Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology 34 (2022) 507–513

Contents lists available at ScienceDirect

Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology

journal homepage: www.elsevier.com/locate/jomsmp

Original Research

Immunohistochemical investigation of biomarkers for predicting adipose tissue invasion in oral squamous cell carcinoma*

Yibing Han [a], Shin-ichi Yamada [a,*], Makiko Kawamoto [a], Takahiko Gibo [a], Masao Hashidume [a], Hiroki Otagiri [a], Hirokazu Tanaka [a], Atsushi Takizawa [a,b], Eiji Kondo [a], Hironori Sakai [a], Takeshi Uehara [c], Hiroshi Kurita [a]

[a] Department of Dentistry and Oral Surgery, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto, 390-8621, Japan
[b] Department of Dentistry and Oral Surgery, Shinshu Ueda Medical Center, 1-27-21, Midorigaoka, Ueda, Japan
[c] Department of Laboratory Medicine, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto, 390-8621, Japan

ARTICLE INFO

Article history:
Received 24 October 2021
Received in revised form
10 November 2021
Accepted 16 November 2021
Available online 28 November 2021

Keywords:
Oral squamous cell carcinoma
Adipose tissue
Invasion
FABP4
Predictive marker

ABSTRACT

The purpose of the present study was to identify histological biomarkers that could be used to predict adipose tissue invasion by oral squamous cell carcinoma (OSCC). The medical records and preoperative computed tomography scans of patients with primary OSCC with suspected buccal fat pad invasion were retrospectively reviewed, and an immunohistochemical study of candidate predictive biomarkers of adipose tissue invasion (α-SMA, E-cadherin, N-cadherin, FABP4, Col VI, and MMP-11) was carried out. Thirty OSCC patients whose tumors were suspected to be in contact with the buccal fat pad based on preoperative imaging were included in this study. Of these, infiltrative adipose tissue invasion was histopathologically confirmed in 6 patients (20.0 %). The significant higher immunoreactivity of candidate predictive biomarkers was detected in the tumor-buccal fat pad contact area compared to in the tumor surface area.(Pearson's correlation coefficient test: α-SMA: 0.422, $p < 0.05$; N-cadherin: 0.476, $p < 0.01$; FABP4: 0.467, $p < 0.01$;). In the tumor-buccal fat pad contact area, Col VI immunoreactivity was significantly higher in the OSCC with buccal fat pad invasion than without buccal fat pad invasion (median test, $p < 0.01$). Furthermore, high α-SMA expression exhibited a tendency towards an association with OSCC with buccal fat pad invasion, as did high FABP4 expression (median test, α-SMA: $p = 0.06$, FABP4: $p = 0.07$). These results suggest that the expression levels of α-SMA and FABP4 may be useful biomarkers for predicting aggressive adipose tissue invasion in OSCC.
© 2021 Asian AOMS, ASOMP, JSOP, JSOMS, JSOM, and JAMI. Published by Elsevier Ltd. All rights reserved.☆

1. Introduction

Among head and neck malignancies, oral squamous cell carcinoma (OSCC) is the most common malignant neoplasm, and squamous cell carcinoma (SCC) accounts for >90 % of oral cancers [1]. The treatment outcomes of OSCC patients have improved due to recent advances in treatments for OSCC. However, standard radical therapy involving wider resection often causes reductions in function and quality of life in OSCC patients [2].

Wider tumor resection improves the prognosis of OSCC. However, it is difficult to remove tumors in the head and neck region, including the oral cavity, with appropriate surgical margins due to anatomical limitations. Regarding the resection of OSCC, surgical margin status significantly affects prognosis [3]. The surgical removal of such tumors can be carried out using both metric and barrier approaches [4]. Although OSCC are resected with appropriate surgical margins (usually 1 cm beyond the visible and palpable margins of the tumor based on measurements) in the metric approach, the surgeon may decide to resect the tumor up to or including an anatomico-pathological barrier, such as the periosteum [4]. The optimal width of surgical margins and the ability to obtain tumor-free margins may vary depending on the anatomical site in cases of OSCC. As for the barrier approach to OSCC surgery, it was reported that bone and the periosteum can function as anatomical barriers in OSCC, and some reports have investigated predictive molecular markers of bone invasion [4–10]. However, there have not been reports about the significance of adipose tissue as an anatomical barrier in OSCC.

Since adipose tissue is present in various organs, the cancer cells of many solid malignant tumors come into close contact with

* AsianAOMS: Asian Association of Oral and Maxillofacial Surgeons; ASOMP: Asian Society of Oral and Maxillofacial Pathology; JSOP: Japanese Society of Oral Pathology; JSOMS: Japanese Society of Oral and Maxillofacial Surgeons; JSOM: Japanese Society of Oral Medicine; JAMI: Japanese Academy of Maxillofacial Implants.

