Bull Yamaguchi Med Sch 66(1-2):15-27, 2019

Overexpression of Runt-Related Transcription Factor-1 at Invasive Front in Oral Squamous Cell Carcinoma is Associated with Lymph Node Metastasis and Poor Prognosis

Takahiro Hisano,[1,2] *Dan Cui,*[1] *Koji Harada,*[2] *Tokuhiro Kimura,*[1,3] *Katsuaki Mishima*[2] *and Eiji Ikeda*[1]

[1] Department of Pathology, Yamaguchi University Graduate School of Medicine, 1-1-1 Minami-Kogushi, Ube, Yamaguchi 755-8505, Japan
[2] Department of Oral and Maxillofacial Surgery, Yamaguchi University Graduate School of Medicine, 1-1-1 Minami-Kogushi, Ube, Yamaguchi 755-8505, Japan
[3] Department of Diagnostic Pathology, Saiseikai Yokohamashi Tobu Hospital, Yokohama, Kanagawa 230-8765, Japan
(Received December 3, 2018, accepted January 17, 2019)
Correspondence to Eiji IKEDA, M.D., Ph.D. E-mail: ikedae@yamaguchi-u.ac.jp

Abstract This study aims to examine clinical significance of Runt-Related Transcription Factor-1 (RUNX1) in oral squamous cell carcinoma (OSCC). RUNX1 expression was analyzed by immunohistochemistry in 43 patients with OSCC. The clinical significance of RUNX1 expression was evaluated by Chi-square test, Kaplan-Meier method and Cox regression model. The expression of RUNX1 at invasive front was significantly higher than that at central part of tumor ($P < 0.01$). OSCC patients with high RUNX1-Labeling Index (LI) at invasive front had significantly shorter overall survival period ($P < 0.05$) as compared with low RUNX1-LI at invasive front. Poorer prognosis of patients with higher RUNX1-LI at invasive front was supported by the data that the RUNX1-LI at invasive front was statistically correlated with poor differentiation ($P < 0.05$), invasiveness ($P < 0.01$), and post-operative lymph node metastasis ($P < 0.05$). Moreover, high RUNX1-LI at invasive front was demonstrated to be an independent prognostic factor for unfavorable overall survival ($P < 0.05$). Our findings suggest for the first time that overexpression of RUNX1 at the invasive front in OSCC can be a valuable marker to evaluate lymph node metastasis as well as a promising predictor of poor prognosis.

Key words: lymph node metastasis, oral squamous cell carcinoma, prognosis, runt-related transcription factor-1

Introduction

Oral squamous cell carcinoma (OSCC) is a typical malignant neoplasm of the oral cavity and occupies about 90% of all oral malignancies.[1] OSCC is the 8th common cancer in humans, which accounts for approximately 4% of all carcinomas in men and 2% in women worldwide.[2,3] The incidence of OSCC is increasing gradually. Approximately 300,000 patients are annually estimated to get oral cancer in the world,[4-6] whereas about 11,000 new patients are estimated in Japan.[7] In spite of the recent advancement of cancer therapeutic methods and the use of improved chemotherapeutic agents and molecular targeting agents, the 5-year survival rate of OSCC is approximately 50% in the advanced stage of OSCC.[8,9] OSCC is an inhomogeneous disease and responds in differential fashion to the same treatment, which could be the reason for its poor outcome in the advanced stage

of OSCC. Therefore, we must develop useful markers for early detection of OSCC. In addition, the mortality rate of OSCC has been increasing due to the metastasis at the early stage.[10] The standard treatment for OSCC is still surgical resection. However, 20-40 % of patients finally die of metastasis in spite of curative surgical resection of the primary tumor.[10,11] Therefore, elucidation of the processes of metastasis is necessary to find the new treatment modalities and improve outcomes of patients with OSCC.

The processes of metastasis are complicated. They include various phases and several molecular events at invasive front of malignant tumor. Their molecular events and sequential phases often play an important role in the formation of metastatic lesions. Also, invasive front is defined as three to six layers of tumor cells at the front edge or scattered tumor nests between tumor and host tissue. In addition, invasive front is for recognized as tumor budding sites. It is thought that OSCC presents cellular dedifferentiation at invasive front. This phenomenon is characterized by acquisition of a mesenchymal phenotype as well as forfeit of an epithelial phenotype. Moreover, it leads to the invasion and metastasis of original differentiated cancer cells. Malignant progression is thought to be related with an epithelial-mesenchymal transition deeply.11,12 We have sought to investigate the useful biomarker for evaluating the level of malignant progression at the invasive front in OSCC. We have also supposed that some of cancer stem cell-related factors must be the candidate biomarker, since epithelial-mesenchymal transition is one of typical characteristics of cancer stem cells.

