

Supplementary Table S1. Clinicopathological data of Oropharyngeal cancer patients under study.

Patient and tumor characteristics	Full panel (n = 85)	Oropharyngeal cancer	
		HPV positive (n = 48)	HPV negative (n = 37)
<i>Age</i>			
Mean ± S.D.	63.4 ± 10.2	62.7 ± 10.9	64.5 ± 9.3
<i>Gender</i>			
Female	20 (23.5%)	16 (33.3%)	4 (10.8%)
Male	65 (76.5%)	32 (66.7%)	33 (89.2%)
<i>Smoking status</i>			
Smoker	59 (69.4%)	29 (60.4%)	30 (81.1%)
Non smoker	26 (30.6%)	19 (39.6%)	7 (18.9%)
<i>Alcohol exposure</i>			
Ever	66 (77.6%)	33 (68.7%)	33 (89.2%)
Never	19 (22.4%)	15 (31.3%)	4 (10.8%)
<i>Tumor size</i>			
T1	17 (20.0%)	11 (22.9%)	6 (16.2%)
T2	39 (45.9%)	28 (58.3%)	11 (29.7%)
T3	7 (8.2%)	3 (6.3%)	4 (10.9%)
T4	22 (25.9%)	6 (12.5%)	16 (43.2%)
<i>Lympho-node status</i>			
N0	24 (28.2%)	12 (25.0%)	12 (32.4%)
N+	61 (71.8%)	36 (75.0%)	25 (67.6%)
<i>Stage</i>			
I	41 (48.2%)	37 (77.1%)	4 (10.8%)
II	7 (8.3%)	5 (10.4%)	2 (5.4%)
III	9 (10.6%)	6 (12.5%)	3 (8.1%)
IV	28 (32.9%)	0 (0.0%)	28 (75.7%)
<i>Treatment</i>			
Surgery	43 (50.6%)	25 (52.1%)	18 (48.6%)
Surgery + CRT or BRT or RT	26 (30.6%)	14 (29.2%)	12 (32.4%)
CRT or BRT or RT	9 (10.6%)	5 (10.4%)	4 (10.8%)
Chemotherapy	7 (8.3%)	4 (8.3%)	3 (8.1%)
<i>Recurrence events</i>			
Positive	20 (23.5%)	9 (18.8%)	11 (29.7%)
Negative	65 (76.5%)	39 (81.2%)	26 (70.3%)

S.D.:standard deviation; CRT: chemoradiation therapy; BRT: bioradiotherapy;
RT: radiation therapy

Supplementary Table S2. Clinicopathological data of liquid biopsy patients under study.

Patient number	Age (years)	Gender	Primary HPV status	ctDNA HPV status	Smoking status	Alcohol exposure	TNM stage	Primary site	Initial treatment	DFS (months)	OS (months)
1-L	42	Male	positive	positive	Smoker	Ever	T4N2M0	R-Tonsil	oropharyngectomy, B-ND	12	27
2-L	72	Female	positive	negative	Smoker	Ever	T4N2M0	R-Tonsil	CRT (CDDP+70Gy)	6	6
3-L	45	Male	positive	negative	Smoker	Ever	T4N0M0	BOT	oropharyngectomy, B-ND	12	12
4-L	73	Female	positive	negative	Non smoker	Ever	T2N2M0	L-Tonsil	oropharyngectomy, L-ND	22	27
5-L	66	Female	positive	positive	Non smoker	Never	T2N0M0	L-Tonsil	oropharyngectomy, L-ND	25	25
6-L	47	Male	positive	positive	Non smoker	Never	T2N1M0	L-Tonsil	oropharyngectomy, L-ND	4	13
7-L	60	Male	positive	positive	Smoker	Ever	T2N2M0	L-Tonsil	oropharyngectomy, B-ND	12	12
8-L	61	Male	positive	positive	Smoker	Ever	T2N1M0	R-Tonsil	CRT (CDDP+66Gy)	12	12

DFS, disease-free survival; OS, overall survival; BOT, base of tongue; ND, neck dissection; CRT, chemoradiotherapy; B, bilateral; R, right; L, left.

