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Prognostic significance of hypoxia-inducible factor-1α expression in advanced pharyngeal cancer without human papillomavirus infection

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Abstract

Objective. This study aimed to clarify the association between both hypoxia-inducible factor-1α and glucose transporter type-1 expression and survival outcome in advanced pharyngeal cancer without human papillomavirus infection.

Method. Twenty-five oropharyngeal and 55 hypopharyngeal cancer patients without human papillomavirus infection were enrolled. All patients had stage III–IV lesions and underwent concurrent chemoradiotherapy or surgery. Hypoxia-inducible factor-1α and glucose transporter type-1 expression were investigated in primary lesions by immunohistochemistry.

Results. There were 41 and 39 cases with low and high hypoxia-inducible factor-1α expression, and 28 and 52 cases with low and high glucose transporter type-1 expression, respectively. There was no significant correlation between hypoxia-inducible factor-1α and glucose transporter type-1 expression. In univariate analysis, nodal metastasis, clinical stage and high hypoxia-inducible factor-1α expression, but not glucose transporter type-1 expression, predicted significantly worse prognosis. In multivariate analysis, hypoxia-inducible factor-1α overexpression was significantly correlated with poor overall survival, disease-specific survival and recurrence-free survival.

Conclusion. High hypoxia-inducible factor-1α expression was an independent risk factor for poor prognosis for advanced human papillomavirus-unrelated pharyngeal cancer.

Main Article

Dr M Suzuki takes responsibility for the integrity of the content of the paper

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Introduction

The head and neck regions are closely associated with quality of life and social activity, so their functional preservation is important in the treatment of head and neck cancer as well as for fair prognosis. For organ preservation, concurrent chemoradiotherapy has been successfully introduced for the treatment of head and neck cancer.[1,2] However, patients sometimes have severe early and late adverse toxic reactions such as mucositis, disturbance of salivary secretion, dysphagia, laryngeal necrosis and mandibular osteomyelitis.[3–5] These adverse events decrease the treatment completion rate and increase the mortality rate. Salvage surgery is used to control tumours after concurrent chemoradiotherapy failure. However, surgical treatment after concurrent chemoradiotherapy can cause a number of complications, such as local infection and suture breakage because of scarring and decreased local blood flow.[6–9] Thus, biomarkers for predicting the effects of concurrent chemoradiotherapy have been examined.

Because cancer cells proliferate chaotically, angiogenesis in a tumour cannot maintain cancer growth and the vascular network, leading to hypoxia. Cancer cells in a heterogeneously hypoxic environment acquire an adaptive capacity to the hypoxic environment through changes in their signalling system, in which a key molecule is hypoxia-inducible factor-1.[10–13]

Hypoxia-inducible factor-1 is a transcription factor composed of two subunits, hypoxia-inducible factor-1α and hypoxia-inducible factor-1β. Hypoxia-inducible factor-1β is constitutively expressed not only in cancer cells but also in normal cells, and hypoxia-inducible factor-1α levels are extensively regulated by the concentration of oxygen.[12] Under normal oxygen conditions, hypoxia-inducible factor-1α is hydroxylated by a prolyl hydroxylase and is degraded through the ubiquitin pathway via binding to the von Hippel–Lindau tumour suppressor.[10] However, under low oxygen conditions, hypoxia-inducible factor-1α is stabilised without being degraded and moves into the nucleus. In the nucleus, it subsequently binds to hypoxia-inducible factor-1β to function as a transcription factor[14] that promotes the expression of many hypoxic adaptation-related factors, including glucose transport proteins such as glucose transporter type-1. Because these adaptations to a hypoxic environment influence treatment resistance,[11,14–17] the expression levels of hypoxia-inducible factor-1α and glucose transporter type-1 could be used to predict therapeutic effect, recurrence and prognosis in advanced head and neck cancer.[15,18,19]

Hypoxia-inducible factor-1α is overexpressed in various types of cancer, including head and neck cancer.[20,21] In head and neck cancer, hypoxia-inducible factor-1α overexpression has been investigated extensively in patients with oral squamous cell carcinoma (SCC).[18,22,23] Although hypoxia-inducible factor-1α overexpression has been linked with poor prognosis in oral SCC,[18,22] the findings of these reports have not been conclusive.[24] By contrast, there are a relatively small number of reports examining the relationship between hypoxia-inducible factor-1α expression and disease prognosis in oropharyngeal or hypopharyngeal SCC. Hypoxia-inducible factor-1α is overexpressed in the vast majority of patients with oropharyngeal SCC, and its degree of expression has predictive and prognostic significance in patients undergoing radiation therapy.[25] However, one report demonstrated that human papillomavirus (HPV) status, and not hypoxia-inducible factor-1α expression, was a predictor of survival outcome in patients with oropharyngeal SCC.[26]

Patients with advanced hypopharyngeal and HPV-unrelated oropharyngeal carcinoma have a poor prognosis among patients with head and neck cancer. Thus, the aim of this study was to clarify the association between both hypoxia-inducible factor-1α and glucose transporter type-1 expression and survival outcome in advanced pharyngeal cancer patients without HPV infection.

