


Mitochondrial assembly receptor expression is an independent prognosticator for patients with oral tongue squamous cell carcinoma

Journal of the Renin-Angiotensin-Aldosterone System
 July-September 2017: 1–8
 © The Author(s) 2017
 Reprints and permissions:
sagepub.co.uk/journalsPermissions.nav
 DOI: 10.1177/1470320317717904
journals.sagepub.com/home/jra


Yan-Ye Su^{1,*}, Chang-Han Chen^{2,3,4,*}, Chih-Yen Chien¹,
 Wei-Che Lin⁵, Wan-Ting Huang⁶ and Shau-Hsuan Li⁷

Abstract

Introduction: Recent evidence suggests that the local renin-angiotensin system has been implicated in various malignancies. The mitochondrial assembly receptor is a newly identified receptor for angiotensin peptides, angiotensin-(1-7), and has an important role in the renin-angiotensin system. However, the role of the mitochondrial assembly receptor in the prognosis of cancer patients remains unclear. The aim of this study was to evaluate the significance of mitochondrial assembly receptor signaling in the prognosis of oral tongue squamous cell carcinoma.

Methods: Mitochondrial assembly receptor immunohistochemistry was examined in 151 oral tongue squamous cell carcinoma patients and was correlated with treatment outcome. The functional relevance of the mitochondrial assembly receptor in oral tongue squamous cell carcinoma cell lines was evaluated by 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide reduction and bromodeoxyuridine incorporation assays.

Results: Mitochondrial assembly receptor overexpression was significantly correlated with early pathological T classification ($p=0.029$) and the absence of extracapsular spread ($p=0.039$). Univariate analyses demonstrated that mitochondrial assembly receptor overexpression was significantly associated with superior overall survival ($p=0.012$). In multivariate comparison, mitochondrial assembly receptor overexpression remained independently associated with superior overall survival ($p=0.008$, hazard ratio=1.862). In vitro, angiotensin-(1-7) suppressed the cell growth in oral tongue squamous cell carcinoma cells, and this response was reversed by the mitochondrial assembly receptor antagonist, A779.

Conclusion: Mitochondrial assembly receptor expression is independently associated with the prognosis of oral tongue squamous cell carcinoma patients. These findings suggest that mitochondrial assembly receptor signaling may be a promising novel target for oral tongue squamous cell carcinoma.

Keywords

Tongue cancer, squamous cell carcinoma, mitochondrial assembly receptor, renin-angiotensin system, prognosis

Date received: 24 November 2016; accepted: 3 May 2017

¹Department of Otolaryngology, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan, R.O.C.

²Institute for Translational Research in Biomedicine, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan, R.O.C.

³Department of Applied Chemistry, and Graduate Institute of Biomedicine and Biomedical Technology, National Chi-Nan University, Taiwan, R.O.C.

⁴Center for Infectious Disease and Cancer Research, Kaohsiung Medical University, Kaohsiung, Taiwan, R.O.C.

⁵Department of Diagnostic Radiology, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan, R.O.C.

⁶Department of Pathology, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan, R.O.C.

⁷Department of Hematology-Oncology, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan, R.O.C.

*These authors contributed equally to this work.

Corresponding author:

Shau-Hsuan Li, Department of Hematology-Oncology, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, No.123, Dapi Rd, Niasong Dist., Kaohsiung City 833, Taiwan, R.O.C.
 Email: lee.a0928@msa.hinet.net



Introduction

Oral cancer, the sixth most common cancer worldwide, is a serious and growing problem in many parts of the globe.¹ More than 90% of oral cancers are squamous cell carcinomas.^{1,2} The prevalence of oral cancers is high in Asian countries, especially in South Asia.² Distinct Asian cultural practices such as betel-quid chewing, and varying patterns of tobacco and alcohol use are important risk factors that predispose individuals to oral cancers.^{2,3} In European and US populations, as well as in the populations of many Asian countries including Japan, Taiwan, Thailand, Yemen, India and Iran, the tongue has been reported to be the most frequently affected site, contributing to about 30–50% of all oral cancers.^{1,2} The treatment for early-stage oral tongue squamous cell carcinoma (OTSCC) generally consists of a single modality, either surgery or radiotherapy, while the treatment for locally advanced OTSCC is multimodal, with either surgery followed by adjuvant radiation or chemoradiation.^{4,5} The presence of cervical lymph node metastasis and extracapsular spread lymph nodes provides particularly reliable predictors of regional and distant failure.⁶ Despite advances in treatment, the survival of patients with OTSCC has not significantly improved over the past several decades.⁷ Therefore, the discovery of prognostic biomarkers for OTSCC could improve risk-adapted treatment strategies and provide useful insights that could help to facilitate the development of potential novel therapeutic targets.

