OMTN, Volume *16*

Supplemental Information

miR-31-5p Is a Potential Circulating Biomarker and Therapeutic Target for Oral Cancer

Zhiyuan Lu, Qianting He, Jianfeng Liang, Wuguo Li, Qiao Su, Zujian Chen, Quan Wan, Xiaofeng Zhou, Laurel Cao, Jingjing Sun, Yu Wu, Lin Liu, Xinming Wu, Jinsong Hou, Keqian Lian, and Anxun Wang

Supplementary Table S1 The serum miRNA levels in oral cancer patients detected by qRT-PCR

Factors	miR-99a-5p (△Ct)	*p*	miR-31-5p (△Ct)	*p*	miR-138-5p (△Ct)	*p*	miR-21-5p (△Ct)	*p*	miR-375-3p (△Ct)	*p*
Age≥60	2.61±1.75	0.919	-3.41±1.87	0.394	2.21±1.56	0.332	-3.34±1.46	0.919	2.30±1.48	0.516
Age<60	2.47±1.28		-3.63±1.95		2.35±1.23		-3.40±1.81		2.39±1.22	
Male	2.72±1.51	0.068	-3.48±2.00	0.754	2.40±1.51	0.193	-3.25±1.65	0.290	2.25±1.40	0.075
Female	2.02±1.47		-3.63±1.61		1.94±0.95		-3.70±1.58		1.90±1.10	
T_{1+2}	2.45±1.54	0.141	-3.28±2.42	0.274	2.19±1.39	0.200	-2.95±1.44	0.151	2.23±1.34	0.070
T_{3+4}	2.82±1.47		-3.59±1.73		2.59±1.41		-3.49±1.68		2.72±1.34	
C_{I+II}	2.26±1.48	0.012	-3.12±2.20	0.100	1.92±1.25	0.001	-3.19±1.58	0.545	2.01±1.23	0.001
C_{III+IV}	2.95±1.52		-3.79±1.63		2.81±1.45		-3.49±1.68		2.84±1.39	
LN^-	2.43±1.48	0.135	-2.85±1.78	0.156	2.14±1.35	0.068	-3.34±1.63	0.475	2.20±1.32	0.059
LN^+	2.89±1.65		-3.72±1.90		2.73±1.51		-3.45±1.68		2.28±1.38	
Well	2.86±1.70	0.144	-3.38±2.09	0.530	2.54±1.77	0.590	-2.99±1.89	0.028	2.52±1.68	0.431
Moderate-Poor	2.30±1.35		-3.63±1.76		2.09±1.02		-3.65±1.37		2.22±1.03	

Supplementary Table S2 ROC curve analyses of serum miRNAs for distinguishing pre- from postoperation oral cancer patients in cohort 4.

	AUC	Sensitivity	Specificity	*p* value	95% CI
miR-99a-5p	0.506	18.3%	91.5%	0.908	0.403-0.610
miR-31-5p	0.666	74.5%	52.4%	0.002	0.571-0.761
miR-138-5p	0.547	93.9%	21.3%	0.373	0.440-0.654
miR-21-5p	0.611	59.6%	58.5%	0.037	0.507-0.714
miR-375-3p	0.505	59.6%	45.1%	0.926	0.401-0.609
Combination*	0.780	81.7%	63.8%	0.000	0.693-0.868

*Combination of miR-99a-5p, miR-31-5p, miR-138-5p, miR-21-5p and miR-375-3p.

Supplementary Table S3 Clinical characterization of the cohorts used in this study*

