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Original Article

Vasculogenic mimicry and expression of ALDH1, Beclin1, and p16 correlate with metastasis and prognosis in oral squamous cell carcinoma

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Abstract: Background: Vasculogenic mimicry (VM) is a new blood supply in malignant tumors and has long been considered as an effective factor in the metastasis and prognosis of many cancers. ALDH1 (a marker of cancer stem cells), Beclin1 (a biomarker of autophagy) and p16 (a suppressor gene of tumor) are all useful predictive factors for many cancer metastases. However, the prognostic and metastatic value of VM, ALDH1, Beclin1, or p16 in oral squamous cell carcinoma (OSCC) is unclear. In this study, we analyzed correlations among VM, ALDH1, Beclin1, and p16 in OSCC, and their respective associations with clinicopathological parameters and survival in OSCC. Methods: Positive rates of VM, ALDH1, Beclin1, and p16 in 186 whole OSCC specimens were detected by immunohistochemical and histochemical staining. Patients' clinical information was also collected. Results: Positive rates of VM, ALDH1, and Beclin1 were significantly higher, and levels of p16 were significantly lower in OSCC than in normal oral tissues. Positive rates of VM, ALDH1, and Beclin1 were positively associated with tumor grade, primary tumor (pT), lymph node metastasis (LNM), and tumor-node-metastasis (TNM) stage, and inversely with patients overall survival (OS) time. Levels of p16 was negatively associated with grade, pT, LNM, and TNM stage, and positively associated with smoking and alcohol. The p16+ subgroup had significantly longer OS time than did the p16- subgroup. In multivariate analysis, high ALDH1, VM, Beclin1 levels, tumor grade, pT, LNM, TNM stage, and low p16 levels were potential to be independent prognostic factors for OS time in OSCC patients. Conclusions: VM, and the expression of ALDH1, Beclin1, and p16 represent promising markers for metastasis and prognosis, and potential therapeutic targets for OSCC.

Keywords: Oral squamous cell carcinoma, VM, ALDH1, Beclin1, p16, prognosis

Introduction

In 2012, an estimated 300,400 new cases and cause 145,400 deaths from oral cancers occurred worldwide [1]. The most common source of cells in oral cancer is the squamous cell, accounting for more than 90% of oral cancers [2]. Although there are currently integrated sequential therapies, including surgery, chemoradiation, biotherapy, and gene-targeted therapies, the long-term treatment effects of OSCC remain unsatisfactory [3]. Oral squamous cell carcinoma has a 5-year survival rate in the past few decades that did not significantly improve, remaining at less than 50%, which is a low survival rate for malignant tumors [4]. Metastasis and postoperative recurrence are the major reasons [5, 6]. Routine anti-angiogenic therapy did not significantly improve patient survival time [7]. Some researchers have found that when endothelium dependent angiogenesis was not sufficient to support the rapid growth of tumor tissue, some cancer cells could mimic endothelial cells and form vascular structure is called vasculogenic mimicry (VM) [8, 9]. The VM is a lumen-like structure that provides nutrient and blood supply and promotes metastasis [10]. VM is mainly composed of three structures: stem-like cancer cells, remodeling of the extracellular matrix, and the lumen-like structure which can directly connect with the host microcirculation system [11]. Accumulating studies have shown that cancer-related VM patients are prone to metastasis and have poor prognosis [8-13].

Table 1. Patient characteristics

Patients characteristics	Frequency (n)	Percentage (%)
Age (year)		
<60	76	40.9
≥60	110	59.1
Gender		
Male	104	55.9
Female	82	44.1
Smoking		
No	75	40.3
Yes	111	59.7
Alcohol		
No	66	35.5
Yes	120	64.5
Location		
Tongue	70	37.6
Gingiva	36	19.3
Oral floor	28	15.1
Jaw	25	13.4
Buccal mucosa	15	8.1
Others	12	6.5
Grade		
Well	84	45.2
Moderate	74	39.8
Poor	28	15.0
Primary tumor		
T1	90	48.4
T2	56	30.1
T3	26	14.0
T4a	14	7.5
Lymph node metastasis		
N0	135	72.6
N1	37	19.9
N2	14	7.5
TNM stage		
I	82	44.1
II	48	25.8
III	39	21.0
IVA	17	9.1