The renin-angiotensin system (RAS) plays a key role in the modulation of many functions in the body. The circulating RAS is well known as a hormonal circulating system that increases plasma aldosterone, promotes vasoconstriction, and retains water and sodium, and contributes to the systemic regulation of blood pressure and the maintenance of fluid and electrolyte homeostasis.⁸ Recently, emerging data have suggested that the local RAS plays an important role in tumor angiogenesis, progression, and environments.⁸ The function of the RAS is achieved by a group of enzymes, peptides, and receptors. It is well known that angiotensin II is the main effector of the RAS. Angiotensin II is an octapeptide cleaved from angiotensin I by the angiotensin I-converting enzyme (ACE). The majority of angiotensin II effects are mediated by the angiotensin II type 1 receptor (AT1R). Many studies have shown that the angiotensin II/AT1R axis is generally associated with cell proliferation and tumor angiogenesis, and AT1R has been reported to be associated with cancer patients' prognosis.^{8–10} Besides the classical angiotensin II/AT1R axis, another new regulatory axis in the RAS, the angiotensin-(1-7)/mitochondrial assembly receptor (MasR) axis, has been identified recently. MasR is a receptor of angiotensin-(1-7) which is generated directly from angiotensin II by the enzymatic activity of angiotensin-converting enzyme 2 (ACE2) and elicits a counter-regulatory influence on many of the effects induced by the angiotensin II/AT1R

axis such as its apoptotic, anti-proliferative, and angiogenesis inhibiting effects.¹¹ Until now, the role of angiotensin-(1-7)/MasR signaling in cancer progression has not been deeply investigated. Previous studies in several types of cancer have shown a tumor inhibitory role of MasR.^{7,9,12–16} However, to the best of our knowledge, the significance of MasR signaling on the prognosis of cancer patients remains unclear. In the present study, therefore, we sought to elucidate the prognostic role of MasR signaling in patients with OTSCC.

Methods

Patients and samples

This study included 151 patients with available paraffin blocks who underwent primary surgical resection for the treatment of OTSCC between January 2006–December 2012 at Kaohsiung Chang Gung Memorial Hospital. This retrospective study was approved by the Chang Gung Medical Foundation Institutional Review Board. Patients with synchronous cancers in the organs and those who received preoperative chemoradiotherapy, preoperative chemotherapy, or preoperative radiotherapy were excluded. Clinicopathological information was obtained retrospectively from clinical records and pathology reports. The pathological tumor node metastasis (TNM) stage was determined according to the 7th American Joint Committee on Cancer (AJCC) staging system.¹⁷ Adjuvant therapy, such as radiation alone or concurrent chemoradiation with platinum-based chemotherapy, was used in patients with adverse pathologic features, which was mostly based on the National Comprehensive Cancer Network (NCCN) guidelines. The radiation technique utilized was intensity-modulated radiation therapy. Overall survival (OS) was calculated from the time of surgery to death as a result of any cause. Disease-free survival (DFS) was computed from the time of surgery to the recurrence of the disease or to death from any cause without evidence of recurrence.

Immunohistochemistry

Immunohistochemistry staining was performed using an immunoperoxidase technique. Staining was performed on slides (4 mm) of formalin-fixed, paraffin-embedded tissue sections with primary antibodies against MasR (1:150, rabbit polyclonal, Lifespan Biosciences, cat# LS-A1528). Briefly, after deparaffinization and rehydration, the retrieval of the antigen was performed by treating the slides in 10 mmol/l citrate buffer (pH 6.0) in a hot water bath (95°C) for 20 min. Endogenous peroxidase activity was blocked for 15 min in 0.3% hydrogen peroxide. After blocking with 1% goat serum for one hour at room temperature, the sections were incubated with primary antibodies for at least 18 h at 4°C overnight. Immunodetection was performed using the

LSAB2 kit (Dako, Carpinteria, California, USA) followed by the use of 3-3'-diaminobenzidine for color development and hematoxylin for counterstaining. Incubation without the primary antibody was used as a negative control, and breast carcinoma known for its MasR positivity was used as a positive control.

