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Gene expression profiling reveals biological pathways responsible for phenotypic heterogeneity between UK and Sri Lankan oral squamous cell carcinomas

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SUMMARY

Objectives: It is well recognized that oral squamous cell carcinoma (OSCC) cases from Asia that are associated with betel quid chewing are phenotypically distinct to those from Western countries that are predominantly caused by smoking/drinking, but the molecular basis of these differences are largely unknown. The aim of this study is to examine gene expression, related carcinogenic pathways and molecular processes that might be responsible for the phenotypic heterogeneity of OSCC between UK and Sri Lankan population groups.
Methods: We have compared the gene expression profiles of OSCCs and normal oral mucosal tissues from both Sri Lankan and UK individuals using Affymetrix gene expression arrays. The generated data was interrogated using significance analysis of microarrays and Ingenuity Pathway Analysis (IPA).
Results: The gene expression profiles of UK and Sri Lankan OSCC are similar in many respects to other oral cancer expression profiles reported in the literature and were mainly similar to each other. However, genes involved in tumor invasion, metastasis and recurrence were more obviously associated with UK tumors as opposed to those from Sri Lanka.
Conclusion: The development of OSCCs in both UK and Sri Lankan populations appears largely mediated by similar biological pathways despite the differences related to race, ethnicity, lifestyle, and/or exposure to environmental carcinogens. However, IPA revealed a highly activated "Cell-mediated Immune Response" in Sri Lankan normal and tumor samples relative to UK cohorts. It seems likely, therefore, that any future attempts to personalize treatment for OSCC patients will need to be different in Western and Asian countries to reflect differences in gene expression and the immune status of the patients.

Introduction

Oral squamous cell carcinoma (OSCC) is a major component of a diverse group of neoplasms often referred to as 'head and neck cancer'. Tobacco use and/or alcohol consumption are the two principal risk factors involved in development of head and neck squamous cell carcinoma (HNSCC) [1–3]. OSCC is the most common form of cancer in Sri Lanka [4], accounting for 25% of all cancers in males (in females it accounts for 9% of all cancers and is the fourth most common cancer). By contrast, the incidence of OSCC in Western countries is much lower, accounting for 1–2% of cancer diagnoses [5]. The development of OSCC in Sri Lanka is associated with chewing betel quid containing areca nut [6,7]. Such tumors are phenotypically different from those seen in Western countries

Abbreviations: IPA, Ingenuity Pathway Analysis; qRT-PCR, quantitative real time PCR; OSCCs, oral squamous cell carcinomas; HNSCCs, head and neck squamous cell carcinoma; H&E, haematoxylin and eosin; AJCC/UICC TNM, American Joint Committee on Cancer/Union for International cancer control, Tumour, Nodal, Metastasis; cDNA, complementary DNA; cRNA, complementary RNA; SAM, Significance Analysis for Microarrays; ESTs, expressed sequence tags; qPCR, quantitative PCR; FDR, False discovery rate; AA, arachidonic acid; FC, fold change; SCC, squamous cell carcinoma; ECM, extra-cellular matrix; HCC, hepatocellular carcinoma.

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and clinically display an exophytic "cauliflower" appearance; these differences have significant clinical importance because OSCCs in Sri Lanka rarely metastasize to the loco-regional lymph nodes of the neck [8] and are generally less aggressive than that seen in Western countries, including the UK. In developed countries, potentially malignant lesions are identifiable in only a minority of cases and most oral cancers arise from clinically normal mucosa. These cancers are more aggressive and have a poorer prognosis than those arising in an area of tobacco induced leukoplakia [2,9].

The identification of genes involved in the pathogenesis of OSCCs constitutes a substantial progress in the development of diagnostic markers and/or more effective therapeutic strategies, particularly in the current era of precision medicine. The genetic alterations that drive the development and progression of OSCC are starting to be elucidated, but the disease is characterized by significant clinical and genetic heterogeneity [10]. A considerable number of gene expression microarray studies investigating OSCC in Western populations have been published (60 studies reviewed here) [11], though relatively few studies have reported OSCC cases from Asian countries [12–15], which could be attributed to the cost of the technology or access to suitable clinical material.

In the present study, we observed significant tumor heterogeneity between the samples from the two populations, where OSCC in patients from UK were highly aggressive and metastasising, with early recurrence (less than 12 months) and had poor survival relative to Sri Lankan cohorts (Tables 1 and 2). We have compared the gene expression profiles of OSCCs and normal oral mucosal tissues from both Sri Lankan and UK individuals and attempted to correlate the findings with clinico-pathological variables. Additionally, we have compared the profile of deregulated genes between UK and Sri Lankan OSCCs.

