Appendix A

Sociodemographics of OSCC patients where FFPE specimens were obtained and details of the RNA quantity and quality.

No	Sam-ples	Block#	Diagnosis	Site	Age of sample month	Tissue dep-th mm	Area mm^2	Tum-our volume mm^3	RNA yield ng/ul	Total RNA yield ng	Ct differ-ence	% genes with sig-nificant signal	Pattern of differ-entiation	Age	Gender	Race	Habits	Staging
1	B01	D273/06 C	OSCC	B	24	0.04	39	1.6	128.1	3842.4	12	77.7	Moderate	49	F	Indian	BQ	T4N0M0
2	B02	D119/06(vi)b	OSCC	B	28	0.06	37.8	2.3	208.7	6260.1	14	61.4	Well to moderate	61	F	Malay	No Habit	T1N0M0
3	B03	D474/04- A (VII)a	OSCC	B	45	0.04	41	1.6	74.2	2224.5	10	72.9	Moderate	50	F	Indian	BQ	T4N1M0
4	B04	D108/06	OSCC	B	28	0.04	11.9	0.5	70	2099.1	12	73.7	Moderate	66	M	Indian	NA	Missing data
5	B05	20/04	OSCC	B	54	0.1	13.1	1.3	112.1	3363.3	12	78.1	Moderate	57	F	Indian	NA	T4 N1 Mx
6	B06	520/05	OSCC	B	32	0.1	33	3.3	403.1	12091.5	11	87.8	Moderate	73	F	Indian	Smoke, BQ	T2N0M0
7	B07	154/03	OSCC	B	60	0.1	19.2	1.9	266.6	7997.1	12	90	NA	67	L	Chinese	Smoke, Alcohol	T4 N2a Mx
8	B08	76/06	OSCC	B	29	0.1	41.9	4.2	446.8	13403.7	11	90.4	Moderate	74	F	Indian	BQ	T4 N2c Mx
9	B09	296/06	OSCC	B	24	0.1	24.4	2.4	285.3	8560.2	10	88.4	Well	67	F	Indian	Alcohol, BQ	T4 N1 Mx
10	B10	237/06	OSCC	B	24	0.1	37	3.7	144.2	4326.3	11	81.5	Well	69	F	Indian	BQ	T4 N1 Mx
11	B11	190/06	OSCC	B	26	0.1	52.4	5.2	890.1	26703.6	12	75.7	Well to moderate	53	L	Malay	No Habit	T4 N3 M0
12	B12	138/04	OSCC	B	51	0.1	44.9	4.5	91.5	2743.5	12	86.9	Well	72	F	Indian	NA	T4 N0 Mx
13	B13	551/04	OSCC	B	43	0.1	22.7	2.3	346.6	10399.2	13	33.5	Moderate	48	F	Indian	Alcohol, BQ	T4N1M0
14	B14	120/06	OSCC	B	28	0.1	17.6	1.8	321.7	9651	11	90	Moderate	76	M	Indian	Smoke, Alcohol, BQ	T4 N1 Mx
15	B15	366/06	OSCC	B	23	0.1	20.9	2.1	189.4	5683.2	11	83.5	Well	67	F	Indian	Alcohol, BQ	T4 N1 Mx
16	B16	62/06	OSCC	B	29	0.1	35.1	3.5	172.5	5175.3	11	83.7	Well	52	F	Indian	BQ	T1N0M0
17	B17	84/05	OSCC	B	40	0.1	31	3.1	34.2	1025.4	12	18.9	Moderate	74	F	Indian	BQ	T1N0M0
18	B18	303/05	OSCC	B	36	0.1	19.9	2	54.7	1641	12	85.1	Well	67	F	Indian	BQ	T4N0M0
19	B20	334/03	OSCC	B	57	0.1	30.5	3.1	405.5	12163.8	12	85.9	Well	72	F	Indian	NA	Missing data
20	B21	55/04	OSCC	B	53	0.1	15.9	1.6	185.3	5560.2	12	80.5	Moderate	47	F	Indian	BQ	T4 N1 Mx
21	B22	558/04	OSCC	B	43	0.1	18.1	1.8	284	8520.6	13	84.1	Well	22	F	Indian	BQ	T4 N0 M0
22	B23	335/03	OSCC	B	57	0.1	11.5	1.2	373.7	11209.8	12	85.5	Moderate	55	M	Indian	Smoke, BQ	T1 N0M0
23	B24	D137/04-B1	OSCC	B	51	0.04	56	2.2	38.1	1143	12	68.7	Moderate	63	F	Indian	BQ	T2N2BM0
24	BD25	D113/04 A	OSCC	B	52	0.06	12	0.7	25.5	763.8	NA	NA	Moderate	66	F	Indian	BQ	T4N2BMx
25	BD26	D241/05 I(vi)b	OSCC	B	36	0.04	20	0.8	9.7	292.2	NA	NA	Moderate	38	F	Chinese	Smoke, Alcohol	T1N2AM0
26	BD27	D406/04-1a(ii)	OSCC	B	46	0.04	60	2.4	18.9	566.4	NA	NA	Moderate	58	F	Chinese	No Habit	T2N0M0
27	BD28	D251/06-B(iii)	OSCC	B	24	0.1	20	2	13.2	395.1	NA	NA	Papillary variant	66	F	Indian	BQ	T1N0Mx
28	BD29	139/05	OSCC	B	39	0.1	30	3	22.8	685.2	NA	NA	NA	65	F	Indian	BQ	T2N0M0

Appendix A, continued

No	Sam-ples	Block#	Diagnosis	Site	Age of sample month	Tissue depth mm	Area mm^2	Tum-our volume mm^3	RNA yield ng/ul	Total RNA yield ng	Ct differ-ence	% genes with sig-nificant signal	Pattern of differ-entiation	Age	Gender	Race	Habits	Staging
29	BD30	212/05	OSCC	B	38	0.1	25	2.5	27.4	820.5	NA	NA	NA	59	F	Indian	BQ	T4N0M0
30	BD31	48/05	OSCC	B	42	0.1	40	4	3.9	116.1	NA	NA	NA	50	M	Indian	BQ	T4N0M0
1	AM01	D118/04 b(i)	OSCC	G	52	0.06	100	6	62.2	1867.2	17	58.4	Moderate	51	F	Indian	BQ	T4N0M0
2	AM02	D422/04 B5 (ii)	OSCC	G	45	0.06	100	6	209.5	6283.5	14	72.7	Moderate	41	M	Malay	Smoke	T2N2BM0
3	AM03	D88/05 A(v)a	OSCC	G	40	0.1	66	6.6	42.1	1263	16	55	Well	54	M	Malay	Smoke	T4N0M0
4	AM04	D55/05	OSCC	G	41	0.1	12	1.2	170.9	5125.5	13	86.5	Poor	76	M	Indian	Smoke, Alcohol	T2N0M0
5	AM05	D83/07	OSCC	G	16	0.1	15	1.5	219.3	6579	10	80.7	Well	67	M	Malay	No Habit	T4N0M0
6	AM06	D392/05-1	OSCC	G	35	0.1	20	2	224.1	6723.6	11	87.3	Well	75	F	Indian	BQ	Missing data
7	AM07	D407/06	OSCC	G	22	0.1	12	1.2	162	4860.6	11	87.1	Poor	54	M	Indian	Smoke, Alcohol	T4N2bMx
8	AM08	D135/06-T(i)	OSCC	G	27	0.1	50	5	236.8	7103.1	13	76.1	Well	71	F	Indian	BQ	T4N2BM0
9	AM09	D134/07-8(i)a	OSCC	G	15	0.1	135	13.5	324	9719.7	9	81.5	Well	49	M	Malay	Smoke	T4N0M0
10	AM10	D472/06- B1	OSCC	G	45	0.1	50	5	185.5	5565	19	70.5	Well	58	F	Indian	BQ	T3N0M0
11	AM11	D146.04-A T(ii)	OSCC	G	51	0.1	44	4.4	222.5	6674.4	16	66.7	Well	80	F	Indian	BQ	T4N0M0
12	AM12	D577/06	OSCC	G	19	0.1	40	4	362.2	10867.2	11	80.5	Poor	71	F	Indian	Alcohol, BQ	Missing data
13	AM13	D38/07	OSCC	G	18	0.1	30	3	345	10349.4	14	64.3	Poor	71	F	Indian	Alcohol, BQ	Missing data
14	AM14	D98/07	OSCC	G	16	0.1	19	1.9	379.7	11391.6	8	86.1	NA	57	M	Indian	NA	Missing data
15	AM15	D182/06	OSCC	G	26	0.1	28	2.8	190.3	5708.7	14	67.3	Well to moderate	72	F	Indian	Smoke, Alcohol, BQ	T1N2BM0
16	AM16	12 / -03	OSCC	G	66	0.1	16	1.6	199.6	5988.6	16	33.3	Moderate	64	M	Chinese	NA	Missing data
17	AM17	49/02	OSCC	G	69	0.1	18	1.8	269.4	8081.1	16	14.5	Well	76	M	Malay	NA	Missing data
18	AM18	D442/06	OSCC	G	21	0.1	20	2	342.8	10283.4	14	76.9	Well	66	F	Indian	BQ	T4N0M0
19	AMD19	D498/06	OSCC	G	20	0.1	100	10	23.2	695.4	NA	NA	Well	58	F	Indian	BQ	Missing data
20	AMD20	D472/04 B2 (vi)	OSCC	G	0	0.06	80	4.8	6.3	188.4	NA	NA	NA	58	F	Indian	BQ	T3N0M0
1	T01	D264/06-A1	OSCC	T	24	0.1	37.2	3.7	64.9	1945.5	12	73.5	Moderate	59	F	Sikh	BQ	T1N2BM0
2	T02	D250/05D3	OSCC	T	36	0.1	9.6	1	175.3	5259.3	15	84.9	Moderate	71	M	Indian	Alcohol	T1N0M0
3	T03	D70/06	OSCC	T	29	0.1	36.8	3.7	142.6	4276.8	8	77.3	Moderate	72	M	Indian	Smoke, Alcohol	Missing data
4	T04	B973/05	OSCC	T	0	0.01	80	0.8	40	1200	15	74.1	Moderate				NA	Missing data
5	T04(5)	D33/06	OSCC	T	29	0.1	25	2.5	133.1	3991.5	12	73.9	Moderate	65	M	Chinese	Smoke	Missing data
6	T05(6)	D33/04	OSCC	T	54	0.1	18	1.8	69.1	2073	11	77.3	Moderate	53	M	Malay	NA	Missing data
7	T06(7)	541/05	OSCC	T	31	0.1	10.9	1.1	291.4	8740.5	15	86.1	Moderate	69	M	Indian	Smoke, Alcohol, BQ	T1N0M0
8	T07(8)	D446/05	OSCC	T	34	0.1	20	2	623.1	18693.9	12	81.1	Well	46	M	Chinese	Smoke, Alcohol, BQ	Missing data
9	T08(9)	537/05	OSCC	T	31	0.1	12	1.2	352.9	10586.1	14	80.1	Moderate	45	M	Chinese	Alcohol	T4N1M1
10	T09(10)	D241/06	OSCC	T	24	0.1	45.4	4.5	400.4	12012	14	78.9	Moderate to well	65	M	Chinese	Smoke	Missing data

