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BP180 Is a Prognostic Factor in Head and Neck Squamous Cell Carcinoma

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Abstract. *Background/Aim: Prognosis plays a vital role in head and neck squamous cell carcinoma (HNSCC) patient management and decision-making. This study aimed to identify the role of BP180 as a prognostic factor in HNSCC. Patients and Methods: Protein expression of bullous pemphigoid antigen II (BP180) was verified by immunohistochemistry (IHC) in a tissue microarray study of 202 cases. Results: IHC analysis revealed that protein expression of BP180 among HNSCC patients differed significantly in the presence and absence of neural invasion, and according to T status in laryngeal and pharyngeal cancer subgroups. Overall survival and multivariate analysis showed that positive BP180-IHC and advanced clinical stage were significant independent positive predictors of mortality in HNSCC patients. In addition, in the oral cancer subgroup, independent positive predictors were positive BP180-IHC, advanced N status and neural invasion. In laryngeal and pharyngeal cancer subgroups, predictors were positive BP180-IHC and advanced clinical stage. Conclusion: BP180 is a prognostic factor in head and neck squamous cell carcinoma.*

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Head and neck cancer was reported as the 7th most common cancer worldwide in 2018, and the vast majority of malignant head and neck cancers are head and neck squamous cell carcinomas (HNSCCs) (1). Squamous cell carcinoma (SCC) affects the oral cavity, nasopharynx, oropharynx, hypopharynx and larynx. Early-stage patients with stage I or II disease can be cured with surgery alone and/or adjuvant therapies, improving long-term survival rates in approximately 70-90% of patients (2). About 60% of HNSCC patients present with stage III or IV disease (3). Such locally advanced disease shows high malignancy, easily develops early metastasis, and carries a poor prognosis with an overall 5-year survival rate below 50%. This pathology also displays a local recurrence rate of 15-40% and frequent distant metastasis (4).

Bullous pemphigoid antigen II (BP180; also called collagen XVII, BPA-2 or BPAg2) is not only an epithelial transmembrane protein, but also a hemidesmosome transmembrane adhesion molecule, and likely participates in keratinocyte-matrix interactions in both physiological and pathological conditions (5, 6). The intracellular domain contains binding sites for plectin, integrin b4, and BP230 (7). Hemidesmosomes are adhesion complexes connecting keratin intermediate filaments of stratified and complex epithelia to extracellular matrix components (EMCs) (8, 9). EMCs are responsible for cell communications, adhesion and proliferation, and has also been recognized as playing key roles in both progression and tumor initiation (10). Aberrant expression of BP180 has been reported in many malignant tumors, such as skin (11, 12), esophageal (13), oral (14), pancreatic (11, 15), colorectal (16, 17), lung (18-20), cervical (12, 21, 22), breast (21, 23), nasopharyngeal (24) and

salivary gland cancer (25, 26). A murine monoclonal antibody designated NCC-Lu-226 [immunoglobulin (Ig) G1, K] was obtained from NCC (National Cancer Center, Tokyo, Japan) has been used in previous studies that found aberrant expression of BP180 in solar keratosis, Bowen's disease, invasive skin squamous cell carcinoma, lung squamous cell carcinoma, esophageal squamous cell carcinoma and cervix squamous cell carcinoma (13). According to recent research using BP180 overexpression and knock-out models, this protein is presumed to play roles in cell migration and differentiation under pathological conditions (5, 7). Although many cancers are reportedly associated with BP180, clinical prognostic relationships have not yet been described.

This study evaluated protein expression levels of BP180 immunohistochemically using tissue microarrays (TMAs) for 202 surgical specimens of HNSCC and normal squamous epithelium. In addition, data on prognosis were analyzed using data for overall survival (OS), disease-free survival (DFS) and multivariate analysis. This study may contribute to understanding the prognostic influence of BP180 in malignant phenotypes of HNSCC.

Patients and Methods

Patients and tissue samples. This study investigated 202 tumor specimens from 202 patients who underwent surgical resection with curative intention for head and neck carcinoma at the National Cancer Center Hospital (Tokyo, Japan) between 2006 and 2016. Clinicopathological records were retrospectively reviewed. In this cohort, median follow-up of the 202 patients was 34 months (range=2-74 months). Formalin-fixed paraffin-embedded tissue specimens of the 202 HNSCCs were collected according to the World Health Organization classification (4th edition) (27) of HNSCC (Table I). All study protocols were approved by the ethics committee of the National Cancer Center (approval #2013-247).

TMA construction. TMAs were prepared from formalin-fixed, paraffin-embedded pathological blocks as previously described (28). The blocks were sectioned at a thickness of 4 μM and subjected to immunohistochemical analyses.

Immunohistochemistry (IHC). Serial 4-μM-thick sections were incubated with the mouse monoclonal anti-human BP180 antibody established by our laboratory (collagen XVII) [1:1000, NCC-Lu-226 (immunoglobulin (Ig) G1, K), National Cancer Center Research Institute, Tokyo, Japan] (13) using the Ventana DABMap detection kit and automated slide stainer (Discovery XT) (Ventana Medical Systems, Tucson, AZ, USA) (29, 30). Head and neck normal tissues stained positively for BP180 antibody were recognized as controls. Expression levels of BP180 protein were used to classify two groups. The first was a BP180-negative group in which no tumor cells were stained with BP180 antibody, tumor cells were stained at weaker intensity compared with normal tissue staining, or some tumor cells were more intense compared with normal tissue staining, but comprised less than 10% of the tumor cell area. The other was a BP180-positive group, comprised all other findings. Staining patterns were evaluated by two independent investigators blinded to clinical information. Representative staining patterns are shown in Figure 1.