Mammalian runt-related transcription factor (RUNX) family consists of three members including RUNX1, RUNX2 and RUNX3. They form the core-binding factor (CBF) complex that is a transcription factor complex.[13,14] The RUNX proteins are associated with cellular differentiation, survival and proliferation in various tissues by regulating the gene transcription.[15] In addition, the function of each RUNX protein has high specificity, and RUNX1 and RUNX2 are require for generation of multiple hematopoietic lineages and osteogenesis, respectively, and RUNX3 is intimately associated with neurogenesis and gut development.[15,16] Moreover, various reports have clarified that RUNX proteins might have the oncogenic and tumor suppressive functions. We have focused our study on the role of RUNX1 in neoplastic disease, since the overexpression of RUNX1 has been identified in several human malignancies, including skin cancer,[17] endometrial cancer,[18] ovarian cancer,[18] prostate cancer,[19] breast cancer,[20] colon cancer,[15] and head and neck cancer.[21] In addition, it may act as a transcriptional factor for differentiation of cancer stem cells as well as lymphocytes and myelocytes.[22] The expression patterns and involvement of RUNX1 in OSCC is currently unclear, although it was reported that RUNX1 might activate matrix metalloproteinases (MMPs)-2 and 9, suggesting the role of RUNX1 in the invasive stage of OSCC.[23] Therefore, the purpose of current study is to examine the clinical significance of RUNX1 expression at invasive front in OSCC. Here, we demonstrate the data showing the contribution of RUNX1 to lymph node metastasis. In the meantime, our present findings are expected to lead to the determination of possible therapeutic targets as well as potential candidates for survival predictors of OSCC.

Materials And Methods

Patients and specimens

Forty-three patients with OSCC who had visited Yamaguchi University Hospital from January 2001 to December 2012 are analyzed in this study. They had mainly stage I or II without distant metastasis at the first visit to our hospital. No patients had received any treatments previously. All of the 43 patients were histopathologically diagnosed as squamous cell carcinoma. Clinical data on patients' age, gender, performance status, T classification, N classification, stage of disease, post-operative lymph node metastasis, grade of differentiation, mode of invasion (Y-K classification), smoking history and alcohol intake are shown in Table 1. All patients received surgical operation without any adjuvant or neo-adjuvant chemotherapy. Before the primary treatment, tissue specimens were obtained from all 43 patients by

biopsy. All tissue samples were fixed in 10% phosphate-buffered formalin and were paraffin-embedded. The authors strictly followed the ethical standards of the Institutional review board (IRB) of Yamaguchi University Hospital (IRB approved number H26-43) while performing this study. As this study is a retrospective one, informed consent was waived by the IRB.

Immunohistochemical staining and evaluation

The paraffin-embedded tissues were sectioned into 4-μm slices. Tissue sections were dewaxed in xylene and rehydrated in graded ethanol for 5 min, and washed with distilled water at room temperature. One section from each specimen was stained with hematoxylin (Muto Pure Chemicals, Tokyo, Japan) and eosin (Wako Pure Chemical Industries, Ltd., Osaka, Japan) for histological evaluation. Other sections were heated in an autoclave for 20 min to retrieve the antigenicity with a antigen retrieval buffer (Histofine; Nichirei Bioscience, Japan), pH 9.0. Then, they were cooled to room temperature gradually, and rinsed in distilled water. Endogenous peroxidase activity was blocked using 3% H_2O_2 in methanol for 5 min at room temperature. A 10% normal goat serum was applied to the sections for 30 min as a blocking reagent to reduce nonspecific binding. A mouse monoclonal antibody against RUNX1 protein (1:1000 Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) was used as the primary antibody. The sections were incubated at 4 ℃ overnight, and rinsed in PBS. After applying second antibody (ImmPRESS™ REAGENT Anti-Mouse IgG PEROXIDASE; Vector Laboratories, Burlingame, CA) for 60 min at room temperature, color was developed with 3, 3'-diaminobenzidine (DAB). The sections were then counterstained with hematoxylin. After staining, slides were washed in tap of water, dehydrated in graded alcohol and xylene, mounted and cover slipped. For negative control experiments, the primary antibody was replaced by mouse immunoglobulin (Agilent Technologies Dako, Glostrup, Denmark).

To quantitate the RUNX1 expression, the mean percentage of positive tumor cells (Labeling Index; LI) was determined in three random fields for invasive front, central part

Table 1 Characteristic of patients

Characteristic	Cases	%
Age (years)		
< 65	14	32.6
≧ 65	29	67.4
Gender		
Male	22	51.2
Female	21	48.8
ECOG Performance Status		
0	39	90.7
1	4	9.3
T classification		
T1	28	65.1
T2	13	30.2
T3	1	2.3
T4	1	2.3
N classification		
N0	41	95.3
N1	1	2.3
N2	1	2.3
Stage		
Ⅰ	27	62.8
Ⅱ	12	27.9
Ⅲ	2	4.7
Ⅳ	2	4.7
Post-operative lymph node metastasis		
−	34	79.1
+	9	20.9
Grade		
Well	28	65.1
Moderately	11	25.6
Poorly	4	9.3
Mode of invasion (Y-K criteria)		
Grade 2	9	20.9
Grade 3	27	62.8
Grade 4C	7	16.3
Smoking history		
Never	26	60.5
Past and present	15	34.9
Missing	2	4.7
Alcohol intake		
Never	23	53.5
Past and present	18	41.9
Missing	2	4.7

