Expression of secreted protein acidic and rich in cysteine is an independent prognostic indicator of a poor clinical outcome in oropharyngeal carcinoma patients

Title: Expression of secreted protein acidic and rich in cysteine is an independent prognostic indicator of a poor clinical outcome in oropharyngeal carcinoma patients

Authors and Affiliations:

Shinya Yoshida [1], Naohiro Wakisaka [1*], Satoru Kondo [1], Makiko Moriyama-Kita [1], Nobuyuki Hirai [1], Kazuhira Endo [1], Akira Tsuji [1], Yosuke Nakanish [1], Shigeyuki Murono [1], and Tomokazu Yoshizaki [1]

Division of Otolaryngology-Head and Neck Surgery[1], Graduate School of Medical Science, Kanazawa University, Takara-machi 13-1, Kanazawa 920-8640, JAPAN

Conflict of interest: None declared

Abstract

Objective: The objectives of the study were to clarify the correlation between the expression of secreted protein acidic and rich in cysteine (SPARC) and HPV-status, and to determine the prognostic value of SPARC-expression in oropharyngeal squamous cell carcinoma (OPSCC) patients.

Methods: Fifty-three formalin-fixed and paraffin-embedded tissues were obtained from patients with OPSCC who underwent curative treatment. The SPARC protein was detected by immunohistochemistry. SPARC-expression level was divided into two categories, SPARC-High and SPARC-Low, according to the staining index.

Results: Twenty-two out of the 53 OPSCC patients were HPV-positive. There was no significant correlation between the HPV-status and SPARC-expression level. Multivariate Cox proportional hazards regression analysis revealed that the HPV-status and SPARC-expression are independent prognostic indicator of favorable and unfavorable overall survival (OS) ($p = 0.021$ and $p = 0.012$), respectively. For disease-free survival, the HPV-status was the only predictive factor ($p = 0.022$). After stratification by the HPV-status, high SPARC-expression was a significant predictor of poor OS in HPV-negative OPSCC patients using Kaplan-Meier analysis and the log-rank test ($p = 0.014$). Ten out of 28

SPARC-Low patients relapsed, among which 6 patients (60%) were salvaged. However, 14 out of 25 SPARC-High patients relapsed, and only 3 patients (21.4%) were salvaged.

Conclusion: SPARC-expression is an indicator of the prognosis in terms of OS independent of HPV-infection. HPV-negative patients with SPARC-Low show survival as favorable as HPV-positive patients, probably because of their higher salvage rate after relapse than SPARC-High patients.

Key Words: oropharyngeal carcinoma, human papillomavirus, secreted protein acidic and rich in cysteine, prognostic indicator.

Introduction

Infection with high-risk human papillomavirus (HPV) has been etiologically linked to the development of head and neck squamous cell carcinomas, particularly carcinomas that arise in the oropharyngeal region. Patients with HPV-positive oropharyngeal squamous cell carcinoma (OPSCC) are characterized by an epidemiologic, demographic, and clinical profile that deviates from that of HPV-negative patients [1]. The most important difference is related to the prognosis, which is markedly better for patients with HPV-positive tumors compared to their HPV-negative counterparts [2].

To date, OPSCC patients have presented with few prognostic markers targetable for improving prevention and treatment strategies. The HPV-status is closely associated with the prognosis of OPSCC patients and there is significant heterogeneity in outcomes of HPV-negative OPSCC patients [3]. ~~However, no other reliable biomarkers have been found.~~ If new biomarkers, that can help determine the prognosis and survival of OPSCC patients, can be detected, they may help avoid both over- and under-treatment of OPSCC patients, resulting in improvements in the survival and quality of life of patients with this disease.

Secreted protein acidic and rich in cysteine (SPARC) has attracted marked interest as a tumor-associated protein for its diverse actions and complex functions. The increased expression of SPARC is associated with a highly aggressive tumor phenotype in melanomas

and gliomas, as supported by previous functional studies [4], [5], [6], [7]. However, other studies reported that SPARC acts as a tumor suppressor in pancreatic adenocarcinoma, acute myeloid leukemia, and ovarian and colorectal carcinomas [8], [9], [10], [11]. Actually, recent studies revealedthat SPARC mediates the interaction between cells and the extracellular environment as a matricellular protein, and upregulating its expression enhances chemosensitivity [12]. Functions of SPARC are elucidated in other cancers, but there are no report about relations between OPSCC and SPARC, and viral carcinogenesis and SPARC. The role of SPARC-expression in OPSCC remains to be clarified.