Materials and methods

Patients

Patients were diagnosed with oropharyngeal SCC or hypopharyngeal SCC by pathologic examination of biopsy samples and were treated by surgery or concurrent chemoradiotherapy with curative intent at the Department of Otorhinolaryngology, Head and Neck Surgery, University of the Ryukyus, Japan, between 2006 and 2017.

Clinical tumour staging was performed according to the Union for International Cancer Control tumour–node–metastasis classification (7th edition, 2009). All patients had clinical stage III or IV disease. Because HPV-related oropharyngeal SCC has a fair survival rate, patients with HPV-related oropharyngeal SCC or hypopharyngeal SCC were excluded. The minimum follow-up period was set to six months after completion of treatment. Finally, this study enrolled 80 treatment-naive patients without distant metastasis. Human papillomavirus status was determined by polymerase chain reaction analysis of HPV DNA and immunohistochemistry of p16 protein, as reported previously.[27] No patients in the present study had HPV DNA or p16 overexpression in the primary lesion.[28]

In order to determine clinical stage and to detect concomitant multiple primary cancers, the patients underwent physical and endoscopic examinations of the upper gastrointestinal tract, ultrasonic inspection of the neck, and computed tomography (CT) and ^{18}F-fluorodeoxyglucose-positron emission tomography-CT (PET-CT) imaging. Patient evaluation and the decision-making processes were conducted by head and neck surgeons and radiation oncologists before treatment was initiated.

Treatment protocol

Patients with T_3 or T_4 hypopharyngeal SCC were usually treated with one cycle of induction chemotherapy for organ preservation and prevention of distant metastasis. The basic regimen of induction chemotherapy was 1 or 2 cycles of a combination of 5-fluorouracil (600 mg/m^2 on days 1–5), nedaplatin (60 mg/m^2 on day 2) and docetaxel (60 mg/m^2 on day 2).

The therapeutic response was evaluated using the four categories of the Response Evaluation Criteria in Solid Tumours (version 1.1) guidelines: complete response, partial response, stable disease and progressive disease. The initial CT or magnetic resonance imaging scans were used as reference images. The response to induction chemotherapy was classified into the response (complete response or partial response) and no response (stable disease or progressive disease) groups. Those cases for whom a partial response or complete response to induction chemotherapy was achieved underwent concurrent chemoradiotherapy as organ preservation treatment, and cases with stable disease or progressive disease were recommended to undergo total pharyngo-laryngectomy.

As a general rule, we performed post-operative radiotherapy (RT; 60 Gy) with a triweekly infusion of 80 mg/m^2 cisplatin 3 times within 6 weeks of surgery if the patients had the following pathological high-risk factors for recurrence: lymph node metastasis with extracapsular extension or a positive or close surgical margin (tumour located less than 5 mm from the surgical margin). Hypopharyngeal SCC patients with concurrent chemoradiotherapy underwent definitive RT (either a total of 50.4 Gy with 1.8 Gy per day, or a total of 50 Gy with 2 Gy per day, 5 times per week) that was administered to the primary site and whole neck including the bilateral neck lymph nodes. The primary site and metastatic lymph nodes were subsequently treated with boost doses of a further 16.2 or 20 Gy in 9 or 10 fractions, respectively. Thus, the cumulative dose to the gross primary tumour and metastatic neck lymph nodes was 66.6 Gy or 70 Gy (once daily fraction).

In patients with oropharyngeal SCC, the primary treatment was concurrent chemoradiotherapy. The patients received platinum-based chemotherapy (nedaplatin and 5-fluorouracil, given twice with a 4-week interval) combined with 66.6 Gy RT. The radiological response of the primary lesion was determined at 39.6 Gy irradiation in all patients by CT, according to the revised Response Evaluation Criteria in Solid Tumours guidelines (version 1.1). If the primary lesion showed a partial response, concurrent chemoradiotherapy was continued as per the protocol. When the primary tumour failed to show a partial response regardless of the neck lymph node response, the patients underwent curative surgery for the primary lesion combined with neck dissection. Patients with N_2 and N_3 lesions underwent neck dissection at 2–3 months after concurrent chemoradiotherapy.[29]

Immunohistochemical study

For hypoxia-inducible factor-1α and glucose transporter type-1 immunohistochemistry, 4 µm thick sections from paraffin-embedded block samples were deparaffinised in xylene and hydrated in a graded series of alcohol. Epitope retrieval was achieved by heating at 100°C for 10 minutes in 1 mM ethylene diamine triacetic acid buffer (pH 8.0) for hypoxia-inducible factor-1α immunohistochemistry or in 10 mM citrate buffer (pH 6.0) for glucose transporter type-1 immunohistochemistry.