Appendix A, continued

No	Sam-ples	Block#	Diagnosis	Site	Age of sample month	Tissue depth mm	Area mm^2	Tum-our volume mm^3	RNA yield ng/ul	Total RNA yield ng	Ct differ-ence	% genes with significant signal	Pattern of differ-entiation	Age	Gender	Race	Habits	Stage
11	T10	90/04	OSCC	T	52	0.1	13.2	1.3	209.5	6285.3	16	76.1	Basaloid SCC	70	M	Indian	Smoke, Alcohol	Missing data
12	T11	D552/05	OSCC	T	31	0.1	16.8	1.7	267.2	8016.3	11	83.3	SCC microinvasive	48	F	Chinese	No Habit	T1N0M0
13	T12	D394/05	OSCC	T	35	0.1	8.1	0.8	145.4	4360.8	14	85.5	Well	28	F	Indian	No Habit	T1N0M0
14	T13	D520/04	OSCC	T	43	0.1	12	1.2	274.2	8224.5	10	86.9	Poor	66	F	Indian	BQ	Missing data
15	T14	D393/05	OSCC	T	35	0.1	10	1	217	6510.3	14	83.1	Poor	70	F	Indian	BQ	Missing data
16	T15	D216/04	OSCC	T	48	0.1	14.1	1.4	214.7	6442.2	16	83.5	Moderate	63	M	Malay	NA	Missing data
17	T16	D104/05	OSCC	T	40	0.1	6.6	0.7	99.1	2971.8	14	85.7	Moderate	56	M	Indian	Smoke, BQ	T1N0M0
18	T17	D34/04	OSCC	T	54	0.1	8.6	0.9	47.5	1423.5	16	59.6	Moderate	54	M	Indian	NA	Missing data
19	T18	D427/03	OSCC	T	55	0.1	12	1.2	121.3	3638.7	17	72.7	NA	77	M	Indian	NA	Missing data
20	T19	D53/05	OSCC	T	41	0.1	12	1.2	60.4	1812.6	18	34.5	Well	49	M	Chinese	NA	Missing data
21	T20	D381/05	OSCC	T	35	0.1	10	1	84.2	2525.1	14	73.7	Moderate	59	M	Indian	Smoke, Alcohol	Missing data
22	T21	D463/05	OSCC	T	33	0.1	11	1.1	292.7	8781.9	14	87.3	Moderate	61	M	Indian	Smoke, Alcohol	T1N2CM0
23	T22	D117/07	OSCC	T	16	0.1	12	1.2	238.3	7148.1	12	72.9	Moderate	49	F	Malay	No Habit	T2N0M0
24	T23	D106/07	OSCC	T	16	0.1	13	1.3	270.4	8112	11	81.3	Moderate	52	M	Indian	Smoke, Alcohol	Missing data
25	T24	D432/04	OSCC	T	45	0.1	18	1.8	244.3	7327.5	13	81.7	Papillary variant	65	F	Indian	BQ	T2 N2c Mx
26	T25	D67/07	OSCC	T	17	0.1	22	2.2	402.1	12063.3	13	61.8	Well	49	M	Malay	Smoke, Alcohol	T1N0M0
27	T26	157/04	OSCC	T	51	0.1	30.5	3.1	258.6	7756.8	12	77.9	Well	35	M	Indian	NA	Missing data
1	nB01	D160/03	NCOM	B	60	2	7	8.2	63.9	1916.4	14	81.9	NA	55	M	Chinese	NA	FEP
2	nB02	D196_04	NCOM	B	48	1	8	4.7	203.3	6098.7	13	83.3	NA	53	F	Indian	NA	FEP
3	nB03	D243/04	NCOM	B	48	2	5	5.9	37.2	1114.5	13	77.5	NA	59	M	Malay	NA	FEP
4	nB04	D374/03	NCOM	B	54	1.5	10	8.8	702.7	21081.6	13	83.5	NA	37	F	Chinese	NA	FEP
5	nB05	D409/04	NCOM	B	44	2	30	35.3	598	17940	13	79.3	NA	26	M	Other	NA	FEP
6	nB06	59/02	NCOM	B	67	1.5	24	21.2	127.6	3826.8	15	58.2	NA	27	M	Malay	NA	FEP
7	nB07	52/03	NCOM	B	60	1.5	28	24.7	741.4	22242	15	83.7	NA	52	F	Chinese	NA	FEP
8	nB08	13/04	NCOM	B	48	1	7	4.1	456.4	13691.7	10	89	NA	77	F	Chinese	NA	FEP

Appendix A, continued

No	Sam-ples	Block#	Diagnosis	Site	Age of sample month	Tissue depth mm	Area mm^2	Tum-our volume mm^3	RNA yield ng/ul	Total RNA yield ng	Ct differ-ence	% genes with significant signal	Pattern of differ-entiation	Age	Gender	Race	Ha-bit	Stage
9	nB09	20/01	NCOM	B	84	1.5	6	5.3	545.1	16353.6	0	47.2	NA	25	F	Malay	NA	FEP
10	nB10	8/01	NCOM	B	89	2	10	11.8	368.4	11052	1	52.8	NA	53	F	Chinese	NA	FEP
11	nB11	04/06	NCOM	B	30	2	20	23.6	285.8	8574.3	5	63.5	NA	50	F	Chinese	NA	FEP
12	nB12	D267/05	NCOM	B	36	1.5	12	10.6	347.6	10428.6	5	83.3	NA	70	M	Orang Asli	BQ	FEP
13	nB13	D443/05	NCOM	B	34	2	10	11.8	254.9	7647.9	12	80.9	NA	43	M	Chinese	NA	FEP
14	nB14	D35/04	NCOM	B	42	2	10	11.8	269.9	8096.1	13	82.5	NA	39	M	Indian	NA	FEP
1	nG01	D106/05	NCOM	G	40	2	8	9.4	403.769	12113.1	13	81.1	NA	21	F	Malay	NA	Fibrous epulis
2	nG02	16/05	NCOM	G	40	2	20	23.6	898.98	26969.4	14	62.9	NA	70	F	Malay	NA	Fibrous epulis
3	nG03	D580/05	NCOM	G	31	2	10	11.8	404.09	12122.7	14	74.1	NA	19	M	Chinese	NA	Fibrous epulis
4	nG04	24/06	NCOM	G	26	1.5	10	8.8	188.34	5650.2	15	71.1	NA	33	F	Malay	NA	Fibrous epulis
5	nG05	D227/05 (i)	NCOM	G	36	3	5	8.8	364.9	10947	10	75.1	NA	2	M	Indian	NA	Pyogenic granuloma
6	nG06	D286/05	NCOM	G	36	3	7	12.4	222.5	6675	11	74.3	NA	40	M	Chinese	NA	Fibrous epulis
7	nG07	D471/05	NCOM	G	33	2	10	11.8	724.9	21747	10	73.5	NA	43	F	Chinese	NA	Fibrous epulis
8	nG08	D589/06	NCOM	G	19	2	12	14.1	557.5	16725	9	76.9	NA	47	F	Chinese	NA	Fibrous epulis
9	nG09	D123/07	NCOM	G	16	2	5	5.9	447.3	13419	15	73.5	NA	40	F	Chinese	NA	Fibrous epulis
10	nG10	D39/07	NCOM	G	18	3	10	17.7	271.1	8133	10	76.9	NA	36	F	Chinese	NA	Fibrous epulis
1	IA01	D132/07	NCOM	G	15	2	2	2.4	355.5	10665	10	65.1	NA	25	M	Chinese	NA	I8
2	IA02	D157/07	NCOM	G	15	2	5	5.9	633.5	19005	11	71.3	NA	21	M	Malay	NA	I8
3	IA03	D158/07	NCOM	G	15	2	7	8.2	110.1	3303	10	73.7	NA	20	M	Malay	NA	I8
4	IA04	D245/07	NCOM	G	12	2	3	3.5	345.4	10362	7	61.4	NA	23	F	Malay	NA	I8
5	IA05	D269/07	NCOM	G	12	2	2	2.4	125	3750	8	59	NA	28	F	Malay	NA	I8
6	IA06	D270/06	NCOM	G	12	2	3	3.5	260	7800	6	NA	NA	41	F	Chinese	NA	I8
1	nT01	21/04	NCOM	T	12	1.5	9	7.9	144.33	4329.9	14	79.9	NA	46	M	Chinese	NA	FEP
2	nT02	30/04	NCOM	T	46	2	9	10.6	174.54	5236.2	15	72.1	NA	57	M	Malay	NA	FEP
3	nT03	D570/05	NCOM	T	31	2	12	14.1	604.93	18147.9	10	58.6	NA	45	F	Chinese	Alco hol	Dysplasia
4	nT04	D186/05A	NCOM	T	26	2	2	2.4	203.5	6105	11	72.1	NA	51	F	Indian	BQ	Dysplasia
5	nT05	D469/05	NCOM	T	33	2	7	8.2	213	6390	12	58.6	NA	51	F	Malay	NA	FEP
6	nT06	27/05	NCOM	T	38	2	6	7.1	113.8	3414	10	48.2	NA	22	F	Malay	NA	FEP
7	nT07	D416/05	NCOM	T	34	2	4	4.7	110.1	3303	13	54	NA	50	F	Indian	NA	FEP
8	nT08	D488/05	NCOM	T	33	2	7	8.2	440.3	13209	13	17.5	NA	55	M	Indian	NA	FEP

Appendix B

Sociodemographics of OSCC patients where the fresh frozen samples were obtained and qPCR results of the genes selected for validation. (FC = fold change generated from comparing OSCC to non-cancerous oral mucosa).

