Annals of Diagnostic Pathology 51 (2021) 151695

Contents lists available at ScienceDirect

Annals of Diagnostic Pathology

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

Original Contribution

The prognostic value of USP7 and p53 in advanced hypopharyngeal carcinoma

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ARTICLE INFO

Keywords:
Hypopharyngeal squamous cell carcinoma
Prognosis
USP7
p53

ABSTRACT

Background: Hypopharyngeal squamous cell carcinoma (HPSCC) is a rare malignancy of poor prognosis in head and neck. The aim of the study is to assess the expression and prognostic value of USP7 and p53 in advanced HPSCC.

Methods: A retrospective study was performed on a cohort of 103 patients with advanced HPSCC. The immunohistochemical expression of USP7 and p53 was evaluated in all the patients, and the prognostic value of USP7 and p53 was further evaluated. Overall survival (OS), disease-free survival (DFS), distant metastasis-free survival (DMFS), and local-regional recurrence-free survival (LRFS) were assessed using the Kaplan–Meier method and multivariate Cox regression analysis.

Results: In our study, 78 patients (75.7%) showed low expression of USP7, and the other 25 patients (24.3%) had high expression of USP7; additionally, high USP7 expression was associated with advanced T stage. Low expression of p53 was found in 52 patients (50.5%), while the other 51 patients (49.5%) had a high expression of p53. Our data revealed that low expression of p53 was associated with the advanced N stage (p=0.028). Kaplan–Meier analysis revealed that high expression of USP7 was significantly correlated with the inferior OS, DFS, DMFS, and LRFS, respectively (all p<0.05); additionally, high expression of p53 was correlated with superior OS (p=0.023). The Cox proportional multivariate hazard model revealed that high expression of USP7 was an independent predictor of poor OS, DFS, and LRFS, respectively (all p<0.05).

Conclusions: Our findings suggest that USP7 combined with p53 are reliable prognostic factors in patients with advanced HPSCC.

1. Introduction

Hypopharyngeal squamous cell carcinoma (HPSCC) is a rare malignancy which originates from the upper aerodigestive tract, and accounts for about 3% - 5% of all head and neck squamous cell carcinomas (SCCs) [1,2]. The submucosal spread is a characteristic of HPSCC which leads to the direct invasion of adjacent structures. As the rich lymphatics network of the hypopharynx, cervical lymph node metastasis is frequent in HPSCC. Most patients with HPSCC have advanced disease with lymph node metastasis at initial diagnosis as the symptoms at early stage are nonspecific [1]. It reported that about 60–80% of patients had ipsilateral node metastasis, and 40% patients had bilateral nodal metastases at initial diagnosis [1,3]. Despite aggressive treatment using combinations of surgery, radiotherapy, and chemotherapy, the clinical outcomes of advanced HPSCC remain poor. The reported five-year survival rates in advanced HSCC is only 15–45% [1-4]. Therefore, it is essential to identify valid biomarkers to predict the prognosis of HPSCC patients, which may also be beneficial for therapeutic targeting of HPSCC.

Abbreviations: HPSCC, hypopharyngeal squamous cell carcinoma; OS, overall survival; DFS, disease-free survival; DMFS, distant metastasis-free survival; LRFS, local-regional recurrence-free survival; SCCs, squamous cell carcinomas; USP7, ubiquitin specific peptidase 7; LSCC, laryngeal squamous cell carcinoma; IHC, immunohistochemistry; USPs, ubiquitin specific proteases; MDM2, murine double minute.

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https://doi.org/10.1016/j.anndiagpath.2020.151695

Available online 5 January 2021

Ubiquitin specific peptidase 7 (USP7) is a deubiquitinating enzyme that can remove ubiquitin and protect the substrate protein from degradation to keep stability [5]. USP7 plays a crucial part in several cellular processes, including immune response, tumor inhibition, DNA reparation, and epigenetic regulation [5-8]. The abnormal expression of USP7 has been shown to be associated with tumorigenesis and progression in a variety of tumors [9,10]. The overexpression of USP7 has been observed in a series of tumors, and high expression of USP7 was related to the poor prognosis, such as in lung cancer, colorectal cancer, breast cancer, cervical cancer, esophageal cancer, and so on [11-15]. In the laryngeal squamous cell carcinoma (LSCC), Zhang et al. also reported that LSCC tissues had high expression of USP7 while the normal laryngeal tissues showed negative expression of USP7 protein; additionally, high USP7 level was associated with poor pathologic differentiation, lymphatic invasion, advanced TNM stage and unfavorable clinical outcomes ($p < 0.001$) [16]. However, the expression of USP7 has never been evaluated in HPSCC. Hence, it will be meaningful to evaluate the expression and prognostic value of USP7 in HPSCC.

