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Original Article

Prognostic value of human papillomavirus infection and p53, p16, epidermal growth factor receptor and $p34^{cdc2}$ expression in patients with salivary adenoid cystic carcinoma

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Abstract: The aim of this study was to explore the correlation between HPV infection, p53, p16, epidermal growth factor receptor (EGFR), $p34^{cdc2}$ protein expression and prognosis in patients with adenoid cystic carcinoma of salivary gland. Totally 78 cases of adenoid cystic carcinoma of salivary gland specimens were selected from January 1, 2004 to December 31, 2013 in Tangshan Union Hospital. PCR-reverse dot blot hybridization was used to detect infection of human papilloma virus (HPV), and SP immunohistochemical method was adopted to detect the expression of p53, p16, EGFR and $p34^{cdc2}$ protein in the carcinoma tissues. Clinical data were collected and the patients were followed up. Results showed that the infection rate of HPV in adenoid cystic carcinoma tissues was 0% (0/78). The expression rate of p53, p16, EGFR and $p34^{cdc2}$ protein in carcinoma tissues were 75.6% (59/78), 57.7% (45/78), 60.1% (47/78) and 64.1% (50/78), respectively. Expression of p53, p16, EGFR and $p34^{cdc2}$ proteins was not significantly correlated with patients' age, gender, disease location, TNM classification and histological type ($P > 0.05$). Kaplan-Meier analysis showed that EGFR-positive patients had a lower median overall survival than EGFR-negative ones (58 months vs. 75 months, respectively. $P = 0.001$). The result of median progression-free survival was virtually the same for both EGFR-positive and EGFR-negative patients (43 months vs. 49 months, respectively. $P = 0.002$). $p34^{cdc2}$-positive patients had a lower median overall survival than $p34^{cdc2}$-negative ones (61 months vs. 71 months, respectively. $P = 0.027$). Median progression-free survival was also almost the same for both $p34^{cdc2}$-positive and $p34^{cdc2}$-negative patients (44 months vs. 51 months, respectively. $P = 0.011$). Cox regression analysis showed that expression of EGFR and $p34^{cdc2}$ was independent risk factors for the prognosis of patients with adenoid cystic carcinoma of salivary gland (relative risk = 13.199, 11.466, $P < 0.001$). In conclusion, HPV infection is not detected in salivary adenoid cystic carcinoma tissues. p53, p16, EGFR and $p34^{cdc2}$ protein are positively expressed in most salivary adenoid cystic carcinoma tissues. p16 is unsuitable as a surrogate for HPV infection status in patients with adenoid cystic carcinoma of salivary gland. Expression of EGFR and $p34^{cdc2}$ is independent risk factors in the prognosis of patients with salivary gland adenoid cystic carcinoma. Patients with EGFR or $p34^{cdc2}$ positive expression should be followed up closely.

Keywords: Adenoid cystic carcinoma, HPV, EGFR, Cdc2, prognosis

Introduction

Adenoid cystic carcinoma (ACC) is one of the most common malignant tumors in salivary gland, which is a relatively rare tumor in the parotid gland with a 10% incidence, and its incidence rate in minor salivary glands is about 30% [1]. Histologically, the tumor shows the characteristics of local growth, perineural invasion, distant metastasis and high recurrence rate. Evaluations of the prognosis and tumor markers of recurrence in patients with adenoid cystic carcinoma are ongoing in recent years. Viral infection has become a recognized cause in human head and neck tumors, such as human papilloma virus (HPV) and Epstein-Barr virus (EBV) [2]. HPV infection can induce squamous cell carcinoma at multiple sites, such as the cervix and oral cavity, while its role in the development of salivary gland tumors is still

questionable. The purpose of this study was to evaluate the correlation of HPV infection and cyclin-dependent kinase inhibitor 2A (CDKN2A/p16), tumor protein p53 (TP53), EGFR and cell cycle-dependent kinase $p34^{cdc2}$ protein to determine the relationship between these factors and the occurrence, development and prognosis of salivary gland adenoid cystic carcinoma.

Here, we analyzed the HPV DNA by PCR-DNA reverse dot blot and examined the expression of p53, p16, EGFR and $p34^{cdc2}$ by immunohistochemistry in the adenoid cystic carcinoma of salivary gland, to investigate the relationship between HPV infection, the expression of the four proteins and prognosis of salivary gland adenoid cystic carcinoma patients.