No	IRPA #	Diagnosis	Site	FOLR1 FC	IL1B FC	BCL2A1 FC	CSF3F FC	TRAF4 FC	LYN FC	IL11 FC	TNFRSF1B FC	MXI-1 FC	PNUTL1 FC	MMP1 FC	MMP10 FC	ITGB4 FC	PTHLH FC	TGFBR3 FC	SERPINE1 FC	CTSL FC	Pattern of differentiation	Age	Gender	Race	Habits	Staging
1	01-0005-05	SCC	G	4.36	6.24	8.40	2.54	4.09	8.53	1.75	5.50	-2.46	1.48	2.87	-1.95	3.91	35.77	-3.97	-1.25	5.53	Well	55	M	Malay	Smoke	T4N0M0
2	04-0021-06	SCC	T	1.43	2.21	1.30	1.94	2.36	1.15	-3.29	1.96	-1.46	1.69	1.08	1.30	-1.04	2.60	-1.80	1.71	-1.32	Moderate	48	M	Malay	Smoke	T1N2B Mx
3	11-0005-07	SCC	T	1.46	21.76	10.88	4.24	4.60	5.03	9.87	4.90	-1.79	-1.20	243.70	647.16	4.66	219.74	-6.49	63.67	2.27	Moderate	34	M	Chinese	Smoke	T2N1Mx
4	01-0017-08	SCC	B	3.09	63.70	98.52	45.05	3.66	4.78	80.33	9.38	-3.36	-2.79	149.34	117.35	2.29	374.69	-1.86	76.77	12.17	NA	58	M	Chinese	Alcohol	T4N2bMx
5	01-0024-07	SCC	T	5.25	63.80	124.28	47.48	7.56	23.15	16.68	24.54	-1.39	-3.50	117.71	124.56	14.10	273.79	-2.33	23.22	62.08	Poor	48	M	Malay	No habit	T2 N0 Mx
6	04-0010-06	SCC	T	1.43	5.78	5.26	2.29	2.11	5.65	14.05	3.07	-1.44	2.19	347.00	821.80	7.04	36.79	-3.56	51.93	6.54	Moderate	50	M	Indian	Smoke	T3N2BM0
7	01-0040-07	SCC	B	NA	1.04	2.49	3.53	-1.31	1.45	-1.12	1.85	-1.18	-1.01	-50.00	-18.18	-1.79	1.17	1.69	-2.62	-1.45	Well	73	M	Indian	Smoke, Alcohol	T1N0M0
8	01-0002-07	SCC	G	-2.25	1.18	-1.07	-2.28	1.55	1.01	-2.21	1.01	-1.01	-3.07	-24.39	-11.24	-1.08	-1.11	-2.26	1.23	-1.18	Well	56	F	Indian	BQ	T4N3M0
9	01-0038-05	SCC	T	23.51	12.44	27.35	4.72	4.41	5.21	54.50	6.04	-2.26	1.18	137.84	265.62	2.73	36.21	-1.22	38.90	10.81	Poor	52	M	Chinese	No habit	T2N0Mx
10	04-0017-05	SCC	T	-1.72	3.29	10.62	3.74	3.47	14.98	2.71	8.42	-6.21	-4.42	309.31	119.90	8.17	19.50	-2.43	23.01	8.14	Moderate	66	F	Malay	No habit	T2N1M0
11	01-0005-06	SCC	G	6.77	7.22	12.59	4.18	4.56	8.52	17.35	3.91	2.15	1.68	24.31	4.59	6.26	31.69	1.48	23.95	11.72	Well	55	M	Malay	Smoke	T4N2bM1
12	01-0005-04	SCC	Palate	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5.71	24.37	-6.29	24.15	-1.17	3.09	7.94	Well	55	M	Malay	Smoke, Alcohol	T4N0M0
13	04-0006-06	SCC	B	NA	9.57	3.58	2.63	2.74	1.59	6.05	-1.10	-3.24	-30.30	NA	NA	NA	NA	NA	NA	NA	Well	71	F	Indian	BQ	T4N0Mx
14	04-0002-06	SCC	Mandible	NA	NA	4.05	1.57	NA	1.09	2.02	-1.71	1.31	-2.94	NA	NA	NA	NA	NA	NA	NA	Moderate	46	M	Chinese	None	T3N0Mx
15	04-0004-04	SCC	B	1.11	22.96	2.45	2.71	-1.45	1.19	-1.29	-3.97	-2.38	1.47	51.67	8.69	5.12	109.56	-5.26	19.92	7.54	Well	53	M	Chinese	Smoke, Alcohol	T3NxM0
16	04-0005-04	SCC	B	NA	NA	8.09	1.96	NA	5.07	7.50	-1.05	1.07	1.21	NA	NA	NA	NA	NA	NA	NA	Moderate	70	F	Indian	BQ, Alcohol	T4N0M0
17	02-0005-05	SCC	B	-1.96	3.54	10.75	6.31	NA	-1.83	-1.07	-2.33	-4.85	-1.41	453.64	250.44	9.51	531.94	-7.14	6.13	3.29	NA	60	M	Malay	No Habit	Missing data
18	11-0014-06	SCC	G	NA	14.80	7.81	3.36	NA	2.84	1.01	1.21	1.28	-2.37	321.01	94.14	8.99	115.51	-2.04	28.40	9.63	Moderate	59	F	Iban	No Habit	T4N2bMx
19	11-0031-05	SCC	B	-1.69	22.04	25.16	2.31	NA	7.87	4.70	2.63	1.05	1.04	555.64	69.07	21.38	829.76	1.00		14.00	Well	57	F	Malay	BQ	T3N0Mx
20	01-0010-06	SCC	T	1.43	5.37	1.71	7.14	1.87	2.77	-12.99	-1.03	-5.00	-10.42	5.70	38.08	2.38	208.55	-2.80	7.07	4.47	NA	39	F	Malay	No habit	T4 N1 Mx
21	02-0018-05	SCC	B	1.09	2.93	8.29	4.15	3.33	2.18	9.08	2.56	-2.33	-3.48	15.22	21.43	10.72	354.69	-3.44	44.21	4.67	Well	37	M	Malay	Smoke	T4N2M0

Appendix B, continued

No	IRPA #	Diagnosis	Site	FOLR1 FC	ILIB FC	BCL2A1 FC	CSF3F FC	TRAF4 FC	LYN FC	IL11 FC	TNFRSF1B FC	MXI-1 FC	PNUTL1 FC	MMP1 FC	MMP10 FC	ITGB4 FC	PTHLH FC	TGFBR3 FC	SERPINE1 FC	CTSL FC	Pattern of differentiation	Age	Gender	Race	Habits	Staging
22	01-0017-04	SCC	T	1.38	4.87	-1.16	12.94	1.72	-1.19	4.12	-1.01	-3.80	-1.75	202.54	18.94	10.15	151.39	-1.36	5.27	15.02	Moderate	74	F	Indian	BQ	T2N1Mx
23	11-0005-05	SCC	G	NA	71.27	56.26	20.96	6.87	18.88	15.18	13.76	-1.28	-3.12	247.11	489.24	15.79	369.93	1.45	18.81	7.16	Well	67	M	Malay	None	T4N2bMx
24	03-0004-04	SCC	Missing data	NA	17.29	3.67	3.12	1.24	3.09	-18.18	-2.80	-2.03	-1.43	19.01	16.84	4.52	88.80	-2.10	3.62	5.09	Missing data	56	F	Malay	BQ	Missing data
25	01-0017-05	SCC	Missing data	NA	2.92	2.61	-3.38	1.41	-2.00	-1.16	NA	1.60	NA	-1.06	1.02	1.33	6.41	5.74	1.54	3.71	Missing data			Missing data	Missing data	Missing data
26	01-0018-05	SCC	Missing data	NA	4.99	-1.46	-7.25	1.19	1.11	-3.77	NA	-1.39	NA	-1.37	-8.55	-1.22	17.86	1.91	3.08	2.90	Missing data			Missing data	Missing data	Missing data
27	01-0020-05	SCC	Missing data	NA	1.11	1.20	-2.46	1.50	4.03	-3.08	NA	1.94	NA	-2.24	-3.22	-1.65	1.28	1.54	1.54	4.10	Missing data			Missing data	Missing data	Missing data
28	01-0028-05	SCC	Missing data	NA	3.60	NA	NA	NA	NA	NA	NA	NA	NA	22.47	5.49	NA	NA	30.72	56.73	283.89	Missing data			Missing data	Missing data	Missing data
29	02-000204	SCC	T	6.77	3.30	32.63	15.36	5.89	3.08	52.96	NA	-1.49	NA	3741.5	3055.15	21.57	492.28	4.40	NA	17.48	Moderate	48	M	Malay	Smoke	T4N2CM0
30	02-0004-04	SCC	FOM	9.82	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Poor	60	M	Chinese	Smoke, BQ	T4N2cM0
31	04-0001-05	SCC	Missing data	NA	15.83	41.49	4.34	3.07	1.49	22.13	NA	1.26	NA	44.32	3.64	4.73	223.38	1.39	83.05	5.95	Moderate	75	F	Malay	BQ	T4N0M0
32	02-0009-05	SCC	Missing data	NA	21.76	170.18	11.99	6.91	9.97	105.58	NA	-2.36	NA	NA	NA	NA	NA	NA	NA	NA	Well	59	F	Malay	Smoke, BQ	Missing data
33	01-0059-07	SCC	B	12.49	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Moderate	55	F	Indian	BQ	T1N0Mx
34	04-0004-08	SCC	T	18.85	3.97	2.21	-1.47	2.61	1.97	1.80	NA	-3.13	NA	9.14	2.88	3.08	120.82	-1.68		3.96	Well	50	F	Indian	No habit	T2N0M0
35	04-0023-09	SCC	T	11.04	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Missing data			Missing data	Missing data	Missing data
36	01-0023-09	SCC	T	3.13	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Missing data			Missing data	Missing data	Missing data
37	06-0060-06	SCC	B	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	34.96	12.96	8.14	144.97	-3.53	43.80	2.41	Moderate	58	F	Indian	No habit	Missing data
38	03-0003-04	SCC	B	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	117.53	84.19	3.50	8.67	-1.70	9.12	1.40	NA	68	F	Indian	Smoke, Alcohol, BQ	Missing data
39	04-0005-04	SCC	B	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	26.70	11.78	2.47	1.37	-2.58	7.59	3.53	Well	70	F	Indian	BQ, Alcohol	T4N0M0
40	04-0019-06	SCC	B	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	7.44	1.77	2.10	11.49	-2.29	5.33	1.68	Well	67	M	Indian	BQ	T2N0Mx
41	04-0001-07	SCC	B	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1053.53	450.99	5.98	218.60	-1.74	74.24	4.70	Moderate	68	M	Chinese	No habit	T2N1M0
42	04-0008-04	SCC	B	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	461.66	179.27	10.22	65.61	-1.85	50.86	4.72	Missing data	71	M	Chinese	Missing data	Missing data
43	06-0023-07	SCC	B	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	8.73	-1.59	2.15	3.35	1.21	3.28	1.97	Poor	88	M	Indian	BQ	T2N0M0
44	01-0011-04	SCC	B	8.77	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Moderate to well	50	F	Indian	BQ	T2N1M0
45	01-0048-07	SCC	T	18.63	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Missing data			Missing data	Missing data	Missing data
46	11-0009-07	SCC	T	3.85	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Moderate	51	F	Others	Smoke, BQ	T4N1Mx
47	06-0012-08	SCC	T	6.76	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Well	49	M	Malay	Smoke	T1N0Mx
48	04-0007-08	SCC	T	4.47	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Well	53	F	Chinese	No habit	T2N0Mx

