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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).

Table 2
Patients and tumor related factors in Sri Lankan cohort of oral cancers.

Study no.	Sex	Age	Site	Pathological staging	Differentiation	Early recurrence	Perineural invasion	Smoking	Heavy alcohol consumption	BALT	Lymphocyte Infiltration
KC01	M	72	Soft palate	T2NxMx	Well	No	No	Yes	No	BALT	Moderate
KC02	M	50	Retromolar	T2NxMx	Moderate	Yes	No	Yes	No	BALT	Mild
KC04	M	66	Buccal	T1NxMx	Well/ moderate	No	No	No	No	BALT	Moderate
KC07	M	50	Alveolus	T2NxMx	Moderate	No	No	No	No	BALT	NA
KC09	F	54	Tongue	T1NxMx	Well	No	No	No	No	BALT	Mild
KC13	M	73	Buccal	T2NxMx	Well	No	No	No	No	BALT	NA
KC15	F	40	Tongue	T2NxMx	Moderate	No	No	No	No	No	NA
KC16	F	71	FOM	T2NxMx	Moderate	No	No	Yes	No	BALT	Mild
KC17	M	48	Alveolus	T2NxMx	Well	No	No	Yes	No	BALT	NA
KC19	M	76	Buccal	T2NxMx	Moderate	No	No	Yes	Yes	BALT	Mild
KC20	M	76	FOM	T1NxMx	Well	No	No	Yes	Yes	BALT	Moderate
KC21	F	56	Retromolar	T2NxMx	Well	No	No	No	No	BALT	Moderate
KC24	F	55	Buccal	T1NxMx	Moderate	No	No	No	No	BALT	NA
KC25	M	74	Buccal	T2NxMx	Moderate	No	No	Yes	No	BALT	NA
KC26	F	85	Alveolus	T2NxMx	Moderate	No	No	No	No	BALT	Mild
KC29	M	55	Buccal	T2NxMx	Well	No	No	Yes	Yes	BALT	Mild
KC31	M	95	Buccal	T2NxMx	Well/ moderate	No	No	No	No	BALT	Moderate
KC32	M	52	Buccal	T2NxMx	Well	No	No	Yes	Yes	BALT	Mild
KC38	M	51	Alveolus	T2NxMx	Moderate	No	No	Yes	Yes	BALT	Dense
KC39	M	64	Alveolus	T3NxMx	Moderate	No	No	Yes	Yes	BALT	Mild
KC41	M	82	Tongue	T3NxMx	Moderate	No	No	Yes	No	No	Mild
KC44	M	42	Palate	T3NxMx	Well	No	No	No	No	BALT	Mild
KC45	M	58	Tongue	T2NxMx	Moderate	No	No	Yes	Yes	BALT	Mild
KC46	M	38	Tongue	T2NxMx	Moderate	No	No	Yes	Yes	BALT	Dense
KC47	M	50	Buccal	T2NxMx	Well	No	No	No	No	BALT	NA
KC51	M	78	Buccal	T2NxMx	Moderate	No	No	No	No	BALT	NA
KC53	M	60	Buccal	T3NxMx	Well	No	No	Yes	No	BALT	NA

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

Genome U133 Plus2.0 Array. The probesets that were identically represented on both U133 Plus2.0 and Focus array GeneChip were used for comparative analysis.

Data quality control, pre-processing and analysis

Data analysis was performed using Bioconductor packages [16], http://www.bioconductor.org/). The simpleaffy package was used to assess the data quality of the GeneChips and 6 GeneChips (representing 3 tumor samples and 3 normal samples) of poor quality were removed, leaving 48 tumors and 8 normal arrays for subsequent analyses (details in Supplementary file 1B). Normalization was performed using *gcRMA*. Non-specific filtering was conducted using the function *rowSds* in the *genefilter* package. Data from this study have been deposited in the NCBI Gene Expression Omnibus database under accession no. GSE51010.

Unsupervised clustering was performed and heatmaps generated using Pearson correlation hierarchical clustering in R. Supervised analysis was carried out using the Significance Analysis for Microarrays (SAM) [17] in *siggenes* package available from Bioconductor. Heatmaps were generated using *gplots* package [16].

Biological pathway analysis of significant genes

Ingenuity Pathway Analysis 9.0, IPA, (Ingenuity®Systems, www.Ingenuity.com) was used to highlight the most affected Molecular and Cellular functions, canonical pathways, Diseases and Disorders, Transcriptional Regulators, and most significant pre-generated networks associated with the uploaded group of genes including those related to OSCC development. The database for annotation, visualization, and integrated discovery (DAVID) tool [18,19] was used for conversion of gene identifiers.

Quantitative real-time PCR analysis

A combined thermal cycler and fluorescence detector (Perkin–Elmer Applied Biosystems 7700 sequence detector) was used for quantitative real-time PCR (qPCR) using 4 replicates for

Table 3
Sample information of normal individuals from both UK and Sri Lanka.

Study no.	Sex	Age	Site	Smoking	Heavy Alcohol consumption[b]	Other Habits
M1[a]	M	22	Buccal	No	No	No
M2[a]	F	24	Alveolus	No	No	No
M3[a]	M	23	Buccal	No	No	No
M4[a]	F	20	Alveolus	No	No	No
V2[a]	F	21	Palate	No	No	No
V5[a]	M	20	Tongue	No	No	No
V6[a]	M	26	Tongue	No	No	No
KN1	M	26	Buccal	No	No	No
KN2	F	29	Alveolus	No	No	No
KN3	F	25	Buccal	No	No	No
KN5	M	24	Tongue	No	No	No

[a] UK samples.
[b] Not consume the exceeding national recommended weekly gender allowance of alcohol.

Table 4
Significantly differentially expressed genes discriminating UK tumor from normal samples.[a]