Endogenous peroxidase activity was quenched by incubating the sections in 0.3 per cent hydrogen peroxide in methanol for 20 minutes at room temperature. A SAB-PO Kit (Nichirei Bioscience, Tokyo, Japan) was used to detect immunoreactivity

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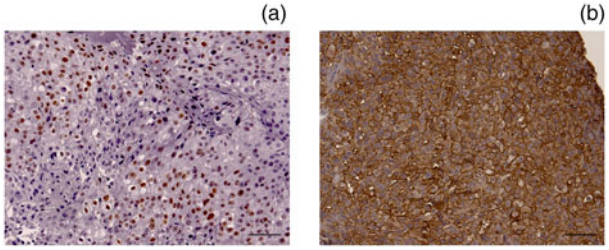

Fig. 1. Representative cases showing (a) hypoxia-inducible factor-1α and (b) glucose transporter type-1 immunohistochemistry. (a) Shows a hypopharyngeal carcinoma case. Strong hypoxia-inducible factor-1α expression was observed in nuclei, and (b) shows an oropharyngeal carcinoma case. There was strong and diffuse glucose transporter type-1 expression in cell membranes. Scale bar, 50 μm.

to hypoxia-inducible factor-1α and glucose transporter type-1, according to the manufacturer's protocol.

After blocking non-specific reactions by incubation in 10 per cent goat serum, the sections were incubated with primary antibodies for 1 hour at room temperature. A mouse monoclonal anti-hypoxia inducible factor-1α antibody (H1α67-immunoprecipitation assay grade; Abcam, Tokyo, Japan) was diluted with Protein Block Serum-Free (Dako; Agilent Technologies, Santa Clara, USA) at 1:100 for hypoxia-inducible factor-1α immunostaining, and a rabbit monoclonal anti-glucose transporter type-1 antibody (ab115730; Abcam, Tokyo, Japan) was diluted with Protein Block Serum-Free at 1:250 for glucose transporter type-1 immunostaining. Subsequently, a biotin-labelled secondary antibody and peroxidase-labelled streptavidin were applied. Immunolabelling was visualised by incubation in 3–3′-diaminobenzidine, and stained slides were counterstained with haematoxylin.

Positive hypoxia-inducible factor-1α expression was defined as having a stained nucleus and cytoplasm (Figure 1a). Positive glucose transporter type-1 expression was defined as having a stained cell membrane of tumour cells as observed for erythrocytes in the same field (Figure 1b). Sample scoring was performed by semi-quantitative microscopic analysis, considering the positive rates of cancer cells and signal intensity in 3 fields of view under ×400 magnification.

Considering the percentage of hypoxia-inducible factor-1α immunopositive tumour cells, the following scores were given: 1 when less than 10 per cent of cells were positive; 2 when equal to or more than 10 per cent and less than 30 per cent of cells were positive; 3 when equal to or more than 30 per cent and less than 70 per cent of cells were positive; and 4 when 70 per cent or more cells were positive. Signal intensity was scored as: negative (0), weak (1), moderate (2) and strong (3). The sum of the two scores was used to categorise hypoxia-inducible factor-1α expression as negative to weak (less than or equal to 2; hereafter, low expression) and moderate to strong (more than 3; hereafter, high expression). A representative case is shown in Figure 1a.

Considering the percentage of glucose transporter type-1 immunopositive tumour cells, the following scores were given: 1 when less than 10 per cent of cells were positive; 2 when equal to or more than 10 per cent and less than 70 per cent of cells were positive; 3 when equal to or more than 70 per cent and less than or equal to 90 per cent of cells were positive; and 4 when more than 90 per cent of cells were positive. Signal intensity was scored as negative (0), weak (1), moderate (2) and strong (3). The sum of the two scores was used to categorise glucose transporter type-1 expression as negative to weak (less than or equal to 3; hereafter, low expression) and moderate to strong (equal to or more than 4; hereafter, high expression). A representative case is shown in Figure 1b. These analyses were performed by S Agena, T Ikegami and N Hasegawa, who were blinded to the patients' clinical information.

Survival estimation

The clinicopathological parameters and treatment outcome of each patient were recorded at scheduled intervals during the observation period. The status of each patient, including information about recurrence and metastasis, was recorded at least every four to six weeks for the first year, every two to three months from two to five years, and thereafter every six months.

Overall survival, disease-specific survival and recurrence-free survival were investigated as prognostic indicators. Survival curves were estimated according to the Kaplan–Meier method, and survival distributions were compared using the log-rank test. Final prognosis was judged in February 2017. Overall survival was defined as the time from the start of treatment to death from any cause (both related and unrelated to oropharyngeal SCC or hypopharyngeal SCC) or to February 2017. Disease-specific survival was defined as the time from the start of treatment to death related to oropharyngeal SCC or hypopharyngeal SCC or to February 2017. Disease-specific survival denotes the probability of remaining free of disease after primary treatment. Recurrence-free survival was defined as the time from the start of treatment to locoregional or distant metastasis or to February 2017. All tests were two-sided, and p-values less than 0.05 were considered statistically significant. The multivariate prognostic significance of tumour variables on overall survival, disease-specific survival and recurrence-free survival was assessed using Cox proportional hazards analysis to identify prognostic parameters. Analyses were performed using the SPSS® (version 25) statistical software. The significance level was set at $p < 0.05$.