Here, we examined the expression of SPARC protein in OPSCC tissues. The purposes of the current study were to explore the correlation between SPARC-expression and the HPV-status, and to determine the prognostic value of SPARC-expression.

Materials and Methods

Patients and tissue samples.

Fifty-three specimens were obtained from patients with OPSCC who underwent curative treatment such as surgery and/or radiotherapy with or without chemotherapy at Kanazawa University Hospital between 1991 and 2012. All specimens were fixed in 10% neutral formalin and embedded in paraffin, and then clinically and histopathologically diagnosed. All patients were staged according to the TNM staging system [13] based on initial radiologic imaging evaluation and endoscopic observation. The follow-up deadline was January 2014. The survival time was counted from the date of starting definitive therapy to the follow-up deadline or date of death, which was primarily caused by carcinoma recurrence or metastasis. All patients signed a letter of informed consent approved by our Institutional Ethics Committee (no. 1666).

All specimens were used to extract DNA for HPV detection using the polymerase chain reaction (PCR), and we also performed p16 immunohistochemistry. DNA was extracted from formalin-fixed, paraffin-embedded (FFPE) tumor specimens using a DNeasy Tissue Kit (Qiagen, Valencia, CA, USA). Sixteen HPV genotypes (genotypes 6, 11, 16, 18, 30, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66) were detected by multiple PCR in a single tube. The details of the procedures are described elsewhere [14]. We defined the specimen as HPV-positive

when both HPV DNA and p16-staining were positive.

Immunohistochemical analysis

Consecutive 4-µm sections were cut from each block. Immunohistochemical staining was performed as described previously [15], [16]. The following primary antibodies were used: monoclonal antibodies for SPARC (dilution 1:200; SantaCruz, USA) derived from rabbits and p16 (dilution 1:100; SantaCruz, USA) derived from mice. The specificities of staining were confirmed using non-immune serum instead of the primary antibodies as negative controls. The degree of immunostaining was reviewed and scored independently by two observers (S.Y. and N.W.) based on the proportion of positively stained tumor cells and intensity of staining. For SPARC evaluation [16], the tumor cell proportion was scored as follows: 0 (≤ 5% positive tumor cells), 1 (6–25% positive tumor cells), 2 (26-50% positive tumor cells), and 3 (> 51% positive tumor cells). The staining intensity was graded according to the following criteria: 0 (no staining), 1 (weak staining, light yellow), 2 (moderate staining, yellow brown), and 3 (strong staining, brown). The staining index (SI) was calculated as the product of the staining intensity score and proportion of positive tumor cells, with scores from 0 to 9 (0, 1, 2, 3, 4, 6, and 9). The cut-off values for high and low expression levels were chosen based on measures of heterogeneity using the log-rank test with respect to overall

survival. Optimal cut-off values were defined as follows: SI of ≥ 4 was used to define tumors with high SPARC-expression, and SI of ≤ 3 was used to indicate low SPARC-expression. For p16 evaluation, the sections were evaluated as p16-positive only when tumor cells were diffusely stained.

Statistical analysis

All statistical analyses were conducted using SPSS 19.0 software (IBM, Armonk, NY, USA). Data were analyzed using Fisher's exact *t*-test. Survival curves were estimated using the Kaplan-Meier method, and the log-rank test was used to investigate differences between the curves. Multivariate analysis using the Cox proportional hazards regression model was carried out to assess the prognostic value of each of the patients' characteristics. Significance was set at a *p*-value less than 0.05.

Results

Expression of SPARC and p16 in OPSCC tissues (Fig. 1)

High-expression of SPARC protein was detected in 25 (47.2%) tumors, and low-expression was detected in 28 (52.8%) tumors. SPARC protein was mainly localized in the cytoplasm of tumor cells and some stromal cells (Fig. 1A, B). Some stromal cells were constitutively positive, even if the tumor cells were stained negative (Fig.1C). Twenty-eight and twenty-five cases were classified as SPARC-Low (Score 0, 3 cases; Score 1, 1 case; Score 2, 14 cases; and Score 3, 10 cases) and SPARC-High (Score 4, 2 cases; Score 6, 11 cases; and Score 9, 12 cases), respectively.

The 28 tumors were categorized as p16-positive, among which high-risk HPV-DNA was positive in 22 patients (41.5%), all of which were HPV type 16-positive. We confirmed that all 22 HPV-DNA-positive tumors were also p16-positive (Fig. 1D). Thus, 22 of the 53 OPSCCs were classified as HPV-related OPSCC.

