Int J Clin Exp Med 2017;10(10):14623-14631
www.ijcem.com /ISSN:1940-5901/IJCEM0057542

Original Article

Expression of ORAOV1, ABCG2, and KiSS-1 associate with prognosis in laryngeal squamous cell carcinoma

Wenqing Song[1,2*], Lei Zhou[1,2*], Xiaomeng Gong[1,2*], Shiwu Wu[1,2], Lan Yu[1,2], Bo Zhu[1,2], Danna Wang[1,2]

[1]Departments of Pathology, The First Affiliated Hospital of Bengbu Medical College, Anhui, China; [2]Department of Pathology, Bengbu Medical College, Anhui, China. [*]Equal contributors.

Received March 22, 2017; Accepted September 24, 2017; Epub October 15, 2017; Published October 30, 2017

Abstract: Background: Oral cancer overexpressed 1 (ORAOV1, a novel candidate oncogene), ATP-binding cassette sub-family G member (ABCG2, a biomarker of cancer stem cells), and KiSS-1 (a suppressor of tumor metastasis) are all usefully predictive factors for metastasis in various cancers. However, the metastatic and prognostic value of ORAOV1, ABCG2, or KiSS-1 in laryngeal squamous cell carcinoma (LSCC) is still unclear. In this study, we analyzed correlations among ORAOV1, ABCG2, and KiSS-1 in LSCC, and their respective correlations with clinicopathological characteristics and survival in LSCC. Methods: Specimens from 130 Chinese patients with follow-up were analyzed for ORAOV1, ABCG2, and KiSS-1 protein expression by immunohistochemical staining. The Pearson Chi's square test was used to assess the associations among of these biomarkers and clinicopathological characteristics. The overall survival was studied by univariate and multivariate analyses. Results: Levels of ORAOV1 and ABCG2 were significantly higher, and levels of KiSS-1 were significantly lower, in LSCC tissues than those in control tissues. Levels of ORAOV1 and ABCG2 were positively correlated with primary tumors (pT), lymph node metastasis (LNM), and tumor-node-metastasis (TNM) stages, and negatively with patients overall survival (OS) time; levels of KiSS-1 was inversely associated with tumor grades, pT, LNM, and TNM stages, and the positive expression of KiSS-1 subgroup had significantly longer OS time than did the negative expression of KiSS-1 subgroup. In multivariate analysis, positive expression ORAOV1, ABCG2, or KiSS-1 and tumor stages, as well as TNM stages were potential to be independent prognostic factors for OS time in patients with LSCC. Conclusions: ORAOV1, ABCG2, and KiSS-1 represent promising metastatic and prognostic biomarkers, as well as potential therapeutic targets, for LSCC.

Keywords: LSCC, ORAOV1, ABCG2, KiSS-1, prognosis

Background

In 2015, laryngeal cancer was reportedly found in 26,400 newly diagnosed cases, caused about 12,600 deaths in China [1]. Laryngeal squamous cell carcinoma accounts for about 90% of all diagnosed laryngeal cancers. As laryngeal cancer is usually asymptomatic in its early stages, many patients diagnosed with laryngeal cancer in China have advanced stage cancer.

Oral cancer overexpressed 1 (ORAOV1) gene which is located on human chromosome region 11q13 was originally considered as a potential candidate oncogene in oral squamous cell carcinoma [2]. ORAOV1 is overexpressed in various tumors, playing an important role in the development of the cancers, such as tumorigenesis, cell cycle, and apoptosis [3-7]. In addition, ORAOV1 could regulate VEGF expression in transplantation tumor to promote tumor angiogenesis [3]. Furthermore, Accumulating evidence has suggested that overexpression of ORAOV1 enhance tumorigenicity, cells proliferation, invasion, and metastasis and could be a novel useful prognosis and treatment target of cancers [3-8].

Cancer stem cells (CSCs), also termed as tumor-initiating cells (TICs), which are thought to be a subpopulation of cancer cells which have ability of self-renewal and responsible for tumor initiation, development, metastasis and resistance to conventional radiotherapy or chemotherapy [9-11]. ATP-binding cassette sub-family G member 2 (ABCG2), also named as breast cancer resistance protein (BCRP), which is an