Table I. *Baseline characteristics of HNSCC patients with positive/negative IHC staining for BP180*.

	Number of cases (%)	BP180 IHC		*p*-Value
		Negative	Positive	
Total	202	79 (39.1)	123 (60.9)	
Age, years				
Median (range)	68.0 (29-95)			0.387
<68	94 (46.5)	40	54	
≥68	108 (53.5)	39	69	
Gender				0.058
Male	156 (77.2)	67	89	
Female	46 (22.8)	12	34	
Smoking status				0.248
No	90 (44.6)	31	59	
Yes	112 (55.4)	48	64	
Drinking status				0.146
No	114 (56.4)	50	64	
Yes	88 (43.6)	29	59	
Disease				0.643[#]
Oral cancer	98 (48.5)	25 (25.5)	73 (74.5)	0.084[#]
Tongue cancer	58 (59.2)	11 (19.0)	47 (81.0)	
Mouth floor cancer	12 (12.2)	2 (16.7)	10 (83.3)	
Gingival cancer	17 (17.3)	8 (47.1)	9 (52.9)	
Others	11 (11.3)	4 (36.4)	7 (63.6)	
Laryngeal cancer	14 (6.9)	8 (57.1)	6 (42.9)	
Oropharyngeal cancer	42 (20.8)	20 (47.6)	22 (52.4)	
Hypopharyngeal cancer	41 (20.3)	20 (48.8)	21 (51.2)	
Other	7 (3.5)	6 (85.7)	1 (14.3)	

IHC: Immunohistochemistry; HNSCC: head and neck squamous cell cancer; BP180: also known as collagen XVII, BPA-2 or BPAg2; [#]Fisher's exact test.

Statistical analysis. SPSS version 23.0 statistical software (IBM, Armonk, NY, USA) was used for analysis. Values of $p<0.05$ were taken as indicating statistically significant results. Significant differences were detected using Student's *t*-test, Pearson's chi-square test or Fisher's exact test. OS and DFS were measured as the period from surgery to date of death or recurrence as estimated by the Kaplan–Meier log-rank test. Uni- and multivariate analyses were performed by Cox proportional regression hazard modeling.

Results

Protein expression of BP180 and baseline characteristics in HNSCC patients. Examples of positive expression of BP180 in HNSCC are shown in Figure 1. BP180-positive staining was evident in tumor cell membranes and faintly in the cytoplasm (Figure 1C, D, F, G), and also as linear staining along the basement membrane in normal tissues (Figure 1H, I). BP180-positive cases usually showed aberrant non-basal expression, such as immunoreaction mainly restricted to the peripheral cells of tumor nests and the invasive front (Figure

