Original paper

CD98 expression can be a predictive factor of resistance to radiotherapy in head and neck squamous cell carcinoma

Hitomi Suzuki[1], Yohei Kawasaki[1], Shinsuke Suzuki[1], Takechiyo Yamada[1], Ayumi Ito[2], Maya Suzuki[2], Masahito Miura[2], Haruka Hatakeyama[2], Yasufumi Omori[2]

[1]Department of Otorhinolaryngology and Head and Neck Surgery, Akita University Graduate School of Medicine, Akita, Japan
[2]Department of Molecular and Tumor Pathology, Akita University Graduate School of Medicine, Akita, Japan

CD98 is a marker of cancer stem cells, and it regulates radiosensitivity in head and neck squamous cell carcinoma (HNSCC). The current study aimed to investigate whether CD98 can be used as a prognostic factor and marker of radioresistance.
CD98 immunostaining was performed using biopsy specimens collected from patients diagnosed with HNSCC. The average period of postoperative monitoring was 31.6 months. The treatment options were radiation therapy with either cisplatin or cetuximab, and surgery. The participants were divided into groups of low and high fluorescence intensity.
CD98 was an independent prognostic factor of radioresistance. In total, 103 patients were treated with chemoradiotherapy or bioradiotherapy. The overall survival rates of patients receiving chemoradiotherapy or bioradiotherapy were 69.2% in the low group and 36.2% in the high group. The progression-free survival rates were 60.0% and 24.6%, respectively. CD98 expression was considered an independent prognostic factor of overall survival and progression-free survival. In total, 99 patients underwent surgical treatment. The surgery group did not differ according to CD98 expression.
Via CD98 immunostaining, sensitivity to radiotherapy can be determined in advance. In HNSCC, knowledge about sensitivity to radiotherapy can significantly improve prognosis.

Key words: head and neck squamous cell carcinoma, radiation tolerance, prognosis, biopsy.

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common type of cancer worldwide, affecting more than 6.6 million people annually [1, 2]. The treatment options for this condition are radiation therapy and surgery. However, the cure rate remains at 50–60% [3, 4]. Radiation failure can lead to recurrence and metastasis and thus contributes to a poor prognosis. This led to the concept of cancer stem cells, which is a possible factor affecting treatment resistance [5–8]. In 2007, Prince *et al*. [9] assessed the characteristics of HNSCC stem cells and found that CD44-positive cells have a high self-renewal and tumorigenicity capacity in tumors transplanted into immunodeficient NOD/SCID and Rag2γDKO mice. Wang *et al*. [10] isolated side population cells from HNSCC cell lines and discovered a population with a high colony-forming and tumorigenic potential. Chiou *et al*. [11] reported that sphere-forming cells isolated from oral cancer cell lines expressed OCT4, Nanog CD117, nestin, CD133, and ABCG2 and

showed high differentiation and invasion potential. Further examining the expression of OCT4, Nanog, and CD133 by immunostaining revealed that marker-positive groups had a low survival rate, thereby indicating the significance of these markers as prognostic factors. Different cancer stem cell markers have been reported, and CD98 is considered a candidate [12, 13]. CD98hc is a marker and regulator of radiosensitivity [14] and was identified as a marker of cancer stem cells in an assessment that used five different HNSCC cell lines [15]. Therefore, the current study investigated whether CD98 can be a marker for successful radiotherapy *via* immunostaining of biopsy specimens.

Materials and methods

Patients

In total, 202 patients diagnosed with HNSCC at our institution between January 2010 and December 2020 were included in the study (Table I, left side). The inclusion criteria were that patients were initially diagnosed, treated, and followed up at our institution. The exclusion criterion was insufficient biopsy specimens for immunostaining. Computed tomography scan, magnetic resonance imaging, and positron emission tomography-computed tomography scan were performed to evaluate clinical staging. Staging was performed using the 8th Union for International Cancer Control staging system. The mean observation period was 31.6 (range: 2–60) months. In total, 103 patients received chemoradiotherapy (CRT) or bioradiotherapy (BRT) (Table I, center). The concomitant drugs were cisplatin and cetuximab. The extent and dose of radiation exposure were determined by the radiologist. In total, 99 patients underwent surgical treatment, and standard techniques were used according to tumor location and progression (Table I, right). Informed consent was obtained from all patients. The study was approved by the Ethics Committee of the institution and was conducted in accordance with the Declaration of Helsinki.

