HPV, p16 and p53 As Indicators of Response to Survival in Larynx Squamous Cell Carcinoma

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RESEARCH

HPV,p16 and p53 As Indicators of Response to Survival in Larynx Squamous Cell Carcinoma

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Abstract

Purpose: To analyze the relationship between the prognosis of patients with larynx squamous cell carcinoma (LSCC) and human papillomavirus (HPV) infection, p16 and p53 protein expression.

Methods: All patients were treated at the department of radiation oncology, Anhui provincial hospital,between May 2005 and May 2012. The 41 consecutive patients with LSCC were treated surgically and received postoperative radiotherapy. Analyses of pathology specimen were surgically removed and performed on formalin-fixed, paraffin-embedded tissue samples. HPV DNA sequences in tumor tissues were screened by a commercial Luminex technique for HPVs and HPV-specific PCR assays. P16 and p53 protein expression were detected by immunohistochemical staining.Overall survival(OS)and progression-free survival(PFS)for HPV-positive and HPV-negative patients, p16-positive and p16-negative patients, p53-positive and p53-negative patients were estimated by Kaplan-Meier analysis. Cox regression model was used for multivariate analysis.

Results: HPV-DNA was detected in 4(9.7%)of all specimens.Among them, 3 were positive for HPV-56,1 for HPV-16. With the follow-up of 3-78 months(a median of 34 months),patients with HPV-positive tumors had better overall survival than patients with HPV-positive tumors(75% vs 61%, $P>0.05$). Multivariate analysis by Cox regression model showed that nodal status was independent prognostic factors for patients with LSCC($P<0.05$).

Conclusions: HPV status is not an independent prognostic factor. Nodal status was independent prognostic factors for patients with LSCC.

Keywords: Larynx Squamous Cell Carcinoma(LSCC); Human papillomavirus (HPV); survival

Background

Laryngeal cancer is one of the most common malignant tumors in head and neck, accounting for about 1/5 of the malignant tumors in head and neck[1, 2]. About 85% of head and neck tumors are squamous cell carcinoma[3]. Globally, there are approximately 177,000 new cases and 94,000 deaths of laryngeal cancer each year[2]. It is well known that smoking and drinking alcohol are two major risk factors for laryngeal cancer[4, 5], and recent studies have found that HPV is also associated with the occurrence of laryngeal cancer, Since 1982, when Syrjanen et al[6]. were first found to have HPV infection in laryngeal cancer, People have used different

detection techniques to detect the expression of HPV in laryngeal cancer, and the positive rate of HPV detection in laryngeal cancer later varies from 3% to 100%, showing a large difference. Moreover, the relationship between HPV status and the prognosis of laryngeal cancer is still controversial. A meta-analysis showed that, the OS of the HPV-positive group was better than that of the HPV-negative group in terms of short-term survival. Compared with the HPV-negative group, the HPV-positive group had a better trend of DFS[7].

Materials and methods

Criteria for inclusion

In this group, laryngeal squamous cell carcinoma patients who visited our hospital and received surgery combined with radiotherapy from June 2005 to May 2012 were selected. Forty-one patients who met the following conditions were analyzed: ① Complete clinical and pathological data are available; ② The pathological specimen was squamous cell carcinoma with sufficient quality and quantity of tissue specimen for sectioning analysis. This study is a retrospective study, the specimens were paraffin-embedded tissue archived by the Department of Pathology, the diagnosis of squamous cell carcinoma and its differentiation degree was determined according to the relevant standards of the World Health Organization and was interpreted by two pathologists with the level of chief physician.

The general situation

The 41 patients, 40 males, 1 female; The age ranged from 38 to 80, with a median age of 56; 19 cases of high differentiation, 19 cases of moderate differentiation, 3 cases of low differentiation. Tumor in clinical stage according to UICC/AJCC2007 tumor clinical staging criteria, staging I period in 2 cases, II period in 11 cases, III 8 cases, 20 cases IV period.

Treatment

All the patients received surgery and postoperative radiotherapy, with a total DT 50-60Gy. Among them, 3 patients with severe adverse reactions were unable to tolerate or had economic difficulties, and the dose was 18∼40Gy; 22 patients needed to receive chemotherapy due to their condition, including 2 patients receiving cisplatin synchronous chemotherapy, 15 patients receiving induced chemotherapy, 4 patients receiving adjuvant chemotherapy, and 1 patient receiving both induced chemotherapy and adjuvant chemotherapy.

The experimental method

Specimen preparation

The surgical specimens were fixed with 10% formaldehyde solution, embedded in paraffin, and stored in the pathology department. The tissue specimens were sliced with "sandwich" technology. A total of 8 wax rolls were cut. The first and last rolls were about 0.4 micron thick, and HE staining was performed, Squamous cell carcinoma was reconfirmed by the pathologist. The six intermediate wax coils, each about 0.8 micron thick, were divided into 2 parts on average and placed into two 1.5ml spiral tubes respectively for DNA extraction.