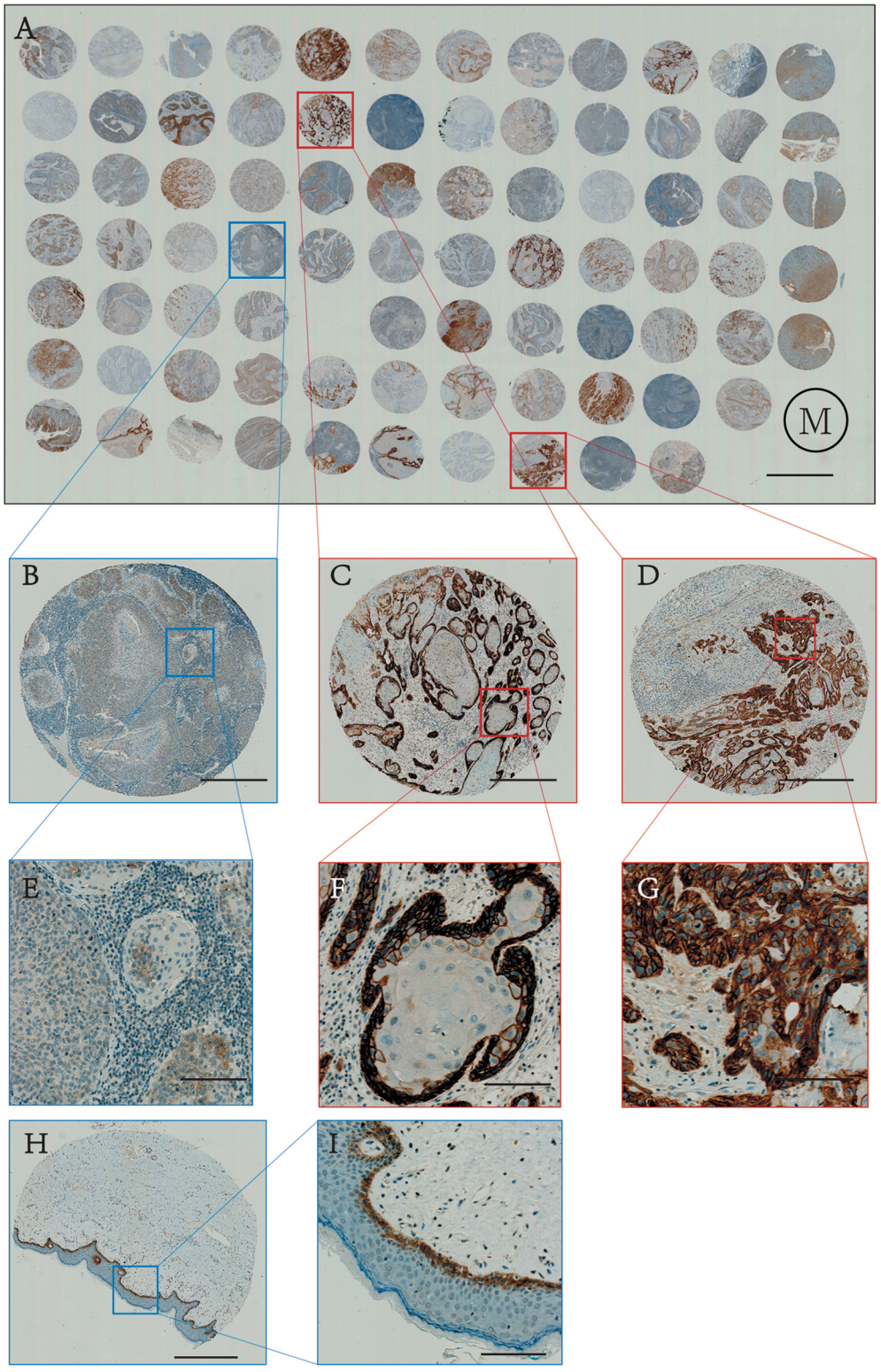

Figure 1. *Immunohistochemistry of BP180 protein expression. A) Representative BP180 protein expression in one HNSCC TMA slide (bar, 5 mm). M, TMA slide marker. B, E) Representative negative expressions of BP180 (bars: 500 μm in B, 100 μm in E). C, D, F, G) Representative positive expressions of BP180 (F: immunoreaction restricted to peripheral cells of cancer nest; G: immunoreaction at invasion front; bars: 500 μm in C and D, 100 μm in F and G). H, I) Representative BP180 protein expression in normal tissue as a positive control (linear staining along basement membrane; bars: 500 μm in H, 100 μm in I).*

Table II. *Association between BP180 IHC status and clinicopathological characteristics in HNSCC patients.*

	Number of cases (%)	BP180 IHC		*p*-Value
		Negative	Positive	
Total	202	79 (39.1)	123 (60.9)	
Clinical stage[a]				0.440
I, II	63 (31.2)	22	41	
III, IV	139 (68.8)	57	82	
N status				1.000
N0	105 (52.0)	41	64	
N1, N2, N3	97 (48.0)	38	59	
T status				0.885
T1, T2	108 (53.5)	43	65	
T3, T4	94 (46.5)	36	58	
Lymphatic invasion				0.301
Absent	123 (60.9)	52	71	
Present	79 (39.1)	27	52	
Neural invasion				0.025*
Absent	156 (77.2)	68	88	
Present	46 (22.8)	11	35	
Vascular invasion				0.925
Absent	98 (48.5)	38	60	
Present	104 (51.5)	41	63	

IHC: Immunohistochemistry; HNSCC: head and neck squamous cell cancer; BP180: also known as collagen XVII, BPA-2 or BPAg2; [a]According to the International Union Against Cancer (UICC) TNM Classification of Malignant Tumors, 7th edition; *$p<0.05$.

Table III. *Association between BP180 IHC status and clinicopathological characteristics in OSCC patients.*

	Number of cases (%)	BP180 IHC		*p*-Value
		Negative	Positive	
Total	98	25 (25.5)	73 (74.5)	
Age, years				
Median (range)	70 (29-95)			0.490
<70	48 (49.0)	14	34	
≥70	50 (51.0)	11	39	
Gender				0.812
Male	64 (65.3)	17	47	
Female	34 (34.7)	8	26	
Smoking status				0.639
No	57 (58.2)	16	41	
Yes	41 (41.8)	9	32	
Drinking status				0.141
No	65 (66.3)	20	45	
Yes	33 (33.7)	5	28	
Clinical stage				1.000
I, II	43 (43.9)	11	32	
III, IV	55 (56.1)	14	41	
N status				0.129
N0	70 (71.4)	21	49	
N1-N3	28 (28.6)	4	24	
T status				0.163
T1, T2	59 (60.2)	12	47	
T3, T4	39 (39.8)	13	26	
Lymphatic invasion				0.129
Absent	70 (71.4)	21	49	
Present	28 (28.6)	4	24	
Neural invasion				0.195
Absent	71 (72.4)	21	50	
Present	27 (27.6)	4	23	
Vascular invasion				0.154
Absent	62 (63.3)	19	43	
Present	36 (36.7)	6	30	

IHC: Immunohistochemistry; OSCC: oral squamous cell carcinoma; BP180: also known as collagen XVII, BPA-2 or BPAg2.

1F, G). In BP180-negative cases, either no tumor cells or less than 10% of tumor cells were stained (Figure 1B, E).