The staining assessment was independently carried out by two pathologists (SLW and WTH) without any information about the clinicopathological features or prognosis for the associated patient. A semi-quantitative immunoreactive score (IRS) was used to evaluate the expression of MasR. The IRS was calculated by multiplying the staining intensity (graded as: 0=no staining, 1=weak staining, 2=moderate staining, and 3=strong staining) and the percentage of positively stained cells ($0 \leq 10\%$ of stained cells, $1=11-50\%$ of stained cells, $2=51-80\%$ of stained cells and $3 \geq 81\%$ of stained cells).^{18,19} The criterion for positive staining was a specimen with an IRS scoring ≥ 4 .

Cell culture and cell viability assays

The OTSCC cell lines HSC-3 and Cal27 were obtained from the American Type Culture Collection (ATCC) and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mmol/l glutamine, 100 U/ml penicillin, and 100 μ g/ml streptomycin. The viability of sub-confluent cells was analyzed by 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay. Cells were seeded at 5×10^3 cells/well in 96-well plates. Serum-starved cells were treated with A779 (a MasR antagonist, 100 nM) or vehicle control for 24 h following angiotensin-(1-7) (100 nM) stimulation. The cells were then cultured for 72 h. Next, the culture medium was removed and the cells were washed with fresh culture medium without FBS. Cells were then incubated with 0.5 mg/ml MTT, in a culture medium without FBS, for 4 h at 37°C in a 5% CO₂ atmosphere. The medium was then removed, 100 μ l dimethyl sulfoxide (DMSO) buffer was added, and the cells were incubated in the dark for an additional 10 min. Absorbance was measured on a microplate reader at 540 nm. The optical density (OD) values were normalized with the value for the control group. For bromodeoxyuridine (BrdU) incorporation assay, analysis was performed as described previously¹⁰ using cell proliferation enzyme-linked immunosorbent assay (ELISA) kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using the SPSS 17 software package. The Chi-square test or Fisher's exact test were employed to compare data between the two groups. For survival analysis, the Kaplan–Meier method was used for univariate analysis, and the difference between survival curves was tested by a log-rank test. In a stepwise

forward fashion, parameters with a p value < 0.1 at the univariate level were entered into a Cox regression model to analyze their relative prognostic importance.

For cell line experiments, a t -test was used for the statistical analysis.

Results

Patient characteristics

The median age for the 151 patients, 138 men and 13 women, was 52 years (range, 26–85 years) (see Table 1). The pathological T classifications of the 151 OTSCC patients were T1 in 41 (27%) patients, T2 in 51 (34%) patients, T3 in 12 (8%) patients, T4a in 42 (28%) patients, and T4b in five (3%) patients. The pathological N classifications were N0 in 83 (55%) patients, N1 in 23 (15%) patients, N2 in 42 (28%) patients, and N3 in three (2%) patients. Additional analyses conducted according to AJCC 7th staging system indicated stages of stage I in 29 (19%) patients, stage II in 32 (21%) patients, stage III in 24 (16%) patients, stage IVA in 59 (39%) patients, and stage IVB in seven (5%) patients. The analyses of histological grading revealed grade 1 in 85 (56%) patients, grade 2 in 60 (40%) patients, and grade 3 in six (4%) patients. Pathological features including vascular invasion, perineural invasion, and extracapsular spread were found in 26 (17%), 69 (46%), and 36 (24%) patients, respectively. With regard to surgical margins, 10 (7%) patients had positive or close margins (a close margin was defined as a margin ≤ 1 mm). Among all 151 patients, there were 124 (82%) smokers, 121 (80%) alcohol drinkers, and 114 (75%) betel-nut chewers. Furthermore, MasR overexpression was identified in 73 (48%) of the total 151 patients (Figure 1). At the time of analysis, the median periods of follow-up were 87 months (range, 61–113 months) for the 76 surviving patients and 61 months (range, 1–113 months) for all 151 patients. The five-year overall and disease-free survival rates for all 151 patients were 50% and 46%, respectively.

Correlation between clinicopathological parameters and MasR expression

The correlations between the clinicopathological parameters and the immunohistochemical expression of MasR are summarized in Table 2. MasR overexpression was significantly correlated with early pathological T classification and the absence of extracapsular spread.

Survival analyses

The correlations of the clinicopathological parameters and MasR expression with overall survival and disease-free survival are shown in Table 3. Univariate analyses demonstrated that MasR overexpression ($p=0.012$, Figure 2(a)),

Table 1. Characteristics of 151 patients with oral tongue squamous cell carcinoma.