of tumor as well as squamous intraepithelial neoplasia (SIN) (Fig. 1A). SIN 1, SIN 2 and SIN 3 are equivalent to mild, moderate and severe dysplasia, respectively. The intensity of the RUNX1-immunoreaction was scored as follows: 0, no staining; 1+, weaker staining than lymphocyte; 2+, similar staining to lymphocyte; and 3+, stronger staining than lymphocyte (Fig. 1B). In addition, we used three different criteria for judgment of RUNX1 positive cells. Briefly, we considered RUNX1 staining as positive by equal to or more than intensity 1 (Intensity 1, 2, 3), equal to or more than intensity 2 (Intensity 2, 3), or intensity 3 only (Intensity 3). Also, we decided the cut off value for high expression or low expression by using ROC curve. These judgments were made by three authors (TH, DC and KH) who had no knowledge of the patient's clinical status.

Statistical analysis

The associations between RUNX1 and clinicopathological parameters were assessed using the Chi-square test, or Spearman's rank correlation coefficient. Overall survival (OS) was calculated using the method of Kaplan-Meier, and comparison between groups was performed with the log-rank test. Cox proportional hazard models were used to assess the prognostic significance of RUNX1 expression and several clinicopathological parameters. All statistical significance was set at $p<0.05$. Statistical analyses were performed using the StatView software (version 5.0J, SAS Institute Inc. Cary, NC, USA).

Results

Patients and tumor characteristics

Clinicopathological data of 43 OSCC patients who participated in this study are summarized in Table 1. All of the patients were received surgical operation without any adjuvant or neo-adjuvant chemotherapy. The median follow-up period was 7.17 years, and the mean age of patients was 68.2 years (range 22-98 years). Clinical stages I, II, III and IV were diagnosed in 27, 12, 2 and 2 patients, respectively. There were adequate histological materials available for immunohistochemical analysis of those patients.

RUNX1 expression at invasive front of tumor was higher than that at central part of tumor

RUNX1 expression was observed in nuclei of tumor cells in OSCC tissues. However, the intensity of nuclear staining of RUNX1 was various (Fig. 1B). So, we had to define the criteria for positive staining of RUNX1. We selected the RUNX1 expression in lymphocytes as a criterial parameter, and set standards for the intensity as below: 0, no staining; 1+, weaker staining than lymphocyte; 2+, similar staining to lymphocyte; and 3+, stronger staining than lymphocyte as written in materials and methods. We considered RUNX1 staining as positive with three different criteria; equal to or more than intensity 1 (Intensity 1, 2, 3), equal to or more than intensity 2 (Intensity 2, 3), or intensity 3 only (Intensity 3). Regardless of the criteria for positive staining of RUNX1, the RUNX1-LI was shown to be higher in invasive front lesions of tumors than central part of tumors (Fig. 1C).

High expression of RUNX 1 at invasive front was associated with short overall survival

A total of 37 patients survived and 6 patients died during the study period with median follow-up time of 7.17 years. The relationship between RUNX1 expression and patients' OS was analyzed by Kaplan-Meier curve. We also investigated them based on three different criteria of immunostained intensity separately in the invasive front or the central part of tumor. Interestingly, there was a significant association between high expression of RUNX1 in tumor cells of the invasive front and short OS when we used intensity 3 as the criteria for positive staining of RUNX1 ($P < 0.05$) (Fig. 2).

High expression of RUNX1 in tumor cells of invasive front was associated with poor tumor differentiation, high degree of invasiveness, and lymph node metastasis

We examined the association between RUNX1 expression and clinicopathological factors based on three different criteria of immunostained intensity separately at the invasive front or the central part of tumor in order to define the importance of high expression of RUNX 1 at the invasive front. Then,