Clinical characteristics of the 202 enrolled HNSCC patients (46 females, 156 males) are shown in Table I. Median age was 68 years (range=29-95 years). In the overall cohort, 79 patients (39.1%) were BP180-negative and 123 (60.9%) were BP180-positive. One hundred and twelve patients (55.4%) were smokers, defined as those currently smoking >20 packs/year of cigarettes. 88 patients were drinkers (43.6%), defined as those currently drinking >10 bottles/week. Rates of BP180 positivity in head and neck cancer types, comprising oral, laryngeal, oropharyngeal and hypopharyngeal cancer, were 74.5% (73/98), 42.9% (6/14), 52.4% (22/42) and 51.2% (21/41), respectively (Table I). In the oral squamous cell carcinoma (OSCC) subgroup, rates of BP180 positivity for tongue cancer and mouth floor cancer were 81.0% (47/58) and 83.3% (10/12), higher than for other anatomical localizations of HNSCC. No significant associations were observed between baseline characteristics of HNSCC patients and BP180 positivity.

Association between BP180 IHC and clinicopathological characteristics in HNSCC patients. IHC was conducted to detect BP180 expression in 202 HNSCC patients. Correlations between clinicopathological characteristics and BP180 expression are summarized in Table II. A significant difference was observed in neural invasion in HNSCC ($p=0.025$) (Table II), but not in clinical stage, N status, T status, lymphatic invasion or vascular invasion. The HNSCCs studied could be classified into two broad subgroups: OSCC (comprising tongue, mouth floor and gingival cancer), and laryngeal/pharyngeal cancer (comprising laryngeal, oropharyngeal and hypopharyngeal cancer). The rate of BP180 positivity in the OSCC group was 74.5% (73/98), higher than that in laryngeal and pharyngeal cancers 50.5% (49/97) (Tables III and IV). In the laryngeal and pharyngeal cancer subgroup, a significant difference in the status of BP180 IHC was observed according to T status ($p=0.026$) (Table IV). However, the OSCC subgroup showed no significant associations in the status of BP180 IHC.

Table IV. *Association between BP180 IHC status and clinicopathological characteristics in laryngeal and pharyngeal cancer patients.*

	Number of cases (%)	BP180 IHC		*p*-Value
		Negative	Positive	
Total	97	48 (49.5)	49 (50.5)	
Age, years				
Median (range)	68 (42-87)			1.000
<68	48 (49.5)	24	24	
≥68	49 (50.5)	24	25	
Gender				0.199
Male	86 (88.7)	45	41	
Female	11 (11.3)	3	8	
Smoking status				0.511
No	30 (30.9)	13	17	
Yes	67 (69.1)	35	32	
Drinking status				0.156
No	45 (46.4)	26	19	
Yes	52 (53.6)	22	30	
Clinical stage				0.803
I, II	19 (19.6)	10	9	
III, IV	78 (80.4)	38	40	
N status				0.664
N0	30 (30.9)	16	14	
N1-N3	67 (69.1)	32	35	
T status				0.026*
T1, T2	47 (48.5)	29	18	
T3, T4	50 (51.5)	19	31	
Lymphatic invasion				0.545
Absent	47 (48.5)	25	22	
Present	50 (51.5)	23	27	
Neural invasion				0.286
Absent	80 (82.5)	42	38	
Present	17 (17.5)	6	11	
Vascular invasion				0.830
Absent	32 (33.0)	15	17	
Present	65 (67.0)	33	32	

IHC: Immunohistochemistry; BP180: also known as collagen XVII, BPA-2 or BPAg2. *$p<0.05$.

Hazard ratios (HRs) for death in HNSCC patients. We calculated HRs for the same factors, including age (median, 68 years), sex, smoking status, drinking status, clinical stage, BP180 positivity, N status, T status, lymphatic invasion, neural invasion and vascular invasion using uni- and multivariate Cox regression analysis.

Univariate Cox regression analyses revealed clinical stage [HR=2.644; 95% confidence interval (CI)=1.444-4.843], BP180 positivity (HR=2.508; 95%CI=1.463-4.300), N status (HR=1.987; 95%CI=1.236-3.194), T status (HR=1.656; 95%CI=1.036-2.645), lymphatic invasion (HR=1.701; 95%CI=1.060-2.729), neural invasion (HR=2.288; 95%CI=1.385-3.778) and vascular invasion (HR=1.730; 95%CI=1.071-2.794) as factors significantly associated with risk of mortality. All significant factors from univariate analyses were entered into multivariate analysis. Multivariate Cox regression analysis indicated clinical stage (HR=2.854; 95%CI=1.558-5.228) and BP180 positivity (HR=2.690; 95%CI=1.569-4.609) remained as significant risk factors for death (Table V).

In the OSCC subgroup, univariate Cox regression analysis showed significant differences in clinical stage, BP180 positivity, N status, smoking status, neural invasion and vascular invasion. Multivariate analysis revealed BP180 positivity (HR=3.936; 95%CI=1.283-12.076), N status (HR=2.492; 95%CI=1.172-5.297) and neural invasion (HR=2.173; 95%CI=1.010-4.675) as prognostic factors for death in OSCC patients (Table VI). In the laryngeal and pharyngeal cancer subgroup, univariate Cox regression analysis, significant differences were found for BP180 positivity (HR=2.146; 95%CI=1.085-4.234) and clinical stage (HR=3.370; 95%CI=1.032-11.005). Multivariate analysis revealed BP180 positivity (HR=2.184; 95%CI=1.103-4.322) and clinical stage (HR=3.442; 95%CI=1.053-11.247) as significant risk factors for death (Table VII).