Malignant tumor metastasis and prognosis may be related to cancer stem cells (CSCs) and the cancer stem cell theory proposes that development of tumors is derived from gene mutations creating tumor stem cells, although these cells in tumor cells only a small part, the cell subsets have a permanent potential for self-renewal and multidirectional differentiation that leads to the formation and growth of tumors [14]. Currently, stem cells have been confirmed as the driving force behind the tumor formation, recurrence, and metastasis of many cancers, including breast cancer, colorectal cancer, and laryngeal squamous cell carcinoma [15-17]. Aldehyde dehydrogenase (ALDH), as one of features of functional stem cells, has been used to identify and analyze many tumor stem cells. Human ALDH1 gene expression is present in the cytoplasm, its gene is cloned and located on chromosome 9q21. ALDH1 is a useful marker of cancer stem cells [18, 19] and high ALDH1 expression may also be correlated with poor prognosis in breast, ovarian, and lung cancers [18, 20, 21].

Autophagy is an evolutionarily conserved and lysosomal degradation pathway where the cell self-digests cell components and recycles useful cytoplasmic material [22]. Beclin1 is a vital protein which can initiate autophagy and play an important role in the nucleation stage of cell autophagy. It was the first tumor suppressor gene in mammals which was directly related to autophagy activation [23]. Furthermore, autophagy also plays a cytoprotective effect in tumor therapy [24].

Several molecular epidemiological research studies have shown that human papillomavirus (HPV) infection could be a subtype which might induce head and neck cancers [25]. It has been reported that the expression of P16 protein, a product of p16 tumor suppressor gene in oral tumors, and is closely related to HPV infection and has been suggested by researchers as an alternative detection method for HPV [26, 27].

Overall, studies of the correlation between tumor metastasis and prognosis suggest that VM, ALDH1, Beclin1, and p16 affect cancer progression. However, associations among VM, ALDH1, Beclin1, and p16 in OSCC have not been widely reported. In our study, we examined the hypothesis that these factors are mutual correlated, and are related to metastasis and prognosis in OSCC.

Materials and methods

Specimens

All 186 patients (median age: 60.5 years; range: 38-79 years) who were treated for OSCC were collected from the First Affiliated Hospital of Bengbu Medical College, (China) from January 2009 to December 2011. Samples of the corresponding adjacent normal tissues

from all 186 patients were removed. We excluded patients who had received preoperative chemotherapy or radiotherapy. All cases were obtained with written consent. The study was approved by the Ethics Committee of Bengbu Medical College and performed in accordance with the guidelines of the Declaration of Helsinki. We collected patients for whom we had completely clincopathological information and follow-up data (at 6-months intervals by phone, e-mail, or mail). Overall survival (OS) time was calculated from the date of surgery of the patients to his/her death date or December 2016 (mean OS: 59.7 months; range: 8-96 months). Tumor-node-metastasis stage was assessed according to the 7th edition of the American Joint Committee on Cancer (AJCC). Tumors were graded according to World Health Organization (WHO) standards. Specific parameters were provided in **Table 1**.

Immunohistochemistry

According to the Elivision™ Plus detection kit instructions (Lab Vision, USA), we performed immunohistochemistry staining. All OSCC and corresponding normal oral tissues were fixed in 10% buffered formalin, and embedded in paraffin. Continuous 4-μm thick sections were cut. All sections were deparaffinized, dehydrated in xylene and graded alcohol, and then washed with phosphate buffer saline (PBS, pH 7.2) for 10 min. The sections were incubated in methanol containing 3% H_2O_2 at room temperature for 10 min, for blocking the endogenous peroxidase activity. Tissue sections were placed in citrate buffer (pH 6.0) and then heated to 95°C for antigen repair for 30 minutes. All sections were then quenched with goat serum at RT for 30 min after several washes with PBS, subsequently tissue sections were incubated with mouse monoclonal antibody against human CD34 (Abcam, USA), Beclin1 (Abcam, USA), ALDH1 (Cell Signaling Technology, USA) and p16 (Abcam, USA) at 37°C for 1 h. Periodic Acid-Schiff (PAS)-CD34 dual staining was used to determine endothelial cells in glycosylated basement membranes of vessels, including vessel-like (VM) structure [13]. Yue's method was used to evaluate VM structure in the OSCC tissues and the control tissues [28]. All sections were counterstained with hematoxylin, dehydrated, air-dried, and mounted. p16 stains were mainly seen in tumor cell nuclear and cytoplasm. Beclin1 and ALDH1 stains were mainly seen in tumor cell cytoplasm.