Gene symbol	Gene ID	Fold change	Gene symbol	Gene ID	Fold change
Genes higher expressed in OSCCC			*Genes lower expressed in OSCC*		
MMP1	204475_at	4.4	CRNN	220090_at	−5.7
MMP3	205828_at	3.2	MAL	204777_s_at	−5.3
HMGA2	208025_s_at	2.9	KRT13	207935_s_at	−5.1
BMP15	221332_at	2.3	KLF5	209211_at	−4.9
REL	206035_at	2.3	SCEL	206884_s_at	−4.6
POSTN	210809_s_at	2.2	CLCA4	220026_at	−4.5
CXCL10	204533_at	2.2	TGM3	206004_at	−4.4
MMP10	205680_at	2.2	EMP1	201324_at	−4.2
SPP1	209875_s_at	2	CLEC3B	205200_at	−4.2
GRIN1	205914_s_at	1.9	RHCG	219554_at	−4.2
LAMC2	202267_at	1.9	CD24	208650_s_at	−3.7
IFI6	204415_at	1.7	SERPINB13	211362_s_at	−3
SLC6A2	215715_at	1.7	CDKN2C	204159_at	−2.9
COL11A1	37892_at	1.6	FGFR2	208228_s_at	−2.8
CXCL11	210163_at	1.5	CLU	208792_s_at	−2.8
PTGS2	204748_at	1.5	CRABP2	202575_at	−2.7
TNC	201645_at	1.5	EHF	219850_s_at	−2.6
			HSP90AB1	214359_s_at	−2.5
			YES1	202933_s_at	−2.5
			CDKN1B	209112_at	−2.5
			IGFBP2	202718_at	−2.5
			RPS6KB1	204171_at	−2.4
			SMAD4	202527_s_at	−2.3
			JUP	201015_s_at	−2.3
			VEGFA	210512_s_at	−2.2
			FGFR3	204379_s_at	−2.2
			HRAS	212983_at	−2.1
			CRYAB	209283_at	−2.1
			DUSP1	201041_s_at	−2.1
			RAD21	200608_s_at	−2
			RAF1	201244_s_at	−1.9
			HSPA1A//HSPA1B	202581_at	−1.9
			KRT19	201650_at	−1.9
			CDH1	201131_s_at	−1.8
			INS–IGF2	202409_at	−1.8
			WNT5A	205990_s_at	−1.8
			ID2	201565_s_at	−1.8
			PTPRC	212588_at	−1.8
			MAPK9	203218_at	−1.7
			MAPK13	210058_at	−1.7
			TNFSF10	202688_at	−1.7
			KRT5	201820_at	−1.7
			EZH2	203358_s_at	−1.7
			TP53	201746_at	−1.7
			RB1	203132_at	−1.5

All genes in the table are previously reported to be related to OSCC and HNSCC.
[a] FDR value <0.005. The complete lists of upregulated and downregulated genes in OSCCs were presented in Supplementary File S2.

each sample. TaqMan® Gene Expression Assays for each gene was purchased from Applied Biosystems (Foster City, CA, USA). Primer sequences used for validation by quantitative real-time PCR were CLU(GAAGAAAGAGGATGCCCTAAATGAG), PTPN13 (ATTCATTCTCC ACGGTTGCTGGGGA) and MAL (GGTCACCTTGGACGCAGCCTACCA).

Results

Gene expression profiles of OSCC versus normal oral mucosa

A total of 59 genes were upregulated and 866 genes (FDR level $\leqslant$0.005 and fold change ±1.5) were downregulated in OSCC compared to normal oral mucosa from UK individuals (Supplementary file 2) including several potential diagnostic biomarkers previously identified to be involved in OSCC and HNSCC (Table 4). Genes associated with tumor invasion, metastasis, and recurrence were significantly differentially expressed in UK tumor relative to normal samples including upregulation of MMP3, PTGS2/COX2, LAMC2, POSTN, COL11A1, and TNC along with downregulation of DUSP1 and SLPI. Ingenuity pathway analysis revealed 'tumorigenesis', 'cellular growth', 'proliferation', 'transformation',' movement', 'migration', 'invasion', 'metastasis', and 'ECM degradation' as functional groups represented by the differentially expressed genes (Supplementary file 3).

Comparing OSCC and normal mucosa from Sri Lankan individuals identified 2070 differentially expressed genes (FDR level $\leqslant$0.005 and fold change ±1.5), including 98 genes upregulated and 1972 genes downregulated in OSCC compared to normal mucosa (Supplementary file 4). Genes differentially expressed in Sri Lankan tumors also included potential biomarkers involved in OSCC and HNSCC (Table 5). These tumors displayed elevated expression of CD80, IL1RL1, GML, CYP27B1, SCN10A, HOXC10, and SH2D2A along with reduced expression of TWIST1, RECK, PSPH, GNG11, LSM7, and ERAP2 relative to their normal comparators. IPA Functional Analysis tool revealed 'cell cycle progression', 'tumor development and colony formation', 'epithelial cell proliferation', and 'cell ploidy and mitosis' as functional groups represented by the differentially expressed genes (Supplementary file 5).

Table 5
Significantly differentially expressed genes discriminating Sri Lankan tumor from normal samples.[a]

Gene symbol	Gene ID	Fold change	Gene symbol	Gene ID	Fold change
Genes higher expressed in OSCCC than normal					
HMGA2	208025_s_at	2.8	SLC6A2	215715_at	1.9
REL	206035_at	2.8	SPP1	209875_s_at	1.9
CXCL10	204533_at	2.8	CXCL11	210163_at	1.8
BMP15	221332_at	2.6	RARB	205080_at	1.8
SCN10A	208578_at	2.2	OPRM1	211359_s_at	1.8
MMP1	204475_at	2.1	MMP10	205680_at	1.7
CHRM2	221330_at	1.9	CNR2	206586_at	1.5
Genes lower expressed in OSCC than normal					
CLEC3B	205200_at	−5.3	CRYAB	209283_at	−2.4
CRNN	220090_at	−5.2	ID2	201565_s_at	−2.4
KLF5	209211_at	−4.8	TGFBR3	204731_at	−2.4
MAL	204777_s_at	−4.8	CASP4	209310_s_at	−2.3
KRT13	207935_s_at	−4.7	FGFR1	211535_s_at	−2.3
SCEL	206884_s_at	−4.3	TP53	201746_at	−2.3
CLCA4	220026_at	−4.2	BAG1	211475_s_at	−2.2
TGM3	206004_at	−4.1	EHF	219850_s_at	−2.1
CDKN2C	204159_at	−4	MAPK9	203218_at	−2.1
CLU	208792_s_at	−4	PTPRC	212588_at	−2.1
EMP1	201324_at	−4	RB1	203132_at	−2.1
PDGFRA	203131_at	−4	EZH2	203358_s_at	−2
RHCG	219554_at	−4	LGALS7	206400_at	−2
TWIST1	213943_at	−4	PTCH1	209815_at	−2
CD24	208650_s_at	−3.7	RAF1	201244_s_at	−2
SERPINB13	211362_s_at	−3.3	SOD2	216841_s_at	−2
INS–IGF2	202409_at	−3.1	LGALS3	208949_s_at	−1.9
HSPA1A//HSPA1B	200800_s_at	−3	PARP1	208644_at	−1.9
IGFBP2	202718_at	−3	BCL2	203685_at	−1.8
RPS6KB1	204171_at	−3	CDC42	208728_s_at	−1.8
KRT19	201650_at	−2.9	SMARCB1	212167_s_at	−1.8
SMAD4	202527_s_at	−2.9	WNT5A	205990_s_at	−1.8
HRAS	212983_at	−2.8	MAPK13	210058_at	−1.7
CDKN1B	209112_at	−2.7	LGALS1	201105_at	−1.7
VEGFA	210512_s_at	−2.7	PIK3CA	204369_at	−1.7
RAD21	200608_s_at	−2.6	TIMP1	201666_at	−1.7
YES1	202933_s_at	−2.6	TNFRSF10B	209295_at	−1.7
CRABP2	202575_at	−2.5	CCND1	208712_at	−1.6
HSP90AB1	214359_s_at	−2.5	JUP	201015_s_at	−1.6
NR3C1	216321_s_at	−2.5	COL1A2	202403_s_at	−1.5

All genes in the table are previously reported to be related to OSCC and HNSCC.
[a] FDR value <0.005. The complete lists of upregulated and downregulated genes in OSCCs were presented in Supplementary File S4.