HPV DNA extraction

DNA fragments of HPV were extracted with the fixed tissue genomic DNA extraction kit (Kang Wei Ji Company). The DNA solution was obtained through dewaxing, centrifugation, debenzenization, vortex oscillation and other steps according to the manufacturer's instructions, and then stored in a refrigerator at -20°C, the extracted DNA was amplified by multiplex PCR, and hybridized with a variety of coded microspheres coated with nucleic acid probes, and the results were detected and analyzed by flow matrix.

Detection of HPV nucleic acid

The PCR system consisted of PCR premix 10.0μL, primer mixture 5.0μL, nucleic acid template (sample) 5.0μL and polymerase 0.8μL. After preparing an appropriate amount of PCR reaction solution according to the sample size, the PCR reaction solution was divided into each PCR reaction tube according to 15μL/tube; The samples were added into the corresponding PCR reaction tubes at the amount of 5μL/tube one by one, and the samples were centrifuged at 2000rpm for 10 seconds after the lid was closed; Put the PCR reaction tube into the PCR instrument, set the hot cover function, and conduct the amplification reaction according to the following conditions: 95°C for 5 minutes;95°C 30 seconds, 58°C 30 seconds, 72°C 30 seconds, a total of 5 cycles; 95°C 30 seconds, 55°C 30 seconds, 72°C 30 seconds, a total of 35 cycles;72°C for 3 minutes. Then the microsphere hybridization liquid reagent bottle was placed on the scroll apparatus and oscillated for 30 seconds. 3μL of PCR amplification products were absorbed from each sample, and added into corresponding hybridization well successively, and then pumped for several times to mix.Cut the corresponding size of the sealing board and cover the sealing plate with the microporous hybrid plate. The hybrid plates were placed in a metal bath at a constant temperature for denaturation at 95°C for 5 min and hybridization at 48°C for 30 min. Remove the sealing sheet, add 75μL fluorescein SA-PE to each well, mix well, glue the sealing sheet again, and incubate at 48°C for 15 min. The microporous hybrid plate was quickly transferred to the preheated Luminex flow analyzer (Shanghai Transview Company) for reading [8].

The whole experimental process was completed in the clean section room. The operators used disposable protective clothing, wearing hats, masks and gloves. At the same time, one negative control (blank wax block) and one positive control (pathological tissue specimens of cervical squamous cell carcinoma untreated in our hospital in the same number of years) were set for every 10 wax blocks.

Detection of p16 and p53

Tissue specimens first underwent three steps: xylene dewaxing, anhydrous ethanol debenzenation and hydration. PH6.0 citric acid buffer was used for high pressure repair for 2min. After cooling, H2O2 is oxidized for 5-6 minutes; PBS washing and adding primary antibody (>1h), PBS rinse after water; then the secondary antibody (20min) was added, and the water was flushed with PBS. DAB staining for 10min; Anhydrous ethanol dehydration, xylene transparent, fixed cover. After the film was finished, the chief physician of the Department of Pathology read the film, and the staining results were evaluated by double-blind method. Dyeing strength: colorless,

0; Light brown yellow, 1 mark; Tan, 2 points; Dark brown, 3 points. Five high-magnification fields (×400) were selected for each section under the light microscope to take the average value: no positive cells, 0 score; The number of positive cells ≤10%, 1 point;11%-50%, 2 points;51%-75%, 3 marks; ≥75%, 4 points. The staining intensity × staining percentage was used to score comprehensively: <3 is negative, ≥3 is positive[9].

Statistical method

Kaplan-Meier survival analysis was performed using SPSS22.0 software, and 3-year overall survival rate and progression-free survival rate (no local recurrence, regional lymph node metastasis, and no distant metastasis) were calculated. Log-rank test was used for univariate analysis, and Cox model was used for multivariate analysis. Chi-square test was used between counting data, P<0.05 indicates that the difference is statistically significant.

results

HPV infection rate and its epidemiological characteristics

The overall rate of HPV infection was 9.7% (4/41), including 3 cases of HPV-56 and 1 case of HPV-16.The positive rate of P16 was 7.3% (3/41), including 25% (1/4) of the HPV positive group and 5.4% (2/37) of the HPV negative group. The P53 positive rate was 56% (23/41), including 25% (1/4) in the HPV positive group and 59% (22/37) in the HPV negative group. Figure 2 In terms of gender, there was a statistically significant difference between HPV-positive and HPV-negative patients (P =0.002), while there was no statistically significant difference between HPV-positive and HPV-negative patients in terms of age, history of alcohol use, smoking history, T grade, N grade, and clinical stage. But our study also showed a trend that HPV-positive patients smoked less than those who were negative. The smoking rate was 50 percent in the HPV-positive group and 64.9 percent in the negative group, respectively.(table 1).

HPV infection status and prognosis

In this study, the follow-up time was from 3 to 78 months.The median follow-up time was 34 months. During the follow-up period, 1 case of HPV positive group died of treatment complications, and 1 case was lost to follow-up. In the HPV-negative group, 9 cases died, including 1 case of uncontrolled local lesions, 5 cases of local recurrence or lymph node metastasis, 1 case of combined with second tumor, and 2 cases of other causes. The 3-year overall survival rate of HPV-positive patients was better than that of negative patients, but the difference was not statistically significant (75% vs 61%, P>0.05), Figure 1, the 3-year progression-free survival of HPV-positive patients was advantageous, but the difference was not statistically significant (75% vs 58.7%, P>0.05), Figure 2.