Evaluation of staining

All staining results were evaluated semi-quantitatively by two experienced pathologists who were blind to patients' clinical information and follow-up data. In order to avoid potential intratumoral heterogeneity of antibody expression, we analyzed ten representative high-power-fields (HPF) from different areas of each OSCC slide. The experimental results were scored according to intensity staining (none staining, 0; weak staining, 1; moderate staining, 2; strong staining, 3) and extent (<11% positive cells mean, 1; 11-50% positive cells mean, 2; 51-75% positive cells mean, 3; >75% positive cells mean, 4). Final scores were obtained by multiplying intensity and extent scores that ranged 0-12. Final scores ≥3 were considered as positive result. For tissue sections that were positive results for all four of VM, ALDH1, Beclin1 and p16, an average value of the final score of each tissue section was taken.

Statistical analysis

Relationships between clinicopathological indices and VM, ALDH1, Beclin1, and p16 were analyzed using Fisher's exact test or Chi-square test. Association between VM, ALDH1, Beclin1, or p16 was evaluated using Spearman's correlate test. Effects of VM, ALDH1, Beclin1, or p16 on survival time were analyzed by univariate and multivariate analyses. Independent prognostic factors were analyzed using the multivariate Cox regression model. We used the Kaplan-Meier method with log-rank test to assess correlations between OS time and VM, ALDH1, Beclin1, or p16 results and clinicopathological characteristics, using SPSS 24.0 software for Windows (New York, IBM, USA). A value of $P<0.05$ was regarded as statistically significant.

Results

Association between VM, ALDH1, Beclin1, and p16 expression and clinicopathological characteristics

To assess the effects of VM, ALDH1, Beclin1, and p16 in OSCC, the experimental results thereof were immunohistochemically detected for both OSCC and corresponding normal oral tissue specimens. Clinicopathological characteristics were compared to these experimental data. The positive result rate of VM findings (small vessel structure, which is a lumen-like in