Immunostaining and evaluation methods

Results were evaluated according to fluorescence intensity and staining range. The examination was performed by a skilled pathologist who was asked to evaluate the findings with clinical data withheld. Based on the percentage of CD98-positive tumor cells, the evaluation method was classified as negative, weak, moderate, and strong, as shown in a previous study [13]. This evaluation method was also used in another study [14]. In brief, ≤ 10% of tumor cells stained negatively; 11–25%, weakly; 25–50%, moderately; and ≥ 50, strongly. Negatively and weakly staining tumor cells were categorized in the low group and moderate and strong tumor cells under the high group (Fig. 1). Then, 3-μm tissue sections collected *via* biopsies were deparaffinized and treated with Tris/EDTA buffer for antigen retrieval and 3% hydrogen peroxide methanol for 10 min to inhibit endogenous peroxidase activity. After washing with the buffer, the cells were reacted with primary antibodies, anti-CD98 antibody (1 : 200, sc-376815, Santa Cruz, USA), anti-SOX2 antibody (1 : 100, ab93689, Abcam, Cambridge, UK), anti-Nanog antibody (1 : 100, ab109250, Abcam, Cambridge, UK), and anti-Oct4 antibody (1 : 100, A7920, ABclonal, Woburn, MA) for 90 min. After rinsing in TBS, the secondary antibody (EnVision+/HRP; DakoCytomation, Glostrup, Denmark) was added, and the sections were incubated for 60 min at room temperature. After rinsing with TBS, the peroxidase reaction was performed using 0.02% 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma-Aldrich, St. Louis, MO, USA) for 3 min at room temperature. The sections were counterstained with Mayer's hematoxylin and then dehydrated and mounted.

Ethics

Informed consent was obtained from all patients, and the study was approved by the Akita University Ethics Committee and conducted in compliance with the Declaration of Helsinki. All procedures used in this research were approved by the Ethics Committee of Akita University Hospital (Approval Number: 2532).

Statistics

Survival was evaluated using the Kaplan-Meier method, and its significance was assessed using the log-rank test. A p-value of < 0.05 was considered statistically significant. Multivariate analysis was performed using the Cox proportional hazard regression model. All analyses were performed using the IBM SPSS Statistics software version 20. P-values of < 0.05 were considered statistically significant.

Results

Resistance to CRT or BRT in the high CD98 expression group

The 5-year overall survival (OS) rates of 202 patients were 46.4% in the high group and 58.0% in the low group ($p = 0.051$) (Fig. 2A). The 5-year progression-free survival (PFS) rates were 34.9% in the high group and 53.2% in the low group ($p = 0.033$) (Fig. 2B). Further, multivariate analysis showed that CD98 was a prognostic factor of PFS ($p = 0.044$) (Table IIB).

Of 103 patients treated with CRT or BRT, 32 were classified in the low group and 71 in the high group. OS rates were 36.2% in the high group and 69.2%

Table I. Patient characteristics

All patients (N = 202)			Patients treated with CRT or BRT (N = 103, CRT = 101, BRT = 2)			Patients treated with surgery (N = 99)		
Characteristics		Values	Characterestics		Values	Characterestics		Values
Age (years)		65.8 ±11.0	Age(years)		65. 6 ±9.4	Age (years)		66.0 ±12.5
Sex (male/female)		168/34	Sex (male/female)		92/11	Sex (male/female)		76/23
T stage (%)	T1	19 (9.4)	T stage (%)	T1	6 (5.8)	T stage (%)	T1	13 (13.1)
	T2	78 (38.6)		T2	42 (40.8)		T2	36 (36.4)
	T3	43 (21.3)		T3	23 (22.3)		T3	20 (20.2)
	T4	62 (30.7)		T4	32 (31.1)		T4	30 (30.3)
N stage (%)	N0	54 (26.7)	N stage (%)	N0	13 (12.6)	N stage (%)	N0	41 (41.4)
	N1	23 (11.4)		N1	16 (15.6)		N1	7 (7.1)
	N2	115 (56.9)		N2	67 (65.0)		N2	48 (48.5)
	N3	10 (5.0)		N3	7 (6.8)		N3	3 (3.0)
M stage (%)	M0	202 (100.0)	M stage (%)	M0	103 (100.0)	M stage (%)	M0	99 (100.0)
Stage (%)	I	17 (8.4)	Stage (%)	I	5 (4.9)	Stage (%)	I	12 (12.1)
	II	29 (14.3)		II	8 (7.8)		II	21 (21.2)
	III	25 (12.5)		III	15 (14.6)		III	10 (10.1)
	IV	131 (64.8)		IV	75 (72.7)		IV	56 (56.6)
Tumor sites (%)	Tongue	38 (18.8)	Tumor sites (%)	Tongue	2 (1.9)	Tumor sites (%)	Tongue	36 (36.4)
	Nasapharynx	10 (5.0)		Nasapharynx	10 (9.7)		Nasapharynx	0 (0.0)
	Oropharynx	64 (31.7)		Oropharynx	49 (47.7%)		Oropharynx	15 (15.2)
	Hypopharynx	76 (37.6)		Hypopharynx	40 (38.8%)		Hypopharynx	36 (36.3)
	Gingiva	14 (6.9%		Gingiva	2 (1.9%)		Gingiva	12 (12.1.)