	Cohort 1		Cohort 2	Cohort 3	Cohort 4	
Factors	OC (82)	NC (53)			Pre-op (82)	Post-op (47)
Age						
≥60	41(50%)	12 (22.6%)	5 (45.5%)	13 (54.2%)	41(50%)	19 (22.6%)
<60	41 (50%)	41 (77.4%)	6 (54.5%)	11 (45.8%)	41 (50%)	28 (77.4%)
Gender						
Male	61 (74.4%)	27 (50.9%)	9 (81.8%)	19 (79.2%)	61 (74.4%)	32 (50.9%)
Female	21 (25.6%)	26 (50.1%)	2 (18.2%)	5 (20.8%)	21 (25.6%)	15 (50.1%)
T classification						
T_{1+2}	63 (76.8%)		8 (72.7%)	17 (70.8%)	63 (76.8%)	36 (76.6%)
T_{3+4}	19 (23.2%)		3 (27.3%)	7 (29.2%)	19 (23.2%)	11 (23.4%)
Clinical stage						
C_{I+II}	49 (59.8%)		5 (45.5%)	10 (41.7%)	49 (59.8%)	27 (57.4%)
C_{III+IV}	33 (40.2%)		6 (54.5%)	14 (58.3%)	33 (40.2%)	20 (42.6%）
LN metastases						
Negative	63 (76.8%)		7 (63.6%)	16 (66.7%)	63 (76.8%)	34 (72.3%)
Positive	19 (23.2%)		4 (36.4%)	8 (33.3%)	19 (23.2%)	13 (27.7%)
Differentiation						
Well	35 (42.7%)		5 (45.5%)	11 (45.8%)	35 (42.7%)	18 (38.3%)
Moderate-Poor	47 (57.3%)		6 (54.5%)	13 (54.2%)	47 (57.3%)	29 (61.7%)

* OC: oral cancer patients, the pathologic diagnosis of all patients was squamous cell carcinoma; NC: normal subjects; Oral cancer cases of cohorts 2, 3 and 4 came from cohort 1; Cohort 2: 11 paired match tumor tissues and adjacent normal tissues; Cohort 3: 24 paired match tumor tissues and preoperation sera.

Supplementary Table S4 The primer sequences of miRNAs used for qRT-PCR.

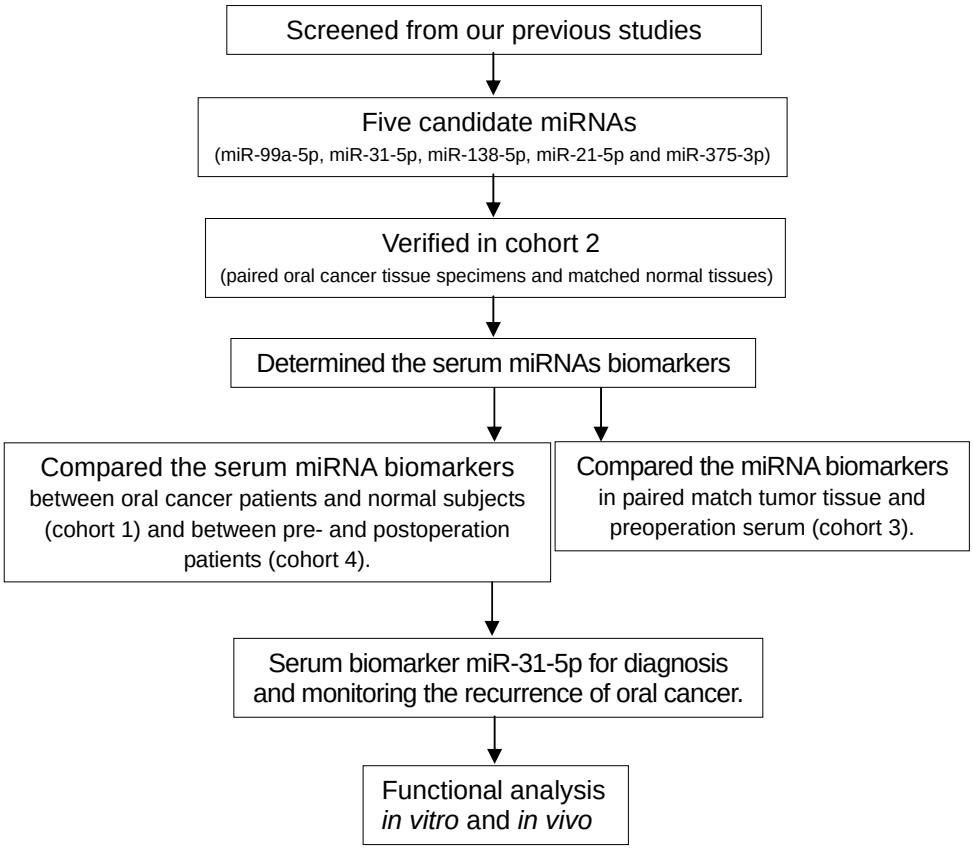
	Primer sequences
hsa-miR-99a-5p	AACCCGTAGATCCGATCTTGTG
hsa-miR-31-5p	AGGCAAGAGGCTGGCATAGC
hsa-miR-138-5p	TGCTGGTGTTGTGAATCAGGC
hsa-miR-21-5p	GCAGCATACCAGACTGATGTTGA
hsa-miR-375-3p	ACTTTGTTCGTTCGGCTCGC
hsa-U6	CTCGCTTCGGCAGCACA