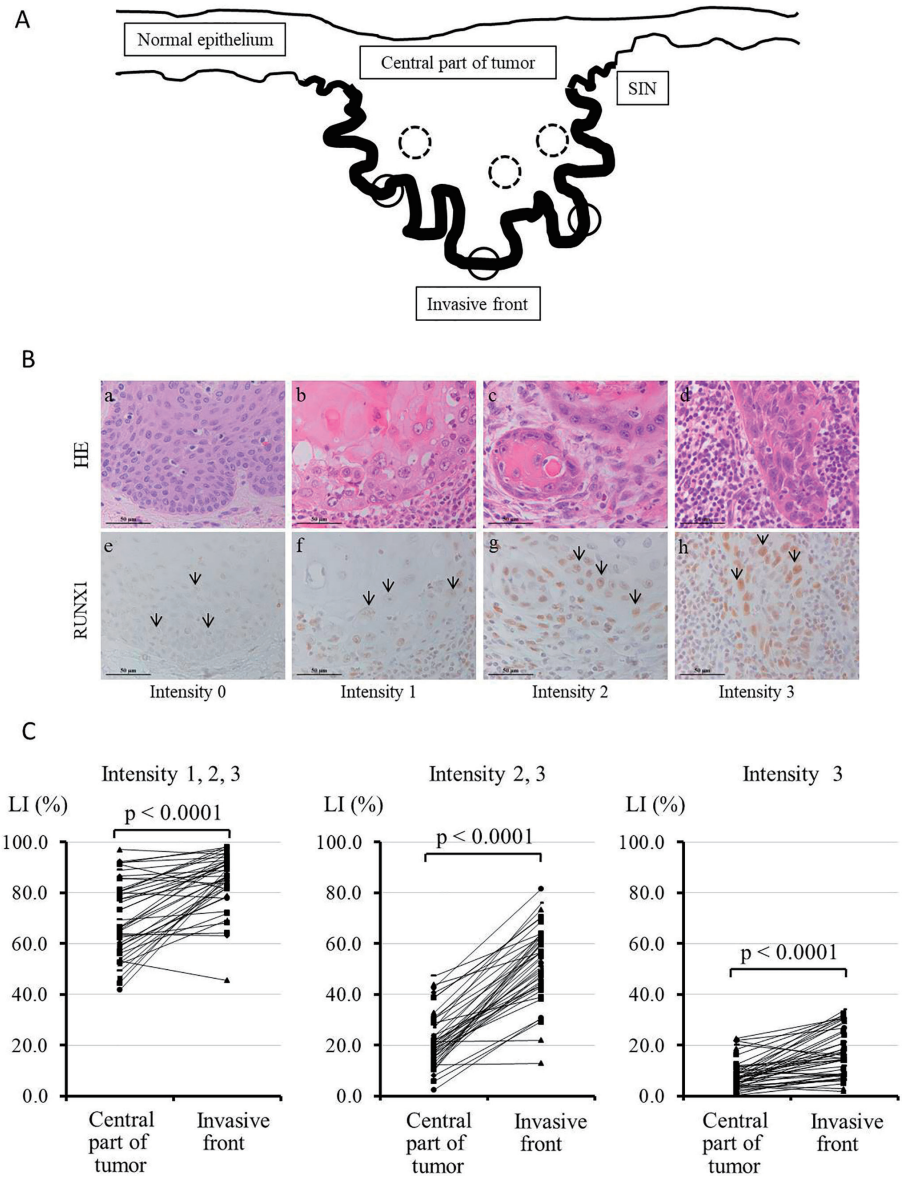

Fig. 1 (A) Schematic description of the invasive front (thick, black, line), central part of tumor and SIN. The mean percentage of positive tumor cells was determined in three random fields in each lesion including invasive front (solid circle), central part of tumor (dotted circle) and SIN. (B) RUNX1 expression in tissue samples stained with hematoxylin-eosin solution (a, b, c, d) or an anti-RUNX 1 antibody (e, f, g, h). RUNX1 positive staining (arrow head) observed in the nucleus of tumor cells (bar; 100 μm). The intensity of the RUNX1-immunoreaction was scored as follows: 0, no staining; 1+, weaker staining than lymphocyte; 2+, similar staining to lymphocyte; and 3+, stronger staining than lymphocyte. (C) RUNX1-LI at invasive front or central part of tumor of OSCC by using three criteria. The RUNX1-LI was significantly increased at invasive front than central part of tumors in each criterion. The statistical analysis performed by Spearman's rank correlation coefficient.