Prognostic significance of BP180 positivity in HNSCC patients. Kaplan–Meier analysis showed significant difference in OS and DFS in the 202 HNSCC patients, who were classified according to BP180 IHC positivity and negativity ($p=0.0005$, HR=2.317, 95%CI=1.443-3.720; $p=0.020$, HR=1.536, 95%CI=1.011-2.333; log-rank test) (Figure 2A, D). In the 98 OSCC subgroup of patients, the same positive results for OS and DFS were found ($p=0.006$, HR=3.700, 95%CI= 1.798-7.611; $p=0.010$, HR=2.349, 95%CI=1.270-4.343; log-rank test), respectively (Figure 2B, E). However, significant results for the 97 patients in the laryngeal and pharyngeal cancer subgroups were only observed in OS ($p=0.024$, HR=2.138, 95%CI=1.109-4.122; log-rank test), not in DFS (Figure 2C, F).

Prognostic significance of BP180 positivity in the late clinical stage. Clinical stages were divided into early (I+II) or late (III+IV). Further OS analysis was carried out in HNSCC patients, OSCC patients and laryngeal and pharyngeal cancer patients (Figure 3). All patient groups showed significant differences in OS between early and late clinical stages ($p=0.0002$, HR=2.967, 95%CI=1.773-4.966; $p=0.015$, HR=4.979, 95%CI=2.076-11.940; $p=0.009$, HR=2.511, 95%CI=1.263-4.989, respectively, log-rank test). In the OSCC subgroup, BP180-positive patients showed a high risk of death (HR=4.979) compared to others.

Five-year survival rates in HNSCC patients. In the present study HNSCC cohort, the mean 5-year overall survival (OS) rate for BP180-positive patients was 42.8±7.7%, lower than that for BP180-negative patients (69.0±7.3%). Compared with different subgroups, OSCC patients showed a slightly

Table V. *Clinicopathological factors and their effect on HNSCC patient mortality and overall survival (OS) by Cox proportional hazards regression modeling.*

	Univariate analysis			Multivariate analysis		
	HR	95%CI	*p*-Value	HR	95%CI	*p*-Value
Age (years)						
≥68 *vs.* <68	1.011	0.634-1.612	0.964			
Gender						
Male *vs.* Female	0.947	0.549-1.633	0.844			
Smoking status						
Yes *vs.* No	1.475	0.913-2.383	0.112			
Drinking status						
Yes *vs.* No	1.215	0.762-1.939	0.413			
Clinical stage						
III, IV *vs.* I, II	2.644	1.444-4.843	0.002**	2.854	1.558-5.228	0.001**
BP180 protein expression						
Positive *vs.* Negative	2.508	1.463-4.300	0.001**	2.690	1.569-4.609	<0.001**
N status						
N1-N3 *vs.* N0	1.987	1.236-3.194	0.005**			
T status						
T3, T4 *vs.* T1, T2	1.656	1.036-2.645	0.035*			
Lymphatic invasion						
Present *vs.* Absent	1.701	1.060-2.729	0.028*			
Neural invasion						
Present *vs.* Absent	2.288	1.385-3.778	0.001**			
Vascular invasion						
Present *vs.* Absent	1.730	1.071-2.794	0.025*			

HR: Hazard ratio; CI: confidence interval; HNSCC: head and neck squamous cell cancer; BP180: also known as collagen XVII, BPA-2 or BPAg2. Age 68 years was used as a cutoff because this was the median age. Values showing $p<0.05$ were entered into multivariate analysis. *$p<0.05$; **$p<0.01$.

Table VI. *Clinicopathological factors and their effects on OSCC patient mortality and overall survival (OS) by Cox proportional hazards regression modeling.*

	Univariate analysis			Multivariate analysis		
	HR	95%CI	*p*-Value	HR	95%CI	*p*-Value
Age (years)						
≥70 *vs.* <70	1.000	0.505-1.982	0.999			
Gender						
Male *vs.* Female	1.116	0.541-2.304	0.766			
Smoking status						
Yes *vs.* No	2.370	1.184-4.742	0.015*			
Drinking status						
Yes *vs.* No	1.606	0.803-3.210	0.180			
Clinical stage						
III, IV *vs.* I, II	2.338	1.108-4.935	0.026*			
BP180 protein expression						
Positive *vs.* Negative	4.267	1.413-12.882	0.010*	3.936	1.283-12.076	0.017*
N status						
N1-N3 *vs.* N0	3.437	1.709-6.910	0.001**	2.492	1.172-5.297	0.018*
T status						
T3, T4 *vs.* T1, T2	1.722	0.868-3.416	0.120			
Lymphatic invasion						
Present *vs.* Absent	1.790	0.858-3.736	0.121			
Neural invasion						
Present *vs.* Absent	3.226	1.580-6.587	0.001**	2.173	1.010-4.675	0.047*
Vascular invasion						
Present *vs.* Absent	2.035	1.012-4.093	0.046*			

HR: Hazard ratio; CI: confidence interval; OSCC: oral squamous cell cancer; BP180: also known as collagen XVII, BPA-2 or BPAg2. Age 70 years was used as a cutoff because this was the median age. Values showing $p<0.05$ were entered into multivariate analysis. *$p<0.05$; **$p<0.01$.