Table 6
The common significantly differentially expressed genes discriminating tumor from normal samples of both UK and Sri Lankan population groups.[a]

Gene symbol	Gene ID	Fold change	Gene symbol	Gene ID	Fold change
Genes higher expressed in OSCC than normal					
MMP1	204475_at	3.2	REL	206035_at	2.3
MAGEA3	209942_x_at	2.9	CXCL10	204533_at	2.2
HMGA2	208025_s_at	2.8	AIM2	206513_at	2.1
BMP15	221332_at	2.3	SPP1	209875_s_at	2
Genes lower expressed in OSCC than normal					
CRNN	220090_at	−5.7	SMAD4	202527_s_at	−2.6
MAL	204777_s_at	−5.4	CDKN1B	209112_at	−2.6
KRT13	207935_s_at	−5.2	CRABP2	202575_at	−2.5
KLF5	209211_at	−5	INS–IGF2	202409_at	−2.5
SCEL	206884_s_at	−4.7	HRAS	212983_at	−2.5
CLCA4	220026_at	−4.6	VEGFA	210512_s_at	−2.5
TGM3	206004_at	−4.5	Rgs5	209071_s_at	−2.5
EMP1	201324_at	−4.3	KRT19	201650_at	−2.4
CLEC3B	205200_at	−4.2	EHF	219850_s_at	−2.3
RHCG	219554_at	−4.2	NR3C1	216321_s_at	−2
FAP1	204201_s_at	−4.0			
CD24	208650_s_at	−3.8	ID2	201565_s_at	−2
CDKN2C	204159_at	−3.5	TP53	201746_at	−2
CLU	208792_s_at	−3.4	CASP4	209310_s_at	−1.9
SERPINB13	211362_s_at	−3.2	SOD2	216841_s_at	−1.8
HSPA1A//HSPA1B	200800_s_at	−3	LGALS3	208949_s_at	−1.8
IGFBP2	202718_at	−2.7	TNFSF10	202688_at	−1.7

The gene list represents the common existed genes expressed as significant between OSCC and normal samples in both population groups at FDR level < 0.005. All genes in the table are previously reported to be related to OSCC and HNSCC.
[a] FDR value <0.005. The complete lists of upregulated and downregulated genes in OSCCs were presented in Supplementary File S6.

Commonly differentially expressed genes between tumor and normal samples from both UK and Sri Lankan patients

Out of the total number of significantly differentially expressed genes, 91.4% (846 genes, 807 downregulated and 39 upregulated) were found to be de-regulated in tumors from both populations (Supplementary file 6). Of the common significant genes, there were a number of potential biomarkers previously reported for OSCC and HNSCC (Table 6). IPA analysis revealed potential tumor-related transcription regulators consistently perturbed in both UK and Sri Lankan populations (MYC, NFE2L2, NR3C1, and TP53). Moreover, tumor related pathways (including fatty acid metabolism followed by glycolysis/gluconeogenesis, citrate cycle, NRF2-mediated oxidative stress response, EIF2 signaling, glucocorticoid receptor signaling, xenobiotic metabolism signaling pathways) were found commonly significant between tumor and normal samples from the two population groups.

Validation of differential gene expression

Three genes downregulated in both UK and Sri Lankan tumors (CLU, MAL, PTP313/FAP1) were selected for quantitative PCR validation. The genes were selected because they have previously been identified as tumor suppressor genes and we found them to play significant roles in the biological pathways and key nodes of the networks. Gene expression levels were compared between normal oral mucosa samples taken from 4 Sri Lankan individuals and 5 UK individuals with that in 12 UK and 12 Sri Lankan OSCCs (representative of patient and tumor characteristics). The qPCR data were repeated and confirmed over four separate rounds of experimental determination which consistently confirmed the expression array findings that these three genes are downregulated in OSCC compared to normal in both UK and Sri Lankan samples (Fig. 1).

Gene expression differences between UK and Sri Lankan tumor samples

It was much more difficult to discriminate between UK and Sri Lankan tumor samples than between tumor and normal samples from each country. Only 21 genes were differentially expressed between the 27 Sri Lankan and 21 UK OSCCs using SAM at FDR $\leqslant$ 0.05 (Supplementary file 7A). A slightly better discrimination was achieved at FDR $\leqslant$ 0.01 with 12 differentially expressed genes (Supplementary file 7B).

The considerable smaller number of differentially expressed genes between UK and Sri Lankan OSCC is evidence that there are greater gene expression differences between tumor and normal samples from either country than between tumors from the two populations. IPA identified activated immune response functions in Sri Lankan relative to UK tumors, including activation, development, differentiation, cell movement, migration, and chemotaxis of T, B-lymphocytes, leukocytes, monocytes, and other immune system cells.