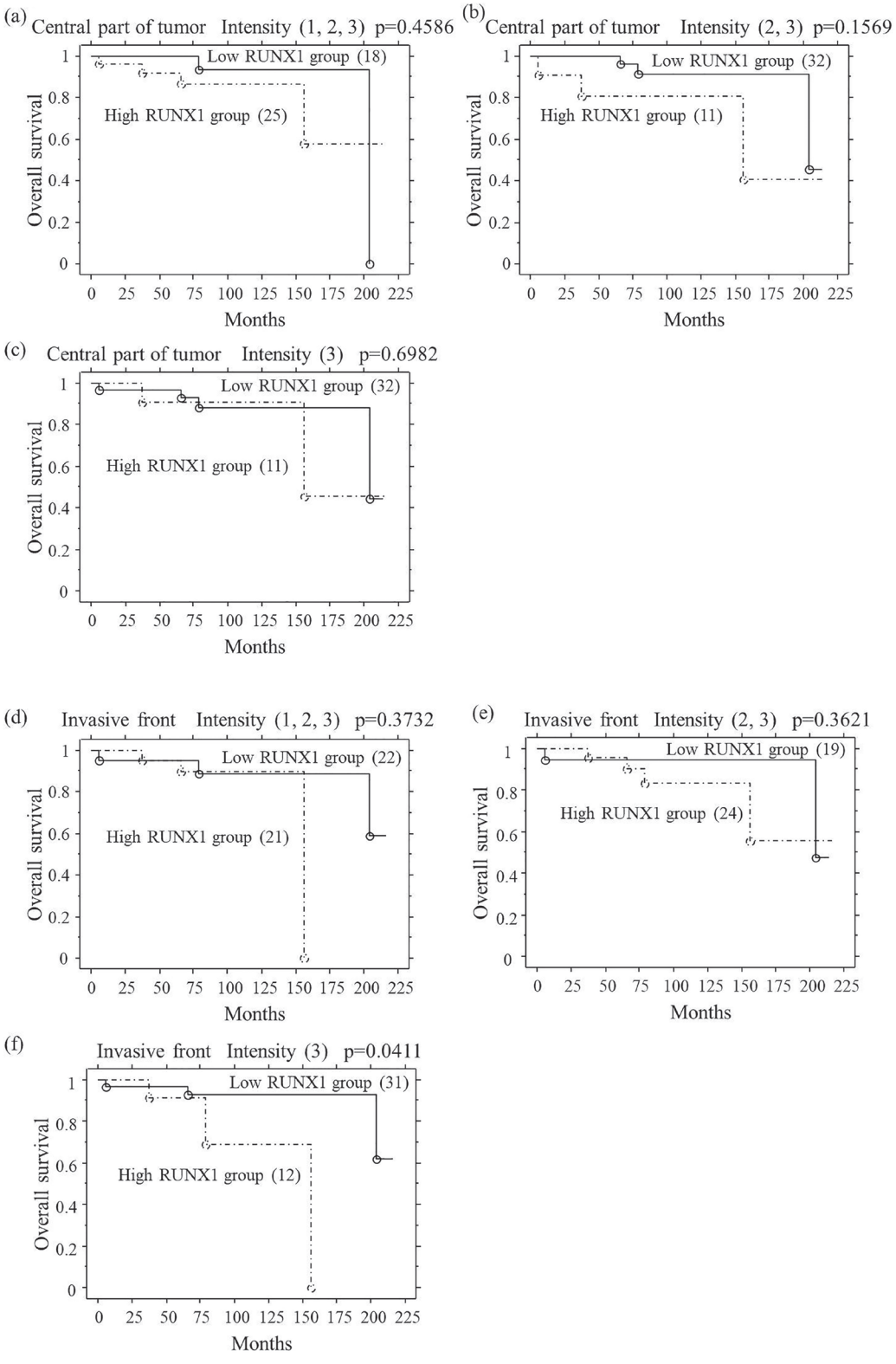

Fig. 2 Correlation of RUNX1 expression and overall survival (OS) in OSCC. The Kaplan-Meier method was used to estimate the probability of OS as a function of time. RUNX1 positivity on tumor cell at central part of tumor, intensity (1, 2, 3) of invasive front and intensity (2, 3) of invasive front were not associated with OS (a, b, c, d, e). However RUNX1 positivity on tumor cell at invasive front was associated with OS when we used with intensity 3 as the criteria for positive staining of RUNX1 ($P < 0.05$) (f).

we could find statistical significances with greatest numbers at the invasive front when we used intensity 3 as the criteria for positive staining of RUNX1. Briefly, no correlation was found between RUNX1 high expression and age, gender, performance status, T classification, N classification, stage, smoking history or alcohol intake of OSCC patients. However, significant correlation was observed between RUNX1 expression and postoperative lymph node metastasis ($P < 0.05$), differentiation grade ($P < 0.05$), and mode of invasion ($P < 0.05$). Association between the status of RUNX1 expression and clinicopathological characteristics of the patients in the invasive front or the central part of tumor, separately is summarized in Table 2.

RUNX1 expression was detected in squamous epithelial cells adjacent to tumors

Regardless of the intensity of the RUNX1 expression, the RUNX1-Labelling Index (LI) was shown to be high in invasive front lesions of tumor, as compared with that of squamous epithelial cells adjacent to tumors, including SIN, as well as that of central part of tumors when we ranged the intensity of the RUNX1-immunoreaction from 0 to 3 as written above (Fig. 3 and Table 3). Interestingly, we could not detect the RUNX1 expression in non-neoplastic squamous epithelial cells adjacent to tumors, while we could detect the RUNX1 expression of intensity 3 in SIN. In addition, the RUNX1-LI in SIN3 was found to be increased as compared with that in SIN1 or SIN2.

High expression of RUNX 1 at invasive front was an independent prognostic factor for unfavorable prognosis

Moreover, according to our univariate analysis by Cox proportional hazard models, the age below 65 years ($P < 0.01$), RUNX1 high expression with intensity 3 at central part of tumor ($P < 0.01$), RUNX1 high expression with intensity 2, 3 at central part of tumor ($P < 0.05$) and RUNX1 high expression with intensity 3 at invasive front ($P < 0.05$) are found to be the predictive factors for shorter survival (Table 4).

Discussion

The present study evaluated the expression of RUNX1 in 43 patients with OSCC. In addition, this work analyzed the correlation between RUNX1 overexpression of the invasive front of OSCC and prognosis. To the best of our knowledge, this paper is the first to investigate the prognostic value of RUNX1 overexpression at the invasive front of OSCC for lymph node metastasis and prognosis.