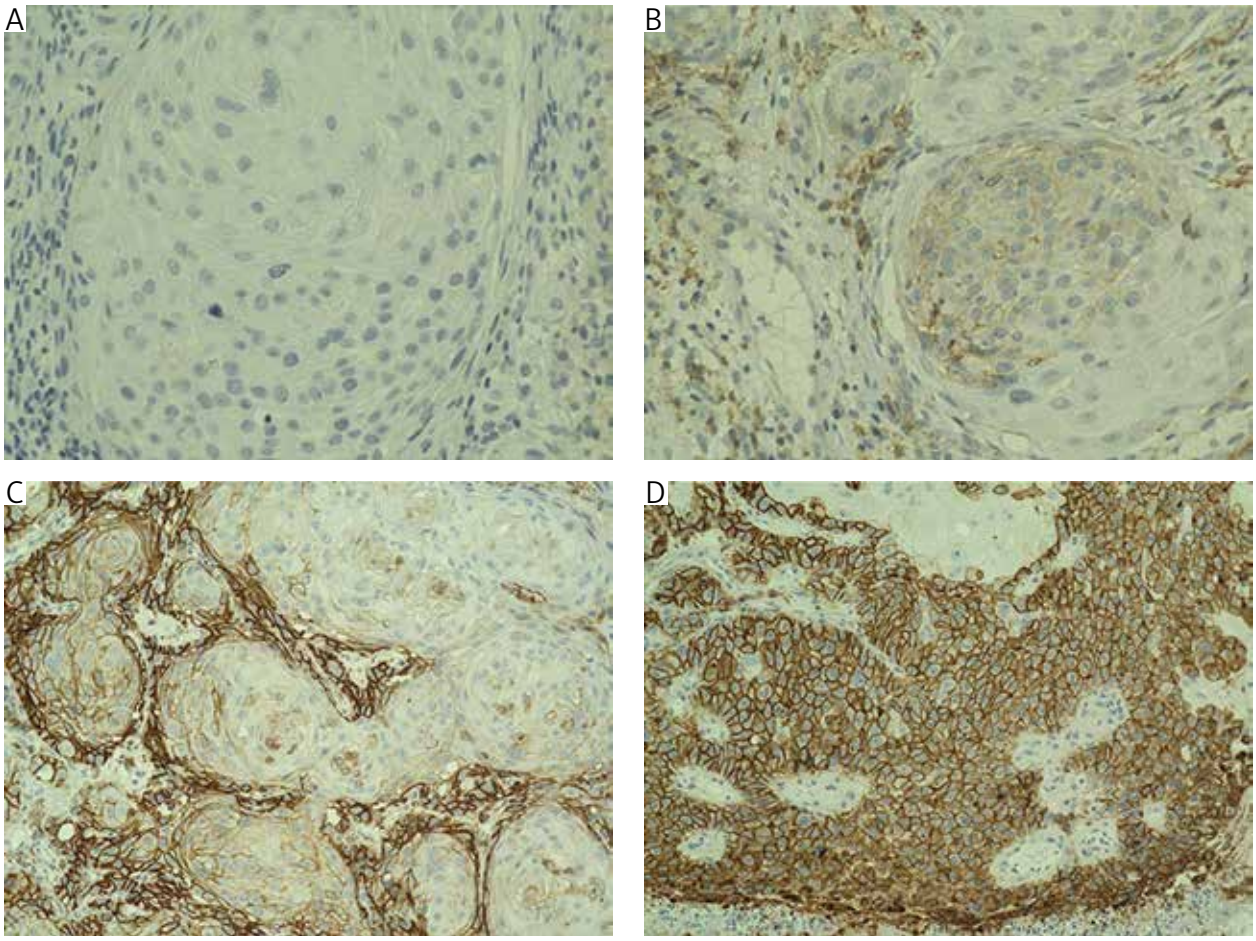
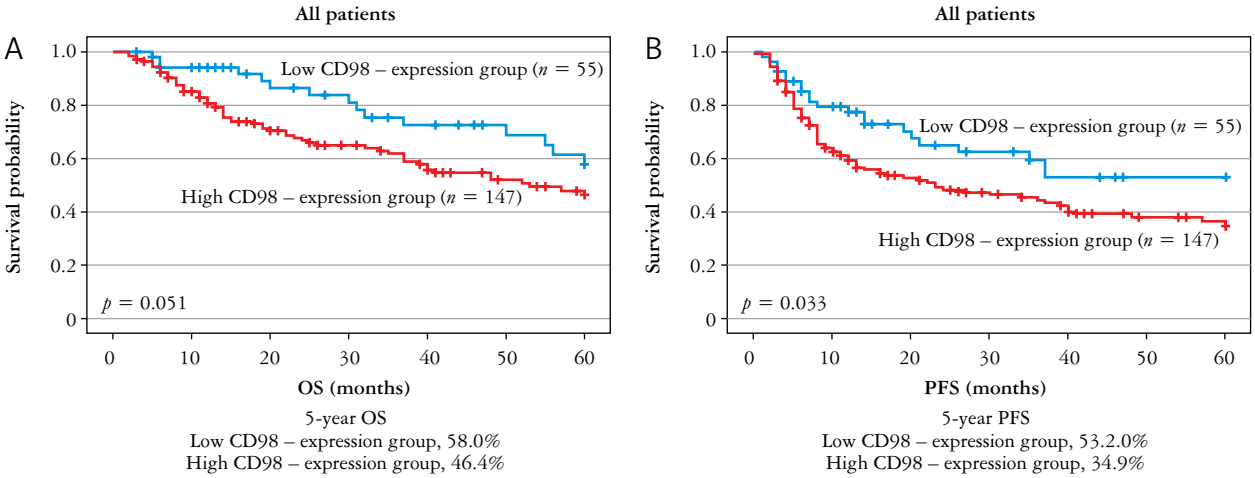