This study was approved by the Institutional Review Board of the University of the Ryukyus and carried out in accordance with the 1975 Declaration of Helsinki, as revised in 2008. Informed consent was obtained from all patients before enrolment. Because the present investigation was a prognostic biomarker study of hypoxia-inducible factor-1α and glucose transporter type-1, we followed the Reporting Recommendations for Tumour Marker Prognostic Studies guideline checklist.

Results

Patient characteristics

The patients included 69 men (86 per cent) and 11 women (14 per cent) with a median age of 66 (range, 39–82) years. The median follow-up period, excluding those patients who died during this time, was 76 (range, 7–132) months. All patients were followed for at least 24 months except for 1 patient who was lost to follow up at 7 months. Of the 80 patients, there were 25 (31 per cent) with oropharyngeal SCC and 55 (69 per cent) with hypopharyngeal SCC. Clinical stage IV was observed in 66 patients (82 per cent). According to T-stage classification, there were 6, 28, 25 and 21 patients with T_1, T_2, T_3 and T_4 stages, respectively. According to N-stage classification, there were 6, 16, 52 and 6 patients with N_0, N_1, N_2 and N_3, respectively (Table 1).

Of the 80 patients, 30 (38 per cent) underwent surgery and 50 (62 per cent) received concurrent chemoradiotherapy as a

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Table 1. Clinical features and survival estimation

Parameter	Patients (*n* (%))	5-year OS (%)	Univariate analysis *p*-value	5-year DSS (%)	Univariate analysis *p*-value	5-year RFS (%)	Univariate analysis *p*-value
Age							
– <65 years	35 (44)	52.8	0.262	64.2	0.086	52.8	0.556
– ≥65 years	45 (56)	69.6		83.4		67.3	
Sex							
– Male	69 (86)	62.7	0.770	71.9	0.221	61.1	0.616
– Female	11 (14)	60.6		90.9		60.6	
Primary site							
– Oropharynx	25 (31)	59.7	0.795	79.3	0.561	56.4	0.938
– Hypopharynx	55 (69)	63.4		72.6		63.4	
Tumour stage							
– T_1 or T_2*	34 (43)	56.1	0.463	72.2	0.550	53.6	0.314
– T_3 or $T_4^{\dagger}$	46 (57)	66.7		76.5		66.7	
Node stage							
– N_0–$N_1^{\ddagger}$	22 (28)	90.9	0.003	95.2	0.012	85.9	0.011
– N_2–N_3**	58 (72)	51.9		66.5		51.9	
Clinical stage							
– III	14 (18)	100.0	0.004	100.0	0.023	91.7	0.026
– IV	66 (82)	54.6		69.0		54.6	
Tumour differentiation							
– Well/moderate	63 (79)	68.4	0.072	78.4	0.235	66.8	0.121
– Poor	17 (21)	40.3		60.1		40.3	
Neoadjuvant chemotherapy							
– No	45 (56)	61.3	0.713	74.6	0.991	59.3	0.882
– Yes	35 (44)	59.2		75.2		62.5	
Primary treatment							
– CCRT with or without surgery	50 (62)	60.1	0.967	73.4	0.720	58.3	0.709
CCRT	36						
CCRT to surgery	4						
CCRT to PND	10						
– Surgery	30 (38)	65.2		77.3		65.2	
Multiple primary cancers							
– No	58 (72)	59.8	0.832	72.4	0.516	58.2	0.528
– Yes	22 (28)	68.2		81.3		68.2	
Brinkman index							
– <800	48 (60)	64.5	0.491	80.1	0.183	62.6	0.544
– ≥800	32 (40)	59.2		66.7		59.2	
Sake index							
– <40	38 (48)	63.3	0.832	77.7	0.581	63.3	0.711
– ≥40	42 (52)	61.4		72.2		58.9	

*T_1 = 6; T_2 = 28; $^{\dagger}T_3$ = 25; T_4 = 21; $^{\ddagger}N_0$ = 6; N_1 = 16; **N_2 = 52; N_3 = 6. OS = overall survival; DSS = disease-specific survival; RFS = recurrence-free survival; CCRT = concurrent chemoradiation therapy; PND = planned neck dissection

primary treatment. Of the 30 patients in the surgery group, 24 had hypopharyngeal carcinoma.

Of the 55 hypopharyngeal SCC patients, 34 had T_3 or T_4 lesions. Of these 34 patients, 27 (79.4 per cent) received induction chemotherapy, and the remaining 7 did not undergo induction chemotherapy because of renal dysfunction (4 patients), previous history of irradiation to the neck (1 patient) and refusal of induction chemotherapy (2 patients).