Up to now, numerous USP7 substrates have been identified, and p53 is the best characterized one [5,9,17]. USP7 can antagonize the functions of p53 through multiple mechanisms [8]. Tumor suppressor p53 is a key protein that participates in apoptosis, DNA repair, and cell cycle control. It plays a vital part in the maintenance of normal cell growth and cellular anticarcinogenic processes [18,19]. It was reported that the inactivation of tumor suppressor gene p53 was related to the oncogenesis of many malignancies [20-22]. Many studies have evaluated the prognostic value of p53 in head and neck SCC. However, its prognostic significance on the basis of immunohistochemistry remains controversial among those studies [22-24]. Hence, the immunohistochemical expression of p53 together with USP7 were evaluated in the HPSCC patients of this study as well.

So far, there were no reports regarding the expression and prognostic value of USP7 in advanced HPSCC. Here, we examine the USP7, p53, clinical information, and treatment results in a series of advanced HPSCC patients. As far as we know, our study is the first one which evaluates the expression and prognostic value of USP7 in advanced HPSCC patients.

2. Materials and methods

2.1. Patients

A cohort of consecutive HPSCC patients who had partial or total laryngectomy between 2014 and 2017 at the Eye, Ear, Nose, and Throat Hospital of Fudan University were included in this retrospective study. Our study was approved by the hospital's institutional review board, and the informed consent was not required as this was a retrospective study. Two senior head and neck pathologists confirmed the diagnoses of HPSCC in all the cases. The American Joint Committee on Cancer 7th staging manual was used, and advanced HPSCC patients with stage III or IV were included. All the patients had complete clinical, image, following up data, and available pathological slices. The exclusion criteria were as follows: distant metastasis at initial diagnosis; surgeries within five years; history of other malignancies; preoperative chemotherapy and/or radiotherapy.

2.2. Immunohistochemistry (IHC)

Tissue specimens were fixed in formalin and embedded in paraffin, and then sliced into sections of 4-μm thick. Rabbit anti-USP7 polyclonal antibody (ab4080; Abcam, UK; 1:100) and mouse anti-p53 antibody (Clone DO-7; Gene Tech, Shanghai, China; ready to use) were used. The sections were dewaxed and rehydrated according to the standard protocols. The sections were rinsed with phosphate-buffered saline, then immersed in 3% H_2O_2 solution (10 min) to block endogenous peroxidases. Incubation with 5% normal goat serum (20 min) was performed to block the non-specific antibody binding. The rabbit anti-USP7 antibody and mouse anti-p53 antibody were then used for incubation at 4 °C overnight. For the detection of antibody binding, incubation with DAB Horse-radish Peroxidase Color Development Kit (Fuzhou MaixinBiological Technology Development Co., Ltd.) for 1 h at 37 °C was performed. Diaminobenzidine solution and hematoxylin were used for the visualization and counterstain.

Table 1
The clinicopathological characteristics of the 103 patients with advanced HPSCC.

Variable	Number of patients (%)
Age at diagnosis	
<60y	54 (52.4)
≥60y	49 (47.6)
Sex	
Male	102 (99.0)
Female	1 (1.0)
Smoke	
No	14 (13.6)
Yes	89 (86.4)
Drink	
No	21 (20.4)
Yes	82 (79.6)
Site	
Pyriform sinus	91 (88.3)
Not pyriform sinus	12 (11.7)
Pathological grade	
G1+G2	80 (77.7)
G3	23 (22.3)
Stage	
III	31 (30.1)
IVA/IVB	72 (69.9)
T stage	
T1–3	66 (64.1)
T4a	37 (35.9)
N stage	
N0	11 (10.7)
N1–3	92 (89.3)
Laryngectomy	
Total	57 (55.3)
Partial	46 (44.7)
USP7	
Low	78 (75.7)
High	25 (24.3)
p53	
Low	52 (50.5)
High	51 (49.5)
Ki-67	
≤30%	57 (55.3)
>30%	46 (44.7)

Abbreviations: HPSCC, hypopharyngeal squamous cell carcinoma.
Note: G1, highly differentiated; G2, moderately differentiated; G3, poorly differentiated.

2.3. Immunohistochemical assessment

All the immunohistochemical slices were assessed by the two pathologists who were blinded to the clinicopathological information. They should reach a consensus on the result when there was a discrepancy. The most representative paraffin block was selected for immunohistochemical analysis. To overcome the intra-tumoral heterogeneity, ten fields were chosen under 400× magnification randomly, and the positive cells in the selected fields were counted. Nuclear staining for USP7 and p53 was qualitatively as well as semi-quantitatively evaluated. Staining intensity was categorized as follows: 0, no staining; 1,