* Corresponding author.
E-mail address: yshinshin@shinshu-u.ac.jp (S.-i. Yamada).

https://doi.org/10.1016/j.ajoms.2021.11.004
2212-5558/© 2021 Asian AOMS, ASOMP, JSOP, JSOMS, JSOM, and JAMI. Published by Elsevier Ltd. All rights reserved.☆

adipocytes due to tumor growth and/or local invasion [11]. In breast, ovarian, and prostate cancer, various cytokines and extracellular matrix molecules, such as fatty acid-binding protein 4 (FABP4), interleukin 6 (IL-6), and collagen type VI (Col VI), play important roles in adipocyte/cancer cell crosstalk [11–17]. However, the significance of adipocyte/cancer cell crosstalk in OSCC remains unknown, and no predictive markers of adipose tissue invasion in OSCC have been identified. Our goal is to identify predictive biomarkers of adipose tissue invasion in OSCC. Such biomarkers may facilitate treatment decisions and improve the prognosis and quality of life of OSCC patients. If the ability of OSCC to infiltrate adipose tissue could be assessed, it would be possible to determine the optimal excision range using the fat layer as an anatomical barrier. Therefore, in this study immunohistochemical (IHC) examinations were conducted to find biomarkers that are significantly associated with histological adipose tissue invasion in OSCC.

2. Materials and methods

2.1. Patients

The medical records of primary OSCC patients whose tumors were found to be in contact with adipose tissue on preoperative computed tomography (CT) scans and magnetic resonance imaging (MRI) and who were treated with surgery without preoperative adjuvant therapy at Shinshu University Hospital between January 2003 and December 2017 were retrospectively reviewed, and IHC examinations of tissue samples were performed to identify biomarkers that could be used to predict adipose tissue invasion in OSCC patients.

The protocol for the present study was approved by the ethics committee of Shinshu University School of Medicine (No. 4182). We published a research plan and guaranteed an opt-out opportunity on the homepage of our hospital.

2.2. Histology and immunohistochemistry

Thirty OSCC patients whose tumors had been shown to be in contact with adipose tissue (the buccal fat pad) on preoperative examinations were enrolled in the present study. The data collected included information regarding age, sex, demographics, histological differentiation, the TNM stage at diagnosis, the mode of invasion, and the treatment strategies employed. Tumor stages were classified according to the TNM classification of the International Union against Cancer [18]. The mode of invasion was defined as described by Yamamoto E et al. [19]. Histological differentiation and the mode of invasion were assessed using biopsy tissue samples obtained from the tumor surface.

In order to identify predictive biomarkers of adipose tissue invasion in OSCC, the IHC staining of surgical specimens was performed. Four-micrometer-thick serial sections were sliced from paraffin-embedded tissue blocks. The sections were deparaffinized in xylene; soaked in target retrieval solution buffer (pH 6.0, Dako, Carpentaria, CA); and then were subjected to antigen retrieval, which was performed at 600 W for 25 min in a microwave. Endogenous peroxidase activity was blocked by incubating the sections with 0.3 % H_2O_2 in methanol for 30 min. IHC staining was manually performed using the Envision + system (Dako, Carpentaria, CA). As the candidate biomarkers of adipose tissue invasion, the following primary antibodies were used: anti-matrix metalloproteinase (MMP)-11 (MS-1035-R7; Thermo Fisher Scientific, Waltham, Massachusetts, USA; 1:500) and Col VI (NB120-6588; Novus Biologicals, Centennial, CO, USA; 1:200) antibodies were used as markers of extracellular matrix destruction; anti-E-cadherin (610181; BD Biosciences, San Jose, CA, USA; 1:1000), N-cadherin (MA5-16324; Thermo Fisher Scientific, Waltham, Massachusetts, USA; 1:125), and anti α-smooth muscle actin (α-SMA) (A2547; Sigma Aldrich, St. Louis, Missouri, USA; 1:2000) antibodies were used as markers of epithelial-mesenchymal transition (EMT); anti-FABP4 (AF3150; R&D Systems, Minneapolis, MN, USA; 1:200) antibodies were used as a marker of adipose metabolism; and anti-IL-6 (sc-28343; Santa Cruz Biotechnology, Dallas, Texas, USA; 1:100) antibodies were used as a marker of inflammation. Sections were incubated with the relevant primary antibody at 4 °C overnight. The reaction products were visualized by immersing the sections in diaminobenzidine (DAB) solution, and then the sections were counterstained with Meyer's hematoxylin and mounted. Negative controls were prepared by replacing the primary antibody with phosphate-buffered saline. All slides were evaluated by two independent authors (T.G. and M.K. supervised by T.U.), who did not have any knowledge of clinical outcomes. Each specimen was examined with an optical microscope at a magnification of ×100 (at three locations on the tumor surface and three locations in the tumor-adipose tissue contact areas). Immunoreactivity was assessed using the H-score, which is based on staining intensity and the percentage of immunoreactive cells.[20] The final H score was determined by multiplying the quality and intensity scores: H score = 0×% of non-stained tumor cells +1×% of weakly stained tumor cells +2×% of moderately stained tumor cells +3×% of strongly stained tumor cells (range, 0–300) [20]. Adipose tissue invasion was assessed histologically on hematoxylin and eosin-stained sections.

2.3. Statistical analysis

To investigate the relationships between the expression of the candidate biomarkers and adipose tissue invasion, the immunoreactivity level of each biomarker was compared between the tumor surface and tumor-adipose tissue contact areas. The immunoreactivity of each biomarker was also compared between the tumors with and without adipose tissue invasion. Statistical analyses were conducted using JMP® 13.0 (SAS Institute Inc., Cary, NC, USA), and Fisher's exact test, Pearson's correlation coefficient test, and the median test were used to test for significance. P-values of <0.05 were considered to indicate significance.