Age (years) (median: 52, range: 26–85)		
Sex		
	Male	138 (91%)
	Female	13 (9%)
Pathological T classification		
	T1	41 (27%)
	T2	51 (34%)
	T3	12 (8%)
	T4a	42 (28%)
	T4b	5 (3%)
Pathological N classification		
	N0	83 (55%)
	N1	23 (15%)
	N2	42 (28%)
	N3	3 (2%)
Pathological 7 th AJCC stage		
	I	29 (19%)
	II	32 (21%)
	III	24 (16%)
	IVA	59 (39%)
	IVB	7 (5%)
Depth of invasion (mm)		
	<4 mm	18 (12%)
	≥4 mm	133 (88%)
Histologic grade		
	1	85 (56%)
	2	60 (40%)
	3	6 (4%)
MasR expression		
	Low expression	78 (52%)
	Over expression	73 (48%)
Vascular invasion		
	Absent	125 (83%)
	Present	26 (17%)
Perineural invasion		
	Absent	82 (54%)
	Present	69 (46%)
Extracapsular spread		
	Absent	115 (76%)
	Present	36 (24%)
Margin status		
	Negative	141 (93%)
	Positive/close	10 (7%)
Smoking		
	Absent	27 (18%)
	Present	124 (82%)
Alcohol		
	Absent	30 (20%)
	Present	121 (80%)
Betel-nut chewing		
	Absent	37 (25%)
	Present	114 (75%)

AJCC: American Joint Committee on Cancer; MasR: mitochondrial assembly receptor.

pathological T classification, T1/2 ($p=0.001$), pathological N classification, N0 ($p<0.001$), pathological 7th AJCC Stage I/II ($p<0.001$), absence of vascular invasion ($p=0.045$), absence of perineural invasion ($p=0.018$), absence of extracapsular spread ($p<0.001$), negative surgical margin ($p=0.024$), and absence of smoking history ($p=0.047$) were significantly associated with superior overall survival. Additionally, MasR overexpression ($p=0.007$, Figure 2(b)), pathological T classification, T1/2 ($p=0.008$), pathological N classification, N0 ($p<0.001$), pathological 7th AJCC Stage I/II ($p<0.001$), histologic grade 1 ($p=0.04$), absence of perineural invasion ($p=0.003$), and absence of extracapsular spread ($p<0.001$) were significantly associated with superior disease-free survival.

In a multivariate comparison, MasR overexpression ($p=0.008$, hazard ratio=1.862, 95% confidence interval: 1.176–2.949) remained independently associated with superior overall survival, together with pathological 7th AJCC Stage I/II ($p=0.011$, hazard ratio=2.029, 95% confidence interval: 1.178–3.495), histologic grade 1 ($p=0.02$, hazard ratio=1.700, 95% confidence interval: 1.087–2.658), and absence of extracapsular spread ($p=0.047$, hazard ratio=1.694, 95% confidence interval: 1.006–2.854). For disease-free survival, MasR overexpression ($p=0.006$, hazard ratio=1.847, 95% confidence interval: 1.190–2.864), pathological 7th AJCC Stage I/II ($p=0.006$, hazard ratio=2.073, 95% confidence interval: 1.231–3.490), and histologic grade 1 ($p=0.008$, hazard ratio=1.785, 95% confidence interval: 1.165–2.736) represented independent adverse prognosticators. The five-year overall and disease-free survival rates were 59% and 55%, respectively, in patients with overexpression of MasR, and 42% and 37%, respectively, in patients with low expression of MasR.

MasR antagonist reversed the inhibition of tongue cancer cell growth induced by angiotensin-(1-7)

To further demonstrate the biological function of MasR in vitro, the growth dependence of MasR was evaluated under angiotensin-(1-7) stimulation in OTSCC cell lines. Using MTT and BrdU assays, we found that the cell proliferation rates of both OTSCC cell lines, HSC-3 and Cal27 were significantly suppressed upon angiotensin-(1-7) treatment (Figure 3(a) and 3(b)). Furthermore, the MasR antagonist, A779, significantly reversed the inhibition of OTSCC cell growth induced by angiotensin-(1-7) (Figure 3(a) and 3(b)). These results suggest that angiotensin-(1-7)/MasR signaling suppresses tongue cancer cell growth and that such effects could be reversed by A779, the MasR antagonist.