Patients and methods

Clinical and pathological data

Our study involved 78 cases of salivary gland adenoid cystic carcinoma resection specimens selected at Tangshan Union Hospital from January 1, 2004 to December 31, 2013. All the cases underwent HPV-DNA, p53, p16, EGFR and $p34^{cdc2}$ protein detection, including 41 males and 37 females, aged 41 to 70 years, with median age 51 years. Lesional location was in the parotid gland (26 cases), submandibular gland (24 cases), pars palatalis (21 cases) and sublingual gland (7 cases), respectively. Histological classification included solid type, sieve type and tubular type. According to the sixth edition of the American Joint Committee on Cancer (AJCC) in 2002, they were all divided into four stages: including 6 cases in stage I, 16 cases in stage II, 20 cases in stage III and 36 cases in stage IV. All the 78 patients involved in the study have been informed content and approved by the Ethics Committee of Tangshan Union Hospital. All the patients were followed up every 3 months by telephone to record the survival outcomes. Follow-up rate was 100% and date was up to January 6, 2016.

HPV DNA detection by PCR-DNA reverse dot blot hybridization

Human Papillomavirus Subtype Nucleic Acid Detection Kit (Shenzhen Yaneng Biotechnology Co., Ltd., China) was used to detect the HPV infection with PCR-DNA reverse dot blot hybridization. The paraffin-embedded salivary adenoid cystic carcinoma specimens were cut to extract HPV-DNA, PCR amplification, hybridization, filter washing and color development according to kit instructions. The positive quality control was colored (blue spots) at the corresponding and the IC membrane sites and other sites were not colored. In accordance with the order of the probe sequence and the color on the membrane the HPV genotypes were determined.

p53, p16, EGFR and $p34^{cdc2}$ expression by immunohistochemistry

All the surgically removed tissue specimens were fixed in 10% neutral formalin, embedded in paraffin, and one representative block from each patient was sectioned at 4 μm, stained with hematoxylin and eosin (HE) and evaluated by immunohistochemistry according to the protocol described in the manufacturer's guide accompanying the kit. The mouse anti human p53 (1:100), EGFR (1:100) and $p34^{cdc2}$ (1:300) monoclonal antibody were all purchased from Santa Cruz Biotechnology (USA). Mouse anti human p16 monoclonal antibody (1:100) and SP immunohistochemistry kit were all purchased from Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd. Known positive samples of p53, p16, EGFR and $p34^{cdc2}$ were used as positive control. For the negative control, the primary antibodies were replaced with phosphate-buffered saline (PBS).

Evaluation of immunohistochemical analysis

Positive signals of the immunohistochemical staining were brown or brownish yellow in color. Nuclear staining was considered positive on the immunostaining for p53 and p16, membrane and cytoplasm immunostaining was considered positive for EGFR, while the cytoplasmic and/or nuclear immunostaining was considered positive for $p34^{cdc2}$. 10 high magnification visions were selected randomly in each stained section, 10 high power field representatives were observed and the brown staining cells were counted. Positive staining in more than 10% of the cells was considered positive, while less than 10% or colorless were defined as negative.

Statistical analysis

The statistical analyses were performed with PASW Statistics 22.0 (SPSS Inc., Chicago, IL,