3. Results

3.1. Patient's characteristics

Thirty OSCC patients whose tumors were suspected to be in contact with the buccal fat pad based on preoperative imaging were included in the study population. The characteristics of these patients are shown in Table 1. The median age at diagnosis was 74 years (range: 56−91 years). The most common primary site was the lower gums (16 patients, 53.3 %) followed by the buccal mucosa (14 patients, 46.7 %). Regarding TNM staging (as defined by the Union for International Cancer Control 7th edition) [18], T4 and T2 were the most common stages (10 patients, 33.3 % for both), followed by T1 (7 patients, 23.3 %) and T3 (3 patients, 10.0 %). As for the histological grade, well-differentiated, moderately differentiated, and poorly differentiated SCC was seen in 19 (63.3 %), 6 (20.0 %), and 5 (16.7 %) patients, respectively. Regarding the mode of invasion [19], grade 3 was the most common grade (14 patients, 46.7 %), followed by grade 4c (8 patients, 26.7 %), and grade 2 (6 patients, 20.0 %). Among the 30 patients, histological buccal fat pad invasion by OSCC was detected in 6 patients (20.0 %). There were no significant correlations between the characteristics of this cohort and histological buccal fat pad invasion except for the primary site of tumor (Table 2).

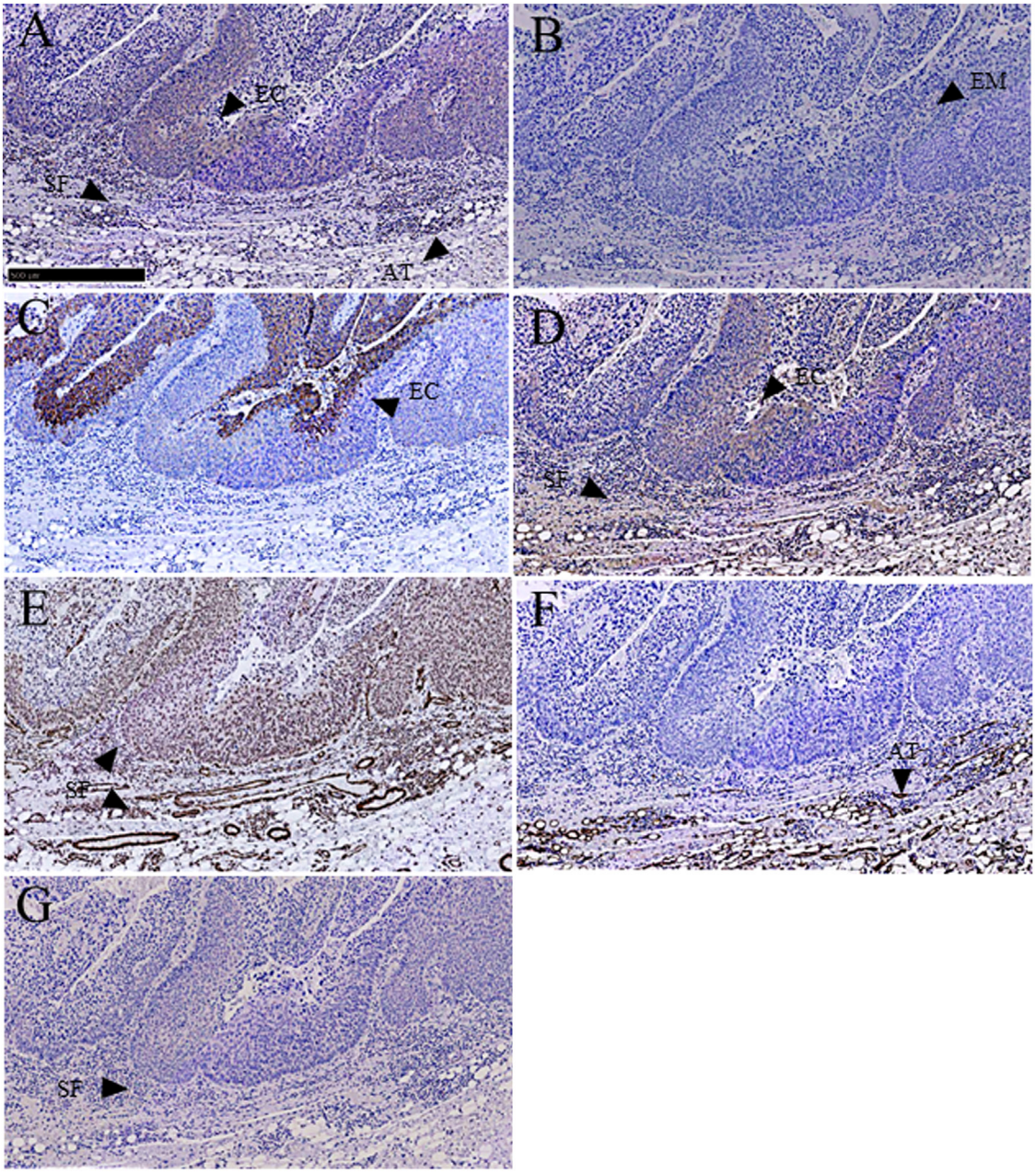
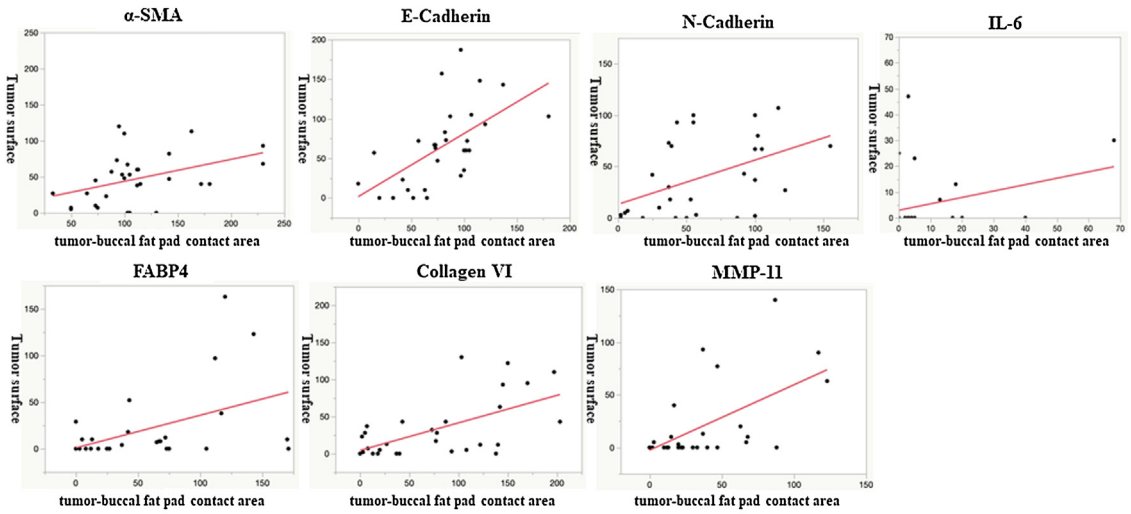


