Title: Salivary DNA methylation of DAPK-1, RASSF1a and micro RNA 9 as biomarkers for head and neck cancer.

Kai Yen Lim [(1)], Yunxia Wan [(1)], Dmitry A. Ovchinnikov [(2)], Chris Perry [(3)], William B Coman [(4)], Paul D Slowey [(5)] and Chamindie Punyadeera [(1)(*)].

[(1)] The University of Queensland Diamantina Institute, The University of Queensland, Level 6, TRI, 37 Kent St, Woolloongabba QLD 4102, [(2)] The Australian Institute for Bioengineering and Nanotechnology [(3)] Princess Alexandra Hospital, Woolloongabba, QLD [(4)] The School of Medicine, [(5)] Oasis Diagnostics® Corporation, USA.

Abstract:

With an increasing recognition of the link between oral and systemic disease, attention has turned to saliva as an alternative diagnostic medium for a diverse array of health conditions [1]. Compared with blood, saliva collection is non-invasive, easy sampling with multiple sampling opportunities, does not need pre-processing and is ideal for 3rd world countries [2]. It is well established that tumour cells secrete biomolecules into the saliva [3]. Head and neck squamous cell carcinoma (HNSCC) encompasses a diverse group of aggressive tumours. HNSCC is the most distressing and disfiguring tumour for the patient to endure, for health workers to manage, and families to cope with. HNSCC patients, particularly those with a history of smoking, often develop secondary tumours. Currently, there are no diagnostic tests to detect these cancers at an early stage; as such, most patients present with metastatic disease at the time of diagnosis (regional nodal involvement in 43% and distant metastasis in 10%), leading to 5-year survival rates of less than 60% [4]. DNA methylation and microRNAs (miRNAs) are the most extensively studied epigenetic biomarkers in HNSCC [5]. We collected saliva (resting saliva and buccal swabs, DNA•SAL™) from HNSCC patients and healthy controls and interrogated CpG hypermethylation events in tumour suppressor genes using a sensitive methylation-specific PCR (MSP) assay. *RASSF1a*, *DAPK1* and *p16*, showed an overall specificity of 87% and sensitivity of 80%. The test panel performed extremely well in the detection of the early stages of HNSCCs, with a sensitivity of 94% and specificity of 87%, and a high κ value of 0.8, indicating an excellent overall agreement between the presence of HNSCC and a positive MSP panel result. In addition, miR-9 and miR-191 provided a good discriminative ability with AUC values of 0.76 and 0.73 respectively ($p<0.01$) for discriminating HNSCC patients from healthy controls. In conclusion, we demonstrate that salivary DNA methylation and miRNA biomarkers are clinically useful in detecting HNSCC in a non-invasive manner.