Table 1. Patients characteristics

Patients characteristics	Frequency (n)	Percentage (%)
Age (years)		
<60	59	45.4
≥60	71	54.6
Gender		
Male	87	66.9
Female	43	33.1
Location		
Supraglotic	43	33.1
Glotic	80	61.5
Subglotic	7	5.4
Size (cm)		
<2.0	64	49.2
≥2.0	66	50.8
Smoking		
No	51	39.2
Yes	79	60.8
Alcohol		
No	50	38.5
Yes	80	61.5
Grade		
Well	45	34.6
Moderate	73	56.2
Poor	12	9.2
primary Tumor		
T1	47	36.2
T2	66	50.8
T3	12	9.2
T4a	5	3.8
Lymph node metastasis		
N0	82	63.1
N1	40	30.8
N2	8	6.2
FIGO stage		
I	44	33.8
II	35	26.9
III	44	33.8
IVA	7	5.4

important member of ATP-binding cassette transporter superfamily. ABCG2 was originally found in human MCF-7 breast cancer cells and is involved in multidrug resistance (MDR) by dimerizing itself (homodimerize) or with other members of the ABCG sub-family (heterodimerize) as an efflux transporter [10, 12-15]. Recently, accumulating studies have been found that ABCG2 is overexpressed in many cancers and correlated with tumor initiation, development, metastasis, relapse, and MDR, which may due to its CSCs characteristics [10, 11, 13, 16]. Therefore, ABCG2 is also identified as a potential biomarker for prognosis and treatment target of cancers.

KiSS-1 gene was originally identified as tumor metastasis suppressor in human melanoma through analysis of subtractive hybridization [17]. This gene is located on human chromosome 1q32 and 6151 base pairs in length. KiSS-1 can promote E-cadherin expression and inhibit MMP9 expression by NF-κB binding inhibition to the promoter [18-20]. KiSS-1 expression can inhibit chemotaxis and invasion through attenuating the metastasis of cancers [21]. Some evidence suggested that KiSS-1 could inhibit metastasis of cancer without affecting tumorigenicity [18, 22]. KiSS-1 gene is reported to bind with an orphan G-protein-coupled receptor named as GPR54 [23]. KiSS-1 encodes a 145-amino-acid peptide which is further cleaved into a KiSS-1 family peptins [24]. However, the precise mechanism in tumor metastasis of KiSS-1 is still unclear. Accumulating studies have demonstrated that KiSS-1 should been considered as a useful biomarker of metastasis and prognosis [20, 24-26].

Overall, studies of ORAOV1, ABCG2, and KiSS-1 expression in relation with tumor metastasis and prognosis demonstrate that these biomarkers can affect tumor progression. However, the relationships among ORAOV1, ABCG2, and KiSS-1 in LSCC have not been widely reported. In this study, we investigated the hypothesis that these biomarkers are mutual related and are related to metastasis and prognosis in LSCC.

Materials and methods

Specimens

We collected samples from all 130 patients (median age: 60.2 years; range: 43-78 years) who were treated for LSCC at the First Affiliated Hospital of Bengbu Medical College, from January 2009 to December 2011, along with 130 samples of the corresponding normal mucosa tissues. Patients who had received any preoperative anticancer therapy were excluded. All tissue samples were obtained with patients writing consent. The study was approved by the ethics committee of Bengbu

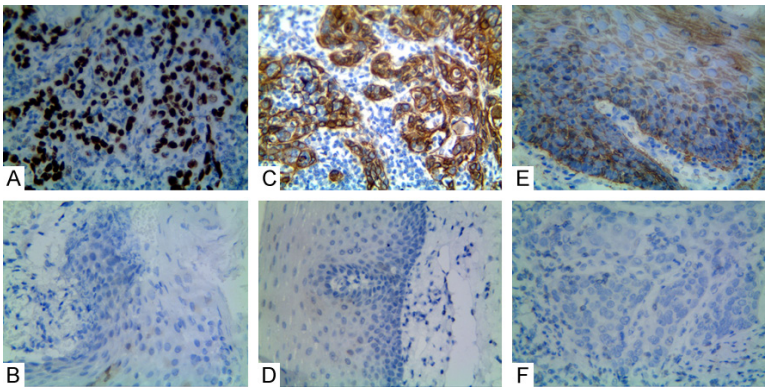

Figure 1. Expression of the ORAOV1, ABCG2, and KiSS-1 in patients with laryngeal squamous cell carcinoma (×400 magnification). A. Positive ORAOV1 expression in the nucleus of cancer cells. B. Negative ORAOV1 expression in the normal laryngeal mucosa epithelial cells (P<0.001). C. Positive ABCG2 expression in the membrane and cytoplasm of cancer cells. D. Negative ABCG2 expression in the normal cells (P<0.001). E. Positive KiSS-1 expression in the cytoplasm of normal cells. F. Negative KiSS-1 expression in the cancer cells (P<0.001).

Medical College and performed in accordance with the guidelines of the Declaration of Helsinki. We collected patients who had completely demographic, clinicopathological, and follow-up data (at 6-month intervals by phone, mail, and social application). Overall survival (OS) was evaluated from the patients surgery date to his or her death date or December 2016 (mean OS: 55.0 months; range: 10-94 months). T stages and tumor-node-metastasis stages were calculated according to the 7th edition of the American Joint Committee on Cancer (AJCC). Tumor grades were according to World Health Organization (WHO) standards. Specific characteristics see **Table 1**.