Fig. 1. Immunohistochemical investigation of potential predictive biomarkers in the tumor-adipose tissue contact area in OSCC.
(A)–(G) The results regarding the immunohistochemical expression of (A) MMP-11, (B) Col VI, (C) E-cadherin, (D) N-cadherin, (E) α-SMA, (F) FABP4, and (G) IL-6 are shown. The strong expressions of MMP-11 and FABP4 were detected in the tumor-buccal fat pad contact area. Col VI expression was detected in the tumor surface. E-cadherin expression was observed in the the tumor surface. The expression of α-SMA was detected in the tumor-buccal fat pad contact area.
(A)–(G) Magnification: ×100.
Arrowhead indicated below in each figure;
EC: epithelial cell.
EM: extracellular matrix.
SF: stroma and fibroblast.
AT: adipose tissue.

3.2. Immunohistochemical study

Our findings regarding the expression of MMP-11, E-cadherin, N-cadherin, α-SMA, Col VI, FABP4, and IL-6 at the tumor-buccal fat pad contact area are shown in Fig. 1. MMP-11, N-cadherin, and FABP4 were detected in the cytoplasm of the tumor cells (Fig. 1A, D, and F). The strong expressions of MMP-11 and FABP4 were detected in the tumor-buccal fat pad contact area. Col VI expression was detected in the extracellular matrix at the tumor surface (Fig. 1B). E-cadherin expression was observed in the cell membrane at the tumor surface (Fig. 1C). The expression of α-SMA was detected in fibroblasts and the stroma at the tumor-buccal fat pad contact area (Fig. 1E). IL-6 expression was detected diffusely in inflammatory cells and the stroma (Fig. 1G).

The results of the comparison of IHC reactivity between the tumor surface and tumor-buccal fat pad contact area are shown in Fig. 2. The significant higher immunoreactivity of candidate predictive biomarkers, such as α-SMA, N-cadherin, and FABP4, was detected in the tumor-buccal fat pad area compared to in the tumor surface area (Pearson's correlation coefficient test: α-SMA: 0.422,



	αSMA	E-Cad	N-Cad	IL-6	FABP4	Collagen6	MMP-11
correlation coefficient	0.422	0.601	0.476	0.314	0.467	0.603	0.592
P value	*p<0.05*	*p<0.01*	*p<0.01*	*p=0.09*	*p<0.01*	*p<0.01*	*p<0.01*

Fig. 2. Comparison of the immunohistochemical reactivity of the candidate biomarkers between the tumor surface and tumor-buccal fat pad contact area.
The significant higher immunoreactivity of α-SMA, N-cadherin, and FABP4, was detected in the tumor-buccal fat pad area compared to in the t tumor surface area.

Table 1
Summary of the patients that participated in this study (n = 30).

Characteristics		Number (%)
Sex	Male	11 (37.0)
	Female	19 (63.0)
Age	Median (range)	74 (56–91) years old
Primary site		
	Lower gums	16 (53.3)
	Buccal mucosa	14 (46.7)
T classification	T1	7 (23.3)
	T2	10 (33.3)
	T3	3 (10.0)
	T4	10 (33.3)
Histological grade		
	Well-differentiated	19 (63.3)
	Moderately differentiated	6 (20.0)
	Poorly differentiated	5 (16.7)
Mode of invasion (YK classification)		
	Grade 1	1 (3.3)
	Grade 2	6 (20.0)
	Grade 3	14 (46.7)
	Grade 4c	8 (26.7)
	Grade 4d	1 (3.3)
Histological adipose tissue invasion		
	Absent	24 (80.0)
	Present	6 (20.0)

Table 2
The correlation between the characteristics and histological buccal fat pad invasion.