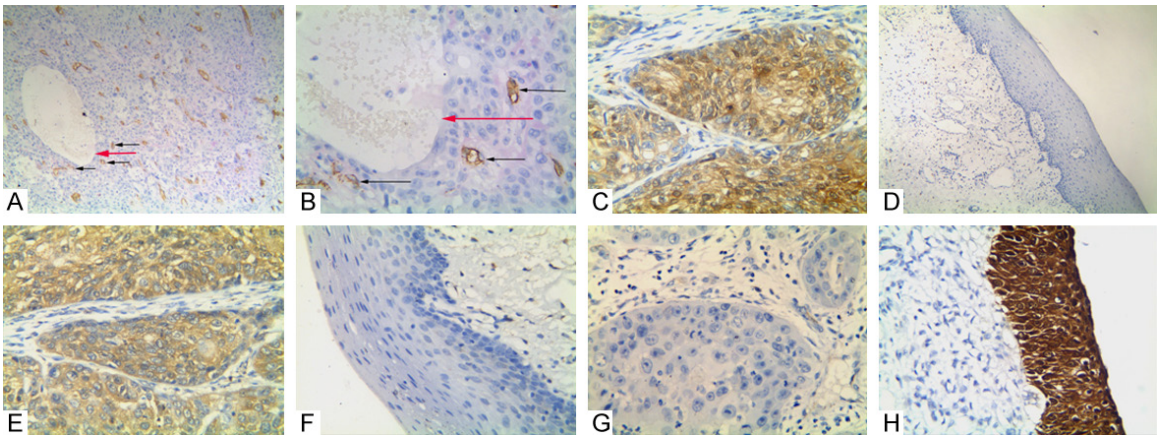

Figure 1. Immunostaining of VM, ALDH1, Beclin1, and p16 in OSCC or the control tissue. A: Positive staining of VM structure in the OSCC tissue (100 magnification, black arrow is microvessel, red arrow is VM structure); B: Positive staining of VM in the OSCC tissue (400 magnification, black arrow is microvessel, red arrow is VM structure); C: Positive staining of ALDH1 in the cytoplasm of cancer cells (400 magnification); D: Negative staining of ALDH1 in the control tissues (100 magnification); E: Positive staining of Beclin1 in the cytoplasm of the cancer cells (400 magnification); F: Negative staining of Beclin1 in the control tissue (400 magnification); G: Negative staining of p16 in the cancer tissue (400 magnification); H: Positive staining of p16 in the cytoplasm and nucleus of the normal oral mucosa epithelial cells (400 magnification).

Table 2. Association between VM and expression of ALDH1, Beclin1, p16, and clinicopathological characteristics of oral squamous cell carcinoma (OSCC)

Variables	VM		*P*	ALDH1		*P*	Beclin1		*P*	P16		*P*
	-	+		-	+		-	+		-	+	
Age (year)			0.952			0.933			0.871			0.716
<60	48	28		32	44		32	44		52	24	
≥60	69	41		47	63		45	65		78	32	
Gender			0.177			0.213			0.130			0.920
Male	61	43		40	64		38	66		73	31	
Female	56	26		39	43		39	43		57	25	
Smoking			0.799			0.796			0.988			0.006
No	48	27		31	44		31	44		44	31	
Yes	69	42		48	63		46	65		86	25	
Alcohol			0.878			0.358			0.602			0.017
No	42	24		31	35		29	37		39	27	
Yes	75	45		48	72		48	72		91	29	
Location			0.733			0.468			0.696			0.201
Tongue	48	22		34	36		29	41		48	22	
Gingiva	21	15		10	26		15	21		30	6	
Oral floor	19	9		12	16		15	13		15	13	
Jaw	14	11		11	14		8	17		19	6	
Buccal mucosa	8	7		6	9		5	10		10	5	
Others	7	5		6	6		5	7		8	4	
Grade			<0.001			<0.001			<0.001			<0.001
Well	69	15		54	30		53	31		45	39	
Moderate	40	34		24	50		19	55		59	15	
Poor	8	20		1	27		5	23		26	2	
Primary tumor			<0.001			<0.001			<0.001			<0.001
T1	74	16		66	24		56	34		46	44	

T2	34	22		10	46		18	38		49	7	
T3	8	18		3	23		1	25		22	4	
T4a	1	13		0	14		2	12		13	1	
LNM			<0.001			<0.001			<0.001			<0.001
N0	110	25		75	60		73	62		83	52	
N1	6	31		4	33		2	35		34	3	
N2	1	13		0	14		2	12		13	1	
TNM stage			<0.001			<0.001			<0.001			<0.001
I	74	8		65	17		56	26		38	44	
II	35	13		10	38		17	31		41	7	
III	7	32		4	35		2	37		35	4	
IVA	1	16		0	17		2	15		16	1	

OSCC, the lumen was PAS-positive result but CD34-negative. The VM structure pattern included linear, tubular, and network, etc.) in the OSCC specimens (37.1%, 69/186) was significantly higher than that in the corresponding normal oral tissues (0%, 0/186; $P<0.001$; **Figure 1A** and **1B**). The positive rate of VM structure in OSCC was positively related to tumor grade, primary tumor, LNM, and TNM stage, but not patients' age, gender, smoking, alcohol, or location (**Table 2**).

Similar to VM, ALDH1+ expression was especially higher in OSCC tissues (57.5%, 107/186) than that in the control oral tissues (6.4%, 12/186; $P<0.001$; **Figure 1C** and **1D**). The positive rate of ALDH1 expression in OSCC was related to tumor grade, primary tumor, LNM and TNM stage, but not patients' gender, age, alcohol, smoking, or location (**Table 2**).