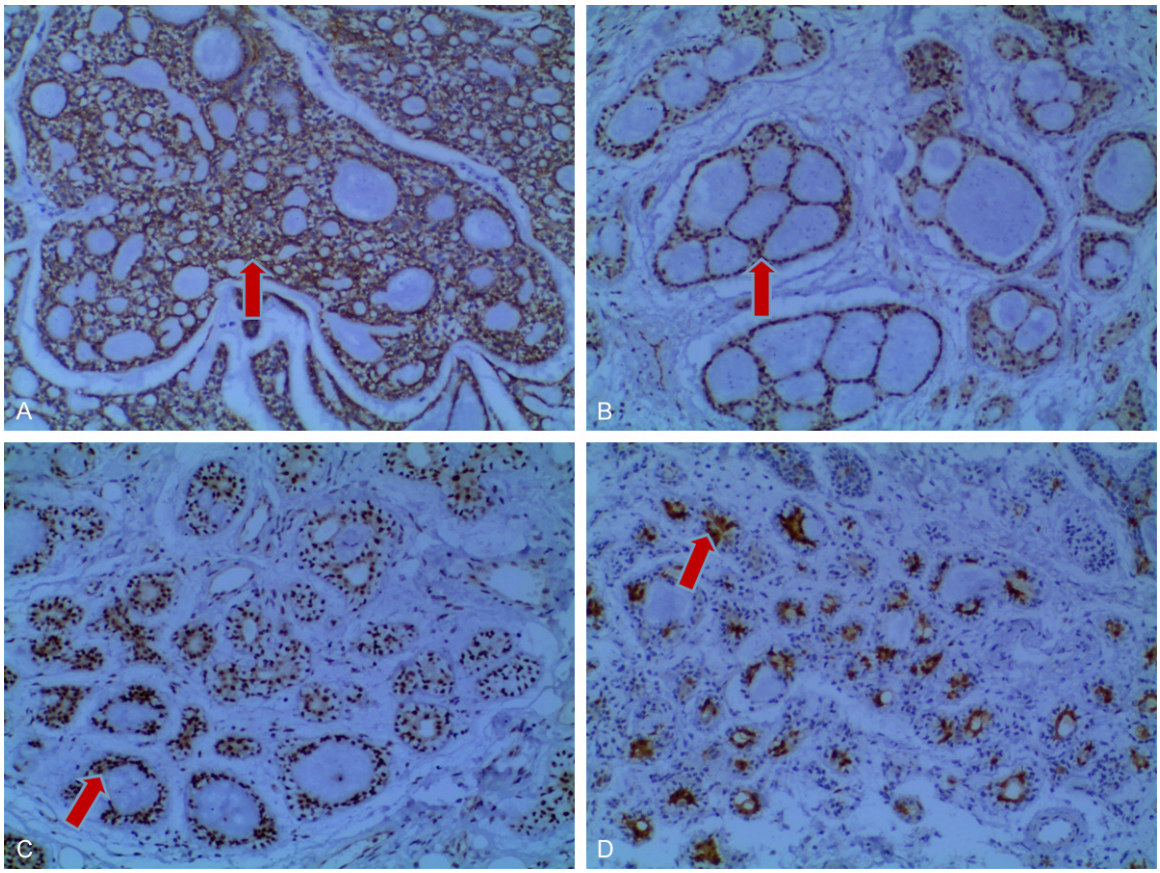

Figure 1. Expression of EGFR, $p34^{cdc2}$, p53, p16 in salivary gland ACC tissues. SP method. A. EGFR staining diffusely positive in ACC tissue (arrow). B. $p34^{cdc2}$ staining is positive in ACC tissue (arrow). C. p53-positive staining in ACC cells (arrow). D. p16 is positive in ACC tissue (arrow). Original magnification, ×100.

USA). The correlations of HPV infection and expression of p53, p16, EGFR and $p34^{cdc2}$ with the various clinicopathological findings were evaluated using the Chi-square test, and the Kaplan-Meier method was used to analyze survival rate. *P* values less than 0.05 were considered to be statistically significant.

Results

Infection rate of HPV and expression rate of p53, p16, EGFR and $p34^{cdc2}$ in the salivary gland adenoid cystic carcinoma tissues

PCR-DNA reverse dot blot hybridization showed that the infection rate of HPV was 0% (0/78) in the salivary gland adenoid cystic carcinoma tissues. Immunohistochemistry showed that expression rate of p53, p16, EGFR and $p34^{cdc2}$ in the salivary gland adenoid cystic carcinoma tissues were 75.6% (59/78), 57.7% (45/78), 60.1% (47/78) and 64.1% (50/78), and the positive rate of p53, p16, EGFR and $p34^{cdc2}$ was all irrelevant to the patient's age, sex, disease location, differentiation, TNM stage and ($P > 0.05$) (**Figure 1**, **Table 1**).

Prognosis of patients with salivary gland adenoid cystic carcinoma

Follow-up to January 6, 2016, in total 78 salivary gland adenoid cystic carcinoma patients, 18 cases were dead, the total mortality rate was 24.36% (19/78). The median overall survival (OS) was 64 months in all patients. The 5-year OS rate was 58.9% (46/78). Kaplan-Meier survival analysis showed that the median OS of EGFR-negative and EGFR-positive patients was 58 months and 75 months respectively, with statistically significant ($P < 0.001$, **Figure 2**). The median OS of $p34^{cdc2}$-negative and $p34^{cdc2}$-positive patients was 71 months and 61 months respectively, and these difference was also statistically significant (*P* =

Table 1. Correlation between expression of p53, p16, EGFR, $p34^{cdc2}$ and clinical features in salivary gland adenoid cystic carcinoma tissues