Table VII. *Clinicopathological factors and their effects on laryngeal/pharyngeal cancer patient mortality and overall survival (OS) by Cox proportional hazards regression modeling.*

	Univariate analysis			Multivariate analysis		
	HR	95%CI	*p*-Value	HR	95%CI	*p*-Value
Age (years)						
≥68 *vs*. <68	0.926	0.480-1.785	0.818			
Gender						
Male *vs*. Female	0.478	0.198-1.154	0.101			
Smoking status						
Yes *vs*. No	0.829	0.413-1.662	0.596			
Drinking status						
Yes *vs*. No	1.258	0.654-2.423	0.491			
Clinical stage						
III, IV *vs*. I, II	3.370	1.032-11.005	0.044*	3.442	1.053-11.247	0.041*
BP180 protein expression						
Positive *vs*. Negative	2.146	1.085-4.243	0.028*	2.184	1.103-4.322	0.025*
N status						
N1-N3 *vs*. N0	1.157	0.569-2.354	0.687			
T status						
T3, T4 *vs*. T1, T2	1.783	0.911-3.491	0.091			
Lymphatic invasion						
Present *vs*. Absent	1.462	0.754-2.836	0.261			
Neural invasion						
Present *vs*. Absent	1.250	0.547-2.855	0.597			
Vascular invasion						
Present *vs*. Absent	1.227	0.591-2.546	0.583			

HR: Hazard ratio; CI: confidence interval; BP180: also known as collagen XVII, BPA-2 or BPAg2. Age 68 years was used as a cutoff because this was the median age. Values showing $p<0.05$ were entered into multivariate analysis. *$p<0.05$.

higher survival rate (57.5±6.8%) than laryngeal and pharyngeal cancer patients (52.1±6.7%). With clinical stage disease from I to IV, 5-year survival rates were 65.6±19.9%, 63.1±9.8%, 52.6±10.4% and 51.5±5.9%, respectively.

Discussion

In this retrospective study, BP180 (collagen XVII) was identified as a novel biomarker for predicting the prognosis of HNSCC, OSCC and laryngeal and pharyngeal cancer. To the best of our knowledge, this is the first study to investigate BP180 as an effective prognostic factor for HNSCC.

BP180 was identified as a prognostic factor in HNSCC, and BP180 expression was also closely associated with OS in HNSCC patients ($p=0.0005$), OSCC patients ($p=0.006$) and laryngeal and pharyngeal cancer patients ($p=0.024$). In particular, positive associations with OS were observed for the clinical late stage (III+IV) of these pathologies. Our findings show that BP180 is strongly predictive of tumor malignancy in HNSCC. BP180 was first found in bullous pemphigoid (BP), which is by far the most common autoimmune blistering dermatosis and mainly occurs in the elderly. BP180 is a transmembrane glycoprotein that acts as a significant autoantigen and is highly immunodominant in BP (31). Our group first reported the relationship between BP180 expression and cancers in 1996 (13). Many studies have focused on the mechanisms of the relationship between BP180 and tumorigenesis, invasion and metastasis in different kinds of cancers, but no studies have investigated BP180 as a biomarker for cancer prognosis. However, many reports have examined the relationship between BP180 and prognosis of BP. In BP, patients with serum concentrations of BP180 autoantibodies ≥61 U/ml showed a 2.4-fold increase in the risk of early death compared with the general population (95%CI=1.81-3.81; $p<0.0001$) (32). Serum levels of IgG1 and IgG4 targeting BP180NC16A were both independent prognostic factors for early death from BP (33). In a retrospective study of BP in 74 young patients, higher expression of anti-BP180 autoantibodies represented a marker of poor prognosis (34). Another multicenter prospective cohort study showed high-titer anti-BP180 ELISA (enzyme-linked immunosorbent assay) score as a predictor of BP recurrence (35). These three studies (33-35) identified high expression of BP180 as a marker of poor prognosis. Such evidence provides some support for our conclusion about prognosis in HNSCC. DFS was also analyzed in our study, showing significant differences in the status of BP180 IHC in HNSCC and OSCC,