	Buccal fat pad invasion: Number (%)		
Characteristics	No	Yes	P value*
Sex			
Male	14(46.7)	3(10.0)	NS
Female	10(33.3)	3(10.0)	(P = 1.000)
Age			
<74	13(43.3)	1(3.3)	NS
≧74	11(36.7)	5(16.7)	(P = 0.176)
Primary site			
Lower gums	8(26.7)	6(20.0)	P <0.01
Buccal mucosa	16(53.3)	0(0.0)	
T classification			
T1−3	14(46.7)	6(20.0)	NS
T4	10(33.3)	0(0.0)	(P = 0.074)
Stage			
I-III	13(43.3)	5(16.7)	NS
IV	11(36.7)	1(3.3)	(P = 0.358)
Histological grade			
Well-differentiated	17(56.7)	2(6.7)	NS
Moderately and poorly-differentiated	7(23.3)	4(13.3)	(P = 0.156)
Mode of invasion (YK classification)			
Grade 1−3	17(56.7)	4(13.3)	NS
Grade 4	7(23.3)	2(6.7)	(P = 1.000)

NS: not significant.
* Fisher's exact test.

p < 0.05; N-cadherin: 0.476, p < 0.01; FABP4: 0.467, p < 0.01;).On the other hand, the higher immunoreactivities of E-cadherin, Coll VI, and MMP-11 were detected in the tumor surface area compared to in the tumor-buccal fat pad area (E-cadherin: 0.601, p < 0.01; Col VI: 0.603, p < 0.01; MMP-11: 0.592, p < 0.01).

The results of the comparison of IHC reactivity between the tumor surface and tumor-adipose tissue contact area with and without buccal fat pad invasion are shown in Table 3. On the tumor surface, although there were no significant differences in the expression levels of the molecular biomarker candidates, N-cadherin expression showed a tendency to be associated with buccal fat pad invasion (median test, p = 0.073). In the tumor-buccal fat pad contact area, Col VI immunoreactivity differed significantly higher in the OSCC with than without buccal fat pad invasion (median test, p < 0.01). In addition, high expression of α-SMA/FABP4 tended to be associated with OSCC with buccal fat pad invasion (median test, α-SMA: p = 0.063, FABP4: p = 0.073).

4. Discussion

The aim of this study was to identify a biomarker that could be used to predict adipose tissue invasion in OSCC. If such a marker were found, it may be possible to use the fatty layer as a barrier during tumor resection in appropriate cases of OSCC. In this study, we used the invasion of OSCC into the buccal fat pad as a model. Although there are many fatty layers in the head and neck region, we chose the buccal fat pad for our study because its morphol-

Table 3
Comparison of immunohistochemical reactivity on the tumor surface and tumor-adipose tissue contact area between tumors with (n = 6) and without (n = 24) buccal fat pad invasion.

	Tumor surface			Tumor-adipose tissue contact area		
Candidate molecule	Median	95 %CI	P-value	Median	95 %CI	P-value
α-SMA	46.0	34.4–59.8	NS (P = 1.000)	103.0	93.5–128.7	NS (P = 0.063)
E-cadherin	66.5	46.2–83.4	NS (P = 1.000)	80.5	65.4–93.4	NS (P = 0.369)
N-cadherin	28.5	24.7–53.0	NS (P = 0.073)	51.5	43.6–74.8	NS (P = 1.000)
IL-6	0.0	0.6–9.1	NS (P = 0.823)	3.0	2.3–13.1	NS (P = 0.342)
FABP4	2.0	4.8–34.4	NS (P = 0.369)	39.5	33.6–73.2	NS (P = 0.073)
Collagen type VI	15.0	17.5–47.0	NS (P = 0.369)	75.0	51.1–99.0	P <0.01
MMP-11	0.0	5.5–32.5	NS (P = 0.717)	22.5	21.9–47.6	NS (P = 0.369)

NS: not significant.
CI: confidence interval.

ogy is well preserved in resected specimens, and histopathological examinations of this site produce reliable results.

Among OSCC, the incidence of buccal mucosa SCC (BSCC) was reported to be 30 % [21]. The recurrence and 5-year overall survival rates of BSCC were reported to be 50 % and 53 %, respectively, and BSCC is considered to be an aggressive disease that exhibits high rates of locoregional recurrence independent of the surgical margin status [21]. Regarding the reasons for local failure in BSCC, since deep surgical margins are usually determined through palpation, accurate assessment of the extent of tumor invasion is difficult due to the anatomical architecture of the cheek wall [22]. On the other hand, the possibility of using the buccinator as an anatomical barrier during the resection of BSCC has been suggested [22]. In a previous study, contrast-enhanced multi-slice CT revealed that 39.4 % of BSCC had invaded the skin and subcutaneous fat tissue [23]. However, the sensitivity and specificity of contrast-enhanced multi-slice CT for diagnosing invasion by primary BSCC were reported to be relatively low [23]. In a study in which MRI was used to assess the depth of invasion (DOI) in BSCC, the optimal cut-off values for the DOI according to MRI and the pathological DOI for predicting buccinator and buccal fat pad invasion were found to be 5 mm and 6 mm, respectively. In addition, the DOI according to contrast-enhanced T1-weighted imaging was reported to be correlated with the pathological DOI and was generally a few millimeters larger than the actual pathological DOI [24].

Since no biomarkers for predicting adipose tissue invasion below the buccinator in OSCC have yet been identified, this study aimed to identify such a marker in order to allow optimal surgical margins to be secured. The expression of α-SMA and FABP4 in the tumor-adipose tissue contact area was found to be correlated with adipose tissue invasion. Therefore, the expression levels of α-SMA and FABP4 may be useful biomarkers for predicting adipose tissue invasion in OSCC.