Clinical Features	Total No.	p53+	*P*	p16+	*P*	EGFR+	*P*	Cdc2+	*P*
Gender									
Male	41 (52.6)	30 (73.1)	0.593	20 (48.8)	0.448	22 (53.7)	0.127	30 (73.2)	0.894
Female	37 (47.4)	29 (78.4)		25 (67.6)		25 (67.6)		20 (54.1)	
Age									
< 50 y	21 (26.9)	15 (71.4)	0.938	9 (42.9)	0.809	12 (57.1)	0.302	10 (47.6)	0.657
≥ 50 y	57 (73.1)	44 (77.2)		36 (63.2)		35 (61.4)		40 (70.2)	
Site									
Parotid gland	26 (33.3)	19 (73.1)	0.922	12 (46.2)	1.000	17 (65.4)	0.807	16 (61.5)	0.234
Submandibular gland	24 (30.8)	18 (75.0)		14 (58.3)		14 (58.3)		15 (62.5)	
Pars palatalis	21 (26.9)	17 (81.0)		14 (66.7)		12 (57.1)		11 (52.4)	
Sublingual gland	7 (9.0)	5 (71.4)		5 (71.4)		4 (57.1)		5 (71.4)	
TNM Staging									
Stages I + II	22 (28.2)	17 (77.2)	0.818	15 (68.2)	0.384	9 (40.9)	0.050	10 (45.5)	0.894
Stages III + IV	56 (71.8)	42 (75.0)		30 (53.6)		38 (67.9)		40 (71.4)	
Histological classification									
Solid type	17 (21.8)	15 (88.2)	0.094	11 (64.7)	0.352	14 (82.3)	0.105	15 (88.2)	0.438
Sieve type	25 (32.0)	18 (72.0)		14 (56.0)		12 (48.0)		16 (64.0)	
Tubular type	36 (46.2)	26 (72.2)		20 (55.6)		21 (58.3)		19 (52.8)	

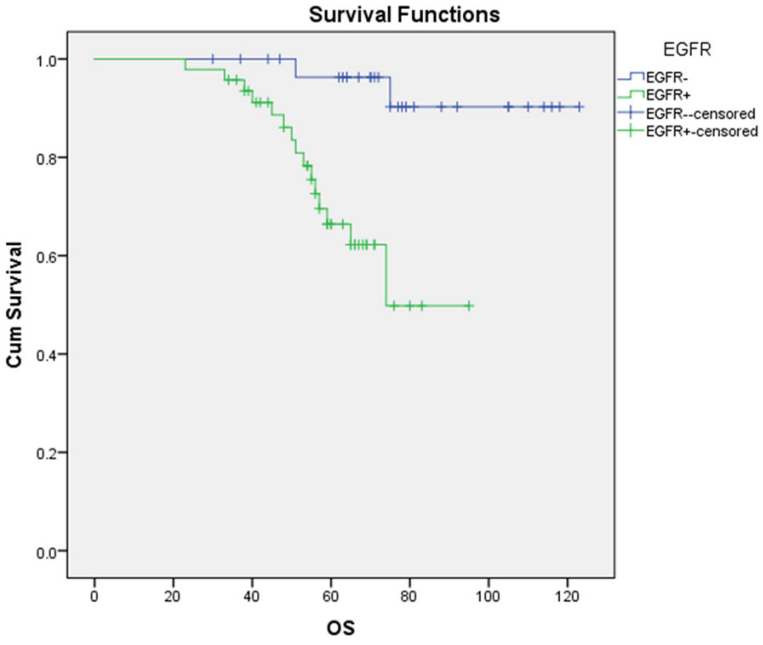

Figure 2. Kaplan-Meier survival analysis showing the impact of EGFR expression on OS. χ^2 = 11.232, *P* = 0.001.

0.027, **Figure 3**). Median OS was irrelevant to patients' gender, age, lesion sites, TNM classification and histological type (*P* > 0.05). The overall median progression-free survival (PFS) of patients was 45 months, Kaplan-Meier survival analysis showed that the PFS of EGFR-negative and EGFR-positive patients was 49 months and 43 months respectively, with statistically significant (*P* = 0.002, **Figure 4**). The median PFS of $p34^{cdc2}$-negative and $p34^{cdc2}$-positive patients was 51 months and 44 months respectively, and these difference was also statistically significant (*P* = 0.011, **Figure 5**). Median PFS was irrelevant to patients' gender, age, lesion sites, TNM classification and histological type (*P* > 0.05). Cox regression analysis showed that positive expression of EGFR and $p34^{cdc2}$ were independent prognostic factors for patients with salivary gland adenoid cystic carcinoma (relative risk = 14.167, 11.322, *P* < 0.001, **Table 2**).