Appendix B, continued

No	IRPA #	Diagnosis	Site	FOLR1 FC	IL1B FC	BCL2A1 FC	CSF3F FC	TRAF4 FC	LYN FC	IL11 FC	TNFRSF1B FC	MXI-1 FC	PNUTL1 FC	MMP1 FC	MMP10 FC	ITGB4 FC	PTHLH FC	TGFBR3 FC	SERPINE1 FC	CTSL FC	Pattern of differentiation	Age	Gender	Race	Habits	Staging
49	01-0005-09	SCC	B	3.10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Missing data	71	F	Malay	No habit	Missing data
50	04-0013-08	SCC	T	2.33	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Moderate	50	M	Indian	BQ	TxN1Mx
51	01-0058-07	SCC	G	4.15	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Moderate	66	F	Chinese	Smoke, Alcohol	T1N0M0
52	11-0004-07	SCC	T	3.22	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Moderate	58	F	Iban	BQ	T3N2cMx
53	04-0009-08	SCC	B	4.29	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Missing data			Missing data	Missing data	Missing data
54	01-0107-08	SCC	G	1.76	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Missing data			Missing data	Missing data	Missing data

Appendix C

Sociodemographics of OSCC patients where the TMaA samples were obtained and immunohistochemistry results of proteins selected for validation

No	Case #	Diagnosis	Site	TMAa	Position within the block	Protein intensity of BCL2A1	Protein intensity of ITGB4	Protein intensity of MMP1	Average CD3	Average FOXP3	% FOXP3	Pattern of differentiation	Gender	Race	Habits	Staging
1	D556/05	OSCC	B	1-1	1	No tissue	NA	NA	NA	NA	NA	Well	F	Indian	BQ,Alcohol	T1N0Mx
2	D366/06	OSCC	B	1-1	2	1	NA	NA	NA	NA	NA	Well	F	Indian	BQ,Alcohol	T1N2bMx
3	D9/06	OSCC	B	1-1	3	2	NA	NA	NA	NA	NA	Moderate	F	Indian	BQ	T4N0Mx
4	OC(S)2	NCOM	G	1-1	4	1	NA	NA	NA	NA	NA	NA				
5	D406/04	OSCC	B	1-2	1	1	1	2	NA	NA	NA	Moderate	F	Chinese	4	T4N0Mx
6	D303/05	OSCC	B	1-2	2	2	2	3	NA	NA	NA	Well	F	Indian	BQ	T4N0Mx
7	D119/016	OSCC	B	1-2	3	2	2	3	NA	NA	NA	Moderate	F	Others	BQ	T2N0Mx
8	OC(S)6	NCOM	G	1-2	4	0	0	0	NA	NA	NA	NA				
9	D48/06	OSCC	B	1-3	1	NA	1	NA	NA	NA	NA	Moderate	F	Indian	BQ,Alcohol	T2N2bMx
10	D152/06	OSCC	B	1-3	2	NA	1	NA	NA	NA	NA	NA	F	Indian	BQ	T2N0Mx
11	D212/02	OSCC	B	1-3	3	NA	0	NA	NA	NA	NA	NA	F	Chinese	BQ,Smoke,Alcohol	NA
12	OC(S)4	NCOM	G	1-3	4	NA	0	NA	NA	NA	NA	NA				
13	D48/05	OSCC	B	1-4	1	NA	2	3	NA	NA	NA	Well	M	Indian	BQ	T4N0M0
14	D62/06	OSCC	B	1-4	2	NA	1	3	NA	NA	NA	Well	F	Indian	BQ	T1N0Mx
15	D134/05	OSCC	B	1-4	3	NA	NA	NA	NA	NA	NA	Moderate	F	Indian	BQ	T4NxM0
16	OC(S)3	NCOM	G	1-4	4	NA	0	0	NA	NA	NA	NA				
17	D84/05	OSCC	B	1-5	1	1	2	3	NA	NA	NA	Moderate	F	Indian	BQ	T1NxMX
18	D273/06	OSCC	B	1-5	2	2	2	3	NA	NA	NA	Moderate papillary variant / verrucous carcinoma	F	Indian	BQ	NA
19	D251/05	OSCC	B	1-5	3	2	2	3	NA	NA	NA		F	Indian	BQ	T1N0Mx
20	OC(S)1	NCOM	G	1-5	4	0	1	0	NA	NA	NA	NA				
22	D517/04	OSCC	B	1-6	2	2	2	3	NA	NA	NA	Moderate	F	Indian	BQ	T4N0Mx
23	D41/05	OSCC	B	1-6	3	2	1	3	NA	NA	NA	Moderate	F	Indian	BQ,Alcohol	T4N1Mx

Appendix C, continued

No	Case #	Diagnosis	Site	TMAa	Position within the block	Protein intensity of BCL2A1	Protein intensity of ITGB4	Protein intensity of MMP1	Average CD3	Average FOXP3	% FOXP3	Pattern of differentiation	Gender	Race	Habits	Staging
24	OC(S)8	NCOM	G	1-6	4	NA	0	1	NA	NA	NA	NA				
25	D359/03	OSCC	B	1-7	1	2	NA	3	142	28	19.40	Moderate	M	Indian	BQ,Smoke	T1N0Mx
26	D137/04	OSCC	B	1-7	2	2	NA	3	93	24	25.20	Moderate	F	Indian	BQ	T2N2bMx
27	D382/03	OSCC	B	1-7	3	2	NA	3	71	28	39.33	Moderate	F	Indian	BQ	T1N0Mx
28	OC(S)5	NCOM	G	1-7	4	NA	NA	0	NA	NA	NA	NA				
29	D212/05	OSCC	B	1-8	1	1	2	3	NA	NA	NA	Moderate	F	Indian	BQ	T1N0M0
30	D68/05	OSCC	B	1-8	2	3	3	3	78	24	31.03	Well	F	Indian	BQ	T4N0Mx
31	D139/05	OSCC	B	1-8	3	2	1	2	61	14	22.49	Moderate	F	Indian	BQ	T4N0Mx
32	OC(S)10	NCOM	G	1-8	4	1	1	0	NA	NA	NA	NA				
33	D48/06	OSCC	B	2-9	1	1	NA	NA	NA	NA	NA	Moderate	F	Indian	BQ, Alcohol	T4N1Mx
34	D406/04	OSCC	B	2-9	2	NA	NA	NA	NA	NA	NA	Moderate	F	Chinese	No habits	T2N0Mx
35	D41/05	OSCC	B	2-9	3	2	NA	NA	NA	NA	NA	Moderate	F	Indian	BQ, Alcohol	T4N1Mx
36	D49/07	OSCC	B	2-9	4	1	NA	NA	NA	NA	NA	Moderate	F	Indian	BQ	T4N1Mx
37	D273/06	OSCC	B	2-10	1	NA	NA	NA	NA	NA	NA	Moderate	F	Indian	BQ	T1N0Mx
38	D21/04	OSCC	B	2-10	2	1	NA	NA	NA	NA	NA	Moderate	F	Indian	BQ	T4N1Mx
39	D79/07	OSCC	B	2-10	3	1	NA	NA	NA	NA	NA	Moderate	F	Indian	NA	T4N1Mx
40	D66/06	OSCC	G	3-1	1	2	NA	NA	NA	NA	NA	Moderate	M	Chinese	NA	T4N0Mx
41	D68/05	OSCC	G	3-1	2	2	2	3	NA	NA	NA	Well	F	Indian	BQ	T4N0Mx
42	D134/07	OSCC	G	3-1	3	NA	2	3	NA	NA	NA	Moderate	F	Indian	BQ	T4N0Mx
43	D135/06	OSCC	G	3-1	4	2	3	3	56	19	33.93	Well	F	Indian	NA	T4N2bM1
44	D182/06	OSCC	G	3-2	1	2	3	2	NA	NA	NA	Moderate	F	Indian	NA	T3N2bM1
45	D303/05	OSCC	G	3-2	2	1	2	2	NA	NA	NA	Well	F	Indian	BQ	T4N0Mx
46	D88/05	OSCC	G	3-2	3	2	2	2	NA	NA	NA	Well	M	Malay	Smoke	T4N0M0
47	D273/06	OSCC	G	3-2	4	1	3	3	NA	NA	NA	Moderate	F	Indian	BQ	T1N0Mx
48	D498/06	OSCC	G	3-3	1	NA	2	1	NA	NA	NA	Well	F	Indian	NA	T3N2aM1
49	D579/05	OSCC	G	3-3	2	1	1	1	NA	NA	NA	Well	M	Indian	NA	T3N0M0