Supplementary Table S3. Q-MSP Primer List

Gene	Forward primer 5'-3'	Reverse primer 5'-3'
ACTB	TGGTGATGGAGGAGGTTTAGAAGT	AACCAATAAAACCTACTCCTCCCTTAA
GALR1	GGTTCGCGGTATTTCGGTAGT	GGTTCGCGGTATTTCGGTAGT
GALR2	CGATTGCGGGGGTTGGAGTTCGGA	CCAACAACGACCGACGACGCTA
GHSR	TAGTATGTGGAACGCGACGT	AACTCGTCGCCCAACGAATC
HCRTR1	CGGGTTTCGGGGTTGGAAGATA	CGGGTTTCGGGGTTGGAAGATA
HCRTR2	CGCGTAGTTTTTTTTATCGTAA	CGAACATCACGAACTCAAATCCG
MLNR	ATCGAGAGGGATTACGCGTT	AAACCGTCGCTACCGTTCCA
NPY1R	TTCGGGTTCGGGTAGCGTTG	AACGAATCTCTAACGAAACCG
NPY2R	CGAGTGAGTGCGGTGTTTAGGCG	CGAACGAACAACCGAAACAATC
NPY4R	AGGTTGGGCGGGCGTAGGCGGGA	CGAAACAAAACCGCGCCTACTT
NPY5R	AGTTACGTGTTTTCGAGACGT	CGTCCTACACCCCGACGATAA
NTSR1	TGCGTTTTAATAGTTTCGCGT	CGTTACCGAAACCCGAAACCAACA
NTSR2	GTCGCGGGAGCGGGATGGAAATTA	AACGAATATCCACGCCCAACCGA
NMUR1	GCGCGGGTTGGGGTCGTTGT	CGCACCTACCATACGACCCG
PTGDR1	TTTCGTACGTTATGAAGTCGT	CGCCCATCACCGCCGAATTAC
PTGDR2	GTTACGTGTAATTCGCGGTAG	CAAACCTCACGACCGCGTAACT
PTGER1	GGGCGAGGCGATTATATGCG	ATTAAACACGACGCCCAACGT
PTGER2	GTAGGCGCGGGAGTTTCGAG	CCGACGACGACTTACCCTAA
PTGER3	AGTGAGTTTTGGCGTCGTCG	CGTTTACCGCGACTAAAATA
PTGER4	AGATGTTGCGGTTTCGCGGAT	ACCACTAAAACCGACGACCGT
PTGFR	TCGCGTTAAGGGAACGAGTG	GCCGCTACCTCAAATTCCACCG
PTGIR	ATGGCGGATTCGTGTAGGAA	CCGATCGCCGTACGCTCAAA
SSTR1	CGGGTGCGCGAGGAGAAAGTT	TAGTTCGGGTAGTTGCGGCGAA
TACR1	TTGGCGTAGTTGTGCGGTTG	CGCGAATTAACCTACGCACGA
TBXA2R	AGAGAGCGAGTCGCGGGTTG	AGCCCCTAAAATCGCGACCTA

Supplementary Table S4. Results of the ROC curve analysis, the sensitivity, specificity, and cut off value.

Genes	ROC Area	Sensitivity (%)	Specificity (%)	Cutoff value
GALR1	0.838	50.0	97.2	0.038
GALR2	0.845	61.1	100	0.038
GHSR	0.810	58.3	93.8	0.563
HCRTR1	0.735	61.1	80.6	0.104
HCRTR2	0.834	69.4	97.2	0.099
MLNR	0.605	25.0	97.2	0.700
NPY1R	0.807	44.4	91.7	0.024
NPY2R	0.697	94.4	13.9	0.160
NPY4R	0.572	86.1	19.4	0.300
NPY5R	0.760	61.1	47.2	0.025
NTSR1	0.622	70.3	50.0	0.045
NTSR2	0.574	75.0	36.0	0.009
NMUR1	0.563	26.5	97.0	0.735
PTGDR1	0.677	86.1	69.4	0.161
PTGDR2	0.627	94.4	66.7	0.123
PTGER1	0.657	88.9	68.1	0.048
PTGER2	0.615	83.3	61.1	0.161
PTGER3	0.477	86.11	55.6	0.502
PTGER4	0.541	100	56.9	0.419
PTGFR	0.629	97.2	68.1	0.368
PTGIR	0.674	86.1	68.1	0.109
SSTR1	0.952	61.1	100	0.012
TACR1	0.628	38.9	97.2	0.008
TBXA2R	0.661	77.8	68.1	0.082

Supplementary Table S5. Results of log-rank tests for effect of number of methylated genes on disease-free survival in 85 oropharyngeal cancer.