P16, P53 expression and prognosis

The expression of P16 and p53 was associated with tumor proteins produced by HPVE7 and E6 genes, respectively. Kaplan-Meier analysis showed that neither of them were independent influencing factors for the prognosis of patients with laryngeal cancer, although the 3-year survival rate of P16-positive patients (66.7% vs

62.2%, P>0.05) and 3-year progression-free survival (88.9% vs 76.9%, P>0.05) was better than P16 negative patients, as shown in Figure 3 and 4.

Univariate analysis showed that N status and age had influence on overall survival rate, while gender, smoking history, pathological differentiation, stage and other clinical factors had no influence on overall survival rate and progression-free survival rate. Cox model analysis of the above related factors showed that N status had a significant impact on overall survival, but no significant impact on progression-free survival (Table 2).

Representative case of HPV-positive LSCC

A 71-year-old man, with no smoking history alcohol drinkin, presented to a referral hospital with a 6-month history of hoarseness. The transglottic lesion was confirmed as moderately-high differentiated squamous cell carcinoma on pathologic examination (Fig. 5a). The patient underwent surgical treatment. Pathological examination of the resected specimen revealed pT3N0M0, the surgical margins were negative. Examination of the lymph nodes revealed no lymph node metastasis. Radiotherapy after surgery was carried out with 60Gy irradiation to the entire neck. As of 4 years since the above treatment, the patient is alive without local recurrence or distant metastasis. PCR for HPV DNA revealed the presence of HPV-56 infection.P16 and P53 expression were observed in primary lesion (Fig. 5b, 5c).

Conclusions

Smoking and drinking alcohol are major risk factors for head and neck cancers, which are linked to about 80 percent of patients.Studies have shown that HPV-positive patients with no smoking history have a better prognosis than patients with smoking history[10]. Etiological studies have confirmed the close correlation between smoking and the occurrence of laryngeal cancer, that is, compared with non-smokers, the incidence of laryngeal cancer and the incidence of the second tumor are significantly increased in smokers, and the survival time after treatment is shortened. Our study showed that smoking was not a major factor affecting the prognosis of patients with laryngeal cancer, but it also showed a trend: smoking rates were lower in the HPV-positive group, and all HPV-positive patients who had never smoked survived. This may be related to the limited sample size of this study.

Currently, it appears that HPV-positive tumor cells are more sensitive to radiation and chemotherapy than HPV-negative tumor cells. Recent research supports this idea from a genetic perspective. HPV-positive tumors have different biological characteristics compared with HPV-negative tumors, including poorer differentiation, less keratinization, and different gene expression and chromosomal variation[11, 12]. Chromosomal aberration rates in HPV-positive tumors were significantly lower than those in HPV-negative tumors ($P = 0.03$), and the same was true for chromosome doubling ($P = 0.039$)[11]. Another study showed that a group of 40 inducible genes and a group of 19 genes were expressed only in HPV-positive tumor cells and not in either HPV-negative tumor cells or normal tissue cells. The functions of these genes mainly include mitosis, DNA repair and transcription[12].

In recent years, with the in-depth study of a large number of HPV and head and neck squamous cell carcinoma, the relationship between HPV and head and neck

squamous cell carcinoma has become increasingly clear, and a number of studies have shown that HPV is an independent risk factor affecting the prognosis of patients with head and neck squamous cell carcinoma[13, 14, 15, 16]. Therefore, HPV infection is a relatively clear prognostic index of head and neck squamous cell carcinoma.However, a number of studies have shown that HPV is not an independent prognostic factor in patients with laryngeal cancer, and the relationship between HPV and laryngeal cancer is still controversial[17, 18, 19]. In this study, HPV-positive patients had an advantage over HPV-negative patients in terms of survival rate between the two groups, but the difference was not statistically significant (P>0.05).

In this study, we also analyzed the relationship between the expression of P16 and P53 and the prognosis of patients with laryngeal cancer. Although P16-positive patients had better 3-year overall survival compared with P16-negative tumors (66.7% vs 62.2%, P>0.05) and 3-year progression-free survival (88.9% vs 76.9%, P>0.05), the difference between the two was not statistically significant. Neither P16 nor p53 expression was an independent risk factor affecting the prognosis of laryngeal cancer. This is consistent with previous studies[20, 21].

The correlation between HPV infection, p16 protein expression and p53 protein expression and prognosis of laryngeal cancer is one of the hot topics in global research. This study preliminarily suggested that the HPV infection status of laryngeal cancer has no significant prognostic significance, and the expression of p16 and p53 is also the same. However, the sample size of this group is limited, and there may be bias due to the large difference in the sample size between positive and negative expression. Therefore, multi-center, multi-region and large-sample in-depth study and cooperative study are still needed for the HPV infection status of laryngeal cancer patients.

Appendix

Acknowledgements
This study was supported by and conducted in cooperation with the pathology department. Text for this section...

Abbreviations
UICC:Union for International Cancer Control; AJCC: American Joint Committee on Cancer; LSCC: Larynx Squamous Cell Carcinoma; HPV: Human papillomavirus; PCR: Polymerase chain reaction; CS: Cumulative survival; PFS: Progression-free survival.

Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate
This study was approved by the Institutional Review Board of the Anhui Provincial Cancer Hospital, the First Affiliated Hospital Of USTC and was carried out in accordance with the 1975 Declaration of Helsinki, as revised in 2008.

Competing interests
The authors declare that they have no competing interests.

Consent for publication

Authors' contributions
KL contributed to the experimental studies, data acquisition, and preparation of the manuscript; JG, ZCT contributed to the experiments and data acquisition. LTQ contributed to the study design, supervision of experiments, and manuscript review. All authors read and approved the final manuscript.

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References

1. Siegel, R.L., Miller, K.D., Jemal, A.: Cancer statistics, 2019. CA: a cancer journal for clinicians **69**(1), 7–34 (2019)
2. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A., Jemal, A.: Global cancer statistics 2018: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians **68**(6), 394–424 (2018)
3. Mehanna, H., Paleri, V., West, C., Nutting, C.: Head and neck cancerpart 1: epidemiology, presentation, and prevention. Bmj **341**, 4684 (2010)
4. Pelucchi, C., Gallus, S., Garavello, W., Bosetti, C., La Vecchia, C.: Alcohol and tobacco use, and cancer risk for upper aerodigestive tract and liver. European journal of cancer prevention **17**(4), 340–344 (2008)
5. Zuo, J.-J., Tao, Z.-Z., Chen, C., Hu, Z.-W., Xu, Y.-X., Zheng, A.-Y., Guo, Y.: Characteristics of cigarette smoking without alcohol consumption and laryngeal cancer: overall and time-risk relation. a meta-analysis of observational studies. European Archives of Oto-Rhino-Laryngology **274**(3), 1617–1631 (2017)
6. Syrjänen, K., Syrjänen, S., Pyrhönen, S.: Human papilloma virus (hpv) antigens in lesions of laryngeal squamous cell carcinomas. ORL **44**(6), 323–334 (1982)
7. Wang, H., Wei, J., Wang, B., Meng, L., Xin, Y., Dong, L., Jiang, X.: Role of human papillomavirus in laryngeal squamous cell carcinoma: A meta-analysis of cohort study. Cancer Medicine **9**(1), 204–214 (2020)
8. Ikegami, T., Uehara, T., Deng, Z., Kondo, S., Maeda, H., Kiyuna, A., Agena, S., Hirakawa, H., Yamashita, Y., Ganaha, A., *et al.*: Detection of human papillomavirus in branchial cleft cysts. Oncology letters **16**(2), 1571–1578 (2018)
9. Marur, S., D'Souza, G., Westra, W.H., Forastiere, A.A.: Hpv-associated head and neck cancer: a virus-related cancer epidemic. The lancet oncology **11**(8), 781–789 (2010)
10. St Guily, J.L., Rousseau, A., Baujat, B., Périé, S., Schultz, P., Barry, B., Dufour, X., Malard, O., Pretet, J.-L., Clavel, C., *et al.*: Oropharyngeal cancer prognosis by tumour hpv status in france: the multicentric papillophar study. Oral oncology **67**, 29–36 (2017)
11. Akagi, K., Li, J., Broutian, T.R., Padilla-Nash, H., Xiao, W., Jiang, B., Rocco, J.W., Teknos, T.N., Kumar, B., Wangsa, D., *et al.*: Genome-wide analysis of hpv integration in human cancers reveals recurrent, focal genomic instability. Genome research **24**(2), 185–199 (2014)
12. Parfenov, M., Pedamallu, C.S., Gehlenborg, N., Freeman, S.S., Danilova, L., Bristow, C.A., Lee, S., Hadjipanayis, A.G., Ivanova, E.V., Wilkerson, M.D., *et al.*: Characterization of hpv and host genome interactions in primary head and neck cancers. Proceedings of the National Academy of Sciences **111**(43), 15544–15549 (2014)
13. Garnaes, E., Frederiksen, K., Kiss, K., Andersen, L., Therkildsen, M.H., Franzmann, M.B., Specht, L., Andersen, E., Norrild, B., Kjaer, S.K., *et al.*: Double positivity for hpv dna/p16 in tonsillar and base of tongue cancer improves prognostication: insights from a large population-based study. International journal of cancer **139**(11), 2598–2605 (2016)
14. Posner, M., Lorch, J., Goloubeva, O., Tan, M., Schumaker, L., Sarlis, N., Haddad, R., Cullen, K.: Survival and human papillomavirus in oropharynx cancer in tax 324: a subset analysis from an international phase iii trial. Annals of oncology **22**(5), 1071–1077 (2011)
15. Tahtali, A., Hey, C., Geissler, C., Filman, N., Diensthuber, M., Leinung, M., Stoever, T., Wagenblast, J.: Hpv status and overall survival of patients with oropharyngeal squamous cell carcinoma–a retrospective study of a german head and neck cancer center. Anticancer research **33**(8), 3481–3485 (2013)
16. Kiyuna, A., Ikegami, T., Uehara, T., Hirakawa, H., Agena, S., Uezato, J., Kondo, S., Yamashita, Y., Deng, Z., Maeda, H., *et al.*: High-risk type human papillomavirus infection and p16 expression in laryngeal cancer. Infectious agents and cancer **14**(1), 8 (2019)
17. Erkul, E., Yilmaz, I., Narli, G., Babayigit, M.A., Gungor, A., Demirel, D.: The presence and prognostic significance of human papillomavirus in squamous cell carcinoma of the larynx. European Archives of Oto-Rhino-Laryngology **274**(7), 2921–2926 (2017)
18. Taberna, M., Resteghini, C., Swanson, B., Pickard, R., Jiang, B., Xiao, W., Mena, M., Kreinbrink, P., Chio, E., Gillison, M.: Low etiologic fraction for human papillomavirus in larynx squamous cell carcinoma. Oral oncology **61**, 55–61 (2016)
19. Sánchez Barrueco, A., González Galán, F., Lora Pablos, D., Villacampa Aubá, J.M., Ballestín Carcavilla, C., Cenjor Español, C., Almodóvar Álvarez, C.: Hpv in larynx squamous cell carcinoma: new serotypes and survival study within 10-year follow-up. Otolaryngology–Head and Neck Surgery **156**(4), 677–682 (2017)
20. Wilson, D.D., Rahimi, A.S., Saylor, D.K., Stelow, E.B., Jameson, M.J., Shonka, D.C., Reibel, J.F., Levine, P.A., Read, P.W.: p16 not a prognostic marker for hypopharyngeal squamous cell carcinoma. Archives of Otolaryngology–Head & Neck Surgery **138**(6), 556–561 (2012)
21. Pinheiro, U.B., de Carvalho Fraga, C.A., Mendes, D.C., Farias, L.C., Cardoso, C.M., Silveira, C.M., DAngelo, M.F.S.V., Jones, K.M., Santos, S.H.S., de Paula, A.M.B., *et al.*: Fuzzy clustering demonstrates that codon 72 snp rs1042522 of tp53 gene associated with hnscc but not with prognoses. Tumor Biology **36**(12), 9259–9265 (2015)