Immunohistochemistry

Immunohistochemical staining was performed according to the Elivision™ Plus detection kit instructions (LabVision, USA). All LSCC and negative mucosa tissues were fixed in 10% buffered formalin and embedded in paraffin. Paraffin sections (4 μm thick) of representative LSCC and control tissues were cut and then were deparaffinized in xylene and dehydrated in a graded series alcohol. Subsequently washed with phosphate buffer saline (PBS, pH 7.2) for 10 min. The endogenous peroxidase activity was quenched through incubation with 3% H_2O_2 in methanol for 10 min at room temperature. Then placed in citrate buffer (pH 6.0) at 95°C for antigen repair for 30 min. After several washes with PBS, all sections were quenched by goat serum for 30 min, then incubated with rabbit polyclonal antibody against human ORAOV1 (Abcam, USA), mouse monoclonal antibody against human ABCG2 (Abcam, USA) and KiSS-1 (Santa Cruz, USA) at 37°C for 1 h. All sections were counterstained with hematoxylin, dehydrated, air-dried, and mounted. Negative controls were prepared by omitting primary antibody from the staining procedure. ORAOV1 staining was mainly seen in cancer cell nuclei; ABCG2 staining was mainly seen in cancer cell membrane and cytoplasm; KiSS-1 staining was mainly seen in cancer cell cytoplasm.

Evaluation of staining

To assess the staining of ORAOV1, ABCG2, and KiSS-1, the number of cancer cells with positive staining at least 10 representative high-power field (HPF) and the extent and intensity of the positive cancer cells were scored. The results were calculated according to intensity (none staining, 0; weak staining, 1; moderate staining, 2; strong staining, 3) and extent (positive cancer cells <11% mean 1; 11%< positive cancer cells <50% mean 2; 51%< positive cancer cells <75% mean 3; positive cancer cells >75% mean 4). The intensity and extent were multiplied to yield final scores that ranged 0-12. Score >2 was considered positive. Immunohistochemical staining was assessed by two independent pathologists who were blind patients' demographic, clinicopathological, and follow-up data.

Statistical analysis

Correlations between clinicopathological characteristics and the expression of ORAOV1, ABCG2, or KiSS-1 were compared using Fisher's exact test or Chi-square test. Correlations between ORAOV1, or ABCG2, or KiSS-1 were compared using Spearman's coefficient test. Effects of ORAOV1, ABCG2, or KiSS-1 on overall survival were determined by univariate and multivariate logistic regression analyses. Survival analysis was determined by the Kaplan-

Table 2. The associations between expression of ORAOV1, ABCG2, and KiSS-1 and clinicopathological characteristics of laryngeal squamous cell carcinoma (LSCC)

Variables	ORAOV1		P	ABCG2		P	KiSS-1		P
	-	+		-	+		-	+	
Age			0.639			0.679			0.414
<60 years	25	34		22	37		29	30	
≥60 years	33	38		29	42		40	31	
Gender			0.117			0.475			0.660
Male	43	44		36	51		45	42	
Female	15	28		15	28		24	19	
Location			0.571			0.371			0.269
Supraglottic	21	22		19	24		21	22	
Glottic	33	47		28	52		46	34	
Subglottic	4	3		4	3		2	5	
Size (cm)			0.290			0.106			0.053
<2.0	32	33		30	35		29	36	
≥2.0	26	39		21	44		40	25	
Smoking			0.417			0.460			0.143
No	25	26		18	33		23	28	
Yes	33	46		33	46		46	33	
Alcohol			0.635			0.015			0.374
No	21	29		13	37		29	21	
Yes	37	43		38	42		40	40	
Grade			0.173			0.107			0.003
Well	25	20		22	23		17	28	
Moderate	29	44		27	46		41	32	
Poor	4	8		2	10		11	1	
Primary Tumor			0.015			<0.001			<0.001
T1	28	19		33	14		10	37	
T2	27	39		16	50		44	22	
T3	3	9		2	10		10	2	
T4a	0	5		0	5		5	0	
LNM			0.010			<0.001			<0.001
N0	43	39		44	38		29	53	
N1	15	25		6	34		33	7	
N2	0	8		1	7		7	1	
TNM stages			0.014			<0.001			<0.001
I	26	18		34	10		7	37	
II	16	19		11	24		20	15	
III	16	28		5	39		35	9	
IVA	0	7		1	6		7	0	

Meier method with log-rank test to assess correlation between ORAOV1, ABCG2, or KiSS-1 immunostaining results or clinicopathological characteristics and OS time, using SPSS 19.0 software for Windows (Chicago, IL). A value of P<0.05 was indicated statistically significant.