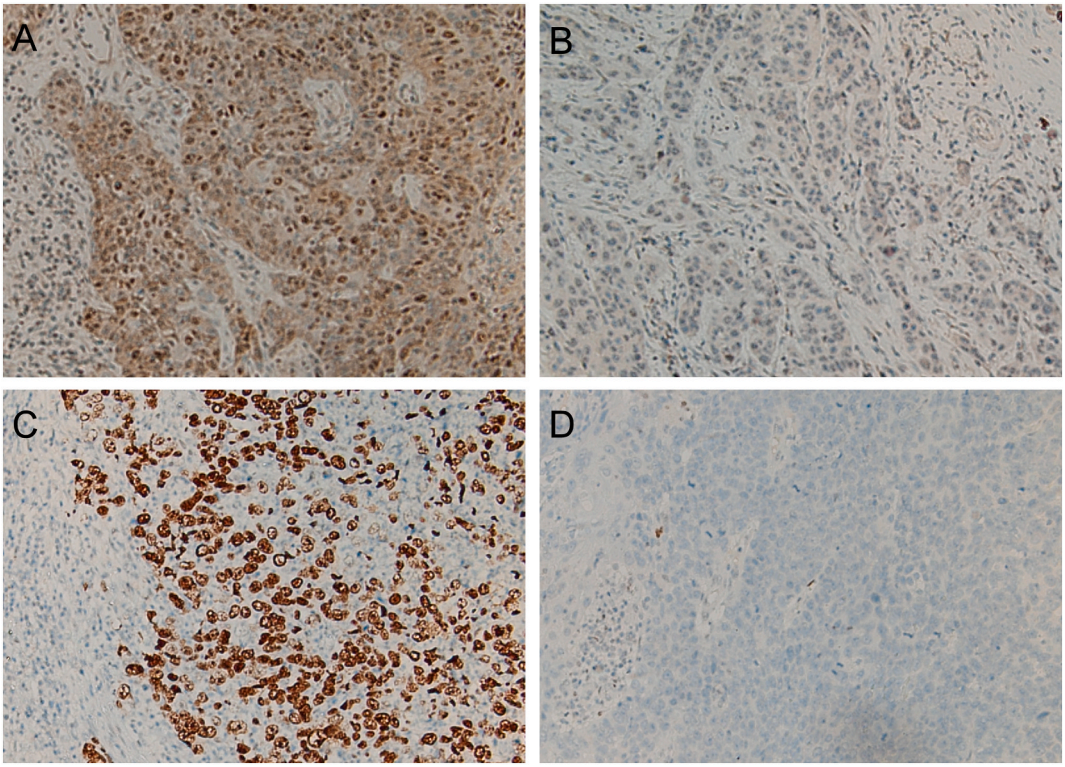

Fig. 1. Representative immunohistochemical staining of USP7 and p53 in patients with advanced HPSCC. (A) high expression level of USP7 (100×); (B) low expression level of USP7 (100×); (C) high expression level of p53 (100×); (D) low expression level of p53 (100×).
Abbreviations: HPSCC, hypopharyngeal squamous cell carcinoma.

weak; 2, intermediate; and 3, strong.

The expression of USP7 was defined as the product of (a) and (b): (a) Percentage of positive cells (0, <25%; 1, 25–50%; 2, 50–75%; 3, >75%); and (b) Immunostaining intensity (0, negative; 1, weak; 2, intermediate; 3, strong). Tumors were defined as USP7 high group when a × b ≥4, and as USP7 low group when a × b <4 [25]. Tumors were defined as p53 high group when > 50% of tumor cells were with intermediate/strong nuclear staining; while tumors in p53 low group showed moderate/strong nuclear staining in no more than 50% of tumor cells or weak nuclear staining or no staining of tumor cells [26].

2.4. Statistical analysis

The endpoints were overall survival (OS), disease-free survival (DFS), distant metastasis-free survival (DMFS), and local-regional recurrence-free survival (LRFS). OS was defined as the time from diagnosis to the last follow up or death from any cause. DFS was calculated as time to relapse of HPSCC or the last follow up or death from any cause. DMFS was calculated as time to detection of distant metastasis. LRFS was calculated as time to detection of local-regional recurrence. Statistical analyses were carried out using IBM SPSS Statistics version 22.0 (IBM, Armonk, NY, USA). The Mann Whitney *U* test was performed for continuous variables, while the Fisher's exact test or chi-square test for categorical variables. OS, DFS, DMFS, and LRFS were evaluated using the Kaplan-Meier method and log-rank test. The univariate and multivariate analyses were carried out with the Cox proportional hazards model. $P < 0.05$ was regarded as significant.

3. Results

3.1. Patients

Finally, 103 patients were included in the study. The primary clinical data are summarized in Table 1. There were 102 men (99.0%) and 1 woman (1.0%). The majority of patients had a history of smoking (*n*=89, 86.4%) or alcohol intake (*n*=82, 79.6%). The subsite of the primary tumor was the pyriform sinus in 91 patients (88.3%), and was the posterior wall or post-cricoid region in the other 12 patients (11.7%). Sixty-six patients (64.1%) were with T1–T3 stage, and the remaining 37 patients (35.9%) were with T4a stage. Eleven patients had N0 stage (10.7%), while the other 92 patients (89.3%) were with N1–N3 stage. All the 103 patients were in the advanced stage, with 31 patients (30.1%) in stage III and the other 72 patients (69.9%) in stage IVA/IVB. Surgery was performed in all the patients, among which 57 patients had total laryngectomy and the others had partial laryngectomy. Table 1 showed the detailed characteristics of patients. The tumors were highly to moderately differentiated in 80 patients (77.7%), and were poorly differentiated in 23 patients (22.3%).