Materials and methods

Sample characteristics and biopsy specimens

All biopsy specimens of OSCC and normal oral mucosa were harvested with appropriate ethical approval and written informed consent for participation in the study was obtained from participants (South Birmingham Research Ethics Committee 0769, UK. Kandy General Hospital and University of Peradeniya Ethical Committee, Sri Lanka). Identical protocols for tissue collection and processing were used in both countries. OSCC samples were obtained from sequential incident cases treated by a single consultant surgeon from 2001 to 2004 at University Hospital of Birmingham, NHS Foundation Trust, Birmingham, UK, and Kandy General Hospital, Kandy, Sri Lanka. A total of 24 UK and 27 Sri Lankan samples yielded RNA of sufficient quality and quantity for microarray analysis (Tables 1 and 2). In addition, 11 normal oral mucosa specimens (seven samples from UK & four samples from Sri Lankan population) were also profiled (Table 3). All normal samples were from non-smokers, who did not chew betel quid and did not consume in excess of the national recommended weekly gender allowance of alcohol. Normal samples were taken from individuals with no history of cancer and had no first degree relatives with a history of cancer.

All tumor and normal samples were processed together in Birmingham and confirmed to be from HPV negative individuals (using Gp5+/Gp6+ primers method; Gp5+: TTT GTT ACT GTG GTA GAT ACT AC, Gp6+: GAA AAA TAA ACT GTA AAT CAT ATT C). Tumor samples were histopathologically confirmed as squamous cell carcinoma and staged according to the AJCC/UICC TNM Classification system. Tumors were pathologically graded into poor/moderate or well differentiated tumor stage.

Gene expression profiling

Each tumor was confirmed histopathologically to contain $\geqslant$60% tumor tissues and <10% necrotic debris by analysis of corresponding H&E sections. RNA was extracted and purified using the TRIzol (Invitrogen) method with RNeasy® (Qiagen) method for RNA purification and was labeled and hybridized to Affymetrix GeneChips using the manufacturer's instructions (Supplementary file 1). Tumor samples of both UK and Sri Lanka were analyzed using the Affymetrix® Human Genome Focus array. Normal samples of both countries were analyzed using the Affymetrix® Human

Table 1
Patients and tumor related factors in the UK cohort of oral cancers.

Study no.	Sex	Age	Site	Pathological staging	Differentiation	Early recurrence	Perineural invasion	Smoking	Heavy alcohol consumption	BALT/snuff	Lymphocyte Infiltration
OCS 001 C	M	59	Buccal	T4N2bMx	Moderate	Yes	No	Smoker	No	None	Moderate
OCS 003 C	F	72	Alveolus	T4N2bMx	Poor	No	No	None	No	Oral Snuff	Dense
OCS 004 C	F	67	Alveolus	T4N0Mx	Moderate	Yes	Yes	None	No	BALT	Moderate
OCS 006 C	F	53	Tongue	T1N0Mx	Moderate	No	No	None	No	None	Dense
OCS 007 C	F	67	Palate	T4N0Mx	Well/moderate	No	No	Smoker	No	None	Dense
OCS 008 C	F	65	Alveolus	T4N0Mx	Moderate	Yes	No	None	No	None	Moderate
OCS 011 C	F	49	Tongue	T1N0Mx	Well/moderate	No	No	Smoker	No	None	Moderate
OCS 012 C	F	72	Tongue	T4N2aMx	Poor	Yes	No	Smoker	Yes	None	Moderate
OCS 013 C	M	43	FOM	T1N0Mx	Moderate	No	No	Smoker	No	None	Moderate
OCS 014 C	M	46	Tongue	T2N2bMx	Poor/moderate	No	Yes	Smoker	Yes	None	Moderate
OCS 015 C	M	51	Tongue	T4N0Mx	Moderate	Yes	Yes	None	No	None	Moderate
OCS 016 C	M	66	Tongue	T2N2bMx	Poor/moderate	Yes	Yes	Smoker	Yes	None	Moderate
OCS 019 C	F	73	Alveolus	T2N0Mx	Well	No	No	None	No	Oral Snuff	Moderate
OCS 020 C	M	64	Retromolar	T4N0Mx	Well	No	No	Smoker	No	None	Dense
OCS 021 C	M	58	FOM	T2N0Mx	Poor/moderate	No	No	Smoker	Yes	None	Moderate
OCS 022 C	F	65	Tongue	T2N0Mx	Well/moderate	No	No	Smoker	Yes	None	Moderate
OCS 023 C	M	70	Alveolus	T2N2bMx	Moderate	No	No	Smoker	No	None	Dense
OCS 024 C	F	68	Alveolus	T2N1Mx	Moderate	Yes	Yes	Smoker	No	None	Moderate
OCS 025 C	F	79	Buccal	T2N0Mx	Well	No	No	None	No	None	NA
OCS 026 C	M	37	FOM	T1N0Mx	Moderate	No	No	Smoker	Yes	None	Mild
OCS 027 C	M	66	FOM	T2N0Mx	Moderate	No	No	Smoker	No	None	Moderate
OCS 029 C	M	52	FOM	T4N0Mx	Moderate	No	No	Smoker	Yes	None	Moderate
OCS 031 C	M	67	FOM	T4N0Mx	Moderate	Yes	No	Smoker	Yes	None	Mild
OCS 032 C	M	60	Retromolar	T2N0Mx	Moderate	No	Yes	Smoker	No	None	Moderate

BALT; Betel quid chewing contents was classified as: Betel nut (B); areca nut (A); lime (L); and Tobacco (T).

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