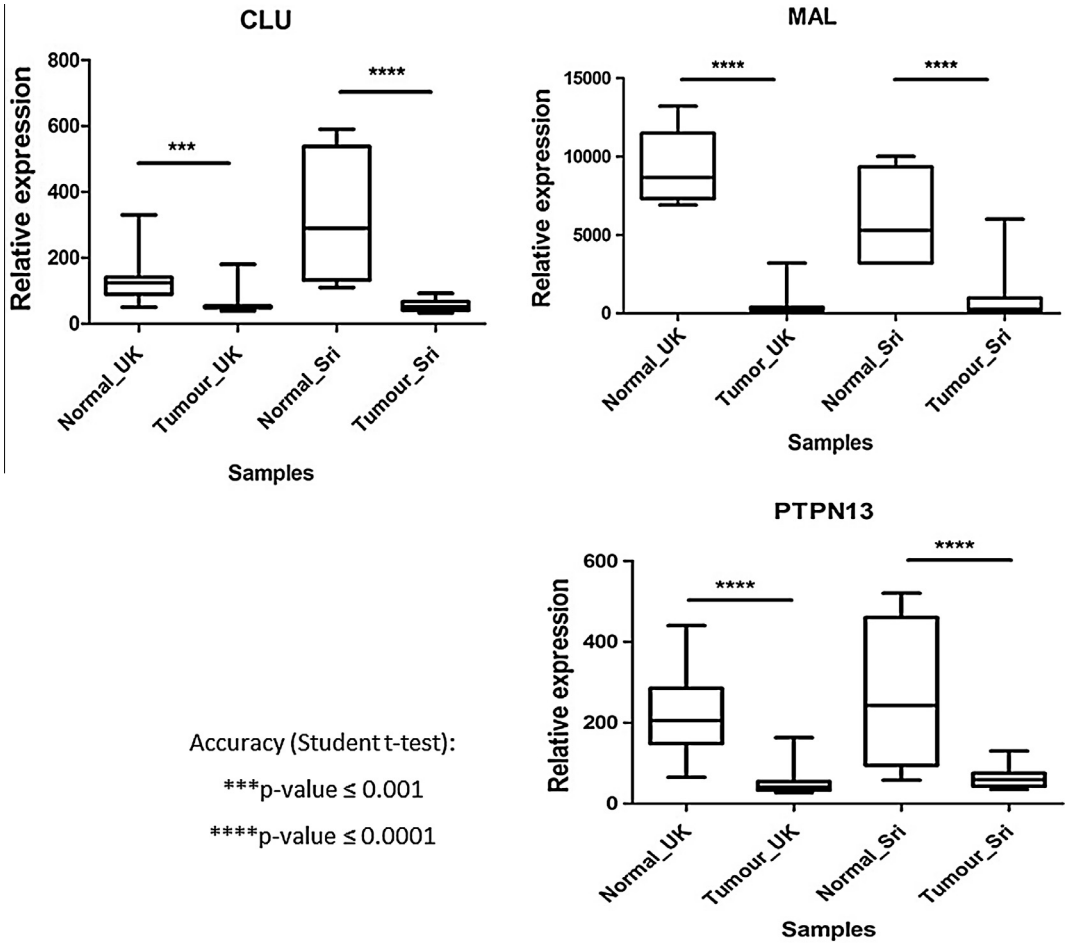

Fig. 1. Box plots displaying results of qPCR analysis for the genes, CLU, MAL, and PTPN13 chosen for validation. $^{**}P$-value $\leqslant$ 0.01, $^{***}P$-value $\leqslant$ 0.001, $^{****}P$-value $\leqslant$ 0.0001 compared tumour to normal samples determined by using unpaired Student *t*-test.

Table 7
Top upregulated and downregulated genes differentially expressed between normal samples of both UK and Sri Lankan groups.[a]

Gene symbol	Gene ID	Fold change	Gene symbol	Gene ID	Fold change
Genes higher expressed in Sri Lankan than UK normal samples					
PHACTR2	244774_at	4.2	LOC399959	232113_at	2.9
SCARA5	229839_at	3.7	POSTN	210809_s_at	2.9
CTHRC1	225681_at	3.5	RECK	205407_at	2.8
ITGBL1	205422_s_at	3.3	P2RY14	206637_at	2.8
IGJ	212592_at	3.3	BCAT1	226517_at	2.8
PSPH	205048_s_at	3.3	COL3A1	232458_at	2.8
FLJ40330	1569040_s_at	3.2	FERMT2	209210_s_at	2.8
GLT8D2	227070_at	3.2	TFPI	213258_at	2.8
FAM46C	226811_at	3.2	LY96	206584_at	2.8
ZBTB38	225512_at	3.1	SLC25A32	221020_s_at	2.8
PLN	204939_s_at	3.1	TYRP1	205694_at	2.8
MGP	202291_s_at	3	MGC29506	223565_at	2.8
FBN1	202766_s_at	3	LAMB1	201505_at	2.8
C12ORF23	224759_s_at	3	TMEM47	209656_s_at	2.8
C13ORF15	218723_s_at	3	LY75//CD302	203799_at	2.8
MAP1B	226084_at	2.9	POSTN	1555778_a_at	2.7
ANGPTL1	231773_at	2.9	IKBIP	227295_at	2.7
DCLK1	229800_at	2.9	EBF1	227646_at	2.7
EVI2B	211742_s_at	2.9	SELM	226051_at	2.7
ERAP2	219759_at	2.9			
MALAT1	223940_x_at	−4.1	ATP6V0D2	1553153_at	−2.2
Genes lower expressed in Sri Lankan than UK normal samples					
MALAT1	224568_x_at	−4	ZNF664	1569935_at	−2.2
EGFR	1565484_x_at	−3.6	SNORA71A	1568249_at	−2.1
EGFR	1565483_at	−3.6	WHAMML2	1557450_s_at	−2.1
PLEKHA5	1558695_at	−3	CUEDC1	1555786_s_at	−2.1
MALAT1	223578_x_at	−2.9	LOC100192378	1559966_a_at	−2.1
NEAT1	238320_at	−2.8	HNRNPD	236000_s_at	−2.1
DEFB106A//B	1552411_at	−2.8	TRIM4	224159_x_at	−2.1
FAM120A	1555944_at	−2.7	NBPF1	214693_x_at	−2
LCE1E	1559224_at	−2.6	NEAT1	214657_s_at	−2
LOC285708	1564281_at	−2.5	CLIP4	242710_at	−2
NEAT1	227062_at	−2.4	EHF	241397_at	−2
CADM3	216535_at	−2.4	MALAT1	227510_x_at	−2
HIF3A	222124_at	−2.3			

All genes in the table are previously reported to be related to OSCC and HNSCC.
[a] FDR ⩽ 0.01. The complete lists of upregulated and downregulated genes in Sri Lankan normal relative to UK normal samples were presented in Supplementary File S8.

Gene expression differences between UK and Sri Lankan normal mucosa

Despite a relatively small number of samples ($n = 8$), a large number of genes (1439, ±1.5 fold change, and FDR levels of ⩽0.01) were differentially expressed when comparing UK and Sri Lankan normal tissues (Supplementary file 8). Increasing the stringency of the SAM approach to FDR level ⩽0.005 reduced the profile to only 10 upregulated and 5 downregulated genes in Sri Lankan compared to UK normal mucosa (Supplementary File 9B). Further confirmation of the discrimination power was obtained by running both supervised and unsupervised analysis using Pearson correlation comparisons (Supplementary file 9A). Among the genes discriminating normal samples of Sri Lanka from UK, we identified biomarkers related to various types of cancer (Table 7). Biomarker analysis (IPA tool), identified vital biomarkers related to OSCC and HNSCC including upregulation of COL1A1, COL4A1, H3F3A/B, BCL2, FGF2, ATM, IGF1, MGMT, FZD7, LGALS1, SPARKL1, CXCL12, TIMP3, MIB1, NBN, NTRK2, and TMEM38B along with downregulation of EGFR and IGF1R in Sri Lankan relative to UK normal controls.