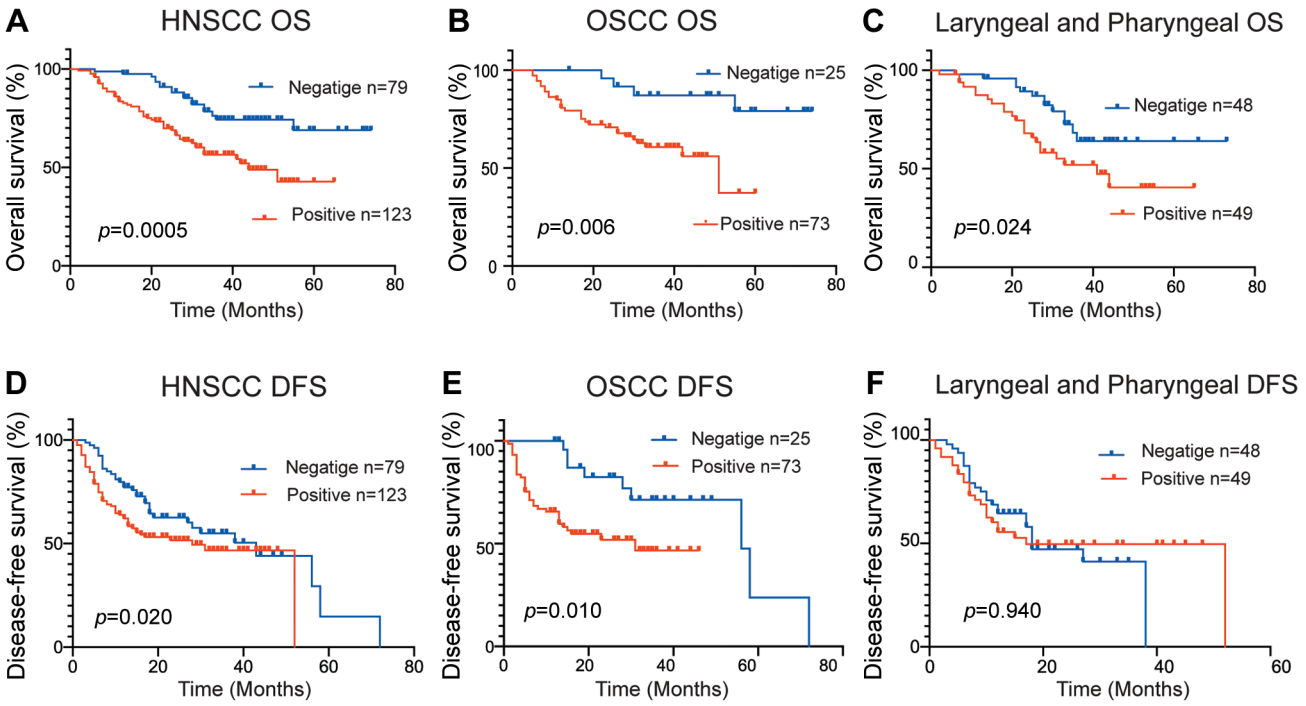

Figure 2. *Kaplan–Meier analyses for overall survival (OS) and disease-free survival (DFS) in 202 HNSCC patients. A) OS curves of 202 HNSCC patients for BP180-positive cases (red line, n=123) and BP180-negative cases (blue line, n=79). B) OS curves of OSCC patients for BP180-positive cases (red line, n=73) and BP180-negative cases (blue line, n=25). C) OS curves of laryngeal and pharyngeal cancer patients for BP180-positive cases (red line, n=49) and BP180-negative cases (blue line, n=48). D) DFS curves of 202 HNSCC patients for BP180-positive cases (red line, n=123) and BP180-negative cases (blue line, n=79). E) DFS curves of OSCC patients for BP180-positive cases (red line, n=73) and BP180-negative cases (blue line, n=25). F) DFS curves of laryngeal and pharyngeal cancer patients for BP180-positive cases (red line, n=49) and BP180-negative cases (blue line, n=48). Values of p<0.05 are considered statistically significant.*

but not in laryngeal and pharyngeal cancer. A key finding was that analysis of OS in laryngeal and pharyngeal cancer revealed a significant difference in the status of BP180 IHC. The possible reason is that there are many relapsed hypopharyngeal cancer patients due to alcohol.

Immunohistochemically, using a mouse monoclonal anti-human BP180 antibody linear staining along the basement membrane and faint cytoplasmic staining in the basal layer of squamous epithelium in limited normal tissues was revealed. BP180 was distributed irregularly or scattered only in layers of the epithelium (13). Parikka *et al*. found similar results in a study of the transformation of oral epithelium to dysplasia and carcinoma, identifying intense staining in carcinoma cells at the invasive front in Grade II OSCC, with signals mainly missing from basal cells and strong signals restricted to the epithelium in cases of dysplasia (5, 14). In our study, the same immune reaction was evident in normal tissues and squamous cell carcinomas. Some studies have proposed that BP180 serves as a cell-matrix adhesion molecule by stabilizing the hemidesmosome complex and mediating anchorage to the underlying basement membranes. Beyond any structural roles, BP180 is presumed to play a role in cell migration and differentiation to pathological states in malignant tumors (5, 7, 36-38).

Head and neck cancer is a wide disease classification that includes oral, salivary gland, thyroid, nasopharyngeal, laryngeal, oropharyngeal and hypopharyngeal cancers. In addition, oral cancers can arise from squamous epithelium of the tongue, gingiva, palate, buccal mucosa, and mouth floor. The present report offers a first demonstration of the expression profile of BP180 in different primary sites. BP180 positivity rates were 57.1-83.3%, with no marked differences apparent between primary sites of HNSCC (p=0.643, Fisher's exact test), but a tendency toward higher expression was noted in the current OSCC cohort (p=0.084, Fisher's exact test). We were surprised to observe that comparing OSCC with laryngeal and pharyngeal cancer, BP180 positivity was 74.5% for OSCC and 50.5% for laryngeal and pharyngeal cancer (p=0.001, Pearson's chi-square test) (Tables III and IV). We hypothesize that BP180 expression in HNSCC correlates with anatomical localization. In a TMA of 124 HNSCCs, BP180 expression was found to be higher in the oral cavity (85.7%) than in other anatomical localizations (39). We found differences in BP180 positivity in some anatomical areas as