Immunohistochemical examinations

Overall, hypoxia-inducible factor-1α expression tended to be weaker than glucose transporter type-1 expression (Figure 1a

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Table 2. Immunohistochemical findings

Expression	Measurement	Patients (*n*)
HIF-1α		
	Positive cell count (%)	
	– 0–30	41
	– 30–70	21
	– 70	18
	Staining degree	
	– Negative	41
	– Weak	18
	– Moderate	13
	– Strong	5
GLUT-1		
	Positive cell count (%)	
	– 0–10	0
	– 10–70	9
	– 70–90	16
	– ≥90	55
	Staining degree	
	– Negative	0
	– Weak	10
	– Moderate	38
	– Strong	32

HIF-1 = hypoxia-inducible factor-1; GLUT-1 = glucose transporter type-1

Table 3. Correlation between HIF-1α and GLUT-1 expression

	GLUT-1 expression	
HIF-1α expression	Low	High
Low	18	23
High	10	29

HIF-1 = hypoxia-inducible factor-1; GLUT-1 = glucose transporter type-1

and b). For hypoxia-inducible factor-1α immunohistochemistry, 41 (51.3 per cent) of 80 samples demonstrated 0–30 per cent positive cell counts, and 59 (73.8 per cent) showed negative-to-weak staining (Table 2). Strong hypoxia-inducible factor-1α expression was observed in only 5 of 80 patients (6.3 per cent). On the other hand, 71 of 80 patients (88.8 per cent) showed weak-to-strong glucose transporter type-1 expression in more than 70 per cent of primary cancer cells, and 70 (87.5 per cent) showed moderate-to-strong staining (Table 2).

High hypoxia-inducible factor-1α expression was observed in 39 cases (49 per cent), and high glucose transporter type-1 expression was found in 52 cases (65 per cent). However, there was no correlation between hypoxia-inducible factor-1α and glucose transporter type-1 expression (Table 3; $p = 0.087$).

Survival estimation

Overall survival, disease-specific survival and recurrence-free survival in relation to the clinical features, and immunoexpression of hypoxia-inducible factor-1α and glucose transporter

Table 4. Immunoexpression and survival estimation

Factor	Patients (*n* (%))	5-year OS (%)	Univariate analysis			5-year DSS (%)	Univariate analysis			5-year RFS (%)	Univariate analysis		
			P-value	Risk ratio	95% CI		*P*-value	Risk ratio	95% CI		*P*-value	Risk ratio	95% CI
HIF-1α expression													
– Low	41 (51)	83.8	0.033	0.476	0.256–0.884	83.8	0.033	0.439	0.185–1.040	77.1	0.015	0.544	0.311–0.949
– High	39 (49)	65.5				65.5				45.2			
GLUT-1 expression													
– Low	28 (35)	76.7	0.729	1.075	0.600–1.927	76.7	0.765	0.857	0.366–2.008	60.0	0.959	1.061	0.618–1.822
– High	52 (65)	73.9				73.9				61.2			

OS = overall survival; DSS = disease-specific survival; RFS = recurrence-free survival; CI = confidence interval; HIF-1 = hypoxia-inducible factor-1; GLUT-1 = glucose transporter type-1

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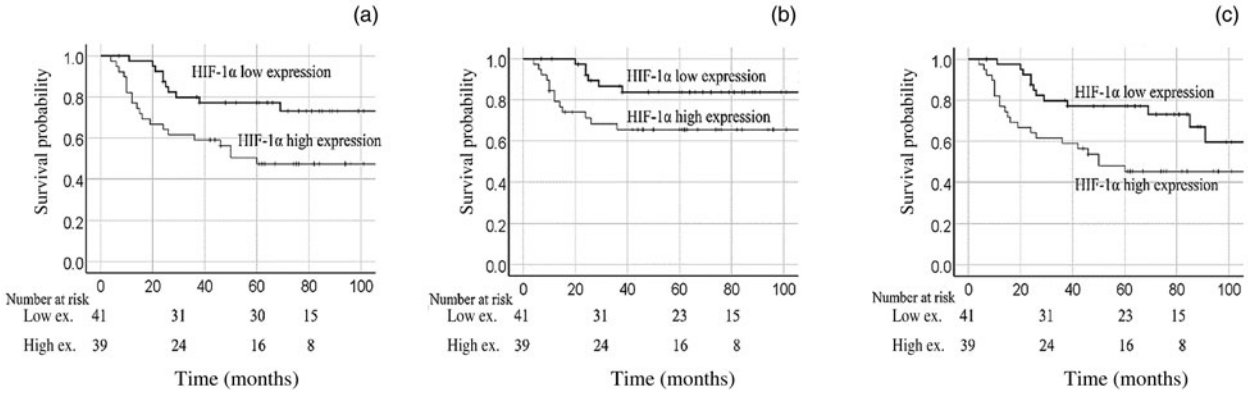