Furthermore, oncogenes with an elevated expression were observed in normal samples from Sri Lanka compared to those from the UK population; BCL2, RUNX1, AXL, ERBB2, RAS, MYC, HTERT, KIT, IGF1, MGMT, TRK, and SRC oncogenes as examples, plus other genes strongly involved in tumorigenesis like POSTN, RECK, COL1A2, COL1A3, COL5A2, WISP1, AGR2. Perhaps surprisingly, elevation of oncogenes was accompanied with downregulation of oncogene receptors, including EGFR, IGF1R, RARA, EPOR, THRA, and NMUR2. Additionally, overexpression of genes coding selenoproteins in normal samples of Sri Lanka was observed including SELM, SELK, SEPP1, and SELT.

IPA functional analysis revealed that differentially expressed genes discriminating Sri Lankan from UK normal samples are predominantly involved in processes such as cell mediated immune response, antigen presentation, cell differentiation, small molecule biochemistry, cell-to-cell signaling and interaction, binding of phagocytes and B-lymphocytes, elevated quantity, development and migration of lymphatic system cells, adhesion of immune cells, cell movement of T-lymphocyte, leukocytes, phagocytes, monocytes, neutrophils, mast cells, T-cell development and differentiation (Fig. 2).

Discussion

It is well recognized that OSCCs in Asian countries tend to be more exophytic and less aggressive than their Western counterparts. However, whilst there have been numerous studies reporting gene expression profiles of Western head and neck tumors, there are relatively few studies using tissues of Asian origin. Therefore, we have attempted to determine the profile of gene expression differences in OSCCs from the UK and Sri Lanka by comparison with relevant control tissues from the respective countries. The gene expression profiles of UK and Sri Lankan OSCC compared to normal oral mucosa were mainly similar to each other. Greater differences in the expression profiles were observed between OSCC and normal tissue than between OSCCs or normal

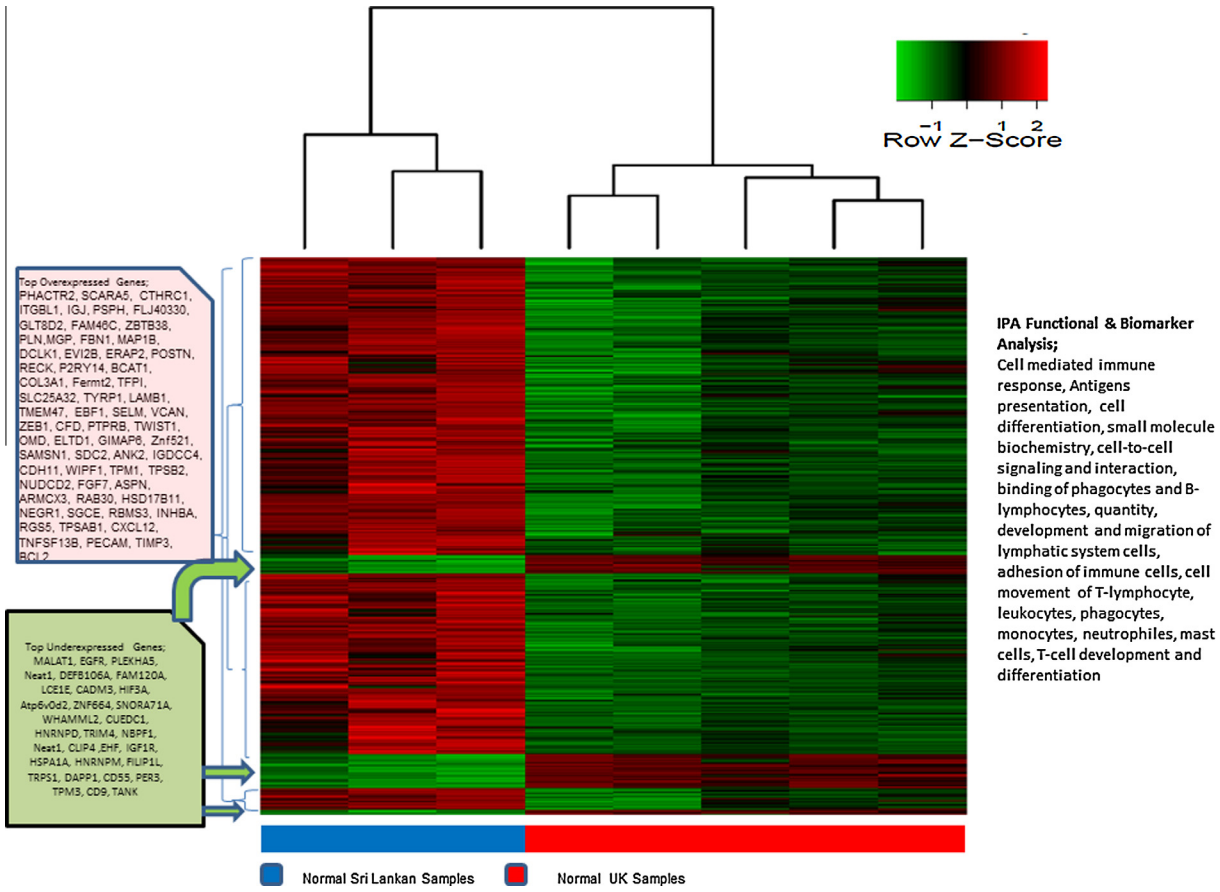

Fig. 2. Hierarchical clustering of 1439 differentially expressed genes separating 3 Sri Lankan and 5 UK normal oral mucosal samples. Red and blue bars represent UK and Sri Lankan normal samples, respectively. Heatmap represents mean-centered expression of genes in rows and samples in columns. Expression (red = high, green = low, black = not changed) is represented on a log2 mean-centered scale. All gene expression values were either above or below fold change magnitudes equal to ±1.5 at FDR ⩽ 0.01 using SAM supervised analysis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

controls from the two populations, with a considerable number of these changes being in common with previous reports. In addition, potential tumor-related transcription regulators were consistently perturbed in tumors of both UK and Sri Lankan populations relative to their normal comparators (MYC, NFE2L2, NR3C1, and TP53). In agreement with previous work, our data indicates that the development of OSCCs is mediated mostly by similar biological pathways despite any differences related to race, ethnicity, lifestyle, and/or exposure to environmental carcinogens [12]. However, certain molecular results noted to have an impact on the phenotypic differences observed between UK and Sri Lankan OSCCs.

Genes associated with tumor invasion, metastasis, and recurrence were significantly differentially expressed in UK but not Sri Lankan tumors including the upregulation of MMP3, PTGS2, LAMC2, POSTN, COL11A1, and TNC along with downregulation of DUSP1 and SLPI. Likewise, significant deregulation of other genes in Sri Lankan, but not UK tumor samples have been reported to play a role in preventing tumor invasion and metastasis (elevated expression of CD80, IL1RL1, GML, CYP27B1, SCN10A, HOXC10, and SH2D2A along with reduced expression of TWIST1, RECK, PSPH, GNG11, LSM7, and ERAP2) [20–54]. Activation of ESR1, SMAD3, and SMAD4 transcription regulators in UK tumors relative to their normal comparators might also facilitate the invasive phenotypic behavior of UK tumors [55–57].