Results

There was a significant difference between the expression of ORAOV1, ABCG2, and KiSS-1 and some clinicopathological characteristics.

To evaluate the contributions of ORAOV1, ABCG2, and KiSS-1 expression to LSCC, the results there of were evaluated for both LSCC and corresponding normal laryngeal mucosa tissues. The data was compared to patients clinicopathological characteristics. The positive expression rate of ORAOV1 in the LSCC specimens (55.4%, 72/130) was significantly higher than that in the corresponding normal laryngeal mucosa tissues (7.7%, 10/130; P<0.001; **Figure 1A** and **1B**). The positive expression rate of ORAOV1 in LSCC was positively correlated with pT, LNM, and TNM stages, but not patients age, gender, location, tumor grades, smoking, alcohol, or tumor size (**Table 2**).

Similar to ORAOV1, ABCG2+ expression was significantly higher in LSCC tissues (60.8%, 79/130) than that in the control normal tissues (11.5%, 15/130; P<0.001; **Figure 1C** and **1D**). The positive expression rate of ABCG2 in LSCC was correlated with pT, LNM, TNM stages, and alcohol, but not patients age, gender, location, tumor grades, smoking, or tumor size (**Table 2**).

The positive expression rate of KiSS-1 expression was significantly lower in LSCC tissues (46.9%, 61/130) than that in the control normal tissues (96.2%, 125/130; P<0.001; **Figure 1E** and **1F**). The positive expression rate of KiSS-1 was inversely correlated with tumor grades, pT, LNM, and TNM stages. No correlation was found between KiSS-1 positive expression and patients age, gender, tumor size, location, smoking, or alcohol (**Table 2**).

Table 3. Correlation among expression of ORAOV1, ABCG2, and KiSS-1 in LSCC

Variable	ORAOV1		r	P	ABCG2		r	P
	-	+			-	+		
ORAOV1							0.261	0.003*
-					31	27		
+					20	52		
KiSS-1			-0.458	<0.001@			-0.634	<0.001@
-	16	53			7	62		
+	42	19			44	17		

*: positive association; @: negative association

Associations among expression of ORAOV1, ABCG2, and KiSS-1 in LSCC

Spearman correlation coefficient analysis demonstrated that negative associations between KiSS1+ expression and that of ORAOV1 (r=-0.458, P<0.001), or ABCG2 (r=-0.634, P<0.001). Expression of ORAOV1 was positive associated with ABCG2 (r=0.261, P=0.003; **Table 3**).

Survival analysis showed that there was a significant difference between the positive expression of ORAOV1, or ABCG2, or KiSS1 and the negative expression of these biomarkers for OS

Follow-up data indicated that OS time was significantly shorter in LSCC patients with ORAOV1+ specimens (43.6±18.4 months) compared with those with ORAOV1- (69.1±15.0 months; log-rank =52.622, P<0.001; **Figure 2A**). Similarly, OS time of ABCG2+ patients (44.6±17.7 months) was significantly shorter than those of ABCG2- patients (71.0±15.1 months; log-rank =60.054, P<0.001; **Figure 2B**). The OS time of KiSS1+ patients (70.2±13.9 months) was significantly longer than those who were KiSS-1- (41.5±16.9 months; log-rank =78.922, P<0.001; **Figure 2C**). In other variable analysis, OS time was significantly correlated with clinicopathological characteristics, including tumor size (log-rank =5.748, P =0.017, **Figure 2D**), grades (log-rank =11.152, P=0.004, **Figure 2E**), pT (log-rank =66.112, P<0.001, **Figure 2F**), LNM (log-rank =43.318, P<0.001, **Figure 2G**), and TNM stages (log-rank =66.832, P<0.001, **Figure 2H**).

Expression of ORAOV1 significantly associated with survival status

Univariate analysis showed that grades of tumor, pT stages, TNM stages, as well as the expression of ORAOV1, ABCG2, or KiSS-1 significantly correlated with survival status of patients with LSCC (P<0.05). In the multivariate logistic regression analysis, the expression of ORAOV1 was significantly associated with survival status of patients with LSCC (**Table 4**).

Discussion

Laryngeal squamous cell carcinoma (LSCC) is a highly heterogeneous disease, which would influence the effectiveness of biomarkers evaluation. Therefore, metastatic and prognostic value of candidate biomarkers should be exhaustively evaluated to make certain their validity. In this study, we demonstrated that ORAOV1 was positively related to LSCC pT, LNM, and TNM stages. In addition, Kaplan-Meier survival curve demonstrated that ORAOV1+ LSCC patients had significantly shorter OS time than did ORAOV1- patients. The above results indicated that ORAOV1 should be involved in the process of progression and metastasis of LSCC, and could be identified as a metastatic biomarker in predicting this disease. Our results are similar to the previous studies [3-8].