Fig. 2. Kaplan–Meier curves showing: (a) overall survival, (b) disease-specific survival and (c) recurrence-free survival in 80 pharyngeal carcinoma patients without human papillomavirus infection. (a) Shows overall survival in relation to hypoxia-inducible factor (HIF)-1α expression. Patients with high hypoxia-inducible factor-1α expression had worse overall survival than those with low hypoxia-inducible factor-1α expression ($p = 0.033$). (b) Shows disease-specific survival in relation to hypoxia-inducible factor-1α expression. Patients with high hypoxia-inducible factor-1α expression had worse disease-specific survival than those with low hypoxia-inducible factor-1α expression ($p = 0.033$). (c) Shows recurrence-free survival in relation to hypoxia-inducible factor-1α expression. Patients with high hypoxia-inducible factor-1α expression had worse recurrence-free survival than those with low hypoxia-inducible factor-1α expression ($p = 0.015$). low ex. = low expression; high ex. = high expression

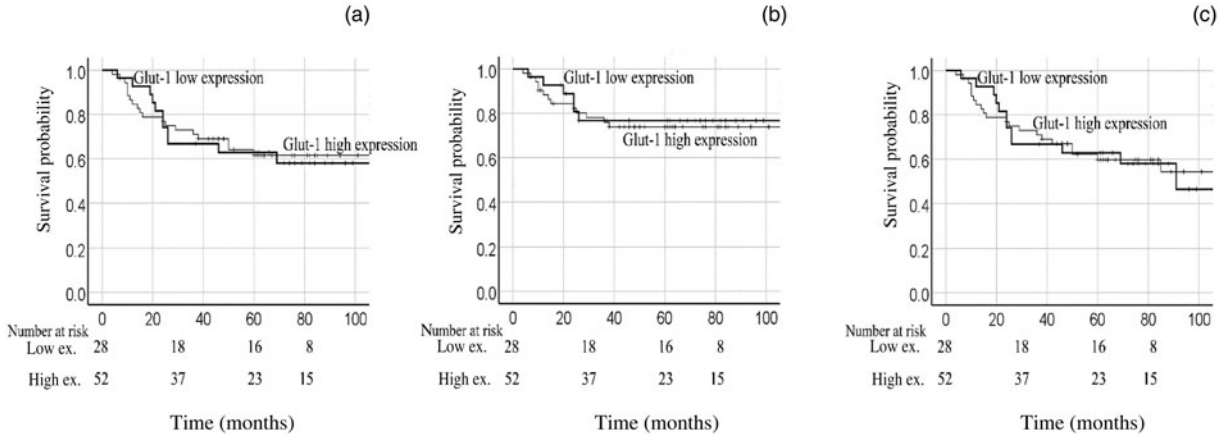

Fig. 3. Kaplan–Meier curves of (a) overall survival, (b) disease-specific survival and (c) recurrence-free survival in 80 pharyngeal carcinoma patients without human papillomavirus infection. (a) Shows overall survival in relation to glucose transporter type (Glut)-1 expression. There was no significant difference in overall survival between patients with high and low glucose transporter type-1 expression. (b) Shows disease-specific survival in relation to glucose transporter type-1 expression. There was no significant difference in disease-specific survival between patients with high and low glucose transporter type-1 expression. (c) Shows recurrence-free survival in relation to glucose transporter type-1 expression. There was no significant difference in recurrence-free survival between patients with high and low glucose transporter type-1 expression. low ex. = low expression; high ex. = high expression

Table 5. Multivariate analysis of survival data

		5-year OS multivariate analysis			5-year DSS multivariate analysis			5-year RFS multivariate analysis		
Factor	Patients (*n* (%))	*P*-value	Hazard ratio	95% CI	*P*-value	Hazard ratio	95% CI	*P*-value	Hazard ratio	95% CI
N		0.221	0.407	0.097–1.714	0.267	0.319	0.043–2.394	0.22	0.407	0.097–1.709
– N_0–N_1*	22 (28)									
– N_2–$N_3^{\dagger}$	58 (72)									
Stage		0.965	0.000	0.000–7.0776e+239	0.973	0.000	0.000–3.124e+304	0.468	0.483	0.068–3.448
– III	14 (18)									
– IV	66 (82)									
HIF-1α expression		0.01	0.367	0.171–0.787	0.037	0.357	0.137–0.942	0.012	0.402	0.196–0.822
– Low	41 (51)									
– High	39 (49)									

*$N_0 = 6$, $N_1 = 16$; $^{\dagger}N_2 = 52$, $N_3 = 6$. OS = overall survival; DSS = disease-specific survival; RFS = recurrence-free survival; CI = confidence interval; HIF-1 = hypoxia-inducible factor-1

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type-1 are shown in Tables 1 and 4, respectively. Node classification and clinical stage classification showed a significant difference in univariate analysis of overall survival, disease-specific survival and recurrence-free survival. However, the other clinical features including T-stage classification and primary sites did not reach significance (Table 1).

For immunohistochemical analysis, patients with high hypoxia-inducible factor-1α expression had significantly worse overall survival, disease-specific survival and recurrence-free survival than those with low hypoxia-inducible factor-1α expression (Table 4 and Figure 2). However, glucose transporter type-1 expression had no significant impact on survival (Table 4 and Figure 3). In multivariate analysis, there was a significant difference in overall survival, disease-specific survival and recurrence-free survival between patients with low and high hypoxia-inducible factor-1α expression (Table 5). Node category and clinical stage classification did not reach significance.