In the present study, we sought to determine whether any of the gene expression changes in Sri Lankan OSCCs might explain the less aggressive behavior of the tumors. Curcumin (diferuloylmethane) has been consumed by Sri Lankan people for centuries as a frequent dietary component [58]; curry and turmeric were described as anti-tumor diets which boost immune surveillance against tumor progression [59,60]. It is possible that curcumin could play a role in the activated immune response functions identified in Sri Lankan OSCCs. Indeed, previous studies on HNSCC suggested that peritumoral lymphocyte infiltration was associated with better prognosis when compared with tumors not exhibiting immune infiltration [61–65]. Results of an additional study [66] demonstrated marked absence of immune response signature in the tumors suggesting that modulation of tumor-specific immune responses may play a role in local treatment failure in HNSCC.

In addition, curcumin has been reported to downregulate various growth regulatory pathways and specific genetic targets including genes for NFκB, STAT3, PTGS2, AKT, anti-apoptotic proteins, and growth factor receptors (as seen in the expression profile of normal tissue from Sri Lankan individuals). The protective effects of curcumin are thought to be mediated through its ability to induce the activation of NRF2 (which was highly activated in Sri Lankan normals relative to both UK normals and Sri Lankan tumors), the expression of antioxidant enzymes (e.g., hemeoxygenase-1 and glutathione peroxidase), increase glutathione, directly quench free radicals, and inhibit p300 HAT activity [67]. Lastly, a number of genes coding selenoproteins were overexpressed in normal tissue from Sri Lankan individuals (including SELM, SELK, SEPP1, SELT) which may be evidence of the cancer protective effect of selenium, which is especially associated with glutathione peroxidase and thioredoxin reductase enzymes [68–70].

In conclusion, the gene expression profiles of UK and Sri Lankan OSCC compared to normal oral mucosa are similar in many respects to other oral cancer expression profiles reported in the literature and were mainly similar to each other. There were greater differences in the profiles of gene expression between OSCC and normal tissue than between OSCCs of the two populations. IPA showed common perturbed canonical pathways which indicate that development of OSCCs is mediated, to some extent by similar biological pathways despite the differences related to race, ethnicity, lifestyle, and/or exposure to environmental carcinogens. However, genes involved in tumor invasion, metastasis and recurrence were more obviously associated with UK tumors as opposed to those from Sri Lanka, which likely reflect their more aggressive nature. Similarly, a highly activated cell-mediated immune response in both Sri Lankan normal and tumor samples relative to UK cohorts, may in part, explain the less aggressive behavior of these betel quid-induced OSCCs. 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. Boosting the immune response of cancer patients, especially from western countries should be considered in future clinical studies. Curcumin and selenium in addition to other nutritional and antioxidant supplements could create a part of the proposed regime.

Conflict of interest statement

None declared.

Author's contributions

The study was conceived by VL, ICP, SSP and PGM. VL carried out the tissue procurement, mRNA isolation/amplification, microarray hybridizations, and participation of data interpretation. AAS provided the bioinformatics analysis and with VL the subsequent interpretation of the data. AHS supervised the bioinformatics and helped draft the manuscript. All authors contributed to writing the manuscript and have read and approved the final version.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.oraloncology.2014.12.004.

References

[1] Moore SR, Johnson NW, Pierce AM, Wilson DF. The epidemiology of mouth cancer: a review of global incidence. Oral Dis 2000;6(2):65–74.
[2] Sankaranarayanan R, Masuyer E, Swaminathan R, Ferlay J, Whelan S. Head and neck cancer: a global perspective on epidemiology and prognosis. Anticancer Res 1998;18(6B):4779–86.
[3] Franceschi S, Bidoli E, Herrero R, Munoz N. Comparison of cancers of the oral cavity and pharynx worldwide: etiological clues. Oral Oncol 2000;36(1):106–15.
[4] Siriwardena BSMS, Tilakaratne A, Amaratunga EAPD, Udagama MNGPK, Ogawa I, Kudo Y, et al. Analysis of histopathological and immunohistochemical differences of oral squamous cell carcinoma in young and old patients in Sri Lanka. J Oral Pathol Med 2007;36(6):357–62.
[5] Thompson L. World Health Organization classification of tumours: pathology and genetics of head and neck tumours. Ear Nose Throat J 2006;85(2):74.
[6] Muir CS, Kirk R. Betel, tobacco, and cancer of the mouth. Br J Cancer 1960;14(4):597.
[7] Ranasinghe AW, Warnakulasuriya K, Johnson NW. Low-prevalence of expression of P53 oncoprotein in oral carcinomas from Sri-Lanka associated with betel and tobacco chewing. Oral Oncol – Eur J Cancer Part B 1993;29B(2):147–50.
[8] Wardbooth RP, Gabriel A, Tanner NSB. Unexpectedly low-frequency of lymph-node spread in oral-cancer in Sri-Lanka. Lancet 1982;1(8264):165.
[9] Sankaranarayanan R, Mathew B, Varghese C, Sudhakaran PR, Menon V, Jayadeep A, et al. Chemoprevention of oral leukoplakia with vitamin A and beta carotene: an assessment. Oral Oncol 1997;33(4):231–6.
[10] Leemans CR, Braakhuis BJM, Brakenhoff RH. The molecular biology of head and neck cancer. Nat Rev Cancer 2011;11(1):9–22.
[11] Lallemant B, Evrard A, Chambon G, Sabra O, Kacha S, Lallemant JG, et al. Gene expression profiling in head and neck squamous cell carcinoma: clinical perspectives. Head Neck 2010;32(12):1712–9.
[12] Dysvik B, Vasstrand EN, Lovlie R, Elgindi OA, Kross KW, Aarstad HJ, et al. Gene expression profiles of head and neck carcinomas from Sudanese and Norwegian patients reveal common biological pathways regardless of race and lifestyle. Clin Cancer Res 2006;12(4):1109–20.
[13] Ibrahim SO, Aarsaether N, Holsve MK, Kross KW, Heimdal JH, Aarstad JH, et al. Gene expression profile in oral squamous cell carcinomas and matching normal oral mucosal tissues from black Africans and white Caucasians: the case of the Sudan vs Norway. Oral Oncol 2003;39(1):37–48.
[14] Suhr ML, Dysvik B, Bruland O, Warnakulasuriya S, Amaratunga AN, Jonassen I, et al. Gene expression profile of oral squamous cell carcinomas from Sri Lankan betel quid users. Oncol Rep 2007;18:1061–75.
[15] Ambatipudi S, Gerstung M, Pandey M, Samant T, Patil A, Kane S, et al. Genome-wide expression and copy number analysis identifies driver genes in gingivobuccal cancers. Genes Chromosom Cancer 2012;51(2):161–73.
[16] Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, et al. Bioconductor: open software development for computational biology and bioinformatics. Genome Biol 2004;5(10):R80.
[17] Tusher VG, Tibshirani R, Chu G. Significance analysis of microarrays applied to the ionizing radiation response (vol. 98, pg 5116, 2001). Proc Natl Acad Sci USA 2001;98(18):10515.
[18] Huang DW, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 2009;37(1):1–13.
[19] Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009;4(1):44–57.
[20] Wen J, Nikitakis NG, Chaisuparat R, Greenwell-Wild T, Gliozzi M, Jin W, et al. Secretory leukocyte protease inhibitor (SLPI) expression and tumor invasion in oral squamous cell carcinoma. Am J Pathol 2011;178(6):2866–78.
[21] Tomasi ML, Ramani K, Lopitz-Otsoa F, Rodríguez MS, Li TWH, Ko K, et al. S-adenosylmethionine regulates dual-specificity mitogen-activated protein kinase phosphatase expression in mouse and human hepatocytes. Hepatology 2010;51(6):2152–61.
[22] Jones FS, Jones PL. The tenascin family of ECM glycoproteins: structure, function, and regulation during embryonic development and tissue remodeling. Dev Dyn 2000;218(2):235–59.
[23] Ziober AF, Patel KR, Alawi F, Gimotty P, Weber RS, Feldman MM, et al. Identification of a gene signature for rapid screening of oral squamous cell carcinoma. Clin Cancer Res 2006;12(20):5960–71.
[24] Schmalbach CE, Chepeha DB, Giordano TJ, Rubin MA, Teknos TN, Bradford CR, et al. Molecular profiling and the identification of genes associated with metastatic oral cavity/pharynx squamous cell carcinoma. Arch Otolaryngol – Head Neck Surg 2004;130(3):295–302.
[25] Kudo Y, Siriwardena BSMS, Hatano H, Ogawa I, Takata T. Periostin: novel diagnostic and therapeutic target for cancer. Histol Histopathol 2007;22(10):1167–74.
[26] Yan W, Shao R. Transduction of a mesenchyme-specific gene periostin into 293T cells induces cell invasive activity through epithelial–mesenchymal transformation. J Biol Chem 2006;281(28):19700–8.
[27] Siriwardena BSMS, Kudo Y, Ogawa I, Kitagawa M, Kitajima S, Hatano H, et al. Periostin is frequently overexpressed and enhances invasion and angiogenesis in oral cancer. Br J Cancer 2006;95(10):1396–403.
[28] Shao R, Bao SD, Bai XF, Blanchette C, Anderson RM, Dang TY, et al. Acquired expression of periostin by human breast cancers promotes tumor angiogenesis through up-regulation of vascular endothelial growth factor receptor 2 expression. Mol Cell Biol 2004;24(9):3992–4003.