Discussion

The RAS is composed of a group of enzymes and peptides, besides the classical arm, whose primary components are

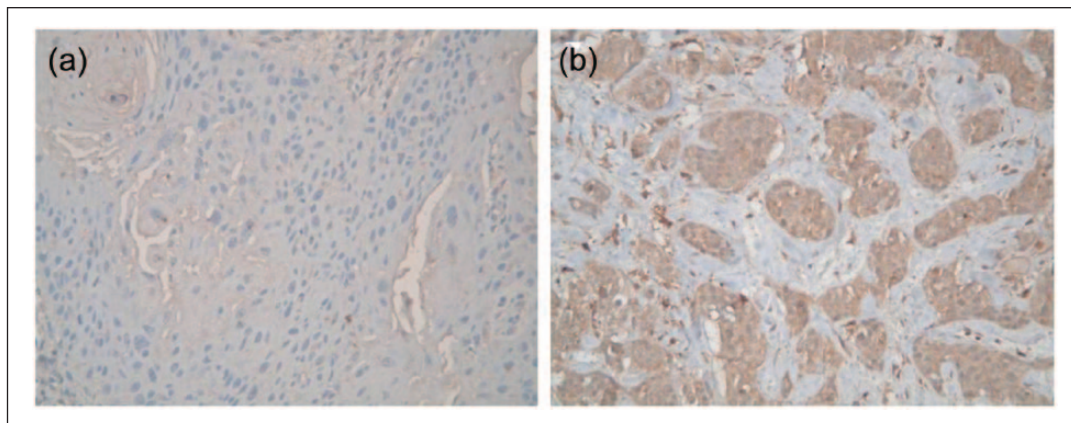


Figure 1. Immunohistochemical staining of mitochondrial assembly receptors (MasRs): (a) low expression of MasR; (b) overexpression of MasR. Original magnification $\times 200$.

Table 2. Associations between mitochondrial assembly receptor (MasR) expression and clinicopathologic parameters in 151 patients with oral tongue squamous cell carcinoma.

Parameters		MasR expression		<i>p</i> Value
		Low	Over	
Age	≤ 52 years	41	38	0.95
	> 52 years	37	35	
Pathological T classification	T1/2	41	51	0.029 ^a
	T3/4	37	22	
Pathological N classification	N0	39	44	0.21
	N1/2/3	39	29	
Pathological 7 th AJCC stage	I/II	27	34	0.13
	III/IV	51	39	
Depth of invasion (mm)	< 4 mm	10	8	0.72
	≥ 4 mm	68	65	
Histologic grade	I	47	38	0.31
	2/3	31	35	
Vascular invasion	Absent	63	62	0.50
	Present	15	11	
Perineural invasion	Absent	39	43	0.27
	Present	39	30	
Extracapsular spread	Absent	54	61	0.039 ^a
	Present	24	12	
Margin status	Negative	73	68	0.91
	Positive/close	5	5	
Smoking	Absent	13	14	0.69
	Present	65	59	
Alcohol	Absent	17	13	0.31
	Present	56	65	
Betel-nut chewing	Absent	18	19	0.67
	Present	60	54	

AJCC: American Joint Committee on Cancer.

^aStatistically significant, Chi-square test or Fisher's exact test was used for statistical analysis.

ACE, angiotensin II, and AT1R, and a recently discovered arm has been characterized. The alternative arm of the RAS includes ACE2, a carboxypeptidase that transforms angiotensin II to angiotensin-(1-7), and angiotensin-(1-7), which

is a peptide possessing the effects opposite to those of angiotensin II, through its specific receptor, MasR.⁸ Recently, increasing evidence has indicated that several components of the RAS might play an important role in cancer

Table 3. Results of univariate log-rank analysis of prognostic factors for overall survival and disease-free survival in 151 patients with oral tongue squamous cell carcinoma.