ABCG2 has been considered as a potential biomarker of CSCs in various cancers [10-12, 27]. Overexpression of ABCG2 cells may possess some stem-like ability to self-renewal, differentiation, resistance therapy, and tumorigenicity [28]. In this study, ABCG2 expression has been positively related to pT, LNM, and TNM stages. Furthermore, Kaplan-Meier survival curve demonstrated that ABCG2+ LSCC patients had significantly shorter OS time than did ABCG2- patients. The above results suggested that overexpression of ABCG2 should play an important role in the process of invasion, metastasis, prognosis, as well as tumorigenesis of LSCC. Thus, our results sustain the notion that ABCG2 should be a trustable biomarker of LSCC, in particularly for predicting metastasis and prognosis [10, 11, 27].

KiSS-1 is widely identified as a suppressor gene of metastasis in diverse cancers [17-26]. KiSS-1 can inhibit cancer cells proliferation, motility, invasion, and metastasis [29]. Results in this study also demonstrated that positive expression rate of KiSS-1 was significantly lower in LSCC tissues than that in control normal tis-

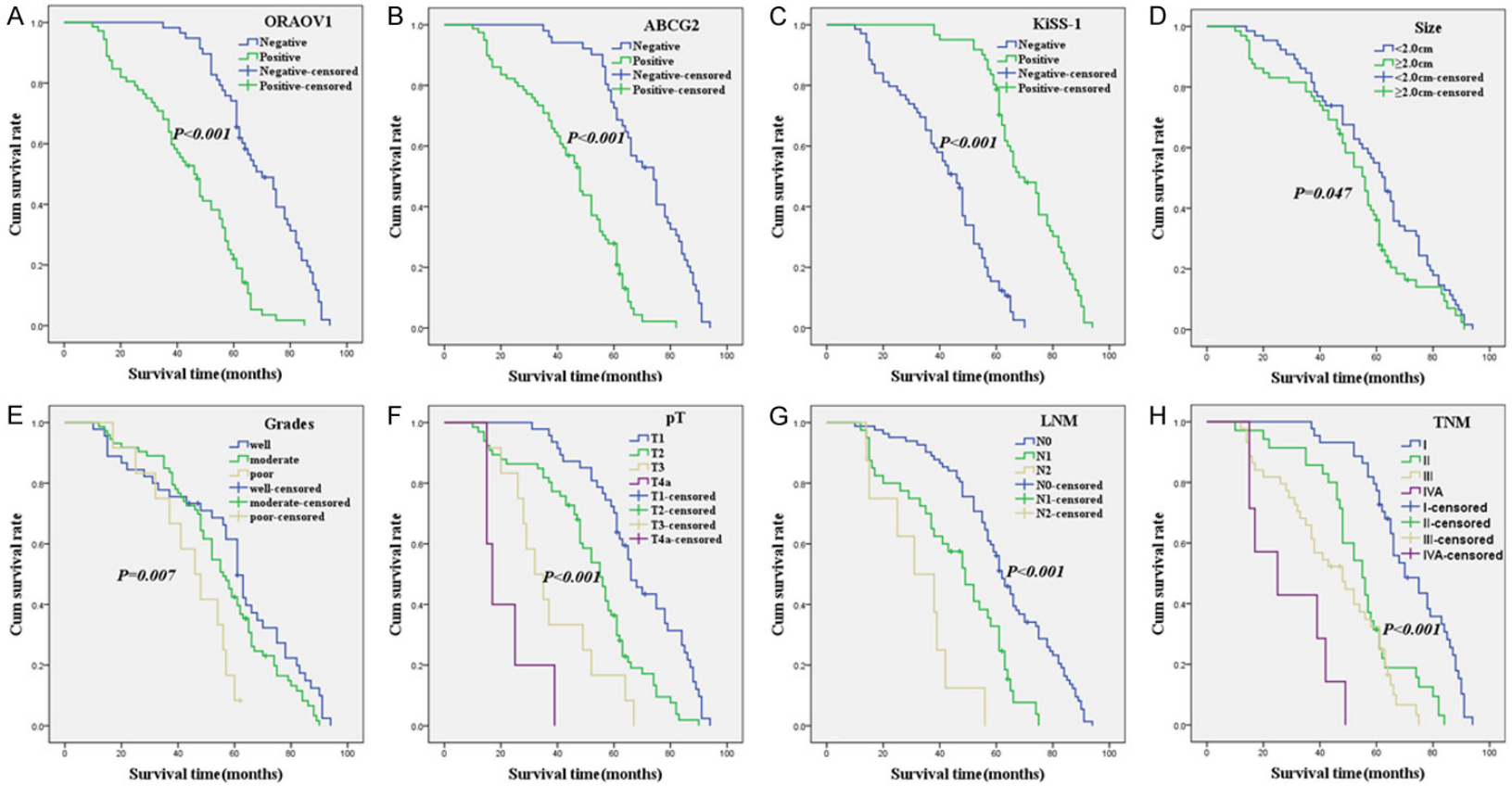

Figure 2. Kaplan-Meier analysis of the overall survival (OS) of patients with laryngeal squamous cell carcinoma. A. OS time of all patients in relation to ORAOV1 expression (log-rank =52.622, P<0.001). B. OS of all patients in relation to ABCG2 expression (log-rank =60.054, P<0.001). C. OS of all patients in relation to KiSS-1 expression (log-rank =78.922, P<0.001). The green line represents positive expression of ORAOV1, ABCG2, and KiSS-1 and the blue line represents negative expression of ORAOV1, ABCG2, and KiSS-1. D. OS of all patients in relation to tumor size (log-rank =5.748, P=0.017, the blue line represents patients with tumor size: <2.0 cm group, the green line represents patients size: ≥2.0 cm group). E. OS of all patients in relation to tumor grades (log-rank =11.152, P=0.004, the blue line represents patients with grade 1, the green line represents patients with grade 2; the brown line represents patient with grade 3). F. OS of all patients in relation to pT stages (log-rank =66.112, 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). G. OS of all patients in relation to LNM (log-rank =43.318, 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 stages (log-rank =66.832, 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 4. Univariate and multivariate logistic regression analysis of factors affecting survival status

Variables	Categories	Univariate analysis	Multivariate analysis		
		P	HR	95% CI	P
Grades	G1/G2/G3	0.020	1.411	0.661-3.014	0.547
pT	T1/T2/T3/T4a	0.022	0.778	0.329-1.836	0.566