Figures

Figure 1 Kaplan-Meier curve of cumulative survival (CS) in Larynx Squamous Cell Carcinoma (LSCC) Kaplan-Meier curve of CS in HPV-positive and HPV-negative.

Tables

Figure 2 Kaplan-Meier curve of Progression-free survival (PFS) in Larynx Squamous Cell Carcinoma (LSCC) Kaplan-Meier curve of PFS in HPV-positive and HPV-negative.

Figure 3 Kaplan-Meier curve of cumulative survival (CS) in Larynx Squamous Cell Carcinoma (LSCC) Kaplan-Meier curve of CS in P16-positive and P16-negative.

Figure 4 Kaplan-Meier curve of Progression-free survival (PFS) in Larynx Squamous Cell Carcinoma (LSCC) Kaplan-Meier curve of PFS in P16-positive and P16-negative.

Figure 5 Representative case data. a HE dyeing of cancer cells($\times$400). b P16 dyeing of cancer cells($\times$400). c P53 dyeing of cancer cells($\times$400).

Table 1 Comparison of general data of HPV positive and negative patients

Clinical factors		HPV-positive	HPV-negative	P values
gender	male	3	37	0.002
	female	1	0	
age(year)	$<$60	1	22	0.303
	$\geq$60	3	15	
smoke	no	2	13	0.615
	yes	2	24	
drink	no	2	24	0.278
	yes	2	13	
T-staging	T1-2	2	15	1.000
	T3-4	2	22	
N-state	N0	4	30	0.586
	N+	0	7	
UICC staging system	I-II	2	12	0.579
	III-IV	2	26	
pathological grading	poorly differentiated	0	3	0.098
	moderately differentiated	3	18	
	high differentiated	1	16	
p16 expression	negative	3	35	0.271
	positive	1	2	
p53 expression	negative	3	15	0.303
	positive	1	22	

Table 2 Multivariate Cox regression analysis of prognosis of patients with laryngeal cancer

	regression coefficient	Standard error	Wald χ^2 value	p value	Odds ratio	95.0% CI of Odds ratio	
						Bottom	upside
N staging	1.611	0.677	5.672	0.017	5.009	1.330	18.863