Correlation between patients' demographic characteristics and the HPV-status (Table 1)

The HPV-positive and HPV-negative OPSCC patients with respect to several clinical features are presented in Table 1. Fifty-three surgical tissues and diagnostic biopsies from OPSCC patients, 22 HPV-positive and 31 HPV-negative, were available for our analysis. The

mean period of follow-up was 27 months (ranging from 2-100 months). In HPV-positive patients, the nodal status had significantly progressed (p = 0.0475). There was no significant correlation between the HPV-status and SPARC-expression (P=0.7280).

Correlation between patients' demographic characteristics and the SPARC-expression (Table 2)

The SPARC-High and SPARC-Low OPSCC patients with respect to several clinical factors are presented in Table 2. No significant correlations were found between any analyzed demographic factors.

Ten out of 28 SPARC-Low patients relapsed, among which 6 patients (60.0%) were salvaged by surgery. On the other hand, SPARC-High patients were prone to relapse (14 out of 25 patients, 56.0%) and salvage was difficult (only 21.4% of patients were salvaged).

Cox proportional hazards survival analysies (Tables 3, 4)

Univariate Cox proportional hazards regression analysis revealed that the HPV-status and SPARC-expression were significant prognostic indicators in patients with OPSCC in terms of the overall survival (OS) ($p < 0.05$) (Table 3). These two parameters were further examined with multivariate analysis. After multivariate adjustment, high

SPARC-expression remained a powerful poor prognostic indicator (p = 0.012, HR = 5.125) independent of HPV infection (p = 0.021, HR = 0.173).

Regarding disease-free survival (DFS), univariate Cox proportinal hazards regression analysis revealed that the HPV-status and N-factor were significant prognostic indicators of DFS ($p < 0.05$) (Table 4). SPARC-expression was not a significant predictor of DFS. After multivariate adjustment, only HPV infection was a significant predictor (p = 0.022, HR = 0.302) (Table 4).

Survival analyses in HPV-negative and HPV-positive patients in relation to the SPARC-expression level (Fig 2)

Kaplan-Meier analyses of the 53 OPSCC patients were carried out. OS and DFS were determined on the basis of the HPV-status and SPARC-expression. Among all 53 patients, the 5-year OS rates differed significantly between HPV-positive and HPV-negative patients (Figure 2A; p = 0.011) and between low and high SPARC-expression patients (Figure 2B; p = 0.006). After stratification by the HPV-status, SPARC-expression remained a significant prognostic indicator in HPV-negative OPSCC patients, but was not a significant predictor in HPV-positive patients (Figure 2C, D).

The disease-free survival rate was significantly more favorable in HPV-positive than

HPV-negative patients (p = 0.003) (Fig.2E). However, SPARC-expression did not show any significance (Figure 2F, G, and H).

Discussion

Higher levels of SPARC-expression have been reported in breast cancer, melanoma, and glioblastoma patients [5], [6], [17]. Based on this pattern of expression, one would hypothesize a potential role of SPARC in tumor promotion or progression. However, lower levels of SPARC-expression have been found in other types of malignancy, such as ovarian, colorectal, and pancreatic cancers and acute myelogenous leukemia [8], [9], [18]. This pattern of decreased SPARC levels suggests an inhibitory role of SPARC in tumor progression. The possible clinical significance of SPARC expression has not been reported in OPSCC patients. Therefore, we investigated the relationships between immunohistochemical SPARC-expression and clinicopathologic parameters, including patient survival. High-expression of SPARC showed a significant correlation with poor OS.

In OPSCC patients, HPV-infection has accounted for a growing proportion of cases, particularly among the middle-aged population. Patients with HPV-positive OPSCC have a more favorable prognosis than HPV-negative patients [3]. Our results also support those of previous reports. It has been reported that aberrant DNA methylation, an important epigenetic mechanism for gene silencing, occurs in HPV-related carcinogenesis [18], and that SPARC is transcriptionally down-regulated by its promoter hyper-methylation in some cancers. Therefore, we examined the association between the HPV-status and

SPARC-expression in OPSCC tissues. However there was no significant correlation between the HPV-status and SPARC-expression. Next, we examined whether SPARC could be a prognostic indicator in OPSCC patients independent of HPV-infection. Cox proportional hazards regression analysis revealed that both SPARC-expression and the HPV-status were independent prognostic indicators of OS. In addition, we performed survival analysis of HPV-positive and HPV-negative OPSCC patients separately. Generally, HPV-positive patients show more favorable survival. Thus, the sample size of the patients involved in this study was not sufficient to yield a significant difference. Among HPV-negative OPSCC patients, the cumulative OS rate of patients with low SPARC-expression was significantly better than in those high expression. Thus, SPARC-expression may be an indicator of a favorable prognosis among HPV-negative OPSCC patients.