Appendix C, continued

No	Case #	Diagnosis	Site	TMAa	Position within the block	Protein intensity of BCL2A1	Protein intensity of ITGB4	Protein intensity of MMP1	Average CD3	Average FOXP3	% FOXP3	Pattern of differen-tiation	Gender	Race	Habits	Staging
50	D473/05	OSCC	G	3-3	3	NA	2	3	NA	NA	NA	Moderate	F	Indian	BQ	T4N2bMx
51	D422/04	OSCC	G	3-3	4	1	2	2	NA	NA	NA	Moderate	M	Malay	Smoke	T4N2bMx
52	D38/07	OSCC	G	3-4	1	NA	1	2	NA	NA	NA	Poor	F	Indian	NA	T4N0M0
53	D146/04	OSCC	G	3-4	2	NA	3	2	NA	NA	NA	Well	F	Indian	NA	T4N0M1
54	D134/07	OSCC	G	3-4	3	NA	1	2	NA	NA	NA	Well	M	Malay	NA	T4NxMx
55	D474/04	OSCC	G	3-4	4	NA	3	3	NA	NA	NA	Moderate	F	Indian	BQ	T2N1M1
56	D303/05 (22)	OSCC	G	4-1	1	NA	2	1	NA	NA	NA	well	F	Indian	BQ	T4 N0 Mx
57	D137/04 (20)	OSCC	G	4-1	2	NA	2	2	NA	NA	NA	moderate	F	Indian	BQ	T2 N2bMx
58	D359/03 (19)	OSCC	B	4-1	3	NA	1	2	NA	NA	NA	moderate	M	Indian	Smoke, BQ	T1 N0 Mx
59	D474/04 (10)	OSCC	G	4-1	4	NA	2	2	NA	NA	NA	moderate	F	Indian	BQ	T2 N1 Mx
60	D212/05 (11)	OSCC	B	4-2	1	NA	2	1	NA	NA	NA	well	F	Indian	BQ	NA
61	D113/04 (12)	OSCC	T/FOM	4-2	2	NA	3	2	NA	NA	NA	basaloid	F	Indian	BQ	T4 N2bMx
62	D251/05 (25)	OSCC	B	4-2	3	NA	1	2	NA	NA	NA	papillary	F	Indian	BQ	T1 N0 Mx
63	D68/05 (16)	OSCC	G	4-2	4	NA	2	2	NA	NA	NA	well	F	Indian	BQ	T4 N0 Mx
64	D62/06 (4)	OSCC	B	4-3	1	NA	NA	2	NA	NA	NA	well	F	Indian	BQ	T1 N0 Mx
65	D366/06 (1)	OSCC	B	4-3	2	NA	2	1	NA	NA	NA	well	F	Indian	BQ, Alcohol	T1 N2bMx
66	D152/06 (7)	OSCC	B	4-3	3	NA	No tissue	No epithelial	NA	NA	NA	Superficially invasive	F	Indian	BQ	T2 Nx Mx
67	D517/04 (38)	OSCC	B	4-3	4	NA	3	2	NA	NA	NA	moderate	F	Indian	BQ	T3 N0 Mx
68	D139/05 (37)	OSCC	G	4-4	1	NA	2	1	NA	NA	NA	moderate	F	Indian	BQ	T4 N0 Mx
69	D501/05 (26)	OSCC	FOM	4-4	2	NA	1	1	NA	NA	NA	basaloid	M	Indian	Smoke, BQ	T1 N2cMx
70	D486/04 (39)	OSCC	B	4-4	3	NA	2	1	NA	NA	NA	poor	F	Indian	BQ, Alcohol	T4 N0 Mx
71	D241/05 (29)	OSCC	T,	4-4	4	NA	2	3	NA	NA	NA	moderate	M	Chinese	Smoke, Alcohol	T1 N2aMx
72	D67/07 (34)	OSCC	T	4-5	1	NA	1	1	NA	NA	NA	well	M	Malay	Smoke, Alcohol	T1 N0 Mx
73	D140/05 (27)	OSCC	T	4-5	2	NA	1	1	NA	NA	NA	poor	M	Indian	BQ, Smoke, Alcohol	T1 N0 Mx
74	D264/06 (36)	OSCC	FOM	4-5	3	NA	3	2	NA	NA	NA	moderate	F	Indian	BQ, Smoke, Alcohol	T1 N2b Mx
75	D34/06 (30)	OSCC	T	4-5	4	NA	1	2	NA	NA	NA	moderate	M	Malay	Smoke	T2 N2b Mx
76	D117/07	OSCC	T	9-9	1	NA	NA	NA	102	25	24.94	Moderate	F	Malay	No habit	NA

Appendix C, continued

No	Case #	Diagnosis	Site	TMAa	Position within the block	Protein intensity of BCL2A1	Protein intensity of ITGB4	Protein intensity of MMP1	Average CD3	Average FOXP3	% FOXP3	Pattern of differen-tiation	Gender	Race	Habits	Staging
77	D154/08-A	OSCC	T/G	9-9	2	NA	NA	NA	NA	NA	NA	Moderate	M	Indian	Smoking, Alcohol	
78	D274/08	OSCC	B	9-9	3	NA	NA	NA	145	51	35.28	Well diff	F	Punjabi		
79	D244/08	OSCC	B	9-9	4	NA	NA	NA	172	63	36.73	NA	F	Indian		
80	D291/08	OSCC	B	9-9	5	NA	NA	NA	53	21	40.49	Moderate	F	Indian		
81	D358/07	OSCC	G	9-10	1	NA	NA	NA	163	38	23.01	Well diff	F	Chinese		
82	D348/07	OSCC	T	9-10	2	NA	NA	NA	NA	NA	NA	Well diff	M	Chinese		
83	D209/07	OSCC	B	9-10	3	NA	NA	NA	39	13	32.80	Well diff	M	Indian		
84	D163/07	OSCC	T	9-10	4	NA	NA	NA	132	38	28.83	Well diff	F	Chinese		
85	D515/07	OSCC	T	9-10	5	NA	NA	NA	127	26	20.49	Poor	M	Indian	Alcohol	
86	D99/10-A	OSCC	T	10-1	1	NA	NA	NA	220	86	38.89	Moderate	M	Chinese		
87	D364/09-B	OSCC	T	10-1	2	NA	NA	NA	95	29	30.43	Well	F	Indian	BQ	
88	D417/09	OSCC	T	10-1	3	NA	NA	NA	99	31	30.88	NA	F	Chinese		
89	D158/09	OSCC	T	10-1	4	NA	NA	NA	35	11	30.95	Moderate	M	Chinese	Alcohol,Smoking	
90	D213/09	OSCC	T	10-1	5	NA	NA	NA	NA	NA	NA	Moderate	M	Malay		
91	D38/08	NCOM	G	9-11	1	0	0	1	NA	NA	NA		F	Malay		
92	D47/08	NCOM	G	9-11	2	No epithelial	0	1	NA	NA	NA		M	Indian		
93	D34/08	NCOM	G	9-11	3	1	2	1	NA	NA	NA		F	Indian		
94	D297/07	NCOM	G	9-11	4	1	1	2	NA	NA	NA		M	Chinese		
95	D570/07	NCOM	G	9-11	5	1	0	1	NA	NA	NA		M	Chinese		
96	D28/08	NCOM	G	9-12	1	NA	2	1	30	0	0.00		F	Indian		
97	D39/08	NCOM	G	9-12	2	NA	0	1	NA	NA	NA		M	Malay		
98	D22/08	NCOM	G	9-12	3	NA	2	1	6	0	0.00		F	Chinese		
99	D569/07	NCOM	G	9-12	4	NA	2	1	27	0	0.00		M	Malay		
100	D21/08	NCOM	G	9-12	5	NA	0	1	17	0	0.00		M	Malay		
101	D312/08	NCOM	G	9-13	1	1	0	1	19	0	0.00		F	Chinese		
102	D325/08	NCOM	G	9-13	2	0	1	1	23	0	0.00		M	Malay		

Appendix C, continued

No	Case #	Diagnosis	Site	TMAa	Position within the block	Protein intensity of BCL2A1	Protein intensity of ITGB4	Protein intensity of MMP1	Average CD3	Average FOXP3	% FOXP3	Pattern of differentiation	Gender	Race	Habit	Staging
103	D324/08	NCOM	G	9-13	3	0	0	1	10	0	0.00		M	Malay		
104	D333/08	NCOM	G	9-13	4	0	1	1	26	0	0.00		F	Malay		
105	D29/08	NCOM	G	9-13	5	1	2	1	15	0	0.00		M	Chinese		
106	D469/08	NCOM	G	9-14	1	1	0	1	NA	NA	NA					
107	D470/08	NCOM	G	9-14	2	0	0	1	NA	NA	NA					
108	D397/08	NCOM	G	9-14	3	0	0	1	NA	NA	NA					
109	D372/08	NCOM	G	9-14	4	1	0	1	NA	NA	NA					
110	D396/08	NCOM	G	9-14	5	0	0	0	NA	NA	NA					