The positive rate of Beclin1 expression was higher in OSCC tissues (58.6%, 109/186) than that in the control tissues (5.3%, 10/186; $P<0.001$; **Figure 1E** and **1F**). The positive rate of Beclin1 expression was significantly associated with primary tumor, tumor grade, LNM, and TNM stage. No correlation was found between Beclin1 expression and patients' gender, age, smoking, alcohol, or location (**Table 2**).

The positive expression rate of p16 expression was significantly lower in OSCC tissues (30.1%, 56/186) than that in the control normal tissues (83.9%, 156/186; $P<0.001$; **Figure 1G** and **1H**). The positive expression rate of p16 was inversely correlated with tumor grade, primary tumor, LNM, TNM stage, smoking, and alcohol. No correlation was found between p16 positive expression and patients' location, gender, or age (**Table 2**).

Univariate and multivariate analyses

Follow-up data suggested that OST was significantly lower in OSCC patients with VM+ specimens (40.6±17.1 months) compared with VM- patients (71.0±13.5 months; log-rank = 128.741, $P<0.001$; **Figure 2A**). Similarly, OST of ALDH1+ patients (47.6±17.9 months) was significantly lower than ALDH1- patients (76.0 ±11.4 months; log-rank = 106.036, $P<0.001$; **Figure 2B**). The OST of Beclin1+ patients (50.0±19.3 months) was significantly shorter than Beclin1- patients (73.4±14.5 months; log-rank = 52.807, $P<0.001$; **Figure 2C**). The OST of p16+ (75.6±14.9 months) was significantly higher than those p16- specimens (52.9±19.4 months; log-rank = 53.718, $P<0.001$; **Figure 2D**). In univariate analysis, OS time was significantly related to clinicopathological information, including tumor grade (log-rank = 97.222, $P<0.001$, **Figure 2E**), primary tumor (log-rank = 369.471, $P<0.001$, **Figure 2F**), LNM (log-rank = 383.472, $P<0.001$, **Figure 2G**), and TNM stage (log-rank = 434.913, $P<0.001$, **Figure 2H**) (**Table 3**).

Multivariate analysis demonstrated that VM+, ALDH1+, Beclin1+, and/or p16+ specimens, and tumor grade, primary tumor, TNM stage and LNM, were independent prognostic factors for OSCC (**Table 4**).

Correlation among VM, and expression of ALDH1, Beclin1, and p16 in OSCC

Spearman correlation coefficient analysis indicated that negative correlations between p16+