It is true that RUNX1 expression was detected in nuclei of squamous epithelial cells of SIN in the peripheral of a tumor as well as invasive lesions of OSCC, but its intensity of staining was various (Fig. 1B and Fig. 3). This variance of staining intensity was considered to be a major problem to determine the clinical significance of RUNX1 in OSCC. So, we had to define the suitable criteria for positive staining of RUNX1. In this study, we selected the RUNX1 expression in lymphocytes as a criterial parameter because RUNX1 expresses widely in lymphatic system24 for lymphocyte differentiation as a transcriptional factor.[25-27] Then, we set standards for the intensity as follows: 0, no staining; 1+, weaker staining than lymphocyte; 2+, similar staining to lymphocyte; and 3+, stronger staining than lymphocyte. This evaluation procedure helped us to do further investigations.

We found the differences of RUNX1 expression between the central part of tumor and the invasive front. RUNX1-LI was significantly increased in the invasive front than in the central part of tumors, regardless of the criteria for the positivity of RUNX1 staining; intensity 1, 2, 3, intensity 2, 3, and intensity of 3 only (Fig. 1C). However, it is noteworthy that we could find a significant association between the expression of RUNX1 in tumor cells of invasive front and the short OS only when we used a criterion regarding intensity 3 as positive RUNX1 staining (Fig. 2). Therefore, in order to determine the concrete roles of RUNX1 in OSCC, we attempted to find out the clinicopathological parameters which were statistically correlated with the RUNX1 expression of intensity 3 in the invasive front. As the results, we found a significantly high RUNX1 expression in patients with postoperative lymph node metastasis ($P < 0.05$),

Table 2 Correlation of RUNX1 expression and clinicopathological factors in OSCC

(A)

Central part of tumor Characteristic	Cases	Intensity 1, 2, 3 (%)	p-value	Intensity 2, 3 (%)	p-value	Intensity 3 (%)	p-value
Age			p=0.8970		p=0.3060		p=0.5510
＜65	14	69.5±14.9		19.7±9.67		7.43±4.56	
≧65	29	70.1±14.5		23.1±10.7		8.88±6.05	
Gender			p=0.0310*		p=0.5560		p=0.3690
Male	22	75.1±13.7		23.4±11.6		9.69±6.81	
Female	21	64.5±13.6		20.5±8.95		7.07±3.64	
Performance Status			p=0.6759		p=0.8672		p=0.9334
0	39	69.7±14.3		21.9±9.58		8.20±5.17	
1	4	72.0±17.6		23.0±17.0		10.5±8.83	
T classification			p>0.9999		p=0.0650		p=0.0650
T1, 2	41	69.9±14.8		22.5±10.5		8.66±5.67	
T3, 4	2	69.9±10.4		11.2±1.19		3.35±0.51	
N classification			p=0.0690		p=0.5450		p=0.9540
N 0	41	69.0±14.3		22.2±10.6		8.46±5.77	
N 1, 2	2	88.7±3.26		16.8±4.90		7.49±1.15	
Stage			p=0.5354		p=0.3168		p=0.0861
Ⅰ, Ⅱ	39	68.9±14.5		22.8±10.6		8.72±5.80	
Ⅲ, Ⅳ	4	79.3±12.2		14.0±4.52		5.42±2.25	
Post-operative lymph node metastasis			p=0.1115		p=0.9378		p=0.6066
−	34	67.5±14.1		22.0±10.9		8.29±5.75	
+	9	79.0±13.0		21.9±8.96		8.89±5.22	
SCC Grade			p<0.0001**		p=0.0053*		p=0.0006**
Well	28	61.9±10.8		18.2±7.58		5.94±3.18	
Moderately, Poorly	15	84.8±7.27		29.1±11.4		13.0±6.33	
Mode of invasion (Y-K criteria)			p=0.3705		p=0.3407		p=0.0733
Grade1, 2	9	66.3±11.5		19.5±9.45		6.58±5.79	
Grade3, 4	34	70.8±15.2		22.6±10.7		8.90±5.51	
Smoking history			p=0.4995		p=0.3523		p=0.4914
Never	28	68.6±14.9		21.3±11.2		8.21±5.72	
Past and present	15	72.3±13.8		23.2±8.95		8.79±5.50	
Alcohol intake			p=0.4166		p=0.5464		p=0.4166
Never	25	67.2±14.9		21.3±10.8		7.78±5.14	
Past and present	18	73.6±13.4		22.9±10.1		9.28±6.18	

* p< 0.05, ** p< 0.01 using the Chi-square test

(B)