Appendix D

Experimental Protocols Used in the Study

1) RNA Extraction

(a) FFPE Specimens

FFPE samples from tumour and non-cancerous mucosal tissues were subjected to RNA extraction using High Pure RNA Paraffin Kit (Roche, Basel, Switzerland). Tissue sections of 80-100 µm and tissue cores (from section 4.3.1) were incubated in a 1.5 ml microcentrifuge tube containing 1 ml of xylene to deparafinized paraffin. A total of 500 µl of absolute ethanol was added to the tube and centrifuged for 2 minutes at 14,000 g. This centrifugation step was then repeated with 1 ml of absolute ethanol and the tissue pellet was air dried for 10 minutes at 55°C. Then, a mixture of 100 µl of Tissue Lysis Buffer, 16 µl of 10% SDS and 40 µl of Proteinase K was added, vortexed briefly in several intervals and incubated overnight at 55°C. Next day, 325 µl of Binding Buffer and 325 µl absolute ethanol were added to the mixture, mixed gently by pipetting, transferred into the filter tube placed in a collection tube and centrifuged for 30 seconds at 8,000 g. Centrifugation was repeated at 14,000 g for 30 seconds to ensure the filter fleece is completely dry. Next, the filter tube was washed with 500 µl of Wash Buffer I, followed by 500 µl and 300 µl of Wash Buffer II by centrifugation for 15 seconds at 8,000 g. The flow-through wasdiscarded and the filter tube with the collection tube was spun at 14,000 g for 2 minutes to ensure complete removal of the Wash Buffer. The filter tube was then placed into a sterile 1.5 ml microcentrifuge tube and 90 µl of Elution Buffer was added and centrifuged

for 1 minute at 8,000 g. Eluted RNA was then subjected to DNAse treatment where 10 µl of DNAse Incubation Buffer and 1 µl of DNAse I working solution were added to the eluate. The mixture was incubated at 37°C for 45 minutes. Next, the cocktail of Tissue Lysis Buffer, 10% SDS and Proteinase K working solution was added to the mixture again (at a different volume of 20 µl, 18 µl and 40 µl respectively), vortexed briefly and incubated for additional hour at 55°C. The filter tube was washed again as described earlier. After the last centrifugation, the filter tube was placed into a nuclease free sterile 1.5 ml microcentrifuge tube. Elution buffer of 38 µl was added to the center of the filter, incubated for 5 minutes at room temperature and centrifuged for 1 minute at 8,000 g to elute the RNA. The RNA was quantitated as described in section 4.5. Only specimens which have a concentration of at least 40 ng/µl concentrations were subjected to FFPE RNA quality control assay.

(b) Fresh Frozen Specimens

For fresh frozen specimens, RNA was extracted from a total of 500 µm cryosections using the RNeasy Micro Kit (Qiagen, Hilden, Germany). Samples were collected into 300 µl of Buffer RLT, which contained β-mercaptoethanol (10 µl β-ME per 1 ml Buffer RLT). The sample volume was adjusted to 350 µl with Buffer RLT and vortexed for 30 seconds to lyse and homogenized the tissues. A total of 350 µl of 70% ethanol was added to the homogenized lysate, mixed by pipetting, transferred into spin column placed in a 2 ml collection tube and centrifuged for 15 seconds at 8000 g to let the RNA bind to the membrane. The flow-through was discarded and the column was washed with 350 µl Buffer RW1. This was followed by DNase treatment to remove traces of DNA. A total of

80 µl of DNase I incubation mix (10 µl DNase I stock solution was added to 70 µl Buffer RDD) was directly applied to the spin column membrane, and placed on the bench top for 15 minutes. Subsequently, several washing steps were carried out with 350 µl Buffer RW1, 500 µl Buffer RPE and 500 µl of 80% ethanol by centrifugation for 15 seconds at 8000 g. Next, the spin column was placed in a new 2 ml collection tube, centrifuged at 14,000 g for 5 minutes to dry the column completely. The column was then placed in a new 1.5 ml collection tube and 14 µl RNase-free water was added directly to the center of the spin column membrane. The spin column was then centrifuged for 1 minute at 14,000 g to elute the RNA.

(c) Cell Lines

RNA extraction from cell lines was done using Tri-Reagent® (Molecular Research Centre Inc., Cincinnati, U.S.A) where 1 ml/cm^2 was added directly into the culture dish. The cell lysate was passed several times through a pipette and incubated for 5 minutes at room temperature. Next, 200 µl of chloroform was added to the homogenate per ml of Tri-Reagent® (Molecular Research Centre, Inc., Cincinnati, U.S.A), covered tightly and vigorously shaken for 15 seconds. The mixture was incubated at room temperature for 15 minutes and centrifuged at 12,000 g for 15 minutes at 4°C. During centrifugation, the mixture was separated into 3 phases. RNA remains exclusively in the upper colourless aqueous phase and was gently removed without touching the layers underneath. A total of 500 µl of isopropanol per ml of Tri-Reagent® (Molecular Research Centre, Inc., Cincinnati, U.S.A) was added to the aqueous phase, mixed, incubated at room temperature for 10 minutes and centrifuged at 12,000 g for 15 minutes at 4°C. Next, the supernatant was removed and the cell pellet was washed with at least 1 ml of 75% ethanol per ml Tri-

Reagent® through vortexing and centrifuged at 12,000 g for 10 minutes at 4 - 25°C. After the last ethanol wash, the RNA pellets were air-dried for 3-5 minutes and a total of 33 µl of RNase free water was added to resuspend the RNA cell pellets.

2) Random genes selection

Genes were selected by first arranging the 502 DASl cancer genes. alphabetically. Every tenth gene were then selected to test the the theory of heterogenous expression of OSCC from the tongue. Through this method, a total of 51 genes were selected.

3) Developing an In Vitro Model to Study the Function of FOLR1 Gene in OSCC

(a) Determining the mRNA Level of FOLR1 in OSCC Cell Lines and Selection of OSCC Cell Line for Gene Transfer

qPCR as described in section 4.9.1 was carried out to determine the mRNA expression of FOLR1 in OSCC cell lines. FOLR1 primer set designed by Primer Blast (NCBI) was used in the qPCR analysis. From the qPCR results, OSCC cell lines with the highest and lowest FOLR1 expression were identified.

(b) Construction of FOLR1 expression construct

i. Polymerase Chain Reaction (PCR)

FOLR1 gene insert was amplified using the cDNA from OSCC cell line 136T which was shown to have high levels of FOLR1. Primers for the PCR were design to include a

translational initiation site (Kozak Consensus Sequence) and a stop codon using Primer-Blast (NCBI). PCR was carried out using 1 µl of cDNA, 200 nM of each primer (Sigma-Aldrich, St. Louis, U.S.A) (Appendix F), 200 µM deoxynucleotriphosphates (dNTPs) (Promega, Fitchburg, U.S.A), 4 µl of 10x PCR Buffer (Promega, Fitchburg, U.S.A) containing 1.5 mM $MgCl_2$ and 1 µl of Taq Polymerase (Promega, Fitchburg, U.S.A). The sample was subjected to initial denaturing step of 95°C for 2 minutes before going through 35 cycles of denaturation 95°C for 30 seconds, annealing 95°C for 2 minutes and extension 72°C for 2 minutes. PCR products were electrophoresed on 1% gel (Appendix E) and maintained in 4°C prior to purification.

ii. Purification of PCR Product

PCR product from section 2)(b)i was purified using Qiaquick Gel Extraction Kit (Qiagen, Hilden, Germany) after agarose gel electrophoresis (Appendix E). Briefly, the gel containing PCR product was excised and weighed. Three volumes of Buffer QG was added to 1 volume of gel (100 mg ≈ 100 µl) before incubating it at 55°C for 10 minutes. This was followed by the addition of 1 gel volume of isopropanol, mixed and transferred into the combined QIAquick spin column and collection tube. The set was centrifuged for 1 minute at 14,000 g. The flow-through was discarded, 750 µl of washing buffer was added, and the column was centrifuged as before. The purified PCR product was eluted in 35 µl of elution buffer, subjected to agarose gel electrophoresis to check the purity and stored in -20°C for further use.

iii. Cloning of PCR product

The PCR product from section 2)(b)ii was then cloned into pLenti 6.3-TOPO® vector at a ratio of 1:3 (vector: insert) (Invitrogen, Carlsbad, U.S.A). A total of 6 µl of TOPO® Cloning reaction was set up as follows: 15 ng of FOLR1 gene insert, 1 µl of salt solution, 1 µl of pLenti6.3-TOPO® vector and sterile water added to the final volume of 6 µl. The reaction was mixed gently and incubated at room temperature for 5 minutes.

iv. Transformation

Frozen One Shot® Stbl3™ (Invitrogen, Carlsbad, U.S.A) chemically induced competent cells were thawed on ice prior to transformation. Two microliters of TOPO® Cloning reaction (as prepared in section 2)(b)iii) was added to the competent cells and mixed gently. As for control, 10 pg of religated vector was added into a separate vial of competent cells and mixed gently. The cells were incubated on ice for 30 minutes and then heat shocked at 42°C for 30 seconds and place on ice for 2 minutes. Next, 225 µl of SOC was added and the competent cells were grown at 37°C for 1 hour with shaking (225 rpm; Innova™ 4430, New Brunswick Scientific) prior to plating. The cells were spread on LB agar plates containing 50 µg/ml of ampicillin (Appendix E). The plates were incubated at 37°C overnight and examined for transformants the next day.

v. Analyzing Transformants by PCR

A total of 10-20 colonies were picked from the plates obtained from an overnight incubation as described in section 2)(b)iv. These colonies were cultured overnight in LB

medium containing 100 µg/ml ampicillin at temperature and shaking condition as mentioned above. The presence and orientation of FOLR1 inserts were determined through PCR using a forward primer specific to the FOLR1 gene insert and a reverse primer that is specific to the vector downstream to the insert (Appendix F). A total of 5 µl of culture media was added to the PCR mix and amplified as mentioned in section (2)b.i. The PCR reaction was then electrophoresed on a 2% agarose gel (Appendix E) to determine which clones contain the FOLR1 gene in the right orientation.

vi. Small Scale Preparation of Plasmid DNA

Plasmid DNA was prepared using QIAprep Spin Miniprep kit (Qiagen, Hilden, Germany). Positive clones with the correct orientation identified in section 2)(b)v were picked and used to inoculate 10 ml of LB medium containing 100 µg/ml ampicillin. A total of 5 ml of this overnight culture was spun down at 800 g for 5 minutes. The pellet was resuspended in 250 µl P1 Buffer and transferred into microcentrifuge tube. Two hundred and fifty microliters of Buffer P2 was added, followed by gentle inversion of the tube 4-6 times to mix. Then, 350 µl of N3 Buffer was added and the tube was inverted 4-6 times to mix prior to centrifugation at 14,000 g for 10 minutes. The supernatant was decanted into a combined spin column with a collection tube and centrifuged for 1 minute at 14,000 g. The flow-through was discarded and the column was washed twice with 500 µl of Buffer PB followed by 750 µl of Buffer PE by centrifugation as above. The column was then placed into a fresh microcentrifuge tube and 50 µl of preheated sterile water (at 65°C) was added to the column. After 2 minutes incubation, the plasmid was eluted by centrifugation as above. Plasmids were then sent for sequencing (1st Base Laboratories, Seri Kembangan,

Malaysia) and analysed for any mutation before the suitable clones were selected for transfection.