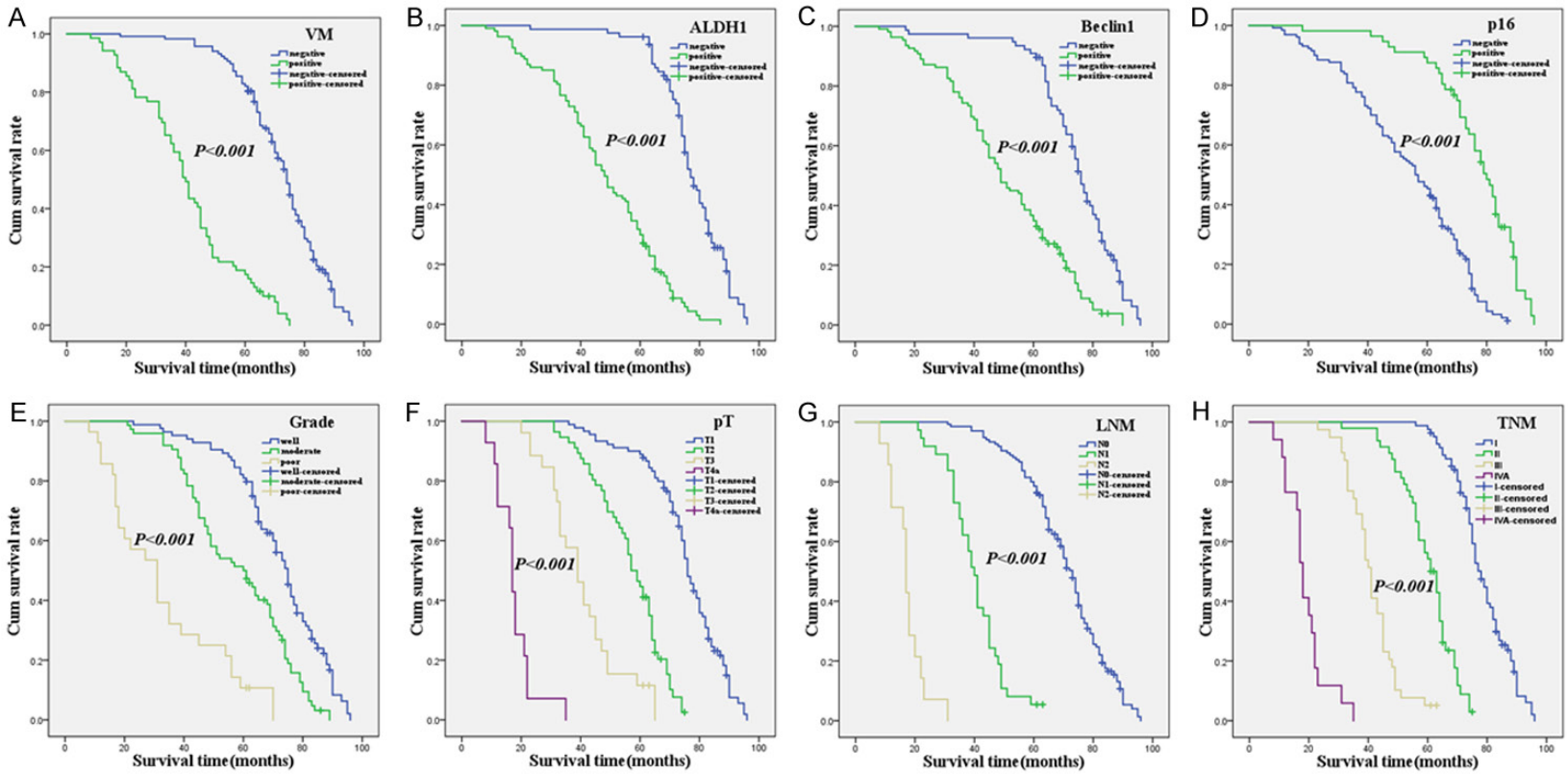

Figure 2. Kaplan-Meier analysis of the survival rate of patients with OSCC. The y-axis represents the percentage of patients; the x-axis, their survival in months. (A) Overall survival of all patients in relation to VM (log-rank = 128.741, *P*<0.001); (B) Overall survival of all patients in relation to ALDH1 expression (log-rank = 106.036, *P*<0.001); (C) Overall survival of all patients in relation to Beclin1 expression (log-rank = 52.807, *P*<0.001); (D) Overall survival of all patients in relation to p16 expression (log-rank = 53.718, *P*<0.001); In (A-D) analyses, the green line represents patients with positive VM, or ALDH1, or Beclin1, or p16, and the blue line representing the negative VM, or ALDH1, or Beclin1, or p16 group. (E) OS of all patients in relation to tumor grade (log-rank = 97.222, *P*<0.001, the blue line represents patients with grade 1 group, the green line represents patients with grade 2 group, the brown line represents patients with grade 3 group). (F) OS of all patients in relation to primary tumor (log-rank = 369.471, *P*<0.001, the blue line represents patients with pT1 group, the green line represents patients with pT2 group, the brown line represents patients with pT3 group, the purple line represents patients with pT4a group). (G) OS of all patients in relation to LNM (log-rank = 383.472, *P*<0.001, the blue line represents patients with N0 group, the green line represents patients with N1 group, the brown line represents patients with N2 group). (H) OS of all patients in relation to TNM stage (log-rank = 434.913, *P*<0.001, the blue line represents patient with I stage group, the green line represents patients with II stage group, the brown line represents patients with III stage group, the purple line represents patients with IV A stage group).