Fig. 1. CD98 staining intensity level. Biopsy tissues immunostained with CD98. The staining intensity was defined as: A) negative; B) weak; C) moderate; D) strong (magnification, 400×)



5-year OS
Low CD98 – expression group, 58.0%
High CD98 – expression group, 46.4%

5-year PFS
Low CD98 – expression group, 53.2.0%
High CD98 – expression group, 34.9%

Fig. 2. Relationship of CD98 expression with overall survival (OS) and progression-free survival (PFS) in all patients. A) OS of the low and high groups was 58.0%, and 46.4%, respectively ($p = 0.051$); B) in terms of PFS, prognosis was significantly better in the low group (53.2%) than in the high group (34.9%) ($p = 0.033$)
OS – overall survival, PFS – progression-free survival

in the low group ($p = 0.006$) (Fig. 3A). Multivariate analysis revealed that CD98 was an independent prognostic factor of radioresistance ($p = 0.023$) (Table IIIA). Progression-free survival rates were 24.6% in the high group and 60.0% in the low group ($p = 0.003$) (Fig. 3B). Moreover, CD98 was considered an independent prognostic factor of PFS ($p = 0.011$) (Table IIIB).

There was no significant difference in terms of OS or PFS in the surgical treatment group (Figs. 4A,B, Table IVA,B).

Table II . Univariate and multivariate analyses. A) Overall survival; B) progression-free survival (all patients)

A

		Univariate analysis			Multivariate analysis		
		HR	95% CI	*p*-value	HR	95% CI	*p*-value
Age	(< 65 vs. ≥ 65)	1.585	0.998–2.518	0.051	1.806	1.125–2.898	0.014*
Sex	(Female vs. male)	1.27	0.670–2.404	0.464	1.273	0.666–2.434	0,464
T stage	(T1-2 vs. T3-4)	2.028	1.273–3.229	0.003**	1.741	1.065–2.848	0.027*
N stage	(N0 vs. N1-3)	1.731	0.997–3.005	0.051	1.586	0.873–2.88	0.13
Stage	(I-II vs. III-IV)	2.057	1.086–3.897	0.027*	NA	NA	NA
CD98	(Low vs. high)	1.736	0.987–3.052	0.056	1.697	0.963–2.990	0.067