TNM	I/II/III/IVA	0.002	1.055	0.504-2.209	0.886
ORAOV1	negative/positive	<0.001	3.703	1.479-9.274	0.005
ABCG2	negative/positive	<0.001	2.223	0.722-6.842	0.164
KiSS-1	negative/positive	<0.001	0.356	0.105-1.209	0.098

sues, and its positive rate was negatively associated with tumor grade, pT, LNM, as well as TNM stages. Moreover, Kaplan-Meier survival demonstrated that KiSS-1+ patients had significantly longer OS time than did KiSS-1- patients. The above results indicated that down- or lose-regulation of KiSS-1 should be considered as a potential biomarker for predicting metastasis and prognosis in LSCC patients, which are consistent with the previous studies [20, 21, 25, 26].

Laryngeal squamous cell carcinoma (LSCC) is the most common type of laryngeal cancer. Moreover, ABCG2 is a biomarker of CSCs, so its involvement in CSCs should play a critical role in the initiation and development of LSCC [28]. CSCs can induce angiogenesis in order to meet the need of nutrient and oxygen for rapid growth and promote tumor cells metastasis [25, 30] During tumorigenesis, abnormal ORAOV1 expression can also induce cells proliferation and promote tumor cell invasion by the activation of Cyclins [4, 5, 7]. It could also regulate VEGF expression to induce tumor angiogenesis [3], thus promote tumor cells metastasis. KiSS-1 suppresses tumor metastasis through the promotion of E-cadherin expression and inhibition of MMP expression which can degrade extracellular matrix [18-20]. Therefore, down- or lost-regulation of KiSS-1 could promote tumor cells invasion and metastasis [25, 31]. In this study, univariate analysis showed that grades of tumor, pT stages, TNM stages, as well as the expression of ORAOV1, ABCG2, or KiSS-1 significantly correlated with survival status of patients with LSCC; multivariate analysis suggested that ORAOV1 was independent prognostic indicator for LSCC patients. These results indicated that ORAOV1, ABCG2, and KiSS-1 expression should be indentified as reliable biomarkers for LSCC, especially in predicting tumor metastasis and prognosis.

Conclusions

Our findings suggested that ORAOV1, ABCG2, and KiSS-1 should be involved in the evolution of LSCC; and the combined detection of ORAOV1, ABCG2, and KiSS-1 were valuable factors for metastasis and prognosis in LSCC's patients.

Acknowledgements

This work was supported by the Nature Science Foundation of Anhui Province (No. 1708085MH230) and the Nature Science Key Program of College and University of Anhui Province (No. KJ2015A269 and No. KJ2016-A488) and Key projects of support program for outstanding young talents in Colleges and Universities of Anhui Province (No. gxyqZD20-16160).