In the comparison of the expression of each candidate molecule between the tumor surface and the tumor-buccal fat pad contact area, it was found that the expression levels of α-SMA, N-cadherin, and FABP4 were higher in the tumor-buccal fat pad contact area, whereas the expression of E-cadherin was lower in the tumor-buccal fat pad contact area. The upregulation of N-cadherin expression followed by the downregulation of E-cadherin expression has been reported to be a characteristic of EMT [25]. EMT induces morphological changes in polarized epithelial cells from the epithelial phenotype to a mesenchymal phenotype characterized by a reduction in the number of cell-cell junctions, cytoskeletal rearrangement, increased cell motility, and extracellular synthesis [26]. During EMT, epithelial cells lose characteristics of the epithelial phenotype, such as E-cadherin expression, which is recognized as a specific epithelial marker, and acquire mesenchymal markers, including α-SMA [26]. Increased expression of mesenchymal markers or decreased expression of epithelial markers has been reported to lead to EMT, and therefore, invasion and metastasis in OSCC [27]. In the analyses of the expression of the candidate molecules in the tumor-buccal fat pad contact area conducted in the present study, a tendency towards the upregulation of α-SMA expression was seen in the OSCC with adipose tissue invasion, and in the analyses of candidate molecule expression on the tumor surface a tendency towards the downregulation of N-cadherin expression was observed in the OSCC with adipose tissue invasion. MMP-11 is an extracellular proteolytic enzyme [28]. At the invasive front of breast cancer, MMP-11 expression is induced in adipocytes by cancer cells [29]. The invading cancer cells aberrantly restore the negative effects of MMP-11 on adipogenesis in proximal adipocytes/preadipocytes, and MMP-11-expressing peritumoral fibroblasts are distinct from α-SMA-expressing myofibroblasts [28]. In OSCC, MMP-11 was suggested to play a role in EMT [30]. In this study, although there was no significant difference, immunoreactivity of MMP-11 at tumor-adipose tissue contact area was higher compared to that at the tumor surface. Therefore, these results indicate that EMT might be induced at sites of adipose tissue invasion and facilitate cancer cell migration and invasion in OSCC.

FABP4 is abundant in mature adipocytes and adipose tissue, and high-fat diets and dietary components influence FABP4 expression and production [31–33]. The upregulation of FABP4 was reported to be associated with cancer angiogenesis and metastatic proliferation in breast cancer, non-small cell lung cancer, and ovarian cancer [34–36]. In ovarian cancer cells, FABP4 expression was detected at the adipocyte-tumor cell interface and promoted metastasis through the direct transfer of lipids from adipocytes to invasive cancer cells [16]. In prostate cancer, FABP4 was reported to enhance cancer progression and invasiveness by upregulating MMP and cytokine production in the prostate cancer stromal microenvironment [17]. FABP expression is significantly upregulated in OSCC, and its expression and activation are mediated by mitogen-activated protein kinase. In addition, it was associated with lymph node metastasis [37]. Regarding the association between FABP4 expression and EMT, in a previous study FABP4 overexpression activated EMT to promote the migration and invasion of colon cancer cells [38]. In the current study, FABP4 expression was significantly higher in the tumor-adipose tissue contact area than on the tumor surface. In addition, in the tumor-adipose tissue contact area FABP4 expression tended to be correlated with OSCC invasion into adipose tissue. These results suggest that FABP4 may be a useful biomarker for predicting adipose tissue invasion in OSCC. On the other hand, in the tumor-adipose tissue contact area Col VI expression was significantly decreased in OSCC cells in cases involving adipose tissue invasion. The upregulation of Col VI expression was reported to be associated with tumor progression in breast and pancreatic ductal cancer [13,39,40]. Our findings were inconsistent with those of previous studies, and hence, Col VI expression in the tumor-adipose tissue contact area in OSCC should be examined further.

The main strength of the present study was that it investigated markers for predicting adipose tissue invasion in OSCC, and found

that the expression levels of EMT markers (α-SMA and FABP4) in the tumor-adipose tissue contact area may be useful for predicting adipose tissue invasion in OSCC. However, it had some limitations, such as its retrospective nature and the fact that it involved a relatively small number of cases including 6 cases with histological buccal fat pad invasion by OSCC and was conducted at a single institution. Further multicenter studies based on a large number of cases are warranted.

5. Conclusion

The expression levels of α-SMA and FABP4 in the tumor-adipose tissue contact area were found to be correlated with infiltrative adipose tissue invasion in OSCC. The expression levels of α-SMA and FABP4 may be useful biomarkers for predicting aggressive adipose tissue invasion in OSCC. In most clinical situations, performing a preoperative biopsy of the tumor-adipose tissue contact area would be difficult, and thus, information about the expression of these biomarkers may be not available to surgeons during the preoperative period. Further studies to identify more effective biomarkers are required.

Funding

None.

Ethical approval

The study protocol was approved by the Ethics Committee of the Shinshu University School of Medicine. (No.4182).

Informed consent

For this type of study, formal consent is not required.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgments

We wish to thank Medical English Service (https://www.med-english.com/) for their help with the English proofreading.