Invasive front Characteristic	Cases	Intensity 1, 2, 3 (%)	p-value	Intensity 2, 3 (%)	p-value	Intensity 3 (%)	p-value
Age			p=0.4140		p=0.6500		p=0.5510
＜ 65	14	83.2±10.7		51.5±10.7		15.9±7.73	
≧ 65	29	85.8±10.8		53.4±15.7		17.8±9.83	
Gender			p=0.6620		p=0.4090		p=0.9320
Male	22	84.5±10.5		50.9±15.5		17.2±9.59	
Female	21	85.4±11.1		54.7±12.6		17.1±8.85	
Performance Status			p=0.2253		p=0.4903		p=0.8344
0	39	84.3±11.1		52.4±14.2		17.3±9.24	
1	4	91.2±4.51		55.9±14.9		15.8±9.16	
T classification			p=0.6037		p=0.3870		p=0.0534
T1, 2	41	84.8±10.8		53.6±13.2		17.8±9.03	
T3, 4	2	88.6±9.90		36.5±23.6		5.10±3.20	
N classification			p=0.4530		p=0.6650		p=0.1340
N 0	41	84.7±11.0		52.6±14.5		16.6±9.12	
N1, 2	2	90.4±3.72		56.4±7.66		28.1±2.31	
Stage			p=0.7029		p=0.3168	p=0.2834	p=0.0861
Ⅰ, Ⅱ	39	84.5±11.0		53.4±13.4		17.2±8.92	
Ⅲ, Ⅳ	4	89.5±7.53		46.4±20.1		16.6±11.9	
Post-operative lymph node metastasis			p=0.3491		p=0.9378		p=0.0394*
−	34	84.8±11.6		53.1±15.6		15.4±8.91	
+	9	85.5±6.87		51.3±7.02		23.9±7.16	
SCC Grade			p=0.0787		p=0.1394		p=0.0378*
Well	28	82.5±12.1		50.0±14.8		14.9±9.06	
Moderately, Poorly	15	89.6±5.57		57.8±11.6		21.5±7.94	
Mode of invasion (Y-K criteria)			p=0.3101		p=0.2154		p=0.0069*
Grade1, 2	9	80.7±14.5		45.3±19.2		10.4±9.16	
Grade3, 4	34	86.1±9.31		54.7±12.0		19.0±8.38	
Smoking history			p=0.1141		p=0.1573		p=0.8986
Never	28	86.5±10.7		54.0±15.7		17.0±9.52	
Past and present	15	82.1±10.4		50.5±10.9		17.5±8.67	
Alcohol intake			p=0.4166		p=0.2787		p=0.9216
Never	25	85.9±10.6		54.4±13.0		17.1±8.78	
Past and present	18	83.7±11.1		50.5±15.7		17.2±9.84	

* p< 0.05, ** p< 0.01 using the Chi-square test

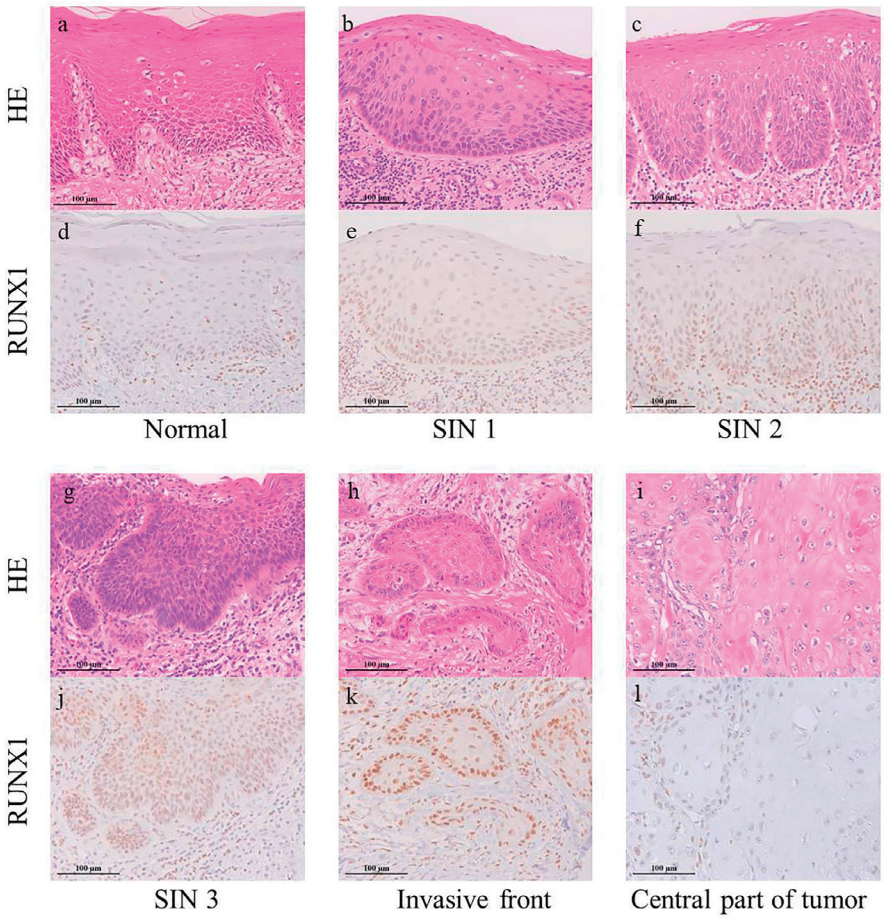

Fig. 3 RUNX1 expression in squamous epithelial cell. SIN1, SIN2, SIN3, invasive front and central part of tumor tissue samples stained with hematoxylin-eosin solution (a, b, c, g, h, i) or an anti-RUNX 1 antibody (d, e, f, j, k, l). RUNX1 positive staining observed in the nucleus of tumor cells (bar; 100 μm).