(c) Transfection and Transduction

i. Harvesting Lentiviruses

To exogenously express FOLR1 in OSCC cell lines, viruses carrying the FOLR1 was engineered by transfecting the FOLR1 construct along with ViraPower™ Packaging mix into the 293FT cells to produce lentiviruses stock using ViraPower™ HiPerform™ Lentiviral Expression System (Invitrogen, Carlsbad, U.S.A). A day before transfection, 5 x 10^6 cells of 293FT cells were plated in 10 ml DMEM High Glucose media with 10% FBS in a 10 cm tissue culture dish so that they will be 90-95% confluent on the day of transfection. On the day of transfection, the culture medium from the 293FT cells was removed and replace with 5 ml of DMEM High Glucose with 10 % FBS. For each transfection sample, DNA-Lipofectamine™ 2000 complexes were prepared as follows. In a sterile 5 ml tube labeled DNA, 9 µg of the ViraPower™ Packaging Mix and 3 µg of pLenti expression plasmid DNA or FOLR1 construct (12 µg total) were diluted in 1.5 ml of DMEM (High Glucose) without serum and mixed gently. In another sterile 5 ml tube labeled Lipofectamine, 36 µl of Lipofectamine™ 2000 (Invitrogen, Carlsbad, U.S.A) was diluted in 1.5 ml of DMEM High Glucose without FBS and gently mixed. Both tubes were incubated separately for 5 minutes. Next, the diluted DNA and Lipofectamine™ 2000 were combined, mixed gently and incubated for another 20 minutes at room temperature to allow the DNA-Lipofectamine™ 2000 complexes to form. The complexes were then added to the 293FT cells in a drop wise manner. The plates were then rocked back and forth before

incubating overnight at 37°C in a humidified 5% CO_2 incubator. The next day, the medium containing the DNA-Lipofectamine™ 2000 complexes were removed, replaced with 10 ml DMEM High Glucose with 10% FBS without antibiotics and incubated at 37°C in a humidified 5% CO_2 incubator. Virus containing media was harvested after 72 hours by removing the supernatant and centrifuged at 8000 g for 15 minutes at 4°C to pellet debris. Supernatant was aliquoted, snap frozen in liquid nitrogen and kept in -80 °C until further use.

ii. Lentiviral Gene Transfer

Selection of suitable OSCC cell lines for FOLR1 gene transfer was carried out using qPCR as described in section 4.9.1. From the qPCR results, 188T cell lines with the lowest FOLR1 expression was selected for lentiviral gene transfer. 188T cell lines were thawed from liquid nitrogen stocks and maintained as described in section 4.3.3. The cells were plated at a density of 5 x 10^5/ml in a 60 mm dish for infection with lentivirus carrying either the pLenti6.3 religated vector as a control or pLenti6.3 FOLR1 construct. The lentiviral containing media were removed from the freezer shortly before use and thawed in the 37°C water bath. A 0.45 µm filter was prepared by flushing it with media which helps in increasing the yield of viruses in the filtered supernatant. Using the same filter, the viral supernatant was filtered and contained in a universal container. The volume of supernatant was measured in order to add the right amount of polybrene to a final concentration of 8 µg/ml. The culture media from 188T cell line was removed and 2.5 ml of virus-containing media was added to each culture dish. The cells were then incubated overnight in 37°C CO_2 incubator. The next day, the media containing virus was removed and replaced with DMEM:F12 with 10% FBS and incubated overnight in 37°C CO_2 incubator. The cells

were then harvested to confirm gene transfer and maintained as described in section 4.3.3. 188T cells infected with pLenti6.3 FOLR1 construct was labeled as 188T_FOLR1 and ORL-188T cells infected with plenti6.3 religated vector were labeled as 188T_pLenti as control.

(d) Confirmation of Gene Transfer

i. qPCR

To confirm whether FOLR1 was successfully transduced into 188T cells, cells from FOLR1_188T and 188T_pLenti were harvested and RNA was extracted as described in section 1)(c) followed by cDNA synthesis in section 4.6. qPCR was conducted as described in section 4.9.1 to confirm expression level of 188T_FOLR1.

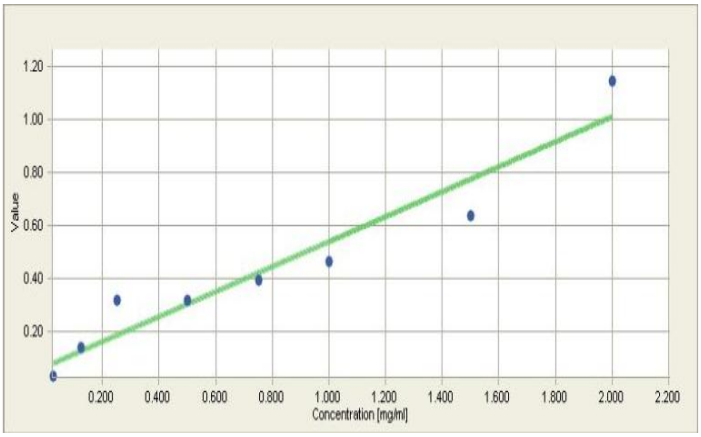
ii. Protein Extraction

To confirm the expression of FOLR1 at the protein level, protein was extracted from cells at 70% confluency. Using suction or a pasteur pipette, culture media was removed from the dish and discarded. The culture dish was washed twice with cold 1X PBS. Protein lysis buffer (Appendix E) ranging from 150-300 µl depending on the culture dish size (40 mm dish: 150 µl, 60 mm dish: 200 µl and 100 mm dish: 300 µl) was added to the adherent cells and left for 5 minutes on ice. Cells were scraped using cell scraper, collected into 1.5 ml tube and sonicated 3 times on ice for 5 seconds each time to disintegrate the tissues. Next the sonicated cells were centrifuged for 20 minute at 14,000 g in 4°C. Using a p1000 pipette (Gilson Inc., Middleton, U.S.A), the supernatant was carefully removed and transferred to a new 1.5 ml tube. A total of 10µl of the supernatant was aliquoted into a 0.5

ml tube for protein quantitation. For the rest of the supernatant, 6x Laemmli loading buffer (Appendix E) was added and the protein was denatured for 10 minutes at 100°C. The denatured protein samples were stored in -20°C until further use.

iii. Protein Quantitation

Protein quantitation was done through the Coomasie (Bradford) Assay (Thermo Scientific, Waltham, U.S.A). Protein samples were ran in triplicate where 10µl of protein was added into the 96-well plate containing 300 µl of Commasie blue. The absorbance of the protein samples along with the standards was determined using the ELISA plate reader (Thermo Scientific, Waltham, U.S.A) at 595 nm. A standard curve was generated and calculations of the unknown protein concentrations were calculated based on the standard curve below.



y = 0.471x + 0.07

Standard Curve for Protein Quantitation

iv. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was performed as described previously by Laemmli *et al*, 1970 . The EC120 mini vertical gel system (Bio-Rad, California, U.S.A) was used for electrophoresis of protein. A 12% resolving gel (Appendix E) was prepared and allowed to polymerize. Prior

to electrophoresis, a 4% stacking gel (Appendix E) was poured and left to polymerize for 20 minutes. Fifty microgram of denatured protein prepared as described in section 3)(c)ii was loaded onto the gel. The gel was run in 1X SDS-PAGE running buffer (Appendix E) at 100 V for approximately 2 hours.

v. Western blot analysis of FOLR1 protein level

Western blotting was performed as described previously by Towbin *et al*, 1979 . Following SDS-PAGE electrophoresis, proteins were transferred on to the 0.45 µm nitrocellulose membrane (Pierce, Illinois, U.S.A) using the EC140 mini blot module (Bio-Rad, California, U.S.A). Transfer was done at 400 mA for 1 hour and post transferred gels and membrane were stained with Coomasie Blue (Appendix E) and Ponceau S (Appendix E) respectively, to check if transfers were successful. After transfer, the membrane was blocked for 1 hour in 5% skimmed milk in PBS at room temperature. This was followed by three 10-minute washes of the membrane in PBS-Tween 20 (0.1%) and incubation with mouse anti human FOLR1 antibody at 1:200 dilution (Abnova, Taipei, Taiwan) overnight in 4°C. Next day, the membrane was washed as mentioned above and incubated with goat anti-mouse IRDye® 680 secondary antibody (LI-COR Biosciences, Lincoln, U.S.A) for 1 hour at room temperature in the dark with constant agitation. The membrane was washed and scanned with the Odyssey® Infrared Imaging System (LI-COR Biosciences, Lincoln, U.S.A).