References

[1] Johnson NW, Jayasekara P, Amarasinghe AA. Squamous cell carcinoma and precursor lesions of the oral cavity: epidemiology and aetiology. Periodontol 2000;2011(57):19–37.
[2] Yamada SI, Kurita H, Tomioka T, Ohta R, Yoshimura N, Nishimaki F, et al. Healthy life expectancy of oral squamous cell carcinoma patients aged 75years and older. Oral Oncol 2017;64:22–6.
[3] Yamada S, Kurita H, Shimane T, Kamata T, Uehara S, Tanaka H, et al. Estimation of the width of free margin with a significant impact on local recurrence in surgical resection of oral squamous cell carcinoma. Int J Oral Maxillofac Surg 2016;45:147–52.
[4] Upile T, Fisher C, Jerjes W, El Maaytah M, Searle A, Archer D, et al. The uncertainty of the surgical margin in the treatment of head and neck cancer. Oral Oncol 2007;43:321–6.
[5] De Wever O, Demetter P, Mareel M, Bracke M. Stromal myofibroblasts are drivers of invasive cancer growth. Int J Cancer 2008;123:2229–38.
[6] Ebrahimi A, Murali R, Gao K, Elliott MS, Clark JR. The prognostic and staging implications of bone invasion in oral squamous cell carcinoma. Cancer 2011;117:4460–7.
[7] Fried D, Mullins B, Weissler M, Shores C, Zanation A, Hackman T, et al. Prognostic significance of bone invasion for oral cavity squamous cell carcinoma considered T1/T2 by American joint committee on cancer size criteria. Head Neck 2014;36:776–81.
[8] Russmueller G, Moser D, Würger T, Wrba F, Christopoulos P, Kostakis G, et al. Upregulation of osteoprotegerin expression correlates with bone invasion and predicts poor clinical outcome in oral cancer. Oral Oncol 2015;51:247–53.
[9] Elmusrati AA, Pilborough AE, Khurram SA, Lambert DW. Cancer-associated fibroblasts promote bone invasion in oral squamous cell carcinoma. Br J Cancer 2017;117:867–75.
[10] Gibo T, Yamada SI, Kawamoto M, Uehara T, Kurita H. Immunohistochemical investigation of predictive biomarkers for mandibular bone invasion in oral squamous cell carcinoma. Pathol Oncol Res 2020;26:2381–9.
[11] Duong MN, Geneste A, Fallone F, Li X, Dumontet C, Muller C. The fat and the bad: mature adipocytes, key actors in tumor progression and resistance. Oncotarget 2017;8:57622–41.
[12] Iyengar P, Combs TP, Shah SJ, Gouon-Evans V, Pollard JW, Albanese C, et al. Adipocyte-secreted factors synergistically promote mammary tumorigenesis through induction of anti-apoptotic transcriptional programs and proto-oncogene stabilization. Oncogene 2003;22:6408–23.
[13] Iyengar P, Espina V, Williams TW, Lin Y, Berry D, Jelicks LA, et al. Adipocyte-derived collagen VI affects early mammary tumor progression in vivo, demonstrating a critical interaction in the tumor/stroma microenvironment. J Clin Invest 2005;115:1163–76.
[14] Finley DS, Calvert VS, Inokuchi J, Lau A, Narula N, Petricoin EF, et al. Periprostatic adipose tissue as a modulator of prostate cancer aggressiveness. J Urol 2009;182:1621–7.
[15] Dirat B, Bochet L, Dabek M, Daviaud D, Dauvillier S, Majed B, et al. Cancer-associated adipocytes exhibit an activated phenotype and contribute to breast cancer invasion. Cancer Res 2011;71:2455–65.
[16] Nieman KM, Kenny HA, Penicka CV, Ladanyi A, Buell-Gutbrod R, Zillhardt MR, et al. Adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth. Nat Med 2011;17:1498–503.
[17] Huang M, Narita S, Inoue T, Koizumi A, Saito M, Tsuruta H, et al. Fatty acid binding protein 4 enhances prostate cancer progression by upregulating matrix metalloproteinases and stromal cell cytokine production. Oncotarget 2017;8:111780–94.
[18] Sobin H, Gospodarowicz K, Wittekind C. TNM classification of malignant tumours. 7th edition Chichester: Wiley-Blackwell; 2009.
[19] Yamamoto E, Miyakawa A, Kohama G. Mode of invasion and lymph node metastasis in squamous cell carcinoma of the oral cavity. Head Neck Surg 1984;6:938–47.
[20] Hirsch FR, Varella-Garcia M, Bunn Jr PA, Di Maria MV, Veve R, Bremmes RM, et al. Epidermal growth factor receptor in non-small-cell lung carcinomas: correlation between gene copy number and protein expression and impact on prognosis. J Clin Oncol 2003;21:3798–807.
[21] Lubek JE, Dyalram D, Perera EH, Liu X, Ord RA. A retrospective analysis of squamous carcinoma of the buccal mucosa: an aggressive subsite within the oral cavity. J Oral Maxillofac Surg 2013;71:1126–31.
[22] Ota Y, Aoki T, Karakida K, Otsuru M, Kurabayashi H, Sasaki M, et al. Determination of deep surgical margin based on anatomical architecture for local control of squamous cell carcinoma of the buccal mucosa. Oral Oncol 2009;45:605–9.
[23] Xu SS, Li D, Zhou Y, Sheng B, Zeng C, Zhong SX. Pathways involved in the spread of buccal carcinoma on contrast-enhanced multislice CT. Dentomaxillofac Radiol 2015;44:20140111.
[24] Baba A, Masuda K, Hashimoto K, Matsushima S, Yamauchi H, Ikeda K, et al. Correlation between the magnetic resonance imaging features of squamous cell carcinoma of the buccal mucosa and pathologic depth of invasion. Oral Surg Oral Med Oral Pathol Oral Radiol 2021;131:582–90.
[25] Loh CY, Chai JY, Tang TF, Wong WF, Sethi G, Shanmugam MK, et al. The E-Cadherin and N-Cadherin switch in Epithelial-to-Mesenchymal transition: signaling, therapeutic implications, and challenges. Cells 2019;8:1118.
[26] Ohbayashi M, Kubota S, Kawase A, Kohyama N, Kobayashi Y, Yamamoto T. Involvement of epithelial-mesenchymal transition in methotrexate-induced pulmonary fibrosis. J Toxicol Sci 2014;39:319–30.
[27] Joseph JP, Harishankar MK, Pillai AA, Devi A. Hypoxia induced EMT: a review on the mechanism of tumor progression and metastasis in OSCC. Oral Oncol 2018;80:23–32.
[28] Tan B, Jaulin A, Bund C, Outilaft H, Wendling C, Chenard MP, et al. Matrix Metalloproteinase-11 promotes early mouse mammary gland tumor growth through metabolic reprogramming and increased IGF1/AKT/FoxO1 signaling pathway, enhanced ER stress and alteration in mitochondrial UPR. Cancers (Basel) 2020;12:2357.
[29] Andarawewa KL, Motrescu ER, Chenard MP, Gansmuller A, Stoll I, Tomasetto C, et al. Stromelysin-3 is a potent negative regulator of adipogenesis participating to cancer cell-adipocyte interaction/crosstalk at the tumor invasive front. Cancer Res 2005;65:10862–71.
[30] Richter P, Umbreit C, Franz M, Berndt A, Grimm S, Uecker A, et al. EGF/TGFβ1 co-stimulation of oral squamous cell carcinoma cells causes an epithelial-mesenchymal transition cell phenotype expressing laminin 332. J Oral Pathol Med 2011;40:46–54.
[31] Makowski L, Hotamisligil GS. Fatty acid binding proteins-the evolutionary crossroads of inflammatory and metabolic responses. J Nutr 2004;134:2464S–8S.
[32] Furuhashi M, Hotamisligil GS. Fatty acid-binding proteins: role in metabolic diseases and potential as drug targets. Nat Rev Drug Discov 2008;7:489–503.
[33] Cao H, Sekiya M, Ertunc ME, Burak MF, Mayers JR, White A, et al. Adipocyte lipid chaperone AP2 is a secreted adipokine regulating hepatic glucose production. Cell Metab 2013;17:768–78.
[34] Tang Z, Shen Q, Xie H, Zhou X, Li J, Feng J, et al. Elevated expression of FABP3 and FABP4 cooperatively correlates with poor prognosis in non-small cell lung cancer (NSCLC). Oncotarget 2016;7:46253–62.