The median follow-up time was 27.0 months (interquartile range 19.0–34.4 months). During the follow-up period, thirty-one patients (30.1%) developed treatment failure. 17 patients (16.1%) had local recurrence, while distant metastasis was observed in 19 patients (18.4%). Twenty-three patients (22.3%) died from HPSCC, and two (1.9%) patients died from other causes. The 3-year OS, DFS, DMFS and LRFS for all the 103 patients were 64.1% (95% confidence interval [CI], 50.6% to 77.6%), 59.9% (95%CI, 46.8% to 73.0%), 74.3% (95%CI, 62.0% to 86.6%) and 83.2% (95%CI, 75.9% to 90.5%), respectively.

3.2. USP7 expression

The expression of USP7 was mainly located in the tumor nucleus in HPSCC. In our study, 78 patients (75.7%) had a low level of USP7, and 25 patients (24.3%) had a high level of USP7 (Fig. 1). In our study, a significant correlation was found between USP7 expression level and T stage (*p*=0.016), and a high USP7 expression level was related to advanced T stage (Table 2).

Table 2
Relationship of USP7 and p53 to clinicopathological characteristics.

Variable	USP7		P	p53		P	USP7/p53				P
	Low	High		Low	High		USP7 low/p53 low	USP7 high/p53 low	USP7 low/p53 high	USP7 high/p53 high	
Age at diagnosis			0.250			1.000					0.547
<60y	38	16		27	27		20	7	18	9	
≥60y	40	9		25	24		20	5	20	4	
Sex			1.000			1.000					0.662
Male	77	25		51	51		39	12	38	13	
Female	1	0		1	0		1	0	0	0	
Smoke			1.000			0.390					0.728
No	11	3		9	5		7	2	4	1	
Yes	67	22		43	46		33	10	34	12	
Drink			0.271			0.329					0.435
No	18	3		13	8		11	2	7	1	
Yes	60	22		39	43		29	10	31	12	
Site			1.000			0.358					0.694
Pyriform sinus	69	22		44	47		34	10	35	12	
Not pyriform sinus	9	3		8	4		6	2	3	1	
Pathological grade			1.000			0.816					0.554
G1+G2	60	20		41	39		30	11	30	9	
G3	18	5		11	12		10	1	8	4	
Stage			0.316			1.000					0.593
III	26	5		16	15		13	3	13	2	
IVA/IVB	52	20		36	36		27	9	25	11	
T stage			0.016*			0.682					0.074
T1–3	55	11		32	34		28	4	27	7	
T4a	23	14		20	17		12	8	11	6	
N stage			0.288			0.028*					0.011*
N0	10	1		2	9		1	1	9	0	
N1–3	68	24		50	42		39	11	29	13	
Laryngectomy			0.066			0.554					0.202
Total	39	18		27	30		18	9	21	9	
Partial	39	7		25	21		22	3	17	4	
USP7						0.821					<0.001
Negative	/	/		40	38		40	0	38	0	
Positive	/	/		12	13		0	12	0	13	
p53			0.821								<0.001
Negative	40	12		/	/		40	12	0	0	
Positive	38	13		/	/		0	0	38	13	
Ki-67			1.000			0.431					0.740
≤30%	43	14		31	26		23	8	20	6	
>30%	35	11		21	25		17	4	18	7	

Note: G1, highly differentiated; G2, moderately differentiated; G3, poorly differentiated.
* $p < 0.05$.

The mean OS, DFS, DMFS, and LRFS time were 35.7, 33.4, 36.9, and 38.0 months in the USP7 low group; and were 29.6, 25.7, 33.1, and 31.0 months in the USP7 high group. Fig. 2A showed that the 3-year OS rate in the USP7 high group was significantly lower than that in the USP7 low group (48.8% vs 68.2%, p=0.005). Similarly, the 3-year DFS rate, DMFS rate, and LRFS rate in the USP7 low group was significantly higher than those in the USP7 high group (62.5% vs 45.5%, p=0.008, Fig. 2B; 77.0% vs 63.6%, p=0.042, Fig. 2C; 89.6% vs 63.1%, p=0.002, Fig. 2D).

3.3. p53 expression

In all the 103 patients, 52 patients (50.5%) had low expression of p53, and the other 51 patients (49.5%) had a high expression of p53 (Fig. 1). Our data showed that low expression of p53 was related to advanced N stage (p=0.028) (Table 2). The mean OS, DFS, DMFS, and LRFS time were 31.7, 29.3, 33.5, and 35.4 months in the p53 low group; and were 39.2, 35.7, 40.5, and 39.8 months in the p53 high group. The 3-year OS rate of the p53 low group was significantly lower compared with that of the p53 high group (54.4% vs 75.8%, p=0.023) (Fig. 2E). The 3-year DFS rate, DMFS rate and LRFS rate in the p53 low and p53 high group were 52.0% vs 67.0%, 61.4% vs 88.1%, and 80.8% vs 86.1%, respectively. However, no significant differences were found in 3-year DFS rate, DMFS rate and LRFS rate between the patients in the two groups (p=0.086, 0.066, 0.369, respectively, Fig. 2.F, G, H).