B

		Univariate analysis			Multivariate analysis		
		HR	95% CI	*p*-value	HR	95% CI	*p*-value
Age	(< 65 vs. ≥ 65)	1.041	0.708–1.530	0.837	1.150	0.774–1.708	0.489
Sex	(Female vs. male)	1.465	0.819–2.619	0,198	1.463	0.813–2.633	0.205
T stage	(T1-2 vs. T3-4)	1.556	1.054–2.297	0.026*	1.408	0.933–2.124	0.103
N stage	(N0 vs. N1-3)	1.530	0.963–2.431	0.072	1.373	0.833–2.263	0.214
Stage	(I-II vs. III-IV)	1.698	1.010–2.855	0.046*	NA	NA	NA
CD98	(Low vs. high)	1.663	1.030–2.683	0.037*	1.636	1.012–2.643	0.044*

CI – confidence interval, HR – hazard ratio, NA – not applicable
** $p < 0.05$*
*** $p < 0.01$*

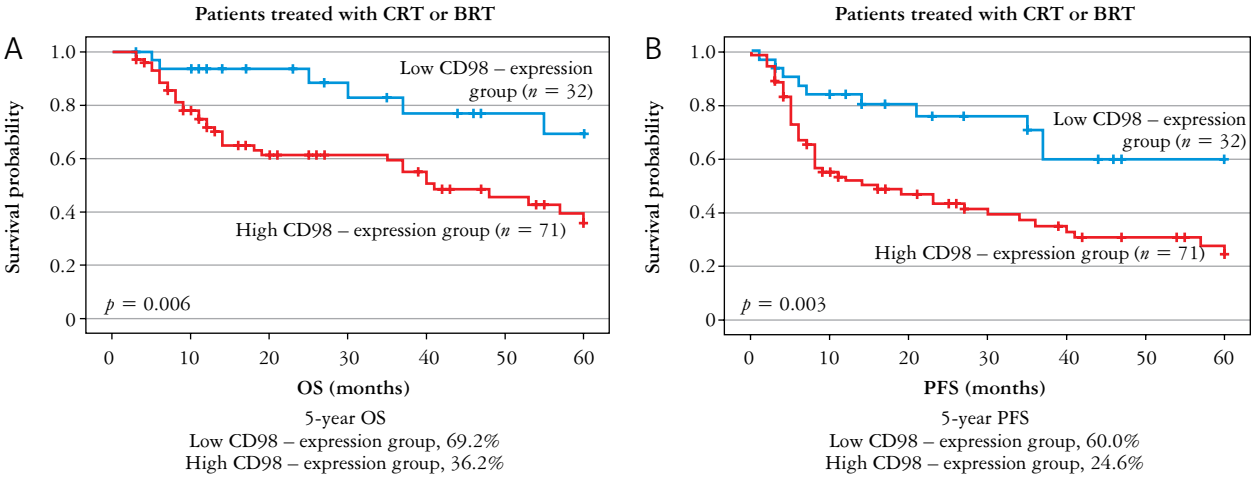


Fig. 3. Relationship of CD98 expression with overall survival (OS) and progression-free survival (PFS) in all patients treated with chemoradiotherapy or bioradiotherapy. A) The OS rates of the low and high groups were 69.2% and 36.2%, respectively ($p = 0.006$); B) the PFS rates of the low and high groups were 60.0% and 24.6%, respectively ($p = 0.003$)

The low group had better prognosis.

BRT – bioradiotherapy, CRT – chemoradiotherapy, OS – overall survival, PFS – progression-free survival

Association between CD98 expression and Nanog, SOX2, and Oct4 expression

If CD98 was a marker of cancer stem cells, it could be associated with transcription factors, such as Nanog, SOX2, and Oct4, which are involved in promoting self-renewal and maintaining an undifferentiated state. Patients with high CD98 expression were more likely to have elevated Nanog, SOX2, and OCT4 expression (Fig. 5). However, those with weak CD98 expression did not present with all cancer stem cell genes.