Table 3. Results of univariate analyses of overall survival (OS) time

Variables	n	Mean OS (months)	Log-rank	*P* value
VM			128.741	<0.001
Negative	117	71.0±13.5		
Positive	69	40.6±17.1		
ALDH1			106.036	<0.001
Negative	79	76.0±11.4		
Positive	107	47.6±17.9		
Beclin1			52.807	<0.001
Negative	77	73.4±14.5		
Positive	109	50.0±19.3		
p16			53.718	<0.001
Negative	130	52.9±19.4		
Positive	56	75.6±14.9		
Age (year)			0.192	0.661
<60	76	59.9±21.3		
≥60	110	59.6±20.7		
Gender			0.095	0.758
Male	104	58.1±21.6		
Female	82	61.8±20.1		
Smoking			0.063	0.802
No	75	59.4±21.7		
Yes	111	59.9±20.5		
Alcohol			0.753	0.386
No	66	59.7±22.3		
Yes	120	59.7±20.2		
Location			8.966	0.110
Tongue	70	65.1±19.5		
Gingiva	36	53.0±20.5		
Oral floor	28	61.2±22.4		
Jaw	25	56.8±18.9		
Buccal mucosa	15	57.2±21.9		
Others	12	53.8±24.5		
Grade			97.222	<0.001
Well	84	70.7±15.1		
Moderate	74	57.5±17.2		
Poor	28	32.5±18.5		
Primary tumor			369.471	<0.001
T1	90	74.1±13.0		
T2	56	56.1±11.5		
T3	26	40.2±12.3		
T4a	14	17.6±6.5		
LNM			383.472	<0.001
N0	135	69.5±13.6		
N1	37	40.2±10.0		
N2	14	17.3±5.8		
TNM stage			434.913	<0.001

expression and that of VM (r = -0.310, *P*<0.001), ALDH1 (r = -0.432, *P*<0.001), or Beclin1 (r = -0.353, *P*<0.001). Expression of ALDH1 was positively associated with a positive rate of VM (r = 0.480, *P*<0.001) and Beclin1 (r = 0.492, *P*<0.001). The expression of VM and Beclin1 showed a positive correlation (r = 0.465, *P*< 0.001) (**Table 5**).

Discussion

As a highly heterogeneous malignant tumor, OSCC can affect the reproducibility of biomarker assessment. In this research, we found that VM was positively correlated with grade, primary tumor, LNM, and TNM stage. Moreover, Kaplan-Meier survival analysis indicates that VM+ OSCC patients had significantly lower OS time than did VM- patients. Our studies suggest that VM should play a vital role in OSCC progression and metastasis, and also should be considered as a very useful biomarker for clinical treatment. Some studies indicate that VM is one of the reasons for the failure of anti-angiogenesis therapy, and should be considered as a potential therapeutic target for OSCC [29, 30]. Some other researchers showed similar results [9-13].

ALDH1, a marker for CSCs, and also an intracellular enzyme that helps detoxify and metabolize many endogenous and exogenous in various cancers [18-21]. In this study, ALDH1 expression was positively associated with tumor grade, primary tumor, LNM, and TNM stage. In addition, Kaplan-Meier survival analysis showed that ALDH1+ OSCC patients had significantly lower OST than did ALDH1- patients. These results demonstrated that overexpression of ALDH1 should promote OSCC invasion, metastasis, and mean poor prognosis. Our findings are consistent

I	82	77.1±9.1
II	48	59.4±9.1
III	39	41.2±8.7
IVA	17	18.8±6.8

Table 4. Results of multivariate analyses of overall survival (OS) time

Variables	B	SE	*P*	RR	95% CI
Grade	0.394	0.158	0.013	1.483	1.087-2.022
pT	0.474	0.195	0.015	1.606	1.095-2.355
LNM	0.829	0.350	0.018	2.291	1.155-4.545
TNM stage	0.865	0.329	0.008	2.376	1.247-4.525
VM	1.220	0.241	<0.001	3.388	2.111-5.438
ALDH1	0.799	0.253	0.002	2.222	1.353-3.651
Beclin1	0.460	0.213	0.031	1.583	1.043-2.405
P16	-0.615	0.236	0.009	0.541	0.341-0.859

with other researches, and including those of oral cancers and other cancers [18-21, 31, 32].