3.4. Combination of USP7 and p53

According to the immunohistochemical expression of USP7 and p53, the patients were divided into four groups. Among the 103 patients, 12 patients were with USP7 high/p53 low expression, 38 patients with USP7 low/p53 high expression, 40 patients were with USP7 low/p53 low expression, while 13 patients with USP7 high/p53 high expression. Our data revealed that high expression of USP7 combined with low p53 was associated with advanced N stage (p=0.011) (Table 2).

The mean OS, DFS, DMFS, and LRFS time were 24.1, 17.5, 28.9, and 24.3 months in the USP7 high/p53 low group; and were 35.4, 32.1, 36.4, and 36.1 months in the USP7 low/p53 high group. The 3-year OS rate in the USP7 low/p53 high group was significantly higher compared with that in the USP7 high/p53 low group (75.3% vs 33.3%, p=0.003) (Fig. 3A). Similarly, the 3-year DFS rate, DMFS rate and LRFS rate in the USP7 low/p53 high group was significantly higher compared with those in the USP7 high/p53 low group (63.1% vs 25.0%, p=0.001, Fig. 3B; 92.1% vs 53.3%, p=0.041, Fig. 3C; 89.4% vs 50.0%, p=0.001, Fig. 3D).

3.5. Univariable and multivariable analyses

Clinicopathologic parameters, including pathological grading, T stage, N stage, USP7 and p53 expression, were assessed to identify predictive factors of HPSCC patients' OS, DFS, and DMFS and LRFS.