Disclosure of conflict of interest

None.

Address correspondence to: Shiwu Wu, Department of Pathology, Bengbu Medical College, No. 287, Changhuai Road, Anhui, China. Tel: +86-13705523357; E-mail: 573448542@qq.com

References

[1] Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-32.

[2] Huang X, Gollin SM, Raja S, Godfrey TE. High-resolution mapping of the 11q13 amplicon and identification of a gene. TAOS1, that is amplified and overexpressed in oral cancer cells. Proc Natl Acad Sci U S A 2002; 99: 11369-74.

[3] Zhao X, Liu D, Wang L, Wu R, Zeng X, Dan H, Ji N, Jiang L, Zhou Y, Chen Q. RNAi-mediated downregulation of oral cancer overexpressed 1 (ORAOV1) inhibits vascular endothelial cell proliferation, migration, invasion, and tube formation. J Oral Pathol Med 2016; 45: 256-61.

[4] Jiang L, Zeng X, Yang H, Wang Z, Shen J, Bai J, Zhang Y, Gao F, Zhou M, Chen Q. Oral cancer overexpressed 1 (ORAOV1): a regulator for the cell growth and tumor angiogenesis in oral squamous cell carcinoma. Int J Cancer 2008; 123: 1779-86.

[5] Togashi Y, Arao T, Kato H, Matsumoto K, Terashima M, Hayashi H, de Velasco MA, Fujita Y, Kimura H, Yasuda T, Shiozaki H, Nishio N. Frequent amplification of ORAOV1 gene in esophageal squamous cell cancer promotes an aggressive phenotype via proline metabolism and ROS production. Oncotarget 2014; 5: 2962-73.
[6] Zhai C, Li Y, Mascarenhas C, Lin Q, Li K, Vyrides I, Grant CM, Panaretou B. The function of ORAOV1/LTO1, a gene that is overexpressed frequently in cancer: essential roles in the function and biogenesis of the ribosome. Oncogene 2014; 33: 484-94.
[7] Jiang L, Zeng X, Wang Z, Ji N, Zhou Y, Liu X, Chen Q. Oral cancer overexpressed 1 (ORAOV1) regulates cell cycle and apoptosis in cervical cancer HeLa cells. Mol Cancer 2010; 9: 20.
[8] Li M, Cui X, Shen Y, Dong H, Liang W, Chen Y, Hu J, Li S, Kong J, Li H, Zhao J, Li F. ORAOV1 overexpression in esophageal squamous cell carcinoma and esophageal dysplasia: a possible biomarker of progression and poor prognosis in esophageal carcinoma. Hum Pathol 2015; 46: 707-15.
[9] Singh SR. Gastric cancer stem cells: a novel therapeutic target. Cancer Lett 2013; 338: 110-9.
[10] Zhao W, Luo Y, Li B, Zhang T. Tumorigenic lung tumorospheres exhibit stem-like features with significant increased expression of CD133 and ABCG2. Mol Med Rep 2016; 14: 2598-606.
[11] Wee B, Pietras A, Ozawa T, Bazzoli E, Podlaha O, Antczak C, Westermark B, Nelander S, Uhrbom L, Forsberg-Nilsson K, Djaballah H, Michor F, Holland EC. ABCG2 regulates self-renewal and stem cell marker expression but not tumorigenicity or radiation resistance of glioma cells. Sci Rep 2016; 6: 25956.
[12] Jabor Gozzi G, Bouaziz Z, Winter E, Daflon-Yunes N, Aichele D, Nacereddine A, Marminon C, Valdameri G, Zeinyeh W, Bollacke A, Guillon J, Lacoudre A,Pinaud N, Cadena SM, Jose J, Le Borgne M, Di Pietro A. Converting potent indeno [1,2-b] indole inhibitors of protein kinase CK2 into selective inhibitors of the breast cancer resistance protein ABCG2. J Med Chem 2015; 58: 265-77.
[13] Wang J, Yunyun Z, Wang L, Chen X, Zhu Z. ABCG2 confers promotion in gastric cancer through modulating downstream CRKL in vitro combining with biostatistics mining. Oncotarget 2017; 8: 5256-67.
[14] Desuzinges-Mandon E, Arnaud O, Martinez L, Huché F, Di Pietro A, Falson P. ABCG2 transports and transfers heme to albumin through its large extracellular loop. J Biol Chem 2010; 285: 33123-33.
[15] Tarapcsák S, Szalóki G, Telbisz Á, Gyöngy Z, Matúz K, Csősz É, Nagy P, Holb IJ, Rühl R, Nagy L, Szabó G, Goda K. Interactions of retinoids with the ABC transporters P-glycoprotein and breast cancer resistance protein. Sci Rep 2017; 7: 41376.
[16] Balbuena J, Pachon G, Lopez-Torrents G, Aran JM, Castresana JS, Petriz J. ABCG2 is required to control the sonic hedgehog pathway in side population cells with stem-like properties. Cytometry A 2011; 79: 672-83.
[17] Welch DR, Chen P, Miele ME, McGary CT, Bower JM, Stanbridge EJ, Weissman BE: microcell-mediated transfer of chromosome 6 into metastatic human C8161 melanoma cells suppresses metastasis but does not inhibit tumorigenicity. Oncogene 1994; 9: 255-262.
[18] Lee JH, Welch DR. Suppression of metastasis in human breast carcinoma MDA-MB-435 cells after transfection with the metastasis suppressor gene, KiSS-1. Cancer Res 1997; 57: 2384-7.
[19] Yan C, Wang H, Boyd DD. KiSS-1 represses 92-kDa type IV collagenase expression by down-regulating NF-κB binding to the promoter as a consequence of IκBα-induced block of p65/p50 nuclear translocation. J Biol Chem 2001; 276: 1164-72.
[20] Kostadima L, Pentheroudakis G, Pavlidis N. The missing kiss of life: transcriptional activity of the metastasis suppressor gene KiSS1 in early breast cancer. Anticancer Res 2007; 27: 2499-504.
[21] Lee JH, Miele ME, Hicks DJ, Phillips KK, Trent JM, Weissman BE, Welch DR. KiSS-1, a novel human malignant melanoma metastasis-suppressor gene. J Natl Cancer Inst 1996; 88: 1731-7.
[22] Lee JH, Welch DR. Identification of highly expressed genes in metastasis-suppressed chromosome 6/human malignant melanoma hybrid cells using subtractive hybridization and differential display. Int J Cancer 1997; 71: 1035-44.
[23] Kotani M, Detheux M, Vandenbogaerde A, Communi D, Vanderwinden JM, Le Poul E, Brézillon S, Tyldesley R, Suarez-Huerta N, Vandeput F, Blanpain C, Schiffmann SN, Vassart G, Parmentier M. The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. J Biol Chem 2001; 276: 34631-6.
[24] Takeda T, Kikuchi E, Mikami S, Suzuki E, Matsumoto K, Miyajima A, Okada Y, Oya M. Prognostic role of KiSS-1 and possibility of therapeutic modality of metastin, the final peptide of the KiSS-1 gene, in urothelial carcinoma. Mol Cancer Ther 2012; 11: 853-63.
[25] Yu L, Zhu B, Wu S, Zhou L, Song W, Gong X, Wang D. Evaluation of the correlation of vasculogenic mimicry, ALDH1, KiSS-1, and MACC1