In contrast to OS, SPARC-expression did not show a significant correlation with DFS. The reason why SPARC-expression was not a prognostic indicator of DFS is considered to be that SPARC-High patients were inoperable at the time of relapse or could not be rescued by salvage surgery. In other words, SPARC-Low patients could be salvaged, so their OS rate was higher than that of SPARC-High patients. It has been reported that SPARC regulates cell-ECM signals which control cell invasion and migration [19], and the formation of matrix metalloproteinases [20]. These roles of SPARC may have influenced the difference in salvage

rates.

Conclusion

SPARC-expression is an indicator of the prognosis in terms of OS independent of HPV-infection. HPV-negative patients with low SPARC-expression show survival as favorably as HPV-positive patients probably because of their higher salvage rate after relapse than SPARC-High patients.

Acknowledgement

This work was supported by a scientific research grant from the Ministry of Education, Science, Sports, Culture and Technology of Japan (C21592189). We thank Dr Seiko Sawada-Kitamura from the Department of Human Pathology, Kanazawa University, Graduate School of Medicine for critical comments and suggestions.

References

[1] Leemans CR, Braakhuis BJ, Brakenhoff RH. The molecular biology of head and neck cancer. Nat Rev Cancer. 2011; 11:9-22.

[2] Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tân PF, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med. 2010; 363:24-35.

[3] Vokes EE, Weichselbaum RR, Lippman SM, Hong WK. Head and neck cancer. N Engl J Med. 1993; 328:184-194.

[4] Horie K, Tsuchihara M, Nakatsura T. Silencing of secreted protein acidic and rich in cysteine inhibits the growth of human melanoma cells with G arrest induction. Cancer Sci. 2010; 101:913-919.

[5] Ledda F, Bravo AI, Adris S, Bover L, Mordoh J, Podhajcer OL. The expression of the secreted protein acidic and rich in cysteine (SPARC) is associated with the neoplastic progression of human melanoma. J Invest Dermatol. 1997; 108:210-214.

[6] Rempel SA, Golembieski WA, Ge S, Lemke N, Elisevich K, Mikkelsen T, et al. SPARC: a signal of astrocytic neoplastic transformation and reactive response in human primary and xenograft gliomas. J Neuropathol Exp Neurol. 1998; 57:1112-1121.

[7] Seno T, Harada H, Kohno S, Teraoka M, Inoue A, Ohnishi T. Downregulation of SPARC expression inhibits cell migration and invasion in malignant gliomas. Int J Oncol. 2009; 34:707-715.

[8] DiMartino JF, Lacayo NJ, Varadi M, Li L, Saraiya C, Ravindranath Y, et al. Low or absent SPARC expression in acute myeloid leukemia with MLL rearrangements is associated with sensitivity to growth inhibition by exogenous SPARC protein. Leukemia. 2006; 20:426-432.

[9] Sato N, Fukushima N, Maehara N, Matsubayashi H, Koopmann J, Su GH, et al. SPARC/osteonectin is a frequent target for aberrant methylation in pancreatic adenocarcinoma and a mediator of tumor-stromal interactions. Oncogene. 2003; 22:5021-5030.

[10] Socha MJ, Said N, Dai Y, Kwong J, Ramalingam P, Trieu V, et al. Aberrant promoter methylation of SPARC in ovarian cancer. Neoplasia. 2009; 11:126-135.

[11] Yang E, Kang HJ, Koh KH, Rhee H, Kim NK, Kim H. Frequent inactivation of SPARC by promoter hypermethylation in colon cancers. Int J Cancer. 2007; 121:567-575.

[12] Bull Phelps SL, Carbon J, Miller A, Castro-Rivera E, Arnold S, Brekken RA, et al.

Secreted protein acidic and rich in cysteine as a regulator of murine ovarian cancer growth and chemosensitivity. Am J Obstet 2 Gynecol. 009; 200:180 e181-187.

[13] Wittekind C. [2010 TNM system: on the 7th edition of TNM classification of malignant tumors]. Pathologe. 2010; 31:331-332.

[14] Wakisaka N, Yoshida S, Kondo S, Kita M, Endo K, Tsuji A, et al. HPV Status Determines the Efficacy of Adjuvant Chemotherapy With S-1, an Oral Fluorouracil Prodrug, in Oropharyngeal Cancer. Ann Otol Rhinol Laryngol. 2014.