Discussion

CD98 is a cell surface antigen comprising a heterodimer of disulfide-linked heavy (CD98hc) and

Table III. Univariate and multivariate analyses. A) Overall survival; B) progression-free survival

A

		Univariate analysis			Multivariate analysis		
		HR	95%CI	*p*-value	HR	95%CI	*p*–value
Age	(< 65 vs. ≥ 65)	2.081	1.059–4.087	0.033*	1.680	0.839–3.361	0.143
Gender	(Female vs. male)	1.838	0.567–5.960	0.311	1.798	0.543–5.954	0.337
T stage	(T1–2 vs. T3–4)	2.777	1.432–5.388	0.003**	2.449	1.203–4.986	0.013*
N stage	(N0 vs. N1–3)	1.498	0.585–3.835	0.399	0.974	0.344–2.759	0.960
Stage	(I–II vs. III–IV)	3.758	0.906–15.583	0.068	NA	NA	NA
CD98	(Low vs. high)	3.107	1.306–7.392	0.010*	2.774	1.150–6.693	0.023*

B

		Univariate analysis			Multivariate analysis		
		HR	95% CI	*p*-value	HR	95% CI	*p*-value
Age	(< 65 vs. ≥ 65)	1.309	0.761–2.251	0.330	1.070	0.611–1.872	0.814
Sex	(Female vs. male)	2.779	0.867–8.913	0.086	2.672	0.826–8.647	0.101
T stage	(T1–2 vs. T3–4)	1.932	1.121–3.331	0.018*	1.748	0.980–3.118	0.058
N stage	(N0 vs. N1–3)	1.602	0.685–3.746	0.277	1.042	0.421–2.579	0.929
Stage	(I–II vs. III–IV)	3.379	1.055–10.821	0.040*	NA	NA	NA
CD98	(Low vs. high)	2.701	1.361–5.364	0.005**	2.481	1.235–4.983	0.011*

Patients treated with chemoradiotherapy or bioradiotherapy.

CI – confidence interval, HR – hazard ratio, NA – not applicable

** $p < 0.05$*

*** $p < 0.01$*

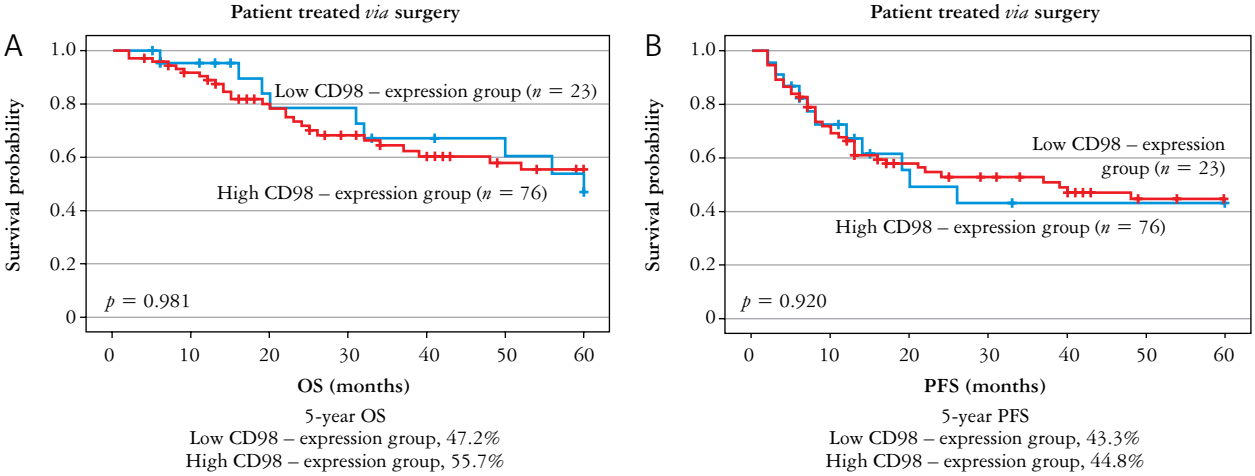


Fig. 4. Relationship of CD98 expression with OS and progression-free survival (PFS) in all patients who underwent surgery. No differences were found in A) OS ($p = 0.981$); B) PFS ($p = 0.920$) in the surgical treatment group

OS – overall survival, PFS – progression-free survival

light chains (CD98lc). CD98hc amplifies integrin signaling and activates AKT, FAK, and PI3K, and it is highly involved in cell survival, anchorage independence, and metastasis. CD98lc is an amino acid transporter, and LAT1 is highly expressed in cancer cells. It is responsible for transporting essential amino acids in rapidly growing tumor cells, activating mTOR signaling, suppressing autophagy, and facilitating cell survival and proliferation. This interaction between CD98hc and CD98lc as well as CD98 pro-