[29] Bao S, Ouyang G, Bai X, Huang Z, Ma C, Liu M, et al. Periostin potently promotes metastatic growth of colon cancer by augmenting cell survival via the Akt/PKB pathway. Cancer Cell 2004;5(4):329–39.
[30] Ouyang G, Liu M, Ruan K, Song G, Mao Y, Bao S. Upregulated expression of periostin by hypoxia in non-small-cell lung cancer cells promotes cell survival via the Akt/PKB pathway. Cancer Lett 2009;281(2):213–9.
[31] Sasaki H, Yu C-Y, Dai M, Tam C, Loda M, Auclair D, et al. Elevated serum periostin levels in patients with bone metastases from breast but not lung cancer. Breast Cancer Res Treat 2003;77(3):245–52.
[32] Sasaki H, Lo KM, Chen LB, Auclair D, Nakashima Y, Moriyama S, et al. Expression of periostin, homologous with an insect cell adhesion molecule, as a prognostic marker in non-small cell lung cancers. Jpn J Cancer Res 2001;92(8):869–73.
[33] Sasaki H, Sato Y, Kondo S, Fukai I, Kiriyama M, Yamakawa Y, et al. Expression of the periostin mRNA level in neuroblastoma. J Pediatr Surg 2002;37(9):1293–7.
[34] Sasaki H, Auclair D, Fukai I, Kiriyama M, Yamakawa Y, Fujii Y, et al. Serum level of the periostin, a homologue of an insect cell adhesion molecule, as a prognostic marker in nonsmall cell lung carcinomas. Cancer 2001;92(4):843–8.
[35] Hensen EF, De Herdt MJ, Goeman JJ, Oosting J, Smit VT, Cornelisse CJ, et al. Gene-expression of metastasized versus non-metastasized primary head and neck squamous cell carcinomas: a pathway-based analysis. BMC Cancer 2008;8:168.
[36] Mohan S, Epstein JB. Carcinogenesis and cyclooxygenase: the potential role of COX-2 inhibition in upper aerodigestive tract cancer. Oral Oncol 2003;39(6):537–46.
[37] Wang Z. The role of COX-2 in oral cancer development, and chemoprevention/treatment of oral cancer by selective COX-2 inhibitors. Curr Pharm Des 2005;11(14):1771–7.
[38] Zhi HY, Zhang J, Hu GX, Lu JY, Wang XQ, Zhou CN, et al. The deregulation of arachidonic acid metabolism-related genes in human esophageal squamous cell carcinoma. Int J Cancer 2003;106(3):327–33.
[39] Kosmehl H, Berndt A, Strassburger S, Borsi L, Rousselle P, Mandel U, et al. Distribution of laminin and fibronectin isoforms in oral mucosa and oral squamous cell carcinoma. Br J Cancer 1999;81(6):1071–9.
[40] Malinoff HL, McCoy JP, Varani J, Wicha MS. Metastatic potential of murine fibro-sarcoma cells is influenced by cell-surface laminin. Int J Cancer 1984;33(5):651–5.
[41] Hintermann E, Quaranta V. Epithelial cell motility on laminin-5: regulation by matrix assembly, proteolysis, integrins and erbB receptors. Matrix Biol 2004;23(2):75–85.
[42] Liotta LA, Wewer U, Rao NC, Schiffmann E, Stracke M, Guirguis R, et al. Biochemical mechanisms of tumor invasion and metastases. Prog Clin Biol Res 1988;256:3–16.
[43] Jeng JH, Ho YS, Chan CP, Wang YJ, Hahn LJ, Lei D, et al. Areca nut extract up-regulates prostaglandin production, cyclooxygenase-2 mRNA and protein expression of human oral keratinocytes. Carcinogenesis 2000;21(7):1365–70.
[44] Grosch S, Tegeder I, Niederberger E, Brautigam L, Geisslinger G. COX-2 independent induction of cell cycle arrest and apoptosis in colon cancer cells by the selective COX-2 inhibitor celecoxib. FASEB J 2001;15(14):2742–4.
[45] Dannenberg AJ, Altorki NK, Boyle JO, Dang C, Howe LR, Weksler BB, et al. Cyclo-oxygenase 2: a pharmacological target for the prevention of cancer. Lancet Oncol 2001;2(9):544–51.
[46] Brown WA, Skinner SA, Malcontenti-Wilson C, Vogiagis D, O'Brien PE. Non-steroidal anti-inflammatory drugs with activity against either cyclooxygenase 1 or cyclooxygenase 2 inhibit colorectal cancer in a DMH rodent model by inducing apoptosis and inhibiting cell proliferation. Gut 2001;48(5):660–6.
[47] Nelson AR, Fingleton B, Rothenberg ML, Matrisian LM. Matrix metalloproteinases: biologic activity and clinical implications. J Clin Oncol 2000;18(5):1135.
[48] Masferrer JL, Leahy KM, Koki AT, Zweifel BS, Settle SL, Woerner BM, et al. Antiangiogenic and Antitumor activities of cyclooxygenase-2 inhibitors. Cancer Res 2000;60(5):1306–11.
[49] Mohammed SI, Knapp DW, Bostwick DG, Foster RS, Khan KNM, Masferrer JL, et al. Expression of cyclooxygenase-2 (COX-2) in human invasive transitional cell carcinoma (TCC) of the urinary bladder. Cancer Res 1999;59(22):5647–50.