[15] Geisler SA, Olshan AF, Weissler MC, Cai J, Funkhouser WK, Smith J, et al. p16 and p53 Protein expression as prognostic indicators of survival and disease recurrence from head and neck cancer. Clin Cancer Res 2002; 8:3445-3453.

[16] Zhao ZS, Wang YY, Chu YQ, Ye ZY, Tao HQ. SPARC is associated with gastric cancer progression and poor survival of patients. Clin Cancer Res. 2010; 16:260-268.

[17] Lien HC, Hsiao YH, Lin YS, Yao YT, Juan HF, Kuo WH, et al. Molecular signatures of metaplastic carcinoma of the breast by large-scale transcriptional profiling: identification of genes potentially related to epithelial-mesenchymal transition. Oncogene. 2007; 26:7859-7871.

[18] Pauline MW, Rob N, Weibel WB, Inge S, Stefan MW, Wilko G. Differences in methylation profiles between HPV-positive and HPV-negative oropharynx squamous cell carcinoma: a systematic review. Epigenetics. 2014; 9:194-203.

[19] Arnold S, Mira E, Muneer S, Korpanty G, Beck AW, Holloway SE, et al. Forced expression of MMP9 rescues the loss of angiogenesis and abrogates metastasis of pancreatic tumors triggered by the absence of host SPARC. Exp Biol Med (Maywood). 2008; 233:860-873.

[20] Lane TF, Sage EH. The biology of SPARC, a protein that modulates cell-matrix interactions. FASEB J. 1994; 8:163-173.

Title: Expression of secreted protein acidic and rich in cysteine is an independent prognostic indicator of a poor clinical outcome in oropharyngeal carcinoma patients

***Corresponding author:**

Naohiro Wakisaka

Division of Otolaryngology-Head and Neck Surgery, Graduate School of Medical Science, Kanazawa University, Takara-machi 13-1, Kanazawa 920-8640, JAPAN

Tel.: +81-76-265-2413

Fax: +81-76-234-4265

E-mail: wakisaka@med.kanazawa-u.ac.jp

Figure Legends

Figure 1 Immunohistochemical detection of SPARC (A, B, and C) and p16 (D) proteins in OPSCC tumors (original magnification, 100x). A and B, SPARC protein was immunolocalized in the cytoplasm of tumor cells. Some stromal cells were also positive. C, Some stromal cells were constitutively positive, even if the tumor cells were stained negative. D, p16 protein expression was evaluated as positive only when diffuse immunoreactivity was observed in the tumor cell nest.

Figure 2 Kaplan-Meier survival curve of overall survival (OS) and disease-free survival (DFS) and log-rank test for patients with a different HPV-status and SPARC-expression levels. A, The 5-year OS rate was significantly different between the HPV-positive and HPV-negative OPSCC patients ($p = 0.011$). B, The 5-year OS rate was significantly different between SPARC-Low and SPARC-High patients ($p = 0.006$). C, Within the HPV-positive stratum, SPARC-expression did not show a significant effect on OS ($p = 0.137$). D, Within the HPV-negative stratum, the SPARC-Low patients showed significantly more favorable OS than the SPARC-High patients ($p = 0.014$). E, The DFS curve of OPSCC patients with a different HPV-status was significantly different between HPV-positive and HPV-negative patients ($p = 0.003$). F, The DFS curve with different SPARC-expression levels did not show

a significant difference (p = 0.157). G and H, In both the HPV-positive and HPV-negative stratum, SPARC-expression did not show any significant correlation with DFS (p = 0.182 and p = 0.420).

Table 1. Correlation between patients' characteristics and HPV-status

Characteristics		Total	HPV-positive	HPV-negative	*p*
No. of cases		53	22	31	
Sex					
	Male	49	20	29	> 0.9999
	Female	4	2	2	
Age at Diagnosis					
	Median	62	61	62	0.8490
	Range	39-89	39-89	45-85	
	Mean	63.7	63.7	63.7	
Staging					
	T1-2	34	15	19	0.7725
	T3-4	19	7	12	
	N0	21	5	16	0.0475
	N1-3	32	17	15	
	I-II	13	4	9	0.5199
	III-IV	40	18	22	
Smoking history					
	Smoker	41	15	26	0.2018
	Non-smoker	12	7	5	
Treatment					
	Surgery±Radiation	20	7	13	0.5689
	Radiation±Chemotherapy	33	15	18	
SPARC					
	High	25	11	14	0.7850
	Low	28	11	17	

Smoker is defined as a patient with smoking history. Non-smoker is defined as a patient without any history of smoking.
HPV, human papillomavirus; SPARC, secreted protein acidic and rich in cysteine.