Factors	No. of patients	Overall survival (OS)		Disease-free survival (DFS)	
		5-Year OS (%)	p Value	5-Year DFS (%)	p Value
Age					
≤52 years	79	53%	0.43	51%	0.19
>52 years	72	47%		40%	
MasR					
Low expression	78	42%	0.012 ^a	37%	0.007 ^a
Overexpression	73	59%		55%	
Pathological T classification					
T1/2	92	60%	0.001 ^a	53%	0.008 ^a
T3/4	59	36%		34%	
Pathological N classification					
N0	83	61%	<0.001 ^a	58%	<0.001
N1/2/3	68	37%		31%	
Pathological 7 th AJCC Stage					
I/II	61	66%	<0.001 ^a	62%	<0.001 ^a
III/IV	90	40%		34%	
Depth of invasion (mm)					
<4 mm	18	72%	0.078	61%	0.18
≥4 mm	133	47%		44%	
Histologic grade					
I	85	55%	0.081	52%	0.04 ^a
2/3	66	44%		38%	
Vascular invasion					
Absent	125	54%	0.045 ^a	49%	0.078
Present	26	35%		31%	
Perineural invasion					
Absent	82	57%	0.018 ^a	55%	0.003 ^a
Present	69	42%		35%	
Extracapsular spread					
Absent	115	57%	<0.001 ^a	52%	<0.001 ^a
Present	36	31%		25%	
Margin status					
Negative	141	52%	0.024 ^a	47%	0.093
Positive/close	10	30%		30%	
Smoking history					
Absent	27	70%	0.047 ^a	63%	0.07
Present	124	46%		42%	
Alcohol history					
Absent	30	53%	0.41	47%	0.49
Present	121	50%		46%	
Betel-nut chewing					
Absent	37	62%	0.056	54%	0.14
Present	114	47%		43%	

AJCC, American Joint Committee on Cancer; MasR: mitochondrial assembly receptor.

^aStatistically significant.

progression.⁸ For example, accumulating evidence has shown that angiotensin-II/AT1R signaling has protumoral effects and enhances cell proliferation, invasion, angiogenesis, and subsequent metastasis.^{8,12–14} AT1R expression in clinical tumor samples has also been reported to be correlated with treatment outcome in several types of cancer.^{8–10} However, the impact of angiotensin-(1-7)/MasR signaling

in cancer progression remains largely undefined. Previous studies have reported that the angiotensin-(1-7)/MasR axis exhibits its anti-tumoral effects on certain cancers.^{9,11,13–16,20} But, to the best of our knowledge, an assessment of the prognostic role of MasR signaling in cancer patients is lacking. Therefore, we conducted the present study to evaluate the prognostic role of MasR in patients with OTSCC.

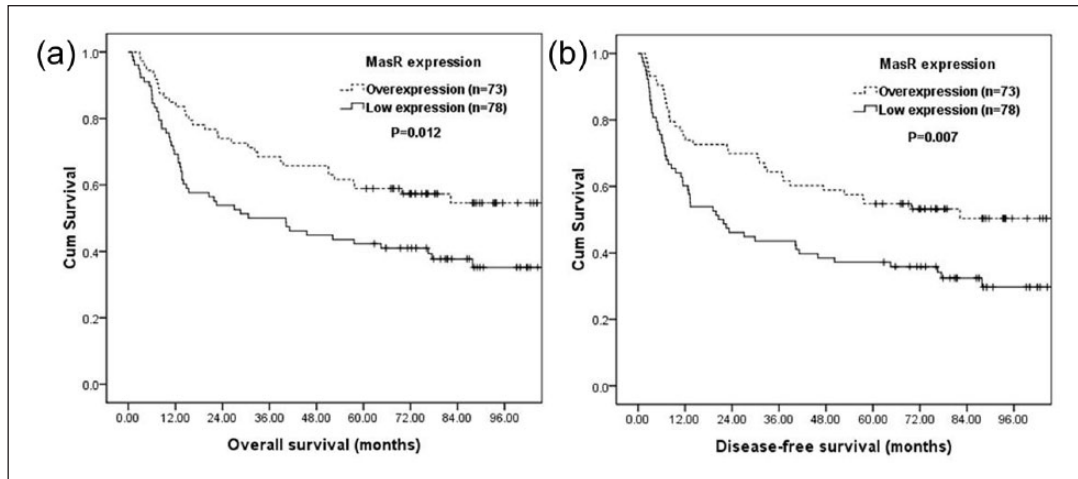


Figure 2. Kaplan–Meier curves according to mitochondrial assembly receptor (MasR) status: (a) overall survival according to MasR status; (b) disease-free survival according to MasR status.

