. India

Oral carcinoma is a major health care problem worldwide, description of the molecular characterization of oral carcinoma with the use of tissue microarrays approach have yielded heterogeneous results.

With the identification, number of novel markers having diagnostic, prognostic and therapeutic significance application of tissue microarray has become a valuable tool for validating markers in oral cancer research.

Tissue microarray helps to analyze multiple molecular targets simultaneously without causing any morphological alteration to tissue specimen. This will enhance diagnosis, provide better prognosis and will improve cancer treatment for individual patients.

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P134. Osteopontin modulates Aurora-A expression and promotes migration and invasion of OSCC via PI3K/AKT pathway

H.-T. Tsai **a,b, C.-H. Chen c, C.-Y. Chien c

a Department of Otolaryngology, Chang Gung Memorial Hospital–Kaohsiung Medical Center Kaohsiung Taiwan, Taiwan, ROC
b Center for Translational Research in Biomedical Sciences, Chang Gung Memorial Hospital–Kaohsiung Medical Center Kaohsiung Taiwan, Taiwan, ROC
c Kaohsiung Chang Gung Head and Neck Oncology Group, Chang Gung Memorial Hospital–Kaohsiung Medical Center Kaohsiung Taiwan, Taiwan, ROC

Purpose: The function of Aurora-A in oral carcinogenesis is largely unexplored. The aim of this study is to investigate the role of Aurora-A in human oral cancer development, to examine the expression levels of Aurora-A in oral carcinomas, and to correlate the results with clinico-pathologic variables and survival.

Methods: The semiquantitative RT-PCR/western blot and IHC approaches were used to evaluate the RNA and protein expression of Aurora-A in paired OSCC patients' specimens. Immunohistochemistry analysis of Aurora-A expression was assessed in 256 OSCC patients who underwent tumor resection between 1996 and August 2005 without previous radiotherapy. Results were correlated with clinico-pathologic characteristics using univariate and multivariate analyses. Human oral cancer cell lines with overexpressing Aurora-A or Aurora-A-mediated siRNAs to repress endogenous Aurora-A were generated by transfection to further elucidate the mechanism of Aurora-A in oral cancer cell lines.

Results: Here, we showed that the mRNA and protein levels of Aurora-A were significantly up-regulated in OSCC compared to adjacent non-cancerous tissues by semiquantitative RT-PCR, western blot and IHC. The cumulative 5-year survival rate was significantly correlated with a relatively advanced tumor stage, positive nodal status, TNM stage, and strong expression of Aurora-A. Among the novel targets identified, Aurora-A was overexpressed in OSCC tissues and is associated with cancer metastasis. By Q-RT-PCR and IHC approaches, we found that Aurora-A exhibits positive correlations with osteopontin/p-AKT in OSCC specimens. Stimulation of cells with osteopontin results in (1) an increase in Aurora-A protein expression, (2) promoting Aurora-A-expressing oral cancer cell metastasis, and (3) an enhance in AKT activity, further supporting the participation of Aurora-A in the osteopontin/PI3K/AKT pathway.

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