Table 2. Correlation between patients' characteristics and SPARC-expression

Characteristics		Total	SPARC-High	SPARC-Low	*p*
No. of cases		53	25	28	
Sex					
	Male	49	23	26	> 0.9999
	Female	4	2	2	
Age at Diagnosis					
	Median	62	66	60.5	0.1808
	Range	39-89	39-89	48-81	
	Mean	63.7	65.2	62.4	
Staging					
	T1-2	34	17	17	0.7748
	T3-4	19	8	11	
	N0	21	9	12	0.7793
	N1-3	32	16	16	
	I-II	13	7	6	0.7508
	III-IV	40	18	22	
Smoking history					
	Smoker	41	17	24	0.1896
	Non-smoker	12	8	4	
Treatment					
	Surgery±Radiation	20	6	14	0.0876
	Radiation±Chemotherapy	33	19	14	
HPV					
	positive	22	11	11	0.7280
	negative	31	14	17	

Smoker is defined as a patient with smoking history. Non-smoker is defined as a patient without any history of smoking.
HPV, human papillomavirus; SPARC, secreted protein acidic and rich in cysteine.

Table 3. Overall survival analyses using univariate and multivariate Cox proportional hazards model

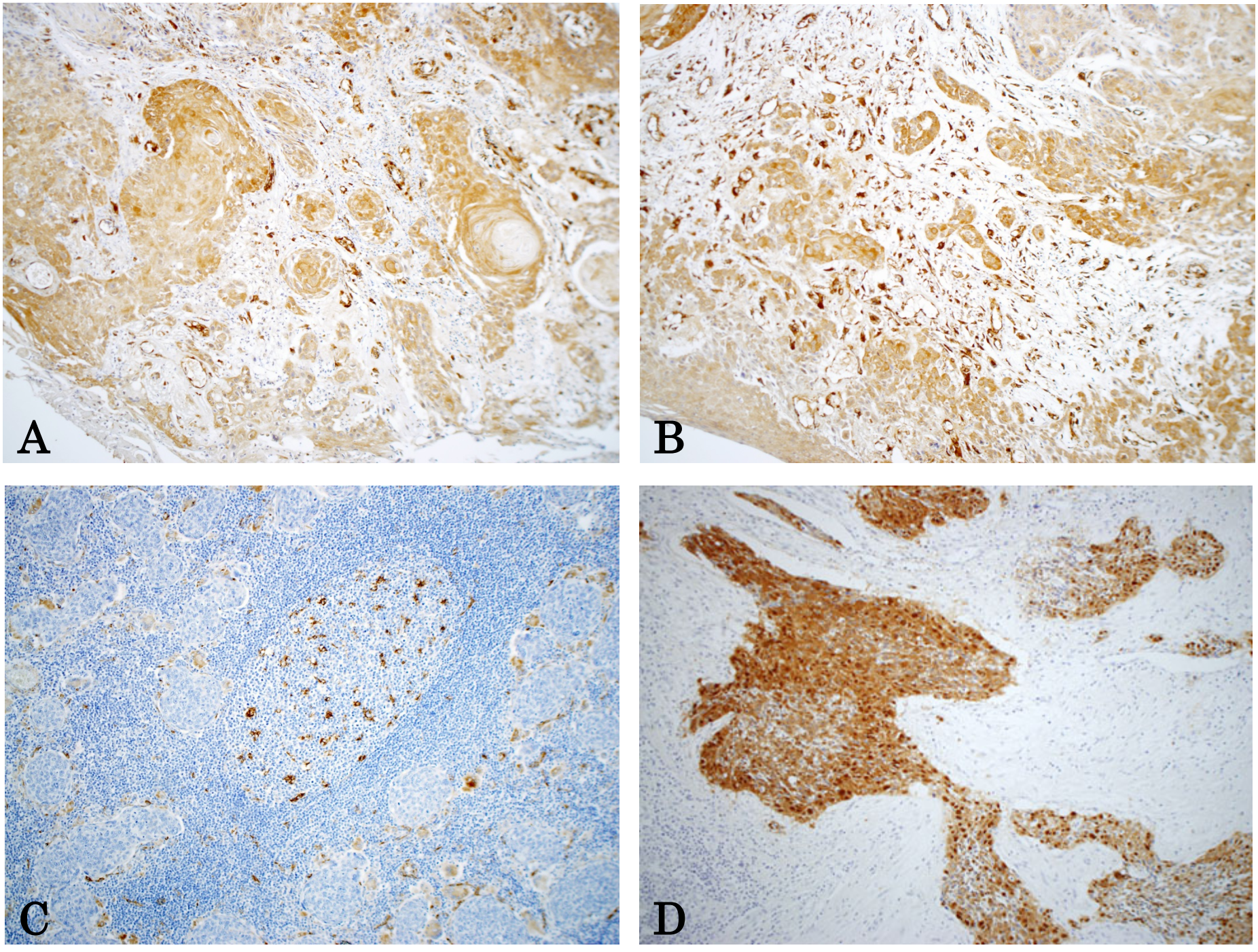
Characteristics	Overall survival			
	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p*	HR (95% CI)	*p*
Age (≧62 vs. <62)	1.016 (0.368-2.806)	0.976		
Sex (Male vs. Female)	1.741 (0.224-13.565)	0.596		
Smoking history (Smoker vs Non-smoker)	1.095 (0.343-3.497)	0.878		
T factor (T3,4 vs. T1,2)	2.068 (0.717-5.963)	0.179		
N factor (N1-3 vs. N0)	0.546 (0.198-1.511)	0.244		
Stage (III,IV vs. I,II)	1.411 (0.396-5.026)	0.595		
Therapy (Radiation vs. Surgery)	2.501 (0.705-8.864)	0.156		
HPV (Positive vs. Negative)	0.181 (0.041-0.805)	0.025	0.173 (0.039-0.769)	0.021
SPARC (High vs. Low)	4.911 (1.277-17.516)	0.014	5.125 (1.437-18.276)	0.012