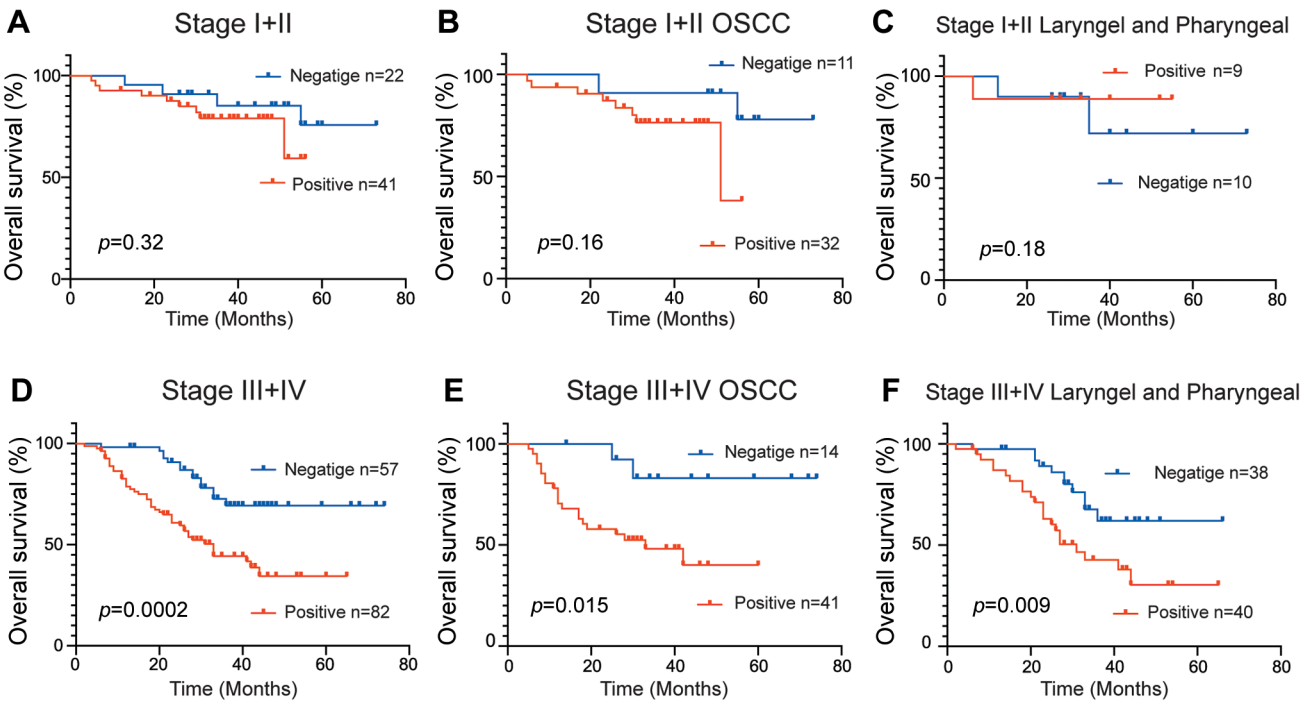

Figure 3. *Kaplan–Meier analyses for overall survival (OS) in early and late clinical stage HNSCC patients. A) OS curves of 63 HNSCC patients in stage I+II for BP180-positive cases (red line, n=41) and BP180-negative cases (blue line, n=22). B) OS curves of 43 OSCC patients in stage I+II for BP180-positive cases (red line, n=32) and BP180-negative cases (blue line, n=11). C) OS curves of 19 laryngeal and pharyngeal cancer patients in stage I+II for BP180-positive cases (red line, n=9) and BP180-negative cases (blue line, n=10). D) OS curves of 139 HNSCC patients in stage III+IV for BP180-positive cases (red line, n=82) and BP180-negative cases (blue line, n=57). E) OS curves of 55 OSCC patients in stage III+IV for BP180-positive cases (red line, n=41) and BP180-negative cases (blue line, n=14). F) OS curves of 78 laryngeal and pharyngeal cancer patients in stage III+IV for BP180-positive cases (red line, n=40) and BP180-negative cases (blue line, n=38). Values of p<0.05 are considered statistically significant.*

novel data, and attributed this to differences in mucosal structure and function. The mucous membrane in the oral cavity is mostly used for maintaining an environment suitable for chewing and ingesting food, whereas the mucous membranes of the pharynx and larynx play important roles in immune function and vocalization. These results provide a clinical basis for future research into the pathological mechanisms of and drug-targeted therapies for HNSCC.

Based on the current research, a possible tumorigenesis mechanism is that the structural extracellular domain (ECD) of BP180 connects cytoplasmic structural components with the extracellular matrix (ECM). The ECD is essential for proper basement membrane formation. In the absence of normal regulation, changes in the ECM may contribute to the first steps toward cancer. Recent data have demonstrated that alterations in BP180 exert profound effects on cancer tumorigenesis, progression, invasion and migration in different kinds of cancers, as mentioned above (40-42). Our findings provide clinical data in support of this notion that BP180 is a factor associated with poor prognosis. However, several potential limitations must be considered. First, the sample size was quite limited and data from more cases is needed. In addition, the molecular mechanisms underlying the effects of BP180 on HNSCC need to be clarified. At last, we did not have an in-depth analysis of surgical related factors.

In conclusion, the present study suggests that BP180 is a prognostic factor for HNSCC. Moreover, multivariate analysis suggested BP180 as a significant independent prognostic factor along with clinical stage in patients with HNSCC. Overall, the prognostic value of BP180 expression in this study provides an important experimental foundation for closer examination of this potentially significant biomarker in targeted treatments for patients with HNSCC.

Conflicts of Interest

The Authors declare that there are no conflicts of interest in relation to this study.

Authors' Contributions

X.M. performed experiments, analyzed the data and wrote the article. F.M. and K.I. analyzed the data and revised the article. T.M.

provided pathological tissue. N.M. revised the article. Y.I. supplied BP180 antibody. K.O. and Y.M. performed experiments. K.K. provided clinical data and analysis. S.Y. provided clinical data. K.H. provided the conceptual and technical guidance, designed the study and revised the article. All Authors read, reviewed, and approved the manuscript.

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