There were no significant differences in clinical characteristics between low and high hypoxia-inducible factor-1α expression (Table 6). However, the high hypoxia-inducible factor-1α expression group contained a relatively large number of patients with oropharyngeal SCC ($p = 0.066$). Figure 4 shows the difference in overall survival between low and high hypoxia-inducible factor-1α expression in oropharyngeal SCC and hypopharyngeal SCC patients. Hypopharyngeal SCC patients with high hypoxia-inducible factor-1α expression had worse overall survival than those with low hypoxia-inducible factor-1α expression (Figure 4b; $p = 0.026$). Oropharyngeal SCC patients also showed the same tendency for overall survival, despite the small number of samples (Figure 4a; $p = 0.114$).

Discussion

In this study, high hypoxia-inducible factor-1α expression was found to be an independent risk factor for poor prognosis in patients with advanced oropharyngeal SCC or hypopharyngeal SCC. This is the first report of hypoxia-inducible factor-1α expression focusing on advanced pharyngeal cancer without HPV infection.

A meta-analysis of 1474 oral cancers demonstrated that hypoxia-inducible factor-1α was associated with tumour size, clinical stage and lymph node metastasis, and high hypoxia-inducible factor-1α expression was an indicator for worse survival outcome.[18] In subgroup analysis, this phenomenon was observed exclusively in Asian patients. According to another systematic review of hypoxia-inducible factor expression in head and neck cancer, hypoxia-inducible factor-1α overexpression was also significantly associated with poor prognosis in Asian patients, but not in European patients.[30] Regarding tumour location in head and neck cancer, oral carcinoma, nasopharyngeal carcinoma and oropharyngeal carcinoma, but not laryngeal carcinoma, showed an association between hypoxia-inducible factor-1α overexpression and worse overall survival. A previous report on the association between hypoxia-inducible factor-1α overexpression and the survival rate in hypopharyngeal SCC demonstrated no clear relationship between hypoxia-inducible factor-1α and locoregional control and disease-specific survival.[31]

In the present study, all patients were Japanese (i.e. Asians) with hypopharyngeal SCC or oropharyngeal SCC, and a clear association was seen between hypoxia-inducible factor-1α expression and overall survival, disease-specific survival and recurrence-free survival. Although only a small number of reports have examined hypoxia-inducible factor-1 expression in patients with oropharyngeal SCC or hypopharyngeal SCC, the findings of the present study are in line with those of previous reports. Given that racial differences in hypoxia-inducible factor-1 expression have been observed between Asian and

Table 6. Clinical characteristics of HIF-1α-positive cases

Parameter	Low HIF-1α expression (*n*)	High HIF-1α expression (*n*)	*P*-value
Age			
– <66 years	18	17	0.978
– ≥66 years	23	22	
Sex			
– Male	36	33	0.679
– Female	5	6	
Primary site			
– Oropharynx	9	16	0.066
– Hypopharynx	32	23	
T-stage			
– T_1 or T_2*	17	17	0.848
– T_3 or T_4†	24	22	
Node stage			
– N_0–N_1‡	12	10	0.716
– N_2–N_3**	29	29	
Clinical stage			
– III	8	6	0.627
– IV	33	33	
Tumour differentiation			
– Well/moderate	32	31	0.875
– Poor	9	8	
Neoadjuvant chemotherapy			
– No	23	22	0.978
– Yes	18	17	
Primary treatment			
– CCRT with or without surgery	26	24	
CCRT	16	20	
CCRT to surgery	3	1	
CCRT to PND	7	3	
– Surgery	15	15	
Multiple primary cancers			
– No	30	28	0.89
– Yes	11	11	
Brinkman index			
– <800	26	22	0.523
– ≥800	15	17	
Sake index			
– <40	23	15	0.114
– ≥40	18	24	

*T_1 = 6, T_2 = 28; †T_3 = 25, T_4 = 21; ‡N_0 = 6, N_1 = 16; **N_2 = 52, N_3 = 6. HIF-1 = hypoxia-inducible factor-1; CCRT = concurrent chemoradiation therapy; PND = planned neck dissection