Table 3 Expression of RUNX1 in Squamous epithelial cell, SIN and OSCC

RUNX1 Intensity	RUNX1 LI (%)					
	Squamous epithelial cell	SIN 1	SIN 2	SIN 3	Invasive front	Central part of tumor
RUNX1 Intensity 1	4.4±8.20	28.6±21.8	31.9±22.6	53.5±23.7	32.2±10.6	47.9±12.4
RUNX1 Intensity 2	0.2±0.42	3.3±3.24	1.9±2.49	6.0±4.04	35.6±11.3	13.6±5.74
RUNX1 Intensity 3	0±0	0.7±0.85	0.7±1.24	2.5±2.01	17.2±9.24	8.4±5.65
RUNX1 Intensity 2, 3	0.2±0.42	3.9±3.84	2.6±3.68	8.5±5.87	52.8±14.3	22.0±10.5
RUNX1 Intensity 1, 2, 3	4.6±8.31	32.5±23.4	34.6±24.6	62.0±26.5	84.9±10.8	69.9±14.6

Table 4 Univariate and multivariate analysis of overall survival

Variable	Univariate analysis			Multivariate analysis		
	Hazard ratio	95%CI	p-value	Hazard ratio	95%CI	p-value
Age						
＜ 65 vs ≧ 65	0.922	0.168 − 5.053	p = 0.009**	0.491	0.072 − 3.325	p= 0.4658
Gender						
Male vs Female		−			−	
Performance Status						
0 vs 1		−			−	
T classification						
T3, 4 vs T1, 2	1.673	0.267 − 10.477	p = 0.5822		−	
N classification						
N 0 vs N 1, 2		−			−	
Stage						
Ⅰ, Ⅱ vs Ⅲ, Ⅳ		−			−	
Post-operative lymph node metastasis						
− vs +		−			−	
SCC Grade						
Well vs Moderately, Poorly	0.787	0.487 − 47.081	p = 0.1794		−	
Mode of invasion (Y − K criteria)						
Grade3, 4 vs Grade1, 2	1.250	0.134 − 11.244	p = 0.8424		−	
Smoking history						
Past and present vs Never	1.554	0.235 − 9.478	p = 0.6325		−	
Alcohol intake						
Never vs Past and present		−			−	
RUNX1 expression at central part of tumor (Intensity 3)						
High vs Low	2.501	1.915 − 77.630	p = 0.0081**	1.807	0.129 − 5.042	p = 0.8187
RUNX1 expression at central part of tumor (Intensity 2, 3)						
High vs Low	7.743	1.271 − 47.176	p = 0.0264*	4.364	0.533 − 35.760	p = 0.1697
RUNX1 expression at central part of tumor (Intensity 1, 2, 3)						
High vs Low	1.905	0.337 − 10.761	p = 0.4654		−	
RUNX1 expression at invasive front (Intensity 3)						
High vs Low	5.549	0.890 − 34.608	p = 0.0411*	6.022	0.845 − 42.943	p = 0.0732
RUNX1 expression at invasive front (Intensity 2, 3)						
High vs Low	3.931	0.653 − 23.669	p = 0.1350		−	
RUNX1 expression at invasive front (Intensity 1, 2, 3)						
High vs Low	2.343	0.344 − 15.944	p = 0.3841		−	

CI: Confidence Interval

* p< 0.05, ** p< 0.01

differentiation grade ($P < 0.05$) and mode of invasion ($P < 0.01$).

Contrary to our expectations, we could not detect stage or post-operative lymph node metastasis as a prognostic factor by univariate and multivariate analysis of OS though some RUNX1 expression and age were detected by univariate analysis of OS (Table 4). It must be due to the small number of investigated patients. In fact, our clinical laboratory often selected neo-adjuvant chemotherapy or adjuvant chemotherapy in these years, and 43 patients were all patients who received surgical operation without any adjuvant or neo-adjuvant chemotherapy though the number of patient is small.

Based on our above findings, it is concluded that RUNX1 expression may be useful for predicting the prognosis, invasion or metastasis of OSCC. Moreover, the present study provides a novel insight into the mechanisms involved in the malignant behavior of OSCC cells. It can be true that squamous epithelial lesions require the sensitive management even if pathological diagnosis was SIN1, when we detect the high RUNX1 expression in the specimen especially in tumor cells at invasive front. Evaluation of RUNX1 expression in tumor cells would enable us to predict the malignant potential of dysplastic lesions. Furthermore, it is possible to say that RUNX1 is a candidate for a novel target molecule to regulate cancer cell invasion and metastasis by molecular targeting therapy.

Acknowledgements

This study was supported in part by a Grant-in-Aid (Grant No: 15K11292) from the Japanese Ministry of Education, Science and Culture.

Conflict of Interest

The authors declare no conflict of interest.

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