Supplementary Table S5 The sequences of the miR-31-5p mimic and antagomiR-31-5p

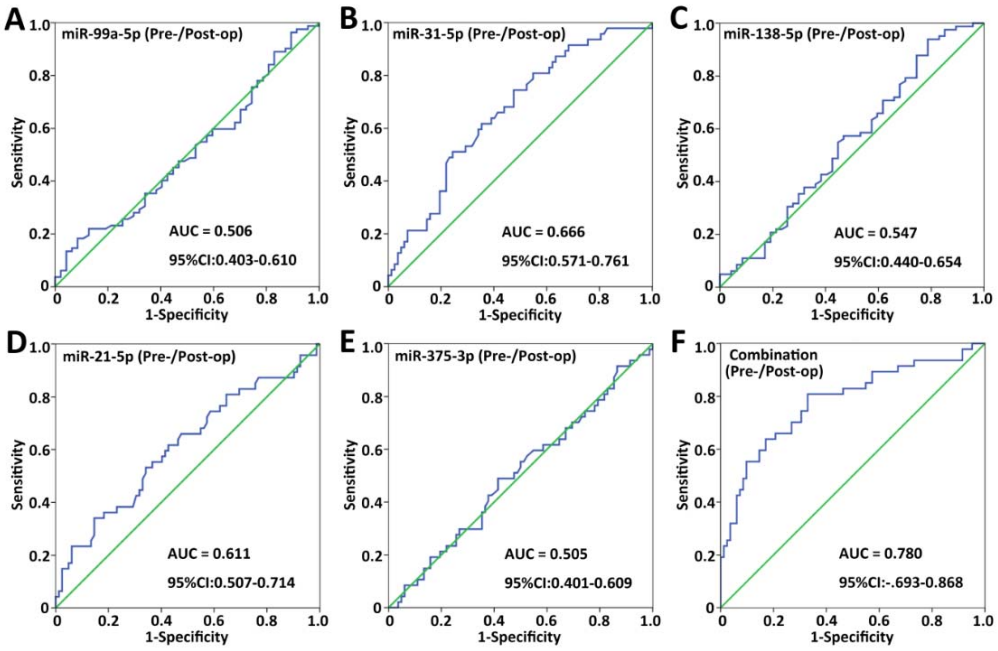
	Sequences
miR-31-5p mimic	5’-AGGCAAGAUGCUGGCAUAGCU-3’
antagomiR-31-5p	5’-CAGCUAUGCCAGCAUCUUGCCU-3’

Supplementary Table S6 Clinicopathological characterization of two oral cancer patients for PDX models

	Case 1	Case 2
Age (years)	41	42
Gender	Male	Male
T classification	T_4	T_2
Clinical stage	C_{IV} (T4N0M0)	C_{IV} (T2N2M0)
LN metastases	Negative	Positive
Differentiation	Moderate	Moderate
Molecular pathology	Ki-67(40%+); E-cad(+); Vim(+); c-Myc(+); P53(80%+); HPV(-)	Ki-67(15%+); E-cad(+); Vim(-); c-Myc(+); P53(1%+); HPV(-)