Beclin1 is a representative tumor gene of autophagy that can be either monoallelically deleted or display reduced expression in various cancers [23]. Furthermore, autophagy may also promote the survival of starved tumor cells in regions of the tumor with a poor blood supply [33], Autophagy may also play a tumor cytoprotective role during anticancer therapy [34]. In this study, Beclin1 expression was positively related to tumor grade, primary tumor, LNM, and TNM stage. Moreover, Kaplan-Meier survival curve demonstrated that Beclin1+ OSCC patients had significantly lower OST than did Beclin1- patients. The above results suggested that overexpression of Beclin1 should play an important role in the process of invasion, metastasis and prognosis of OSCC. Our results are similar to other studies [24, 35-37].

P16 is thought to be a tumor suppressor gene which can inhibit cell proliferation by blocking the cell cycle [38-40]. Moreover, p16 and HPV infection are closely related to the extent and it can be used as an alternative HPV test [26, 27]. Findings in this study also showed that p16/HPV expression was significantly lower in OSCC tissues than that in control tissues, and its expression was negatively correlated with smoking, alcohol, tumor grade, primary tumor, LNM and TNM stage. Furthermore, Kaplan-Meier survival demonstrated that OSCC patients with p16/HPV+ samples had significantly longer survival time than did p16/HPV- patients. These findings suggest that down-regulation of p16 should promote OSCC progression and metastasis, and previous research is similar to our experimental results [38-42].

TNM stages of OSCC provide therapeutic strategies for patients but not provide exhaustive biological behavior information about OSCC. Therefore, it is vital to find effective and novel biomarkers to predict OSCC biological behavior, metastasis, and patients' prognosis. In our study, multivariate analysis showed that VM, expression of ALDH1, Beclin1, and p16, as well as TNM stages, tumor grade, primary tumor, and LNM, are independent prognostic factors for OSCC patients (**Table 4**). Our findings thus demonstrate that VM, ALDH1, Beclin1, and p16 are reliable biomarkers for OSCC, and especially in predicting metastasis and prognosis.

Oral squamous cell carcinoma (OSCC) is the most common pathological type of oral cancer. When the tumor grows to a certain size, tumor cells can induce angiogenesis. But when the blood supply by angiogenesis cannot meet the need of tumor rapid growth, some stem-like cancer cells can mimic endothelial cells and form vasculogenic mimicry [9, 13]. CSCs can induce angiogenesis to provide adequate nutrition and oxygen for rapid tumor growth [43], and can apparently differentiate tumor cells and endothelial cells [44], thus, CSCs can mimic endothelial cells to form VM to overcome environment stress in tumor tissue. At the same time, CSCs protect the genome from damage and thus maintain their own self-renewal capacity. In the process of tumorigenesis, autophagy protects the stability of the genome by delaying the injury/repair cycle and protecting CSCs homeostasis [45, 46]. Thus, autophagy contributes to the stability of CSCs, and promotes VM formation. p16 can inhibit the cell cycle and promote cell senescence [38-40, 47]. Cell senescence can inhibit the proliferation of cells damaged by DNA, so p16 can also inhibit the proliferation of tumor stem cells by inducing cellular senescence [48]. Damage

Table 5. Correlation among VM, expression of ALDH1, Beclin1, and p16 in OSCC

Variables	VM		r	*P*	ALDH1		r	*P*	P16		r	P
	-	+			-	+			-	+		
Beclin1			0.465	<0.001[@]			0.492	<0.001[@]			-0.353	<0.001[*]
-	69	8			55	22			39	38		
+	48	61			24	85			91	18		
VM							0.480	<0.001[@]			-0.310	<0.001[*]
-					71	46			69	48		
+					8	61			61	8		
ALDH1											-0.432	<0.001[*]
-									37	42		
+									93	14		

[*]: negative association; [@]: positive association.

to tumor stem cells further leads to decrease in VM formation in the tumor tissue. Overall, our results indicate that a complex relationship between the VM, ALDH1, Beclin1, and p16 in tumor progression. Combined with the results of our present study, we have reason to believe that the interrelationships between these factors are related to metastasis and prognosis in OSCC.

Conclusions

In summary, low expression of p16 combined with high expression of VM, ALDH1, and Beclin1 was found to be associated with metastasis and poor prognosis in OSCC. Furthermore, combined detection of VM, ALDH1, Beclin1, and p16 are valuable factors of metastasis and prognosis in OSCC.

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Disclosure of conflict of interest

None.

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