Figures

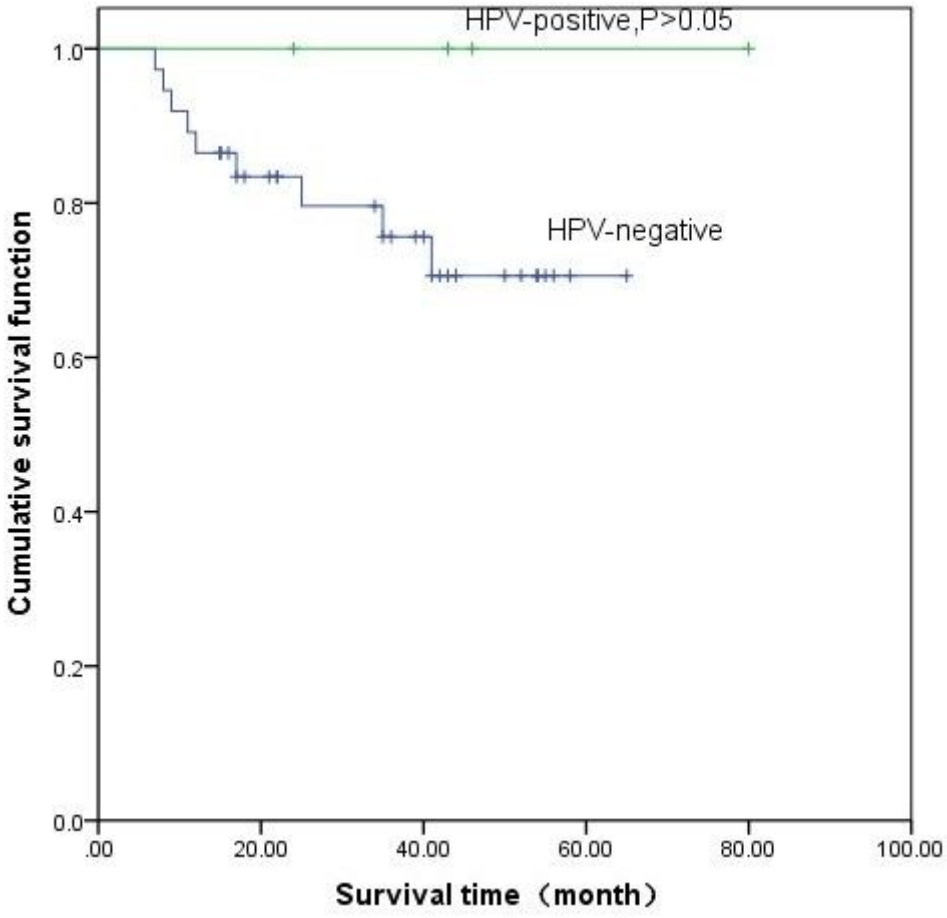


Figure 1

Kaplan-Meier curve of cumulative survival (CS) in Larynx Squamous Cell Carcinoma (LSCC) Kaplan-Meier curve of CS in HPV-positive and HPV-negative.

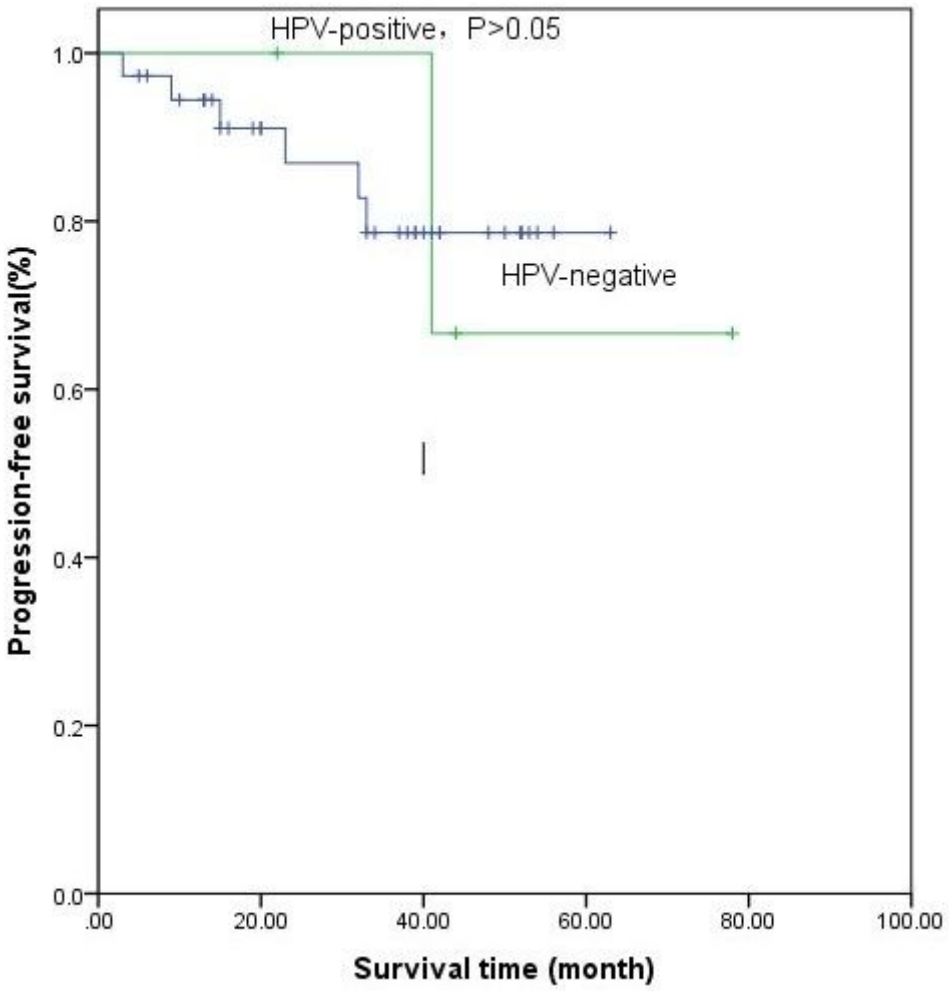


Figure 2

Kaplan-Meier curve of Progression-free survival (PFS) in Larynx Squamous Cell Carcinoma (LSCC) Kaplan-Meier curve of PFS in HPV-positive and HPV-negative.