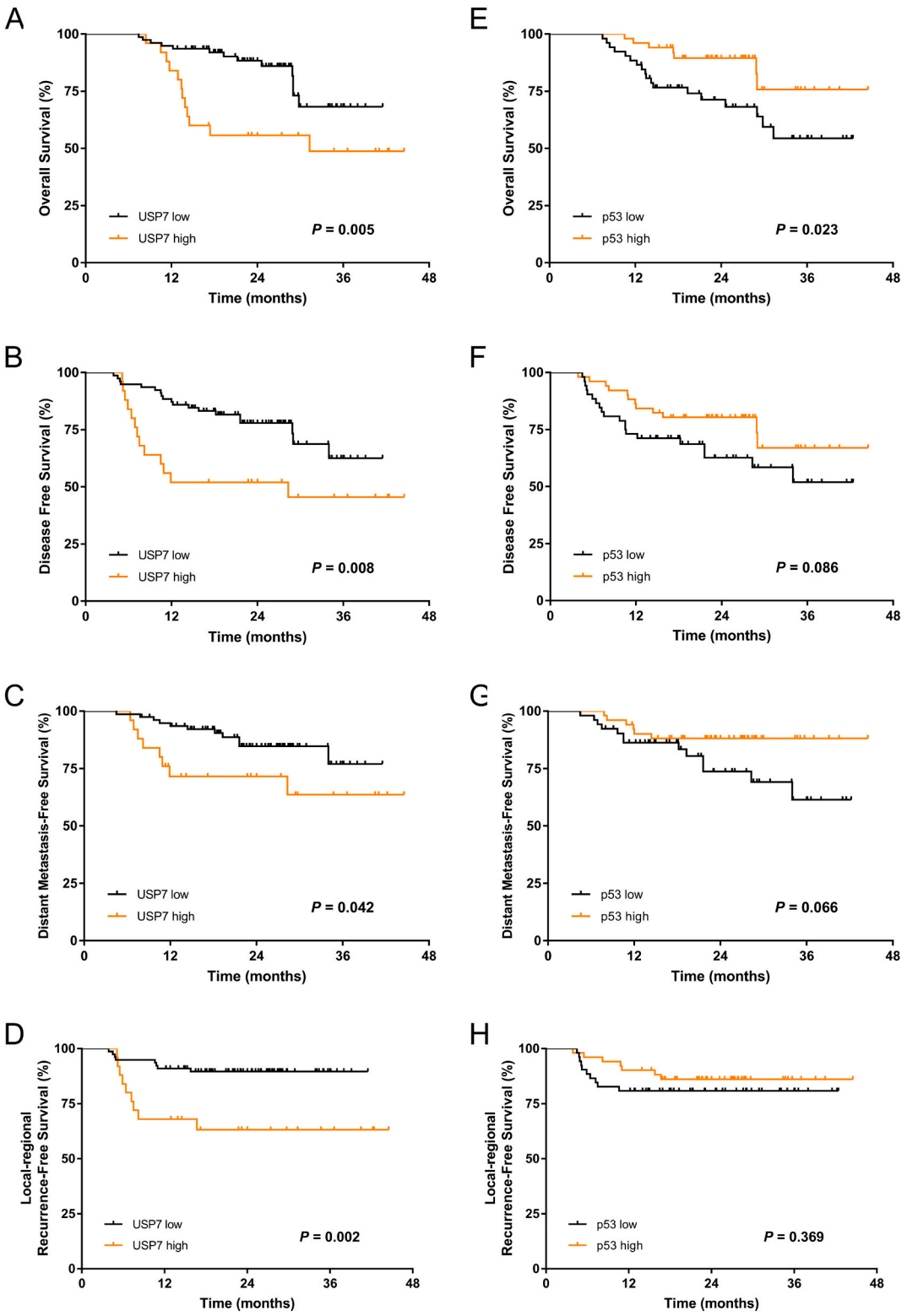

Fig. 2. Kaplan–Meier survival curves of OS (A), DFS (B), DMFS (C), and LRFS (D) according to the expression level of USP7; survival curves of OS (E), DFS (F), DMFS (G), and LRFS (H) according to the expression level of p53. *P*-values were calculated by the log-rank test.
Abbreviations: HPSCC, hypopharyngeal squamous cell carcinoma; OS, overall survival; DFS, disease-free survival; DMFS, distant metastasis-free survival; LRFS, local-regional recurrence-free survival.

Table 3 displayed the statistically significant predictors for OS, DFS, and DMFS and LRFS, which were identified by univariate analyses. The predictors of poor OS included old age, smoking history, posterior hypopharyngeal wall or postcricoid space location of primary tumor, T4a stage, high USP7, and low p53. The multivariate analyses showed that old age and high USP7 were independently correlated with worse OS (p=0.016, HR: 0.304, 95% CI: 0.115–0.804; p=0.004, HR: 3.389, 95% CI: 1.480–7.756) (Table 4). The univariate analyses identified that

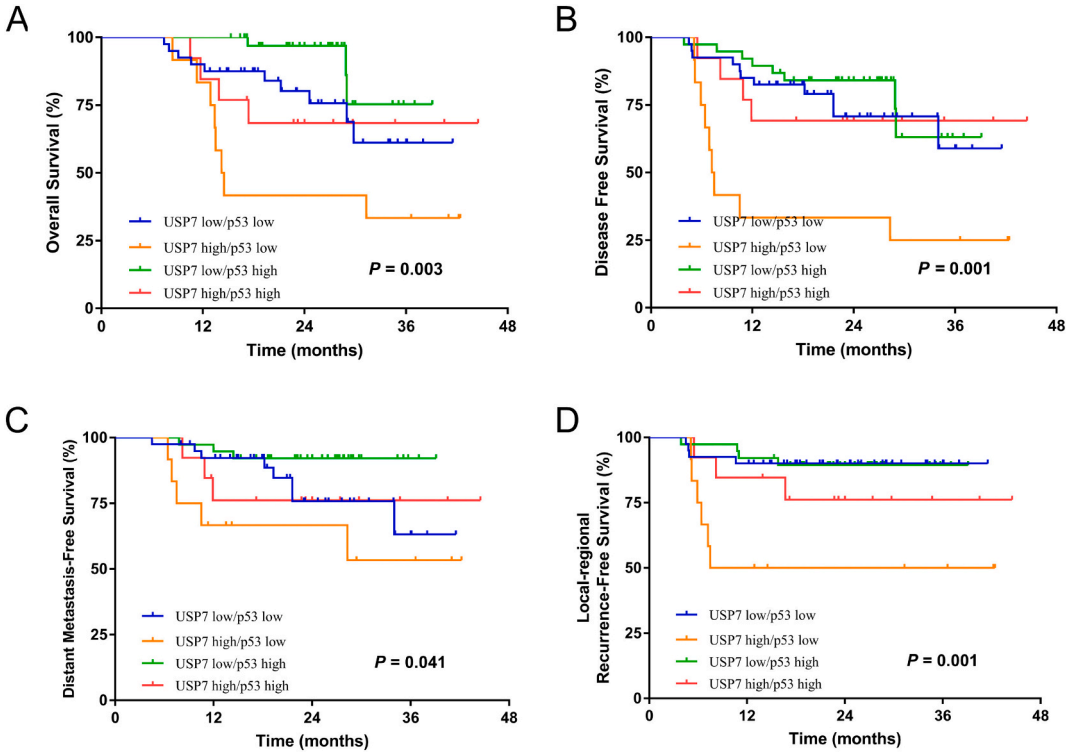

Fig. 3. Kaplan–Meier curves showed that HPSCC patients with USP7 low/p53 high expression had superior OS (A), DFS (B), DMFS (C), and LRFS (D) compared with those having USP7 high/p53 low expression.
Abbreviations: HPSCC, hypopharyngeal squamous cell carcinoma; OS, overall survival; DFS, disease-free survival; DMFS, distant metastasis-free survival; LRFS, local-regional recurrence-free survival.

Table 3
Univariate analyses of factors in relation to the 3-year OS, DFS, DMFS, and LRFS using the Cox proportional hazards model (n=103).