[50] Itoh S, Matsui K, Furuta I, Takano Y. Immunohistochemical study on overexpression of cyclooxygenase-2 in squamous cell carcinoma of the oral cavity: its importance as a prognostic predictor. Oral Oncol 2003;39(8):829–35.
[51] Gallo O, Masini E, Bianchi B, Bruschini L, Paglierani M, Franchi A. Prognostic significance of cyclooxygenase-2 pathway and angiogenesis in head and neck squamous cell carcinoma. Hum Pathol 2002;33(7):708–14.
[52] Wiegand S, Dunne AA, Muller HH, Mandic R, Barth P, Davis RK, et al. Metaanalysis of the significance of matrix metalloproteinases for lymph node disease in patients with head and neck squamous cell carcinoma. Cancer 2005;104(1):94–100.
[53] Ye S, Eriksson P, Hamsten A, Kurkinen M, Humphries SE, Henney AM. Progression of coronary atherosclerosis is associated with a common genetic variant of the human stromelysin-1 promoter which results in reduced gene expression. J Biol Chem 1996;271(22):13055–60.
[54] Gallo O, Franchi A, Magnelli L, Sardi I, Vannacci A, Boddi V, et al. Cyclooxygenase-2 pathway correlates with VEGF expression in head and neck cancer. Implications for tumor angiogenesis and metastasis. Neoplasia 2001;3(1):53–61.
[55] Tchatchou S, Jung A, Hemminki K, Sutter C, Wappenschmidt B, Bugert P, et al. A variant affecting a putative miRNA target site in estrogen receptor (ESR) 1 is associated with breast cancer risk in premenopausal women. Carcinogenesis 2009;30(1):59–64.
[56] Pradhan M, Prasad N, Palakal M. A systems biology approach to the global analysis of transcription factors in colorectal cancer. BMC Cancer 2012;12(1):331.
[57] Pradhan M, Nagulapalli K, Palakal M. Cliques for the identification of gene signatures for colorectal cancer across population. BMC Syst Biol 2012;6(Suppl. 3):S17.
[58] Mishra S, Palanivelu K. The effect of curcumin (turmeric) on Alzheimer's disease: an overview. Ann Indian Acad Neurol 2008;11(1):13–9.
[59] Pirasath S, Thayaananthan K, Balakumar S, Arasaratnam V. Effect of dietary curries on the glycaemic index. Ceylon Med J 2010;55(4):118–22.
[60] Jayasekera R, Freitas MC, Araujo MF. Bulk and trace element analysis of spices: the applicability of k_0-standardization and energy dispersive X-ray fluorescence. J Trace Elem Med Biol 2004;17(4):221–8.
[61] Wolf GT, Hudson JL, Peterson KA, Miller HL, McClatchey KD. Lymphocyte subpopulations infiltrating squamous carcinomas of the head and neck: correlations with extent of tumor and prognosis. Otolaryngol Head Neck Surg 1986;95(2):142–52.
[62] Hiratsuka H, Imamura M, Kasai K, Kamiya H, Ishii Y, Kohama G, et al. Lymphocyte subpopulations and T-cell subsets in human oral cancer tissues: immunohistologic analysis by monoclonal antibodies. Am J Clin Pathol 1984;81(4):464–70.
[63] Guo M, Rabin BS, Johnson JT, Paradis IL. Lymphocyte phenotypes at tumor margins in patients with head and neck-cancer. Head Neck Surg 1987;9(5):265–71.
[64] Hirota J, Ueta E, Osaki T, Ogawa Y. Immunohistologic study of mononuclear cell infiltrates in oral squamous cell carcinomas. Head Neck 1990;12(2):118–25.
[65] Slootweg PJ, Depagter M, Deweger RA, Dewilde PCM. Lymphocytes at tumor margins in patients with head and neck-cancer – relationship with tumor size, human lymphocyte antigen molecules, and metastasis. Int J Oral Maxillofac Surg 1994;23(5):286–9.
[66] Ginos MA, Page GP, Michalowicz BS, Patel KJ, Volker SE, Pambuccian SE, et al. Identification of a gene expression signature associated with recurrent disease in squamous cell carcinoma of the head and neck. Cancer Res 2004;64(1):55–63.
[67] Goel A, Aggarwal BB. Curcumin, the golden spice from Indian saffron, is a chemosensitizer and radiosensitizer for tumors and chemoprotector and radioprotector for normal organs. Nutr Cancer – Int J 2010;62(7):919–30.
[68] Gromadzinska J, Reszka E, Bruzelius K, Wasowicz W, Akesson B. Selenium and cancer: biomarkers of selenium status and molecular action of selenium supplements. Eur J Nutr 2008;47(Suppl. 2):29–50.
[69] Yoo MH, Xu XM, Carlson BA, Gladyshev VN, Hatfield DL. Thioredoxin reductase 1 deficiency reverses tumor phenotype and tumorigenicity of lung carcinoma cells. J Biol Chem 2006;281(19):13005–8.
[70] de Sousa Almondes KG, da Silva Leal GV, Franciscato Cozzolino SM, Philippi ST, de Carvalho Rondo PH. The role of selenoproteins in cancer. Rev Assoc Med Bras 2010;56(4):484–8.