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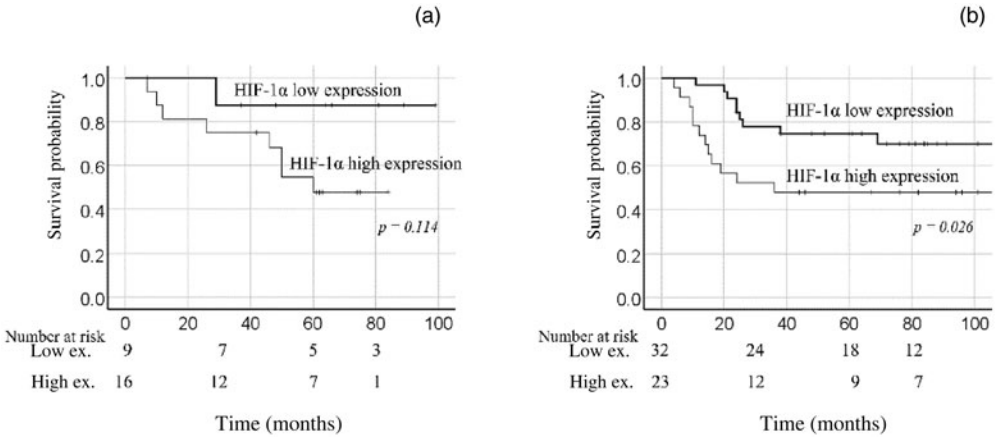

Fig. 4. Kaplan–Meier curves of overall survival in patients with (a) oropharyngeal carcinoma and (b) hypopharyngeal carcinoma. (a) Shows oropharyngeal cancer patients with high hypoxia-inducible factor (HIF)-1α expression tended to have worse overall survival than those with low hypoxia-inducible factor-1α expression, but the difference did not reach significance ($p = 0.114$). (b) Shows hypopharyngeal cancer patients with high hypoxia-inducible factor-1α expression had worse overall survival than those with low hypoxia-inducible factor-1α expression ($p = 0.026$). low ex. = low expression; high ex. = high expression

European countries,[18,30] further studies in different ethnic groups are needed to confirm our observations.

Because this study focused on advanced pharyngeal cancer, clinical tumour, node and stage classifications were not found to be significant prognostic factors in multivariate analysis. Oropharyngeal carcinoma[32] and head and neck cancer[33] with radiotherapy showed worse local control and survival rate when high hypoxia-inducible factor-1α expression was observed.[32] On the other hand, hypoxia-inducible factor-1α expression in surgically treated patients with head and neck SCC (31 oral cavity, 23 oropharynx, 16 larynx and 9 hypopharynx) was associated with improved disease-free survival and overall survival,[21] while the results of the present study were similar to those of previous reports.[32,33]

Elevated hypoxia-inducible factor-1α protein levels have been shown to be associated with increased hypoxic radiation resistance in FaDu human pharyngeal carcinoma cell line *in vitro*.[34] The discrepancies between these contradictory results may reflect different treatment modalities, and hypoxia-inducible factor-1α-overexpressing head and neck cancer might be treated by surgery, and not by radiation-based therapy. Further study is needed to clarify an appropriate treatment modality for hypoxia-inducible factor-1α-overexpressing in head and neck cancer.

- Hypoxia-inducible factor-1α is overexpressed in various types of cancer, including head and neck cancer
- Few reports have examined the relationship between hypoxia-inducible factor-1α expression and disease prognosis in pharyngeal squamous cell carcinoma (SCC)
- No study has reported the association between hypoxia-inducible factor-1α and prognosis in advanced pharyngeal cancer without human papillomavirus (HPV) infection
- High hypoxia-inducible factor-1α expression was an independent risk factor for poor prognosis in advanced HPV-unrelated oropharyngeal or hypopharyngeal SCC
- High glucose transporter type-1 expression was not an independent factor for poor prognosis in advanced HPV-unrelated oropharyngeal or hypopharyngeal SCC

Glucose transporter type-1 is a cell membrane transport protein that determines glucose uptake and is abnormally expressed in head and neck cancer.[19] Although glucose transporter type-1 shows only weak expression in normal mucosal lesions, it is strongly expressed in dysplasia and SCC.[35] Glucose transporter type-1 expression is considered to be a prognostic marker in head and neck cancer.[36,37,38] However, in the present study, glucose transporter type-1 expression did not demonstrate a clear correlation with disease prognosis. We found that glucose transporter type-1 expression was much stronger than hypoxia-inducible factor-1α expression (Table 2). Because PET-CT studies have indicated that glucose uptake is highly increased in head and neck cancer,[19,39,40] the immunohistochemical approach used in the present study might not detect differences in glucose uptake ability in advanced pharyngeal carcinoma. In addition, although a significant correlation between glucose transporter type-1 and hypoxia-inducible factor-1α expression in head and neck cancer has been reported,[40] we found no such association. Other methods, such as metabolic tumour volume in PET-CT, might be more appropriate than glucose transporter type-1 immunohistochemistry for evaluating glucose metabolism.

Conclusion

In conclusion, high hypoxia-inducible factor-1α expression, but not high glucose transporter type-1 expression, was an independent risk factor for poor prognosis in advanced HPV-unrelated oropharyngeal SCC and hypopharyngeal SCC patients who underwent concurrent chemoradiotherapy as a primary treatment. Racial differences in the association of hypoxia-inducible factor-1α expression with survival outcome between Asian and European countries and treatment modality in cases with high hypoxia-inducible factor-1α expression should be examined to facilitate the design of better treatment protocols.

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Competing interests. None declared

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