[35] Guaita-Esteruelas S, Bosquet A, Saavedra P, Guma J, Girona J, Lam EW, et al. Exogenous FABP4 increases breast cancer cell proliferation and activates the expression of fatty acid transport proteins. Mol Carcinog 2017;56:208–17.
[36] Harjes U, Bridges E, Gharpure KM, Roxanis I, Sheldon H, Miranda F, et al. Antiangiogenic and tumour inhibitory effects of downregulating tumour endothelial FABP4. Oncogene 2017;36:912–21.
[37] Lee D, Wada K, Taniguchi Y, Al-Shareef H, Masuda T, Usami Y, et al. Expression of fatty acid binding protein 4 is involved in the cell growth of oral squamous cell carcinoma. Oncol Rep 2014;31:1116–20.
[38] Tian W, Zhang W, Zhang Y, Zhu T, Hua Y, Li H, et al. FABP4 promotes invasion and metastasis of colon cancer by regulating fatty acid transport. Cancer Cell Int 2020;20:512.
[39] Arafat H, Lazar M, Salem K, Chipitsyna G, Gong Q, Pan TC, et al. Tumor-specific expression and alternative splicing of the COL6A3 gene in pancreatic cancer. Surgery 2011;150:306–15.
[40] Wishart AL, Conner SJ, Guarin JR, Fatherree JP, Peng Y, McGinn RA, et al. Decellularized extracellular matrix scaffolds identify full-length collagen VI as a driver of breast cancer cell invasion in obesity and metastasis. Sci Adv 2020;6:eabc3175.

正誤表

1. Abstract , 11〜12 行目

Col VI immunoreactivity was significantly higher in the OSCC with buccal fat pad invasion than without buccal fat pad invasion (median test, $p < 0.01$).

【誤】 higher → 【正】 lower

2. Result, 510 ページ 13〜15 行目

Col VI immunoreactivity differed significantly higher in the OSCC with than without buccal fat pad invasion (median test, $p < 0.01$).

【誤】 higher → 【正】 lower

3. Table1

【誤】 Male 11, Female 19 → 【正】 Male 19, Female 11

4. Table2

【誤】 Male No 14 (46.7) Yes 3 (10); Female No 10 (33.3) Yes 3 (10); buccal mucosa No 16 Yes 0; lower gums No 8 Yes 6

→

【正】 Male No 15 (78.9) Yes 4 (21.1); Female No 9 (81.8) Yes 2 (18.2); buccal mucosa No 8 Yes 6; lower gums No 16 Yes 0

修正された部分は結論に影響がありません。