HPV, human papillomavirus; SPARC, secreted protein acidic and rich in cysteine.

Table 4. Disease-free survival analyses using univariate and multivariate Cox proportional hazards model

Characteristics	Disease-free survival			
	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p*	HR (95% CI)	*p*
Age (≧62 vs. <62)	1.245 (0.557-2.784)	0.593		
Sex (Male vs. Female)	3.102 (0.415-23.203)	0.270		
Smoking history (Smoker vs Non-smoker)	1.366 (0.510-3.650)	0.535		
T factor (T3,4 vs. T1,2)	1.323 (0.584-2.999)	0.502		
N factor (N1-3 vs. N0)	0.378 (0.169-0.843)	0.018	0.480 (0.211-1.094)	0.081
Stage (III,IV vs. I,II)	0.621 (0.267-1.443)	0.268		
Therapy (Radiation vs. Surgery)	0.905 (0.405-2.019)	0.807		
HPV (Positive vs. Negative)	0.257 (0.095-0.694)	0.007	0.302 (0.109-0.839)	0.022
SPARC(High vs. Low)	1.744 (0.781-3.895)	0.175		

HPV, human papillomavirus; SPARC, secreted protein acidic and rich in cysteine.

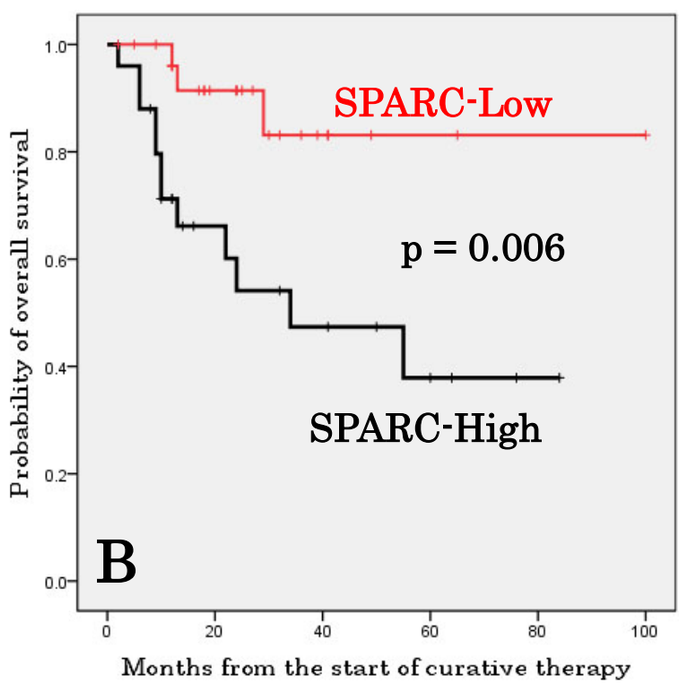

A
B
C
D

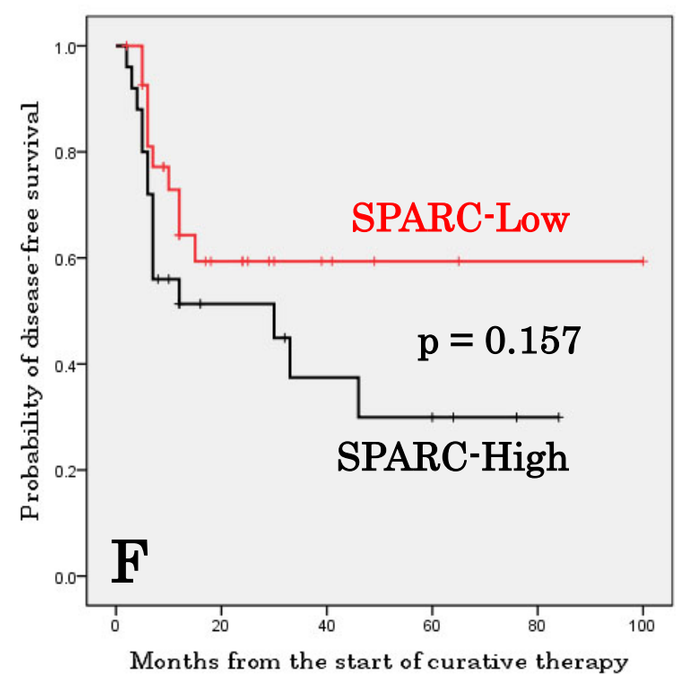
Overall survival

Disease-free survival

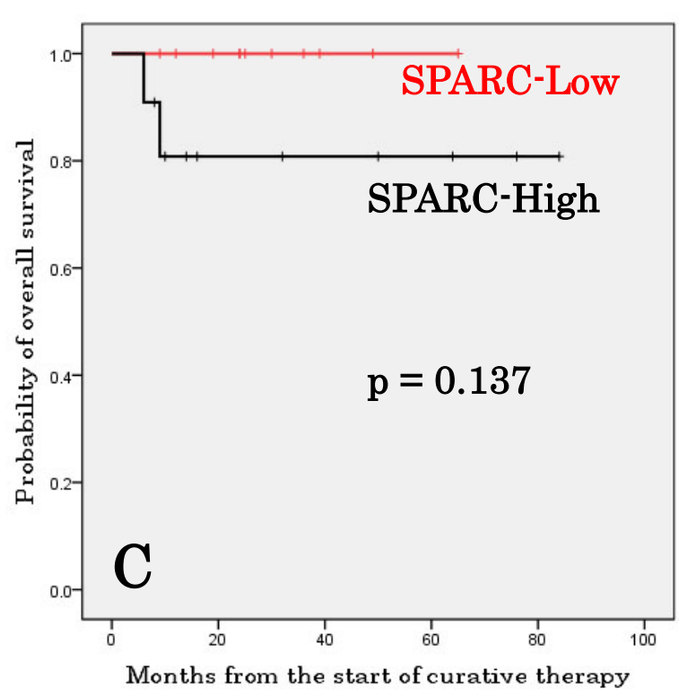
All patients

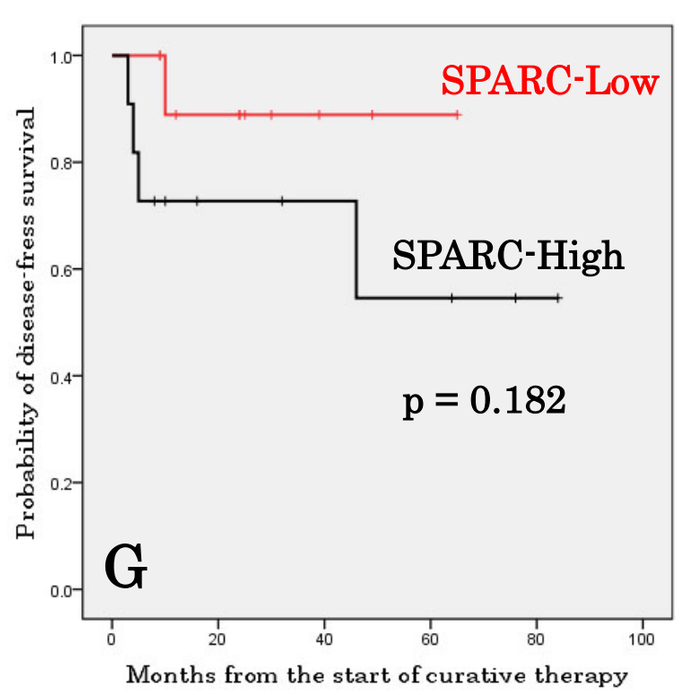
All patients





HPV-positive patients





HPV-negative patients