No. methylated genes	No. patients with profile	<i>P</i>
≥4	74	0.2678
≥5	69	0.2619
≥6	64	0.2165
≥7	57	0.0931
≥8	52	0.0676
≥9	46	0.0271*
≥10	36	0.0301*
≥11	32	0.0035*
≥12	30	0.0094*
≥13	26	0.0015*
≥14	20	0.0003*
≥15	16	0.0019*
≥16	13	0.0251*
≥17	7	0.3885
≥18	6	0.3825

* $p < 0.05$

Supplementary Table S6. Methylation status and expression levels of the GPCRs genes in TCGA cohort of HNSCC.

Gene	Promoter methylation			Gene expression		
	Tumor	Normal	<i>P</i> †	Tumor	Normal	<i>P</i> †
GALR1	0.325±0.134	0.143±0.040	< 0.001*	0.349±0.673	0.702±1.067	0.002*
GALR2	0.385±0.097	0.271±0.032	< 0.001*	14.27±73.04	10.48±9.567	0.734
GHSR	0.434±0.015	0.170±0.002	< 0.001*	0.191±1.204	0.011±0.069	0.327
HCRTR1	0.285±0.073	0.269±0.256	< 0.001*	0.562±1.136	0.609±1.059	0.795
HCRTR2	0.366±0.120	0.273±0.260	< 0.001*	1.152±7.235	0.337±0.465	0.461
MLNR	0.310±0.009	0.207±0.001	< 0.001*	0.695±1.410	0.238±0.330	0.034*
NPY1R	0.216±0.106	0.155±0.147	< 0.001*	8.030±15.37	73.82±112.5	< 0.001*
NPY2R	0.333±0.129	0.110±0.105	< 0.001*	0.260±0.900	2.414±4.468	< 0.001*
NPY4R	0.278±0.113	0.234±0.223	< 0.001*	14.61±18.95	5.677±6.473	0.002*
NPY5R	0.294±0.156	0.109±0.104	< 0.001*	0.430±1.114	6.679±10.04	< 0.001*
NTSR1	0.166±0.022	0.069±0.002	< 0.001*	42.03±137.4	4.281±6.320	0.073
NTSR2	0.368±0.010	0.300±0.002	< 0.001*	0.942±9.403	0.567±0.920	0.794
NMUR1	0.167±0.008	0.106±0.000	< 0.001*	4.549±11.16	4.975±6.064	0.598
PTGDR1	0.382±0.152	0.164±0.052	< 0.001*	12.80±11.25	7.476±6.384	0.002*
PTGDR2	0.481±0.117	0.554±0.057	< 0.001*	1.851±0.964	2.650±1.023	< 0.001*
PTGER1	0.197±0.112	0.144±0.031	< 0.001*	5.072±15.42	200196±3.108	0.223
PTGER2	0.226±0.093	0.158±0.026	< 0.001*	33.21±51.07	41.88±49.14	0.284
PTGER3	0.221±0.076	0.180±0.022	< 0.001*	80.56±134.0	263.0±283.6	< 0.001*
PTGER4	0.032±0.007	0.033±0.004	0.711	251.4±207.8	419.7±447.8	< 0.001*
PTGFR	0.313±0.145	0.128±0.053	< 0.001*	52.93±131.4	182.9±306.2	< 0.001*
PTGIR	0.752±0.112	0.789±0.035	0.02*	56.53±79.67	34.07±57.51	0.071
SSTR1	0.206±0.124	0.077±0.024	< 0.001*	2.902±5.797	15.35±21.20	< 0.001*
TACR1	0.157±0.090	0.074±0.021	< 0.001*	19.66±35.03	95.01±100.7	< 0.001*
TBXA2R	0.178±0.052	0.166±0.030	0.118	36.87±32.15	19.33±22.18	< 0.001*

† Student t test

* *P*<0.05

Original set of tissue analysis of Q-MSP

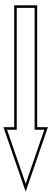
48 OPC (HPV positive) samples
37 OPC (HPV negative) samples



Statistic evaluation

Statistic analysis of aberrant DNA methylation

- (I) Calculation of risk score by multivariate Cox regression and Kaplan-Meier analysis
- (II) Correlation with prognosis in HPV-associated OPC



13 markers

(GALR2, GHSR, NMUR1, NPY1R, NPY2R,
NPY4R, NTSR2, NPY5R, PTGDR1, PTGDR2,
PTGER4, PTGIR and TBXA2R)

ctDNA validation of Q-MSP

- (I) Liquid biopsy from 8 HPV positive OPC cases
- (II) Methylation analysis of 8 pre-treatment ctDNA
- (III) Methylation analysis of 8 after-treatment ctDNA



3 markers (PTGDR1, PTGDR2, and PTGIR)