Variables	OS			DFS			DMFS			LRFS		
	HR	95%CI	P	HR	95%CI	P	HR	95%CI	P	HR	95%CI	P
Age, years (≥60y vs <60y)	0.364	0.152–0.874	0.024*	0.425	0.201–0.898	0.025*	0.552	0.217–1.404	0.212	0.303	0.099–0.931	0.037*
Smoke (yes vs no)	0.387	0.162–0.928	0.033*	0.446	0.200–0.994	0.048*	0.389	0.140–1.083	0.071	1.175	0.269–5.139	0.831
Drink history (yes vs no)	0.474	0.204–1.100	0.082	0.593	0.274–1.283	0.185	0.609	0.219–1.695	0.343	1.172	0.337–4.079	0.803
Site (Not PS vs PS)	2.618	1.044–6.568	0.040*	2.719	1.174–6.299	0.020*	2.556	0.845–7.731	0.096	2.661	0.867–8.166	0.087
Grade (G3 vs G2+G1)	0.856	0.321–2.281	0.755	0.928	0.401–2.146	0.861	1.647	0.626–4.335	0.312	1.416	0.499–4.019	0.514
Stage (IVA/IVB vs III)	1.584	0.631–3.973	0.327	2.154	0.885–5.238	0.091	4.071	0.940–17.627	0.060	3.456	0.790–15.119	0.100
T stage (T4a vs T1–3)	2.727	1.236–6.015	0.013*	2.776	1.378–5.591	0.004*	3.558	1.400–9.047	0.008*	2.811	1.069–7.394	0.036*
N stage (N1–3 vs N0)	3.564	0.481–26.389	0.213	4.557	0.622–33.404	0.136	2.623	0.350–19.681	0.348	24.391	0.039–15.304	0.331
Laryngectomy (total vs partial)	2.366	0.941–5.950	0.067	2.260	1.044–4.891	0.039*	0.573	0.217–1.511	0.260	4.200	1.207–14.62	0.024*
USP7 (high vs low)	2.931	1.328–6.469	0.008*	2.527	1.241–5.145	0.011*	2.498	1.000–6.240	0.050	4.049	1.558–10.522	0.004*
p53 (high vs low)	0.378	0.157–0.907	0.029*	0.539	0.263–1.103	0.091	0.415	0.158–1.094	0.075	0.644	0.245–1.694	0.373

Abbreviations: OS, overall survival; DFS, disease-free survival; DMFS, distant metastasis-free survival; LRFS, local-regional recurrence-free survival; HR, hazard ratio; CI, confidence interval; PS, pyriform sinus.
Note: G1, highly differentiated; G2, moderately differentiated; G3, poorly differentiated.
* $p < 0.05$.

old age, smoking history, posterior hypopharyngeal wall or postcricoid space location of primary tumor, T4a stage, partial laryngectomy, and high USP7 were identified as predictors of poor DFS (Table 3). The multivariate analyses identified that old age (p=0.047, HR: 0.452, 95% CI: 0.207–0.988), smoking history (p=0.034, HR: 0.396, 95% CI: 0.169–0.932), T4a stage (p=0.021, HR: 2.385, 95% CI: 0.908–4.679), and high USP7 (p=0.04, HR: 2.178, 95% CI: 1.036–4.577) were significantly correlated with worse DFS (Table 4). The univariate analyses identified that T4a stage was identified as predictor of poor DMFS (p=0.008, HR: 3.558, 95% CI: 1.400–9.047) (Table 3). The univariate analyses identified that old age, T4a stage, partial laryngectomy, and high USP7 were identified as predictors of poor LRFS (Table 3). The multivariate analyses (Table 4) showed that high USP7 (p=0.010, HR: 3.599, 95% CI: 1.364–9.500) were significantly correlated with worse LRFS.

4. Discussion

HPSCC is a malignancy of male predominance which often occurs in their 50s–70s. The etiology of HPSCC is not fully understood, while high tobacco and alcohol consumption are well-known risk factors. In our study, there was only one female among all the 103 patients who was an old woman (>60 years old) with advanced HPSCC (T2N2M0, IVA) originated from the pyriform sinus. Most patients in our cohort had a

Table 4
Multivariate analyses of factors in relation to the 3-year OS, DFS, and LRFS using the Cox proportional hazards model (n=103).

Variables	OS			DFS			LRFS		
	HR	95%CI	P	HR	95%CI	P	HR	95%CI	P
Age, years (≥60y vs <60y)	0.304	0.115–0.804	0.016*	0.452	0.207–0.988	0.047*	0.353	0.112–1.106	0.074
Smoke (yes vs no)	0.486	0.193–1.228	0.127	0.396	0.169–0.932	0.034*	/	/	/
Site (Not PS vs PS)	1.749	0.662–4.623	0.260	1.971	0.829–4.690	0.125	1.394	0.311–6.255	0.664
T stage (T4a vs T1–3)	1.760	0.745–4.161	0.198	2.385	1.143–4.975	0.021*	3.061	0.970–9.656	0.056
Laryngectomy (total vs partial)	/	/	/	2.061	0.908–4.679	0.084	1.970	0.731–5.311	0.180
USP7 (high vs low)	3.389	1.480–7.756	0.004*	2.178	1.036–4.577	0.040*	3.599	1.364–9.500	0.010*
p53 (high vs low)	0.387	0.149–1.007	0.052	/	/	/	/	/	/

Abbreviations: OS, overall survival; DFS, disease-free survival; LRFS, local-regional recurrence-free survival; HR, hazard ratio; CI, confidence interval; PS, pyriform sinus.
* $p < 0.05$.

history of smoking and/or drinking, and pyriform sinus was the primary location in 88.3% patients. The optimal treatment for advanced HPSCC remains under debate due to its poor prognosis. Generally, there is a lack of histological biomarkers for prognosis in HPSCC. This study was performed to discover new prognostic biomarkers which may be helpful to the clinical treatment decisions.