Table IV. Univariate and multivariate analyses. A) Overall survival; B) progression-free survival

A

		HR	95% CI	*P*-VALUE	HR	95% CI	*P*-VALUE
Age	(< 65 vs. ≥ 65)	1.223	0.634–2.358	0.549	1.581	0.769–3.249	0.213
Sex	(Female vs. male)	0.946	0.431–2.078	0.891	0.979	0.436–2.197	0.959
T stage	(T1-2 vs. T3-4)	1.433	0.739–2.782	0.287	1.141	0.540–2.412	0.730
N stage	(N0 vs. N1-3)	1.802	0.885–3.665	0.104	1.977	0.867–4.508	0.105
Stage	(I-II vs. III-IV)	1.576	0.741–3.354	0.237	NA	NA	NA
CD98	(Low vs. high)	0.991	0.465–2.110	0.981	1.119	0.513–2.441	0.778

B

		HR	95% CI	*P*-VALUE	HR	95% CI	*P*-VALUE
Age	(< 65 vs. ≥ 65)	0.794	0.453–1.392	0.421	0.876	0.468–1.643	0.681
Sex	(Female vs. male)	1.014	0.506–2.033	0.969	0.967	0.471–1.986	0.927
T stage	(T1-2 vs. T3-4)	1.221	0.695–2.145	0.487	1.032	0.543–1.961	0.923
N stage	(N0 vs. N1-3)	1.463	0.812–2.635	0.205	1.375	0.683–2.766	0.372
Stage	(I-II vs. III-IV)	1.249	0.672–2.321	0.483	NA	NA	NA
CD98	(Low vs. high)	0.967	0.494–1.892	0.921	0.945	0.473–1.889	0.873

Patients treated with surgery.

CI – confidence interval, HR – hazard ratio, NA – not applicable

motes anchorage independence and tumorigenesis if CD98 is overexpressed [10].

This study showed that CD98 expression was a poor prognostic factor for resistance to CRT and BRT. This might be correlated with the fact that cells expressing CD98 have the properties of cancer stem cells [12, 13, 16]. Some reports have shown that CD98 expression is associated with radiosensitivity. However, in vitro, they can explain why CD98hc expression regulates radiosensitivity by activating the mTOR/PI3K signaling pathway that promotes survival [14]. CD98hc knockdown HNSCC cells had high radiosensitivity and elevated autophagy levels. Moreover, autophagy activation overcomes nutritional stress caused by the loss of CD98hc and prevents radiation-induced cellular damage [17]. Both findings support the notion that CD98 expression is associated with a poor prognosis and resistance to CRT and BRT in HNSCC.

In the process of identifying cancer stem cells, the expression of stem cell genes, such as Nanog, OCT-4, and SOX2, was found to be correlated with recurrence rate and metastatic and invasive potential [18–20]. Therefore, immunostaining was performed to identify whether there is a correlation between CD98 expression and OCT-4, SOX2, and Nanog expression. In the area with high CD98 expression, OCT-4, Nanog, and SOX2 were also strongly expressed. However, weak CD98 expression did not indicate the absence of OCT-4, Nanog, and SOX2. We believe that if there is negative CD98 expression, all stem cell genes will be negative. However, this was not the case. In fact, the cancer stem cells were unknown. In HNSCC alone, different candidate markers, including CD44 [9], CD133 [21], ALDH1 [22], and ABCG2 [23], have been reported. Although it will be challenging to elucidate the origin of cancer stem cells using CD98 alone, we found that stem cells with strong CD98 expression significantly produce stem cell genes. If CD98 is overexpressed, the cancer stem cell genes are also strongly expressed, and this may be correlated with radioresistance in the high CD98 expression group.

The diagnosis of patients with high CD98 expression before treatment may lead to the provision of individualized treatments, including surgical treatment, and may contribute to a better prognosis.

Furthermore, the establishment of CD98-targeted therapies can increase radiosensitivity, which will significantly improve prognosis. Therefore, it is necessary to elucidate the mechanism of CD98 in vitro and in vivo, to assess more patients, and to increase the population size in future studies in collaboration with other institutions.