Discussion

Adenoid cystic carcinoma is a malignant tumor originated from the gland ducts, its common histology is given priority to with cribriform, tubular, solid three types, with vary in amount

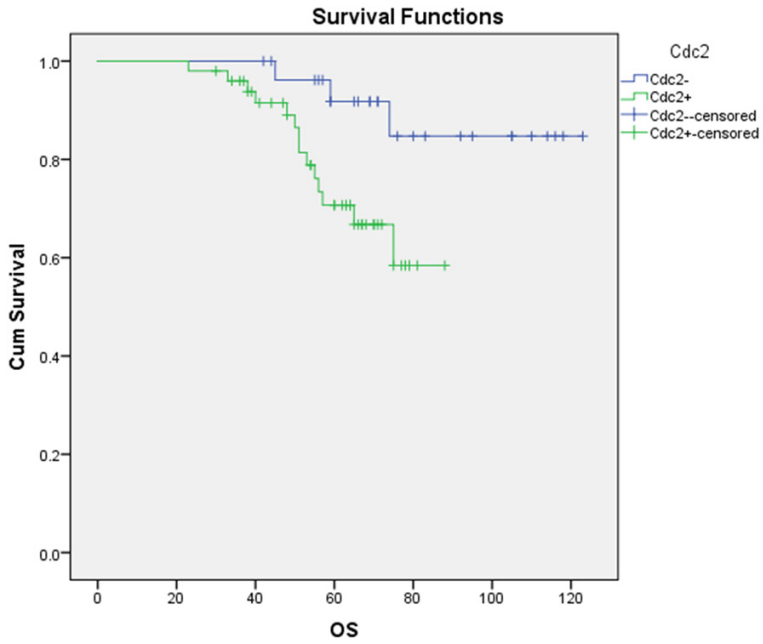

Figure 3. Kaplan-Meier survival analysis showing the impact of $p34^{cdc2}$ expression on OS. $\chi^2 = 4.881$, $P = 0.027$.

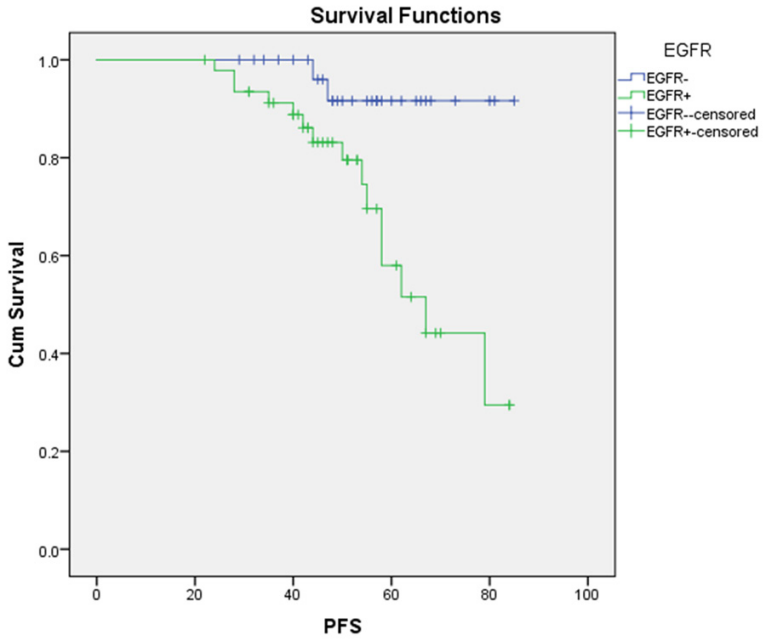

Figure 4. Kaplan-Meier survival analysis showing the impact of EGFR expression on PFS. $\chi^2 = 9.218$, $P = 0.002$.

of mucus and pink colored basement membrane like substance. The tumor cells lined with ductal and myoepithelial epithelial, prone to nerve invasion and distant metastasis with poor prognosis. The related factors of the poor prognosis of the carcinoma are still under investigation. Data from International Agency for Research on Cancer (IARC) in 2012 showed that the incidence of head and neck cancer induced by HPV increased with each passing year, especially in tumors of oromaxillo-facial region [3]. While, there were few studies about the HPV and its related factors in the process of inducing salivary gland adenoid cystic carcinoma and the conclusion was different between researchers. Boland *et al* [4] found that no HPV positive cases detected in salivary gland adenoid cystic carcinoma specimens by in situ hybridization. Bishop *et al* [5] found that HPV was negative in the ACC tissues of nasal cavity and paranasal sinuses. Hühns *et al* [2] in analyzing 17 samples of ACC displayed that the infection rate of HPV was 25%. Huo *et al* [6] reported HPV was negative in 27 cases of lung ACC by in situ hybridization detection. Our study showed that there was no HPV detected in 78 cases of salivary gland ACC, suggesting that the occurrence of ACC in salivary glands may not be associated with HPV infection.