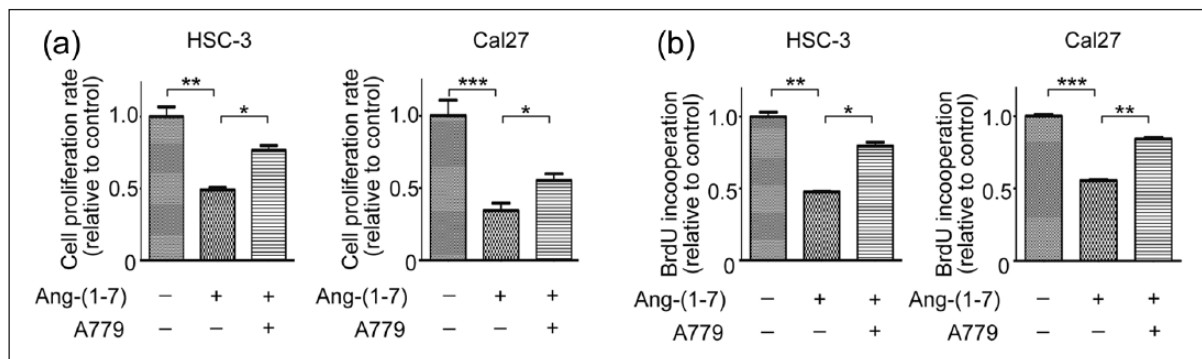


Figure 3. The mitochondrial assembly receptor (MasR) antagonist reversed the inhibition of tongue cancer cell growth induced by angiotensin-(1-7). (a) and (b) Serum-starved HSC-3 and Cal27 cells were pretreated with or without A779 for 24 h following stimulation with angiotensin-(1-7). The cells were then cultured for 72 h and then subjected to 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and bromodeoxyuridine (BrdU) incorporation assays to quantitate cell growth. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

In the present study, MasR overexpression in tumor samples was significantly correlated with early pathological T classification and the absence of extracapsular spread in patients with OTSCC. The previous study by Luo et al.¹⁶ also reported that MasR expression was inversely associated with pathological T classification and N classification in patients with breast cancer. Furthermore, in our survival analysis, MasR overexpression was significantly associated with better clinical outcomes in patients with OTSCC, and it remained an independent prognosticator in multivariate analysis. These clinical findings suggest that MasR signaling may have an inhibitory role in cancer progression.

Although significant progress has been made in preoperative imaging and surgical technique, there is still a proportion of patients with OTSCC who develop recurrences after surgery. Therefore, identification of patients at high risk for recurrence who may benefit from post-operative adjuvant therapy is worthwhile. In the current study, low

MasR expression was highly representative of biological aggressiveness and independently associated with poor overall survival and disease-free survival. The five-year overall and disease-free survival rates were 59% and 55%, respectively, in patients with overexpression of MasR, and 42% and 37%, respectively, in patients with low expression of MasR, implying that MasR status might be employed to select some patients for adjuvant therapy after surgery.

In our in-vitro experiments, angiotensin-(1-7) inhibited the cell proliferation of OTSCC cell lines, and this effect was found to be reversible by a MasR antagonist, A779. Our findings are similar to those of previous studies in lung cancer, breast cancer, and hepatocellular carcinoma. Gallagher et al.¹⁵ showed that angiotensin-(1-7) reduced serum-stimulated growth of human lung cancer cells through activation of MasR receptors. Luo et al.¹⁶ reported that cell growth was suppressed by angiotensin-(1-7) treatment and enhanced by MasR knockdown in breast cancer

cell lines. Using a murine hepatocellular carcinoma cell line, Liu et al.²⁰ found that angiotensin-(1-7) inhibited tumor growth in vitro and in vivo and that this effect was reversed by coadministration with the MasR antagonist, A779. However, Bernardi et al.²¹ did not observe any pro-/antiproliferative effects of angiotensin-(1-7) in colon cancer cell lines. These incongruous findings suggest that the effect of angiotensin-(1-7)/MasR signaling may be different and tumor-specific in a variety of cancer cells. Further studies on different cancer cell lines are thus necessary to fully evaluate the effect of angiotensin-(1-7)/MasR signaling.

Our study has important limitations. First, this was a retrospective analysis, and a prospective study will be necessary in the future in order to define our findings. Second, our observations were limited by the relatively small number of patients included in the study sample.

In conclusion, MasR expression is independently associated with the prognosis of patients with OTSCC. These findings suggest that MasR signaling may be a promising novel target for the treatment of OTSCC.