Conclusions

CD98 expression is a marker of radioresistance. *Via* CD98 immunostaining at the biopsy stage,

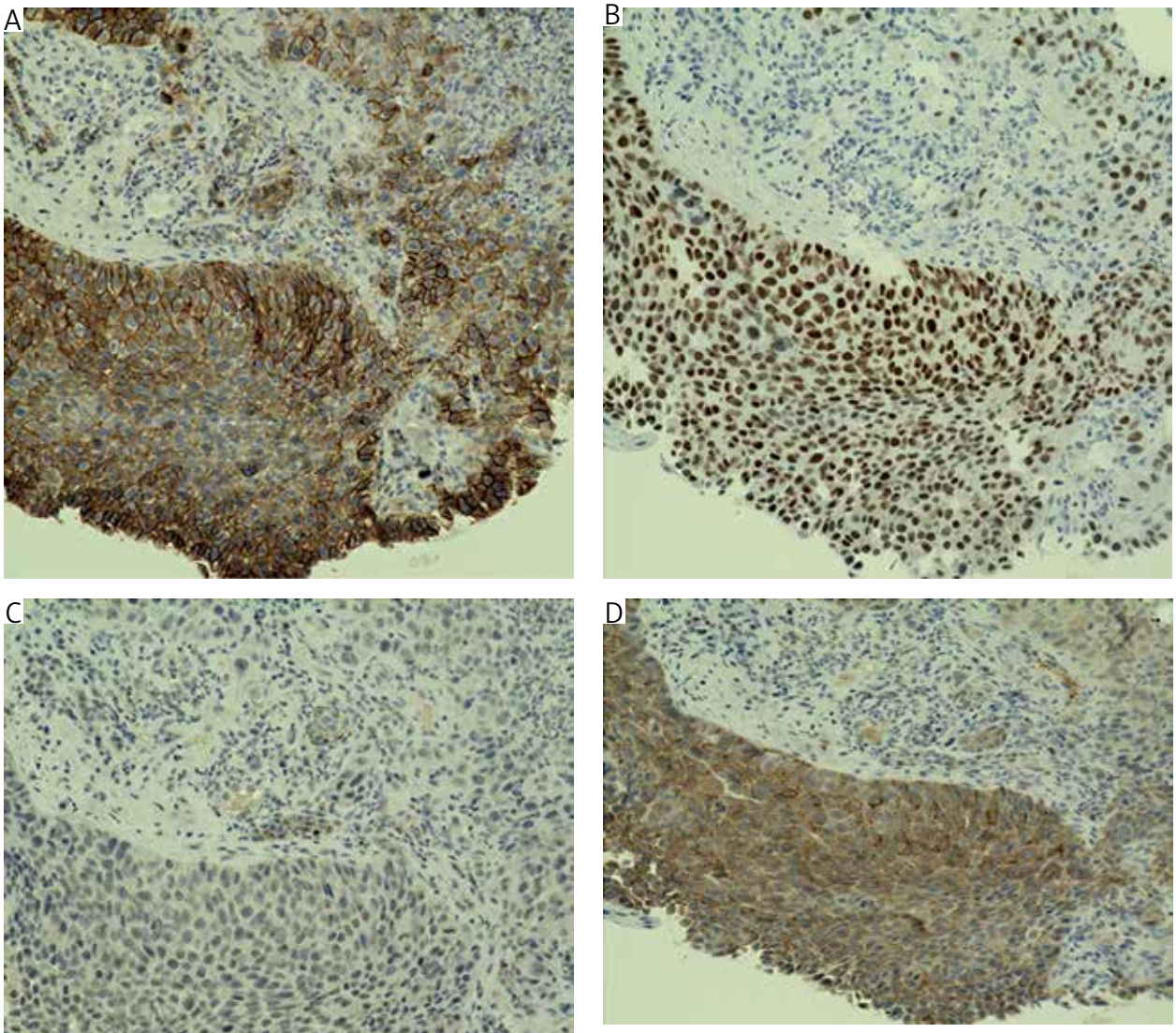


Fig. 5. Association of CD98 expression with cancer stem cell gene expression. When CD98 is highly expressed, cancer stem cell genes are also strongly expressed. The higher the CD98 expression, the stronger the expression of cancer-related genes: A) CD98 strong; B) SOX2; C) OCT-4; D) Nanog (magnification, 200×)

the disease outcomes can be predicted. Furthermore, therapies targeting CD98 should be established, and this can lead to a better prognosis in HNSCC.

The authors declare no conflict of interest.

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Address for correspondence

Yohei Kawasaki
Department of Otorhinolaryngology and Head and Neck Surgery
Akita University Graduate School of Medicine
Akita, Japan
e-mail: kawa0807@med.akita-u.ac.jp