The previous literature showed that p16 has been used as a surrogate marker for HPV infection in a variety of head and neck cancer tissues, but its role in the evaluation of ACC was controversial. Isayeva *et al* [7] reported that the expression rate of p16 in adenoid cystic carcinoma was 64%. Lassen *et al* [8] found that the positive rate of p16 was 22% and was closely related with HPV infection in the pharyngeal and supraglottic carcinoma tissues in Denmark. Patients with p16 positive

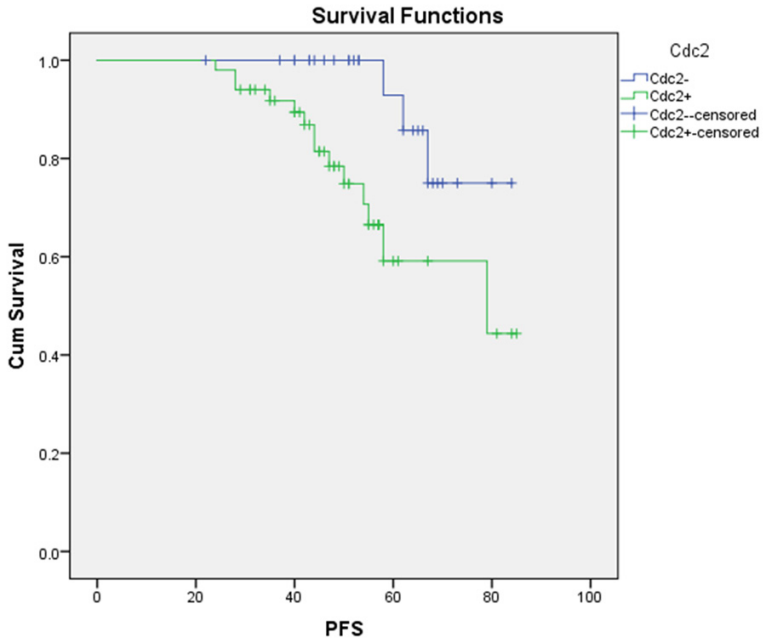

Figure 5. Kaplan-Meier survival analysis showing the impact of $p34^{cdc2}$ expression on PFS. $\chi^2 = 6.418$, $P = 0.001$.

Table 2. Multivariate analysis of prognosis for salivary gland adenoid cystic carcinoma patients by Cox model

	B	SE	Wald	*P*	EXP (β)	95% CI
EGFR	3.260	0.866	14.167	< 0.01	26.042	3.256-55.286
$p34^{cdc2}$	3.601	1.070	11.322	< 0.01	36.632	3.204-53.358

had a higher tumor local control rate (58%:28%, $P = 0.0005$), disease-specific survival rate (72%:34%, $P = 0.0006$) and overall survival rate (62%:26%, $P = 0.0003$). Xing *et al* [9] reported that there was no correlation between high-risk HPV and p16 expression in vulvar and cervical ACC tissues. Boland *et al* [4] also confirmed that the detection of p16 instead of HPV detection was not applicable in ACC specimens. In our study, the expression rate of p16 in 78 cases of salivary ACC tissues was 57.7% (45/78), however, HPV infection was not detected. Therefore, the expression of p16 in salivary gland ACC cannot be used as an indicator of HPV infection.

Tumor induced by HPV is correlation with wild-type p53 tumor suppressor gene. The wild type p53 protein has a short half-life in vivo, which cannot be detected by immunohistochemistry. However, the mutant p53 protein has longer half-life that can be detected by immunohistochemistry. Therefore, the expression of p53 protein was negative in tumors induced by HPV. The conclusion about the expression rate of p53 in adenoid cystic carcinoma is different. Jiang *et al* [10] reported that the positive rate of p53 in the parotid gland ACC was 45.7%, but it was not related to the survival of the patients. Wang *et al* [11] found that the expression rate of p53 in 36 cases of salivary adenoid cystic carcinoma was 69.44%. Our study found that the expression rate of p53 in salivary ACC tissues was 76.3%, p53 positive patients had shorter survival than p53 negative ones, but there was no significant difference in evaluation of the prognosis of patients with ACC and require larger sample size for further research confirmed.

Epidermal growth factor receptor plays an important role in the process of tumor growth and metastasis. Wang *et al* [12] found that the expression of EGFR increased significantly in the ACC tissues, and the expression in cribriform and tubular forms was higher than that in solid forms. Our study showed that the expression rate of EGFR in salivary gland ACC tissues was 60.5%, and the expression rate of EGFR in stage I and II tumor tissues was lower than that in stage III and IV carcinomas, however, the difference was not statistically significant and increase of the sample size was needed to further confirm the finding. Multivariate Cox regression analysis showed that the expression of EGFR was associated with short survival time, which was an independent prognostic factor in patients with salivary ACC.