Acknowledgments

The authors thank Dr. Sheng-Lan Wang (SLW) at the Union pathology center, Kaohsiung, Taiwan, R.O.C for assessment of the immunohistochemical staining.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported in part by grants from the Chang Gung Memorial Hospital (CMRPG8G0081 to YY Su; CMRPG8D0683 to SH Li; CMRPG8E1471-2 to CH Chen) and Kaohsiung Medical University (Aim for the Top Universities Grant, grant No. KMU-TP105E23 to CH Chen). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

- Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. *Oral Oncol* 2009; 45: 309–316.
- Krishna Rao SV, Mejia G, Roberts-Thomson K, et al. Epidemiology of oral cancer in Asia in the past decade—an update (2000–2012). *Asian Pac J Cancer Prev* 2013; 14: 5567–5577.
- Su YY, Chien CY, Luo SD, et al. Betel nut chewing history is an independent prognosticator for smoking patients with locally advanced stage IV head and neck squamous cell carcinoma receiving induction chemotherapy with docetaxel, cisplatin, and fluorouracil. *World J Surg Oncol* 2016; 14: 86.
- Marur S and Forastiere AA. Head and neck squamous cell carcinoma: update on epidemiology, diagnosis, and treatment. *Mayo Clin Proc* 2016; 91: 386–396.
- Li SH, Lin WC, Huang TL, et al. Significance of mammalian target of rapamycin in patients with locally advanced stage IV head and neck squamous cell carcinoma receiving induction chemotherapy with docetaxel, cisplatin, and fluorouracil. *Head Neck* 2016; 38: E844–E852.
- Tong XJ, Shan ZF, Tang ZG, et al. The impact of clinical prognostic factors on the survival of patients with oral squamous cell carcinoma. *J Oral Maxillofac Surg* 2014; 72: 2497.e2491–2410.
- Sano D and Myers JN. Metastasis of squamous cell carcinoma of the oral tongue. *Cancer Metastasis Rev* 2007; 26: 645–662.
- George AJ, Thomas WG and Hannan RD. The renin-angiotensin system and cancer: old dog, new tricks. *Nat Rev Cancer* 2010; 10: 745–759.
- Wegman-Ostrosky T, Soto-Reyes E, Vidal-Millan S, et al. The renin-angiotensin system meets the hallmarks of cancer. *J Renin Angiotensin Aldosterone Syst* 2015; 16: 227–233.
- Li SH, Lu HI, Chang AY, et al. Angiotensin II type I receptor (AT1R) is an independent prognosticator of esophageal squamous cell carcinoma and promotes cells proliferation via mTOR activation. *Oncotarget* 2016; 7: 67150–67165.
- Regulska K, Stanisiz B and Regulski M. The renin-angiotensin system as a target of novel anticancer therapy. *Curr Pharm Des* 2013; 19: 7103–7125.
- Escobar E, Rodriguez-Reyna TS, Arrieta O, et al. Angiotensin II, cell proliferation and angiogenesis regulator: biologic and therapeutic implications in cancer. *Curr Vasc Pharmacol* 2004; 2: 385–399.
- Ender SA, Dallmer A, Lassig F, et al. Expression and function of the ACE2/angiotensin(1–7)/Mas axis in osteosarcoma cell lines U-2 OS and MNNG-HOS. *Mol Med Rep* 2014; 10: 804–810.
- Childers WK. Interactions of the renin-angiotensin system in colorectal cancer and metastasis. *Int J Colorectal Dis* 2015; 30: 749–752.
- Gallagher PE and Tallant EA. Inhibition of human lung cancer cell growth by angiotensin-(1–7). *Carcinogenesis* 2004; 25: 2045–2052.
- Luo Y, Tanabe E, Kitayoshi M, et al. Expression of MAS1 in breast cancer. *Cancer Sci* 2015; 106: 1240–1248.
- Stephen BE DR, Carolyn CC, April GF, et al. Lip and oral cavity. *AJCC cancer staging manual*. 7th ed. New York: Springer-Verlag. 2010: pp.29–40.
- Chen MF, Wang WH, Lin PY, et al. Significance of the TGF-beta1/IL-6 axis in oral cancer. *Clin Sci (Lond)* 2012; 122: 459–472.
- Zhu A, Yuan P, Du F, et al. SPARC overexpression in primary tumors correlates with disease recurrence and overall survival in patients with triple negative breast cancer. *Oncotarget* 2016; 7: 76628–76634.
- Liu Y, Li B, Wang X, et al. Angiotensin-(1–7) suppresses hepatocellular carcinoma growth and angiogenesis via complex interactions of angiotensin ii type 1 receptor, angiotensin ii type 2 receptor and Mas receptor. *Mol Med* 2015; 21: 626–636.
- Bernardi S, Zennaro C, Palmisano S, et al. Characterization and significance of ACE2 and Mas receptor in human colon adenocarcinoma. *J Renin Angiotensin Aldosterone Syst* 2012; 13: 202–209.