in the prediction of metastasis and prognosis in ovarian carcinoma. Diagn Pathol 2017; 12: 23.

[26] Cao F, Chen L, Liu M, Lin W, Ji J, You J, Qiao F, Liu H. Expression of preoperative KISS1 gene in tumor tissue with epithelial ovarian cancer and its prognostic value. Medicine (Baltimore) 2016; 95: e5296.

[27] Niess H, Camaj P, Renner A, Ischenko I, Zhao Y, Krebs S, Mysliwietz J, Jackel C, Nelson PJ, Blum H, Jauch KW, Ellwart JW, Bruns CJ. Side population cells of pancreatic cancer show characteristics of cancer stem cells responsible for resistance and metastasis. Target Oncol 2015; 10: 215-27.

[28] Boesch M, Zeimet AG, Reimer D, Schmidt S, Gastl G, Parson W, Spoeck F, Hatina J, Wolf D, Sopper S. The side population of ovarian cancer cells defines a heterogeneous compartment exhibiting stem cell characteristics. Oncotarget 2014; 5: 7027-39.

[29] Babwah AV, Pampillo M, Min L, Kaiser UB, Bhattacharya M. Single-cell analyses reveal that KISS1R-expressing cells undergo sustained kisspeptininduced signaling that is dependent upon an influx of extracellular Ca2+. Endocrinology 2012; 153: 5875-87.

[30] Hilbe W, Dirnhofer S, Oberwasserlechner F, Schmid T, Gunsilius E, Hilbe G, Wöll E, Kähler CM. CD133 positive endothelial progenitor cells contribute to the tumour vasculature in non-small cell lung cancer. J Clin Pathol 2004; 57: 965-9.

[31] Quevedo EG, Aguilar GM, Aguilar LA, Rubio SA, Martínez SE, Rodríguez IP, Corona JS, Morán MI, Gómez RC, Moguel MC. Polymorphisms rs12998 and rs5780218 in KiSS1 suppressor metastasis gene in Mexican patients with breast cancer. Dis Markers 2015; 2015: 365845.