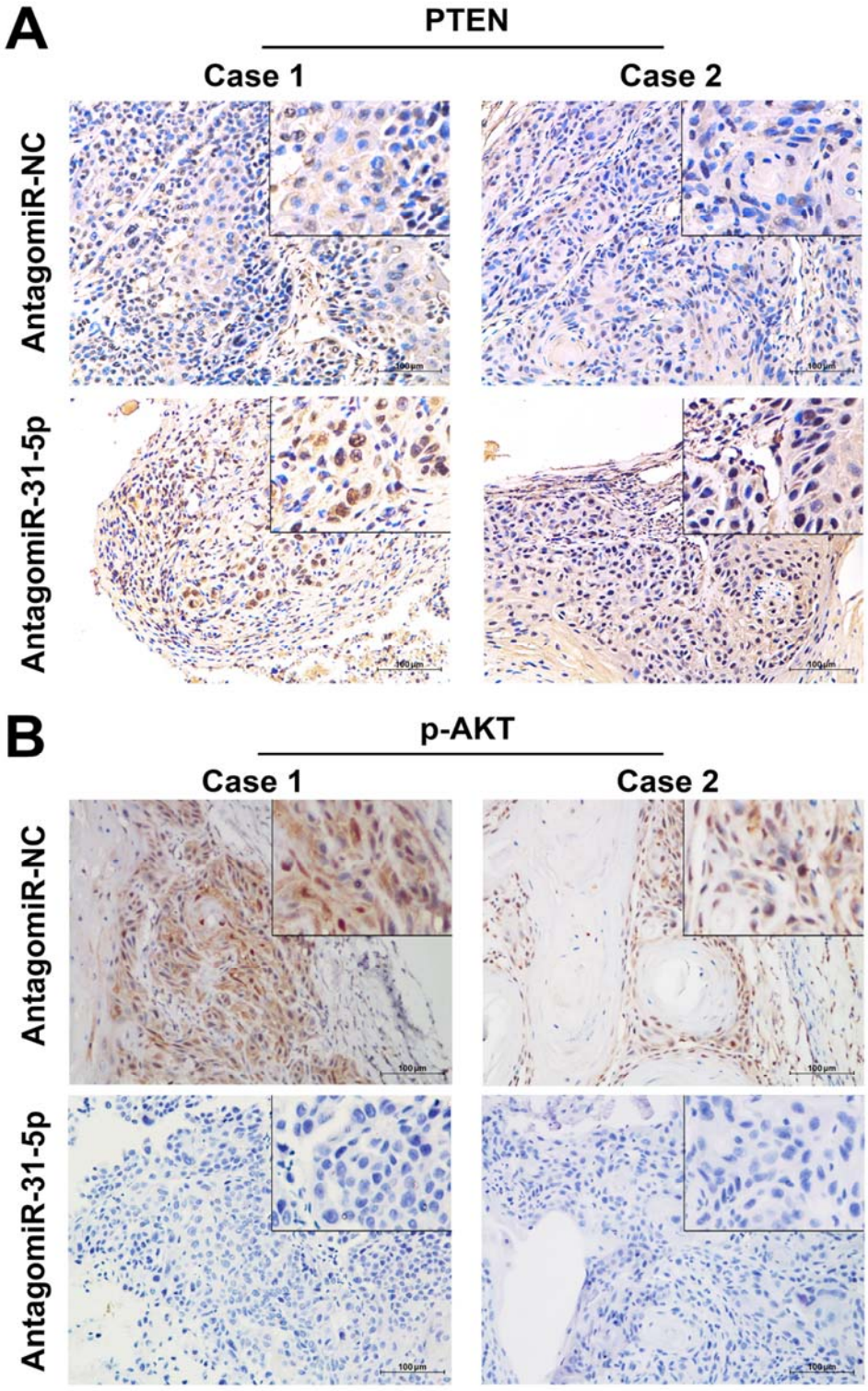


Supplementary Figure S1 Flow-chart of the overall study design.



Supplementary Figure S2 Predictive value of five serum miRNAs in pre- and postoperative oral cancer patients.

ROC curves of serum miR-99a-5p (A), miR-31-5p (B), miR-138-5p (C), miR-21-5p (D) and miR-375-3p (E) levels and the five-miRNA panel (F) were used to differentiate pre- from postoperation oral cancer patients (n=47) in cohort 4.



Supplementary Figure S3 AntagomiR-31 inhibited the growth of PDX through the PTEN/AKT pathway

(A-B) Representative immunohistochemical staining of PTEN (A) and p-AKT (B) expression in xenograft tissues (magnification, ×200).