To date, USP7 is the one of the most often studied deubiquitinating enzyme of the ubiquitin specific proteases (USPs) family which consist of nearly 50 members [5,27]. The overexpression of USP7 could be detected in a series of tumors, and was reported to affect the tumor progression [8,11-16]. In our study, the high expression of USP7 could be detected in 24.3% of the HPSCC patients, and a significant correlation was found between the high USP7 expression level and advanced T stage. Additionally, patients with high USP7 expression had inferior OS, DFS, DMFS, and LRFS compared with those having low USP7 expression (all $p<0.05$). The univariate analyses showed that high expression of USP7 was an independent prognostic factor of poor OS, DFS, and LRFS (all $p<0.05$). Hence, USP7 may be a reliable marker for prognosis in HPSCC patients.

USP7 was initially identified as a cellular factor which was associated with herpes virus, and subsequently was found to deubiquitinate and stabilize p53 [28]. USP7 could regulate the level of p53 through the USP7-MDM2-p53 molecular axis [28]. Murine double minute (MDM2) can inhibit the p53 transcription, and USP7 could promote the p53 degradation through deubiquitination of MMD2 [29]. Hence, once the USP7-MDM2-p53 interaction is activated, the p53 level is down regulated, which will promote the tumor development. The USP7 inhibitors can suppress the tumor growth and invasion in models in vitro as well in vivo [12,17,30], and now they have been explored for clinical antitumor treatment [12,31]. P5091, as the selective inhibitor of USP7, can stabilize the level of p53 and inhibit proliferation of tumors [32,33]. Hence, USP7 may be a potential therapeutic target in HPSCC. In our study, no significant relevance was found between the expression level of USP7 and p53, which may imply that the effects of USP7 do not depend on p53. An et al. [12] also showed that the proliferation of colorectal cancer cells could be inhibited after the knockdown of USP7 despite the different TP53 status. Hence, USP7 may still play a vital part in the regulation of apoptosis even without p53.

p53 was identified as the first substrate of USP7-mediated deubiquitination [28]. The prognostic value of p53 expression has been evaluated by many studies. However, the results in these studies were inconsistent [20,21,23,24]. Studies have reported that p53 overexpression was related to higher tumor grade, TNM stage, as well as poor survival [34]. Hashmi et al. [23] reported that immunohistochemical overexpression of p53 was significantly related to higher tumor grade as well as extra-nodal extension in head and neck SCCs. However, a meta-analysis showed that p53 was not a prognostic biomarker for oral tongue SCCs [35]. It may be explained by the different techniques used for immunohistochemistry, and the variable cutoff values used for the definition of immunohistochemical overexpression of p53 in these studies. In our study, 50.5% of patients had low expression of p53, and low expression of p53 was related to advanced N stage ($p=0.028$). Moreover, the patients with low p53 expression had an inferior 3-year OS rate compared with those with high p53 expression ($p=0.023$). While for the analysis of the combination of USP7 and p53, the 3-year OS, DFS, DMFS, and LRFS rate of the USP7 low/p53 high group were all significantly higher than those of the USP7 high/p53 low group ($p=0.003$, 0.001, 0.041, 0.001, respectively). Therefore, the high expression of p53 is a favorable predictor in advanced HPSCC patients.

5. Conclusion

In summary, our results revealed that high expression of USP7 was related to advanced T stage, and HPSCC patients with high USP7 level had inferior OS, DFS, DMFS, and LRFS. In addition, low expression of p53 was associated with advanced N stage, and patients with low p53 level had an inferior OS rate. Survival analyses identified that high expression of USP7 was independent predictors of poor OS, DFS, and LRFS. Hence, USP7 combined with p53 are reliable markers for prognosis in patients with advanced HPSCC, and USP7 may be a promising target for oncotherapy in HPSCC that needs further research.

Ethics approval and consent to participate

This study was approved by the committee of the Eye & ENT Hospital of Fudan University, Shanghai, China. The study was conducted in accordance with the spirit of Helsinki Declaration. All patients provided written informed consent before surgery. Information of patients were anonymized and de-identified prior to analysis.

Funding

None.

Authors' contributions

JW and CYH participated in the design of the study. JS and JHZ performed the immunostaining techniques. CYH and LL analyzed the data. JW drafted the manuscript. CYH revised the manuscript. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare no competing financial interests.

Acknowledgements

Acknowledgement to the technical staff of Pathology Department of Eye & ENT Hospital of Fudan University.

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