$p34^{cdc2}$ is a member of the Ser/Thr protein kinase family coded by cell division cycle (cdc) gene 2 whose relative molecular weight is 34 KD. $p34^{cdc2}$ is one of the most important regulated kinase of cell cycle and its main function is to monitor spindle microtubule assembly and kinetochore's proper connection of DNA in the G2/M checkpoint. If an error occurs in adjust-

ment mechanism of $p34^{cdc2}$, it will lead directly to cell differentiation disorders, disorder of cell cycle progression and induce abnormal cell proliferation or malignant transformation, thus promoting the occurrence and progression of tumor [13-15]. $p34^{cdc2}$ overexpressed in many cancers, such as oral squamous cell carcinoma [16], tongue squamous cell carcinoma [17], supraglottic cancer [18], esophageal squamous cell carcinoma, esophageal adenocarcinoma [19], gastric cancer [20], liver cancer [21], colorectal cancer [22], breast cancer [23], ovarian cancer [24] and so on. Yang *et al* [25] found that the expression rate of $p34^{cdc2}$ in laryngeal carcinoma tissues was 70.6%, significantly higher than that in adjacent tissues and its negative margins. Patients with $p34^{cdc2}$ positive margins had higher recurrence rate than that of negative patients. However, its expression in salivary gland tissues has not been reported. Our study shows that the expression rate of $p34^{cdc2}$ in salivary gland ACC tissues was as high as 64.1% (50/78), the single factor analysis showed that the median OS of patients with $p34^{cdc2}$ positive was 10 months shorter than that of negative ones, $P = 0.001$. Multivariate analysis showed that the positive expression of $p34^{cdc2}$ was associated with short survival time of ACC, which was an independent factors affecting the prognosis of patients.

In conclusion, HPV was not detected in the salivary gland ACC tissue. p53, p16, EGFR and $p34^{cdc2}$ proteins were expressed in most salivary ACC tissues. p16 could not be used as a surrogate marker for HPV infection in patients with salivary ACC. Patients with positive expression of EGFR and $p34^{cdc2}$ had poor prognosis than the negative ones, and the patients with the expression of EGFR and $p34^{cdc2}$ should be followed up closely.

Disclosure of conflict of interest

None.

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References

[1] Lee SK, Kwon MS, Lee YS, Choi SH, Kim SY, Cho KJ, Nam SY. Prognostic value of expression of molecular markers in adenoid cystic cancer of the salivary glands compared with lymph node metastasis: a retrospective study. World J Surg Oncol 2012; 10: 266.

[2] Hühns M, Simm G, Erbersdobler A, Zimpfer A. HPV Infection, but not EBV or HHV-8 infection, is associated with salivary gland tumours. Biomed Res Int 2015; 2015: 829349.

[3] Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Estimated cancer incidence, mortality and prevalence worldwide in 2012. International agency for research on cancer (IARC), globocan 2012 available online: http://globocan.iarc.fr.

[4] Boland JM, McPhail ED, García JJ, Lewis JE, Schembri-Wismayer DJ. Detection of human papilloma virus and p16 expression in high-grade adenoid cystic carcinoma of the head and neck. Mod Pathol 2012; 25: 529-536.

[5] Justin AB, Takenori O, Edward BS, Christopher AM, Wayne MK, Sara IP, William HW. Human papillomavirus-related carcinoma with adenoid cystic-like features: a peculiar variant of head and neck cancer restricted to the sinonasal tract. Am J Surg Pathol 2013; 37: 836-844.

[6] Huo Z, Meng Y, Wu H, Shen J, Bi Y, Luo Y, Cao J, Liang Z. Adenoid cystic carcinoma of the tracheobronchial tree: clinicopathologic and immunohistochemical studies of 21 case. Int J Clin Exp Pathol 2014; 7: 7527-7235.

[7] Isayeva T, Said-Al-Naief N, Ren ZY, Li R, Gnepp D, Brandwein-Gensler M. Salivary mucoepidermoid carcinoma: demonstration of transcriptionally active human papillomavirus 16/18. Head Neck Pathol 2013; 7: 135-148.

[8] Lassen P, Eriksen JG, Hamilton-Dutoit S, Tramm T, Alsner J, Overgaard J. Effect of HPV-associated $p16^{INK4A}$ expression on response to radiotherapy and survival in squamous cell carcinoma of the head and neck. J Clin Oncol 2009; 27: 1992-1998.