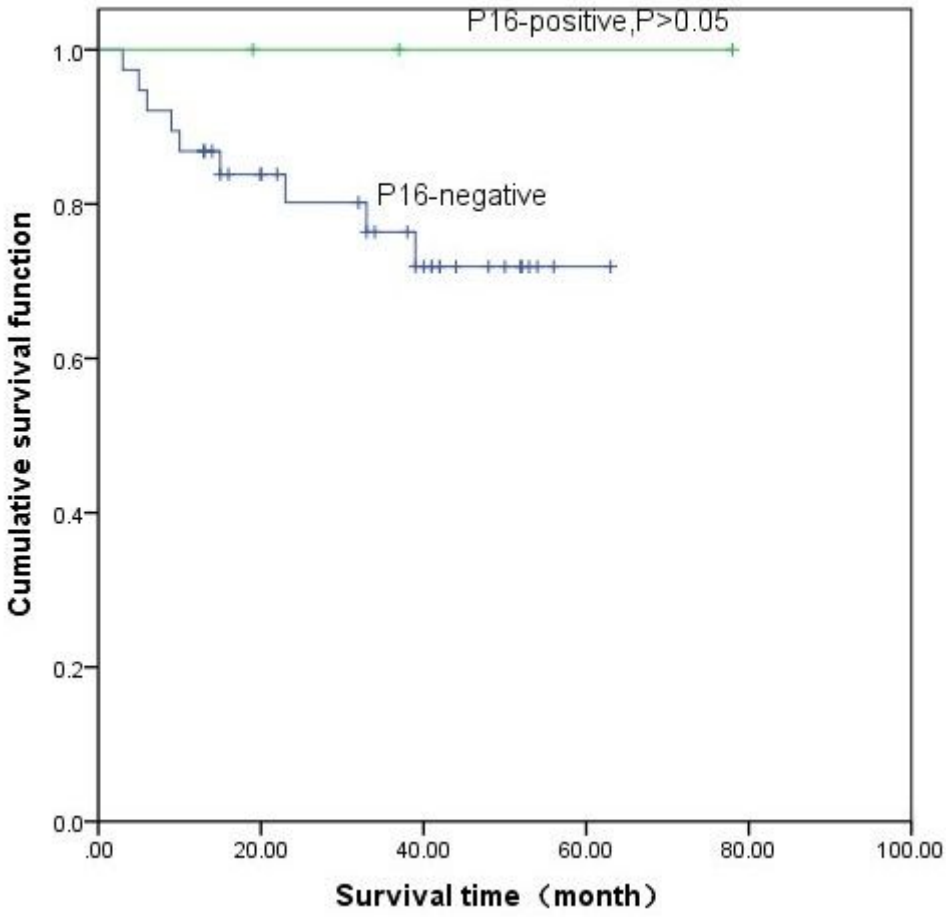


Figure 3

Kaplan-Meier curve of cumulative survival (CS) in Larynx Squamous Cell Carcinoma (LSCC) Kaplan-Meier curve of CS in P16-positive and P16-negative.

