Transcriptional profiling of oral squamous cell carcinoma using formalin-fixed paraffin-embedded samples

A. Saleh a,b, R.B. Zain a, H. Hussaini c, F. Ng d, V. Tanavde a, S. Hamid a, A.T. Chow b, G.S. Lim b, M.T. Abraham e, S.H. Teo a,f, S.C. Cheong a,b,g

a Oral Cancer Research Team, Cancer Research Initiatives Foundation (CARIF), 2nd Floor Outpatient Centre, Sime Darby Medical Centre, 47500 Subang Jaya, Selangor, Malaysia
b Oral Cancer Research and Coordinating Centre and Faculty of Dentistry, University of Malaya, Kuala Lumpur, Malaysia
c Department of Oral Pathology and Oral Medicine, Faculty of Dentistry, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia
d Genome and Gene Expression Analysis Group, Bioinformatics Institute, Agency for Science Technology and Research (A*STAR), Singapore
e Oral Health Division, Ministry of Health, Federal Government Administrative Centre, Putrajaya, Malaysia
f Department of Surgery, University Malaya Medical Centre, Kuala Lumpur, Malaysia
g Department of Oral Maxillofacial Surgery, Faculty of Dentistry, University Malaya, Kuala Lumpur, Malaysia

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Despite the advances in cancer treatment, the 5-year survival rate for oral cancer has not changed significantly for the past 40 years and still remains among the worst of all anatomic sites. Gene expression microarrays have been used successfully in the identification of genetic alterations in cancer development, however, these have hitherto been limited by the need for specimens with good quality intact RNA. Here, we demonstrated the use of formalin-fixed paraffin-embedded tissues in microarray experiments to identify genes differentially expressed between cancerous and normal oral tissues. Forty-three tissue samples were microdissected and gene expression analyses were conducted using the Illumina DASL assay. We report RNA yield of 2.4 and 0.8 μg/mm² from tumour and normal tissues, respectively and this correlated directly with the tissue volume used for RNA extraction. Using unsupervised hierarchical clustering, distinct gene expression profiles for tumour and normal samples could be generated, and differentially expressed genes could be identified. The majority of these genes were involved in regulation of apoptosis and cell cycle, metastasis and cell adhesion including BCL2A1, BIRC5, MMP1, MMP9 and ITGB4. Representative genes were further validated in independent samples suggesting that these genes may be directly associated with oral cancer development. The ability to conduct microarrays on formalin-fixed paraffin-embedded specimens represents a significant advancement that could open up avenues for finding genes that could be used as prognostication and predictive tools for cancer.

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Introduction

It is well established that cancer is driven by a plethora of genetic changes that confer cancer traits to cells such as growth advantage, evasion of apoptosis, sustained angiogenesis and tissue invasion and metastasis. Notably, gene expression profiling using microarrays has been used successfully to identify genes that are differentially expressed between cancer and normal cells, leading to the identification of subtypes of cancers and the development of new methods for the diagnosis, prognosis and therapy of cancer. However, until recently, microarray experiments have been limited by the requirement for high quality RNA, typically extracted from fresh frozen samples. Unfortunately, fresh frozen samples are not always readily available and need to be collected prospectively from patients at clinical centres unless tissue banks with readily available samples are accessible. Despite the effort in systematic banking of tissues for research, prospective collection of patient samples particularly from rare diseases will limit their immediate use. Moreover, the use of fresh frozen tissues particular- larly to look at patient outcome is limited by the availability of clinical and follow-up data associated with the patient. By comparison, formalin-fixed paraffin-embedded (FFPE) tissues are abundant as they are processed and stored routinely in clinical practice and depending on the tissue selection, information on various disease stage associated with these patients can be correlated with molecular findings immediately. Traditionally, FFPE samples are not utilized in microarray experiments due to the chemical modification of nucleic acid by formalin resulting in poor RNA quality. However more recently, technological advances have...