[9] Xing D, Schoolmeester JK, Ren Z, Isacson C, Ronnett BM. Lower female genital tract tumors with adenoid cystic differentiation: P16 expression and high-risk HPV detection. Am J Surg Pathol 2016; 40: 529-536.

[10] Jiang LC, Huang SY, Zhang DS, Zhang SH, Li WG, Zheng PH, Chen ZW, Zhang DS. Expression of beclin 1 in primary salivary adenoid cystic carcinoma and its relation to Bcl-2 and p53 and prognosis. Braz J Med Biol Res 2014; 47: 252-258.

[11] Wang XF, Li SC, Fang DJ. Expression of survivin and p53 in salivary adenoid cystic carcinoma. Kouqiang Yixue Yanjiu Zazhi 2014; 30: 635-637.

[12] Wang WM, Zhao ZL, Zhang WF, Zhang L, Sun SJ. Role of hypoxia-inducible factor-1α and CD146 in epidermal growth factor receptor-

mediated angiogenesis in salivary gland adenoid cystic carcinoma. Mol Med Rep 2015; 12: 3432-3438.
[13] Perez de Castro I, de Carcer G and Malumbres M. A census of mitotic cancer genes: new insights into tumor cell biology and cancer therapy. Carcinogenesis 2007; 28: 899-912.
[14] Barnabas S, Dana B. Premature Cdk1/Cdc5/Mus81 pathway activation induces aberrant replication and deleterious crossover. EMBO J 2013; 32: 1155-1167.
[15] Liu P, Kao TP and Huang H. CDK1 promotes cell proliferation and survival via phosphorylation and inhibition of FOXO1 transcription factor. Oncogene 2008; 27: 4733-4744.
[16] Chang JT, Wang HM, Chang KW, Chen WH, Wen MC, Hsu YM, Yung BY, Chen IH, Liao CT, Hsieh LL and Cheng AJ. Identification of differentially expressed genes in oral squamous cell carcinoma (OSCC): overexpression of NPM, CDK1 and NDRG1 and underexpression of CHES1. Int J Cancer 2005; 114: 942-949.
[17] Wada S, Yue L and Furuta I. Prognostic significance of $p34^{cdc2}$ expression in tongue squamous cell carcinoma. Oral Oncol 2004; 40: 164-169.
[18] Yamamoto Y, Itoh T, Inoue I and Takahashi H. Expression of $p34^{cdc2}$ protein kinase and p53 in supraglottic carcinomas. Auris Nasus Larynx 1996; 23: 105-110.
[19] Hansel DE, Dhara S, Huang RC, Ashfaq R, Deasel M, Shimada Y, Bernstein HS, Harmon J, Brock M, Forastiere A, Washington MK, Maitra A and Montgomery E. CDC2/CDK1 expression in esophageal adenocarcinoma and precursor lesions serves as a diagnostic and cancer progression marker and potential novel drug target. Am J Surg Pathol 2005; 29: 390-399.
[20] Kim DH. Prognostic implications of cyclin B1, $p34^{cdc2}$, p27 (Kip1) and p53 expression in gastric cancer. Yonsei Med J 2007; 48: 694-700.
[21] Ito Y, Takeda T, Sakon M, Monden M, Tsujimoto M and Matsuura N. Expression and prognostic role of cyclin-dependent kinase 1 (cdc2) in hepatocellular carcinoma. Oncology 2000; 59: 68-74.
[22] Nozoe T, Honda M, Inutsuka S and Korenaga D. $p34^{cdc2}$ expression is an independent indicator for lymph node metastasis in colorectal carcinoma. J Cancer Res Clin Oncol 2003; 129: 498-502.
[23] Kourea HP, Koutras AK, Scopa CD, Marangos MN, Tzoracoeleftherakis E, Koukouras D and Kalofonos HP. Expression of the cell cycle regulatory proteins $p34^{cdc2}$, $p21^{waf1}$, and p53 in node negative invasive ductal breast carcinoma. Mol Pathol 2003; 56: 328-335.
[24] Barrette BA, Srivatsa PJ, Cliby WA, Keeney GL, Suman VJ, Podratz KC and Roche PC. Overexpression of $p34^{cdc2}$ protein kinase in epithelial ovarian carcinoma. Mayo Clin Proc 1997; 72: 925-929.
[25] Yang JQ, Liu HX, Liang Z, Sun YM, Wu M. Overexpression of p53, p21 and Cdc2 in histologically negative surgical margins is correlated with local recurrence of laryngeal squamous cell carcinoma. Int J Clin Exp Pathol 2014; 7: 4295-4302.