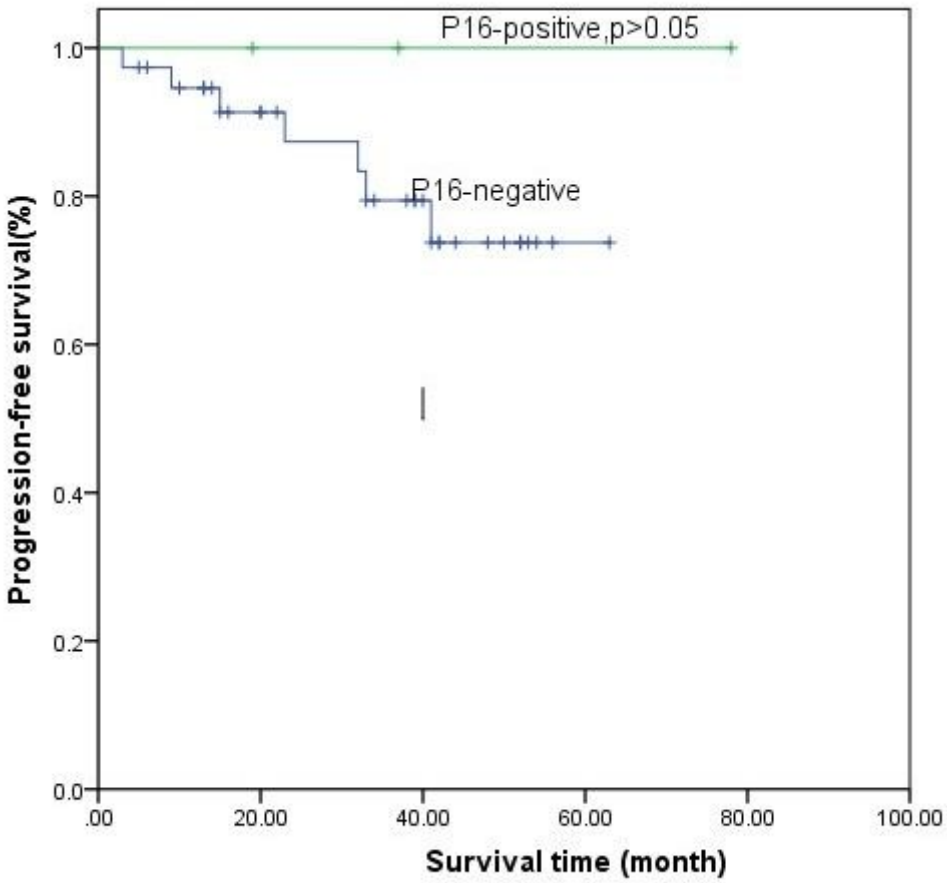
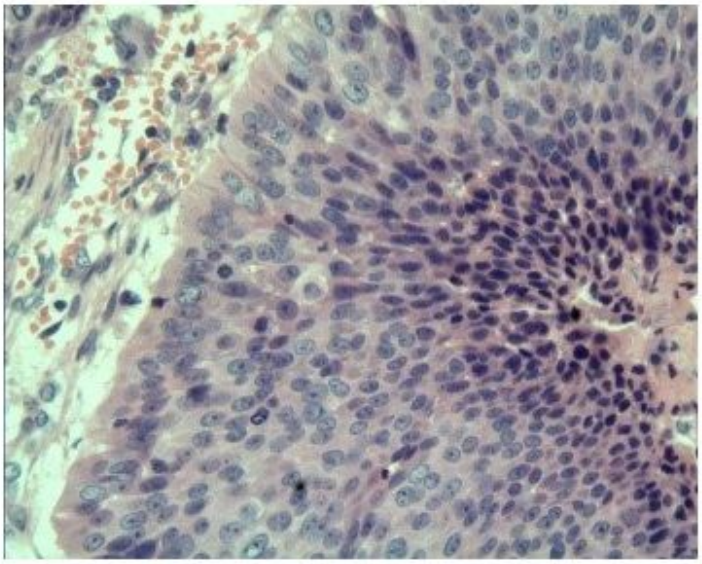


Figure 4

Kaplan-Meier curve of Progression-free survival (PFS) in Larynx Squamous Cell Carcinoma (LSCC) Kaplan-Meier curve of PFS in P16-positive and P16-negative.



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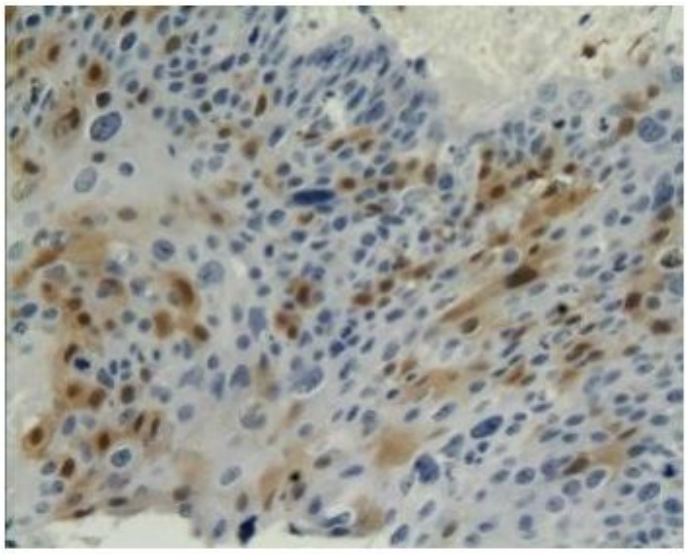

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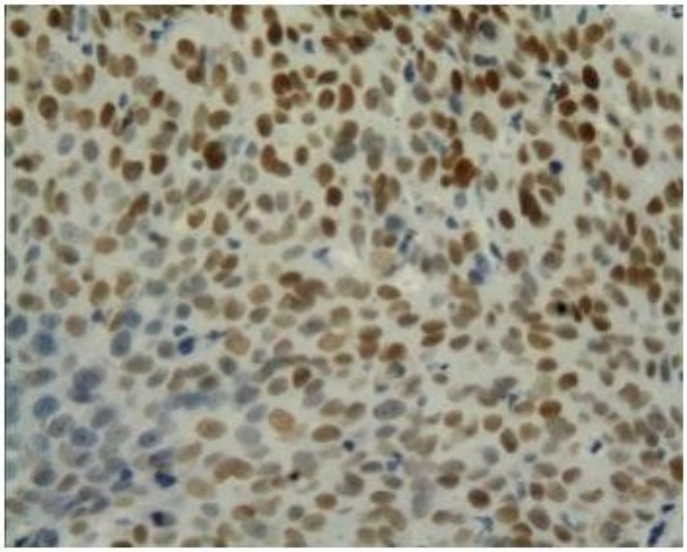

c

Figure 5

Representative case data. a HE dyeing of cancer cells(×400). b P16 dyeing of cancer cells(×400). c P53 dyeing of cancer cells(×400).