Appendix E

Preparation of Buffers and Solutions

Cell culture

Dulbecco's Modified Eagle's Medium F12 (DMEM:F12) supplemented with fetal bovine serum

DMEM-F12 (GIBCO)	17.4 g/L
Sodium Bicarbonate (Sigma- Aldrich)	2.2 g/L
FBS (GIBCO)	10%
Hydrocortisone	20 µl/L

1.74 g/L and 2.2 g/L Sodium Bicarbonate were dissolved in 1 L of distilled water, the pH was adjusted between 6.9-7.2. The media was filter sterilized before the addition of FBS and hydrocortisone

Trypsin

0.25% of Trypsin was prepared as follows: 25% Trypsin was diluted 100x with phosphate buffer saline (PBS) and filter sterilized with 0.22 µM

Phosphate Buffered Saline (PBS)

NaCl	8 g/L
KCl	0.2 g/L
Na_2HPO_4	1.44 g/L
KH_2PO_4	0.24 g/L

pH was adjusted to 6.9-7.2 with either NaOH or HCl. The solution was autoclaved for sterility

Transformation

Luria Bertani (LB) Broth

Bacto-tryptone	10 g/L
Bacto-yeast extract	10 g/L
NaCl	5 g/L

This was autoclaved for sterility

LB Agar with ampicillin

Bacto-tryptone	10 g/L
Bacto-yeast extract	10 g/L
NaCl	5 g/L
Agar	

This was autoclaved for sterility, cooled to approximately 60°C before adding ampicillin and poured into the agar plates.

Protein extraction

Protein Lysis Buffer

Mixture A		Final Concentration
10% NaDOC	5 ml/100 ml	0.5%
1M Hepes	2.5 ml/100 ml	25 mM
5M NaCl	6 ml/100 ml	0.3 M
1M $MgCl_2$	150 µl/100 ml	1.5 mM
0.1 M EDTA	200 µl/100 ml	0.2 mM
100% Triton X-100	1 ml/ 100 ml	1%
1 M β-glycerophosphate	2 ml/100 ml	20 mM

Mixture B	
1 M DTT	1 µl
100x HALT protease cocktail inhibitors	10 µl
200 mM Na_3VO_4	5 µl

1 ml of mixture A was added to mixture B prior to extraction and must be use within 20 minutes of preparation

Western blotting

1.5 M Tris-HCL (pH8.8)

Tris base	181.5/L

pH was adjusted to 8.8 with HCl

0.5 M Tris-HCL (pH 6.8)

Tris base	60 g/L

pH was adjusted to 6.8 with HCl

10% SDS	
Sodium Dodecyl Sulphate	100 g/L
10% Ammonium Persulphate (APS)	
0.3 g was dissolved in 30 ml of distilled water	
10X SDS-PAGE Running Buffer	
Tris base	30 g/L
Glycine	144 g/L
1X SDS-PAGE Running Buffer	
10x stock	80 ml/L
10% SDS (w/v)	8 ml/L
Coomasie Brilliant Blue (R250) Staining Solution	
Coomasie Brilliant Blue (R250)	1 g
Methanol	180 ml
Glacial Acetic Acid	40 ml
This was made up to 400 ml with distilled water and stored at room temperature	
Destaining solution	
Methanol	200 ml
Glacial Acetic Acid	100 ml
This was made up to 1L with distilled water and stored at room temperature	
Ponceau S	
Ponceau S	0.033 g
Glacial Acetic Acid	0.3 ml
This was made up to 30 ml with distilled water and stored at room temperature	

6 x Laemmli loading buffer	
SDS	1.2 g
Glycerol	4.7 ml
Tris 0.5M pH 6.8	1.2 ml
DTT	0.93 g
Bromophenol blue	0.003 g
This was made up to 10 ml with distilled water, aliquoted and stored in -20° C	
12 % Resolving Gel	
dH2O	34 ml
30% Acrylamide/Bis	4 ml
1.5 M Tris (pH 8.8)	2.5 ml
10% SDS	100 µl
10% APS	50 µl
TEMED	5 µl
4% Stacking Gel	
dH_2O	1830 µl
30% Acrylamide/Bis	390 µl
1.5 M Tris (pH 8.8)	750 µl
10% SDS	30 µl
10% APS	15 µl
TEMED	3 µl
cDNA Synthesis	
cDNA synthesis Reaction Mix (Applied Biosystem)	
Mixture A	
10X RT buffer	10 µl
25X dNTP mixture	4 µl
10X Random primers	10 µl
MultiScribe RT (50U/µl)	5 µl
RNase free water	21 µl
Final Volume	50 µl

Mixture B

2 µg of total RNA	X µl
RNase free water	Y µl
Final Volume	50 µl

Mixture A was added to Mixture B to a total of 100 µl and was subjected to cDNA synthesis

Agarose Gel Electrophoresis

50X TAE

Tris base	242 g/L
Glacial Acetic Acid	57.1 ml/L
EDTA(0.5M; pH8.0)	100 ml/L

Immunohistochemistry

Sodium Citrate buffer (pH 6.0)

Tri-Sodium Citrate	2.93/L

pH was adjusted to 6.0 with either NaOH or HCl.

Tris-EDTA Buffer (pH 9.0)

Tris	1.21/L
EDTA	0.37 g/L

pH was adjusted to 6.0 with either NaOH or HCl.

10 X TBS

Trizma base	61 g/L
NaCl	90 g/L

pH was adjusted to 6.0 with either NaOH or HCl and stored at room temperature. Upon using, buffer was diluted 10 X with distilled water and 500 ml of Tween 20/L was added (0.5%).

Organotypic co culture

1% Agar Solution	100 ml
Low melting point Agarose	1 g/100 ml
Formaldehyde	10 ml/100ml
dH_20	to top up 100ml

Formaldehyde with saline	
NaCl	45 g
Formaldehyde	500 ml

Appendix F

List of Primer and Antibodies used in the Study

List of primers.

	Gene	Forward Primer	Reverse Primer
1	RPL13A	GTA CGC TGT GAA GGC ATC AA	GTT GGT GTT CAT CCG CTT G
2	FOLR1	GCT GCT CAG CTG ACC TCC TTT TAC	ATT CAA AGT GGC TGT CAG AG
3	SERPINE1	TGC CCT CAC CAA CAT TCT GA	GAG AGG CTC TTG GTC TGA AAG ACT
4	BCL2A1	TGC AGT GCG TCC TAC AGA TAC C	TCC AAG CAT GAC TTC AGA TTC TTT
5	IL11	TGC TGT GAT CCC AAT TTT GTG	CAA TCC CAC CTC TCT CCT TTG A
6	LYN	GGT GGC TGC CTC ATT TAG AGA	TGT TCT TCC TGA GTA ATT TAG CCT CTA TT
7	TNFRSF1B	GCG AGA GTC TGT CTC AAA AGA AAA	GGC CAC AGC ACT GAA TAT GGT
8	PNUTL1	GCA TTG CAT GAG AAG GTC AAC A	CAT GGA TCC CAA ACT TGT CAA TC
9	MXI1	TGT CTG CGT GGG TCA GAA GA	AGA TGT CAT TTT GGT TTT CAA CAC A
10	CSF3R	CAA CAA GAC CTG GAG GAT GGA	TCC CAT GGT GTC CTG GTA CA
11	IL1B	TGG AAT TTG AGT CTG CCC AGT T	AAT TGC ATG GTG AAG TCA GTT ATA TCC
12	TRAF4	CGA CCC AAA CTG GAA GAA TTT C	CGC ACA TAG TTT CGC TTT CG
13	CTSL	GCT AAT GAC ACC GGC TTT GTG	TTT CAA ATC CGT AGC CAA CCA
14	MMP1	GAA CCC TCG CTG GGA GCA AAC	CCT GGG CCT GGT TGA AAA GC
15	MMP10	GAT CCC ACT GGA ACC CTG AA	TTG GAG GAA AAC CCA GGG TAT
16	ITGB4	GCC GCT ACG AGG GTC AGT T	TCC ATT ACA GAT GCC CCC ATT
17	PTHLH	AGA TTT ACG GCG ACG ATT CTTC	AGC GGC TGC TCT TTG TAC GT
18	TGFBR3	CTG TGT ACG AAG ATG GAG AAG CA	TGA ACG TCT TCT TAT TCT GCA TCAT
19	FOLR1 CLONING	GGG ACA GAC ATG GCT CAG CGG AT	TGT CTG GTT TAT TCA AAG TGG CTG TCA
20	FOLR1 CLONING SELECTION		
	FOLR1	GCT GCT CAG CTG ACC TCC TTT TAC	
	V5		ACC GAG GAG AGG GTT AGG GAT

List of antibodies with the respective dilution factors and antigen retrieval buffers.

Primary Antibody	Manufacturer	Dilution	Antigen retrieval Buffer	Positive Control
BCL2A1	Santa Cruz Biotechnology, Santa Cruz,	1:100	Citrate Buffer pH 6.0	--
CD3	Abcam, Cambridge, England	1:100	Tris/EDTA Buffer pH9.0	Human tonsil tissues
FOXP3	Abcam, Cambridge, England	1:50	Tris/EDTA Buffer pH9.0	Human tonsil tissues
ITGB4	Chemicon, Temecula, U.S.A	1: 200	Citrate Buffer pH6.0	-
MMP1	Chemicon, Temecula, U.S.A	1:300	Citrate Buffer pH6.0	-

Appendix G

Formula for calculation of cell doubling and proliferation ratio

$$\text{Doubling time} = \frac{\ln 2}{\ln(\text{ratio})} \times \text{No of days}$$

$$\text{Ratio} = \frac{\text{Cell number on harvested day}}{\text{Cell number seeded on day-0}}$$

Appendix H

OSCC risk habits data from OSCC patients included in the gene expression analysis

	# of OSCC patients included in the gene expression analysis	Cheek (%)	Gum (%)	Tongue (%)
OSCC Risk Habits				
Single risk habit				
Alcohol only	2	0	0	2(100.0)
Betel quid chewing only	19	9(47.4)	6(31.6)	4(21.0)
Smoking only	4	0	2(50.0)	2(50.0)
Multiple risk habits				
Alcohol and betel quid chewing	4	2(50.0)	2(50.0)	0
Alcohol and smoking	9	1(11.1)	2(22.2)	6(66.7)
Betel quid chewing and smoking	3	2(66.7)	0	1(33.3)
Alcohol, betel quid and smoking	4	1(25.0)	1(25.0)	2(50.0)
No habits	6	2(33.3)	1(16.7)	3(50.0)
NA	11	4(36.4)	1(9.1)	6(54.6)