Cancer Immunol Immunother (2013) 62:481–487
DOI 10.1007/s00262-012-1348-8

ORIGINAL ARTICLE

Up-regulation of Foxp3 participates in progression of cervical cancer

Chao Zeng · Yunhong Yao · Wei Jie · Miao Zhang · Xinrong Hu · Yi Zhao · Sen Wang · Jinbao Yin · Yulan Song

Received: 22 July 2012 / Accepted: 23 August 2012 / Published online: 18 September 2012

Abstract Foxp3 was identified as a key protein in mediating inhibitory functions of regulatory T cell (Treg). Foxp3 was thought to express only in the T cell lineage until recently when some researches reported that Foxp3 was also expressed by cancer cells. In this study, we describe for the first time the expression of Foxp3 in cervical cancer. Progression from cervical intraepithelial neoplasia (CIN) to cervical cancer is a multistep process initiated by persistent infection with high-risk human papillomavirus (HPV). $P16^{INK4a}$ is a crucial marker of HPV integration into host cells. In the present study, expressions of Foxp3 and $P16^{INK4a}$ in CIN and cervical cancer were detected by immunohistochemistry. Our results found expression level of Foxp3 was increased during the progression of cervical neoplasia. Moreover, up-regulation of Foxp3 appeared to be correlated with the expression of $P16^{INK4a}$. Examination of the role of Foxp3 in differentiation by double immunostaining for cytokeratin 10 (CK10) showed significant association between Foxp3 expression and differentiation (Foxp3 vs CK10). Furthermore, positive expression of Foxp3 was correlated with tumor size. These data suggest that Foxp3 may play an important role in differentiation and growth of cervical cancer cells. Our findings provide new insights regarding the role of Foxp3 in differentiation and its association with HPV infection during the development of cervical cancer.

Keywords Foxp3 · Cervical cancer · $P16^{INK4a}$ · Differentiation

C. Zeng (✉) · Y. Yao · W. Jie · X. Hu · S. Wang · J. Yin
Department of Pathology, Guangdong Medical College, Dongguan 523808, China
e-mail: zengchaosysu@yahoo.com.cn

M. Zhang
Department of Pathology, Xin Hui Maternity and Child Health Care Hospital, Jiangmen 529100, China

Y. Zhao
Department of Immunology, GGuangdong Medical College, Dongguan 523808, China

Y. Song (✉)
Department of Pathology, The First Affiliated Hospital of Guangdong Pharmaceutical University, Guangzhou 510080, China
e-mail: songyulan1@126.cn

Introduction

Cervical cancer is the second most frequent cancer among women worldwide [1]. The progression of cervical cancer is a complex process initiated by persistent infection with high-risk human papillomavirus (HPV) [2]. After high-risk HPV infection, cervical lesions advance from cervical intraepithelial neoplasia I (CINI) to cervical intraepithelial neoplasia III (CINIII) to cervical cancer in a small subset. Operation treatment, radiotherapy, and chemical medication are major treatment means of the cervical cancer at present. These treatment options are effective only in limited cases. To improve the treatment for cervical cancer needs a better understanding of molecular events in the immune system from CINs to invasive squamous cell carcinomas (ISCCs).

Forkhead box protein 3 (Foxp3) is a member of the forkhead/winged-helix family of transcription regulators involved in regulating immune system development and function [3, 4]. Foxp3 is widely accepted as the specifical marker for regulatory T cells (Tregs) which play important role in the suppression of tumor immunity [5]. Tregs accumulated in tumors were required in tumor metastasis,

and they were correlated with worse survival in several human cancers [6–8]. Foxp3 is considered to be a critical factor involved in the development and function of Tregs. Loss of Foxp3 function is associated with autoimmune diseases, whereas over-expression of Foxp3 causes immunodeficiency [5]. It had been assumed that Foxp3 participated in immune suppression by regulating Tregs. Interestingly, some researches recently reported Foxp3 was shown to be expressed by cancer cells themselves. Hinz et al. [9] found Foxp3 staining in 60 % of pancreatic carcinoma tissues and in all pancreatic cancer cell lines, and Merlo et al. [10] reported Foxp3 expression was a new independent prognostic factor in breast cancer. These researches indicated that Foxp3 had an important biological function by endowing cancer cells with immune suppressive activity. In contrast, Zuo et al. [11] demonstrated that Foxp3 was an X-linked breast cancer suppressor gene and ectopic expression of Foxp3 in a variety of breast cancer cell lines resulted in cell cycle arrest and cessation of cell growth. Similarly, Foxp3 is an X-linked prostate tumor suppressor in male. As a major transcriptional repressor of c-myc in the prostate, loss of Foxp3 expression is necessary for c-myc over-expression [12]. So far, the exact role Foxp3 plays in tumor cells is still uncertain.

Taking into account the important function of Foxp3 in tumor immunity, in the present study, we hypothesize that Foxp3 may be involved in progression of CIN and carcinogenesis. This study was designed to investigate the expression of Foxp3 in CINs and ISCCs, and their relationship with the expression of $P16^{INK4a}$, which is critical marker for the integration of HPV into host cells, was also evaluated.

Materials and methods

Tumor specimens

A total of 140 formalin-fixed and paraffin-embedded samples were used in this study. Eighty CINs and 40 ISCCs were collected from the Department of Pathology, the first affiliated hospital of Guang Dong Pharmaceutical University. Cases of CINI, CINII, and CIN III were 32, 28, and 20, respectively. ISCCs were diagnosed when the cancer cells broke through epithelial basement membrane. Hysteromyoma is a normal control cervical tissue ($n = 20$) obtained from surgically removed uteruses.

Immunohistochemistry

Four-micrometer sections were cut from the selected paraffin blocks and deparaffinized by routine techniques. The slides were microwaved in citrate buffer for 8 min for antigen retrieval. Subsequently, the slides were incubated overnight with rabbit polyclonal anti-Foxp3 (ab10563, Abcam, USA, 1:100 dilution) and anti-$P16^{INK4a}$ (Gene, China, 1:200 dilution) in a humidified chamber at 4 °C, respectively. Labeling was detected by adding biotinylated secondary antibodies (Maxim-Bio, Fuzhou, China), avidin–biotin complex (Maxim-Bio), and diaminobenzidine (Maxim-Bio). Finally, sections were then counterstained with hematoxylin. Pancreatic carcinoma tissues were used as positive control. Negative control was performed by using appropriate serum controls for the primary antibody.

IHC evaluation

The results of immunohistochemical staining were scored by two pathologists, who were blinded to clinical data. Foxp3 protein was scored using a semiquantitative method by evaluating the number of positive tumor cells over the total number of tumor cells. Foxp3 protein was predominantly expressed in the nuclei of cells. Foxp3 staining was scored according to the intensity and proportion of positive cells as follows: −, no positive staining cells; +, weak intensity with less than 25 % positive staining cells; ++, moderate intensity with 26–50 % positive staining cells; and +++, strong intensity with more than 50 % positive staining cells. $P16^{INK4A}$ immunostaining was shown in the nuclei and/or cytoplasm of cells. $P16^{INK4A}$ staining was also scored on a scale from − to +++. The evaluation standard of $P16^{INK4A}$ was the same as previous.

The double immunostaining of Foxp3 and $P16^{INK4a}$ was detected with DouSP^{TM} double staining kit (Maxim-Bio, Fuzhou, China). $P16^{INK4a}$ immunoreactivity was first examined, and BCIP-NBT was used for visualization. Then Foxp3 immunoreactivity was detected by using AEC. The evaluation standard is the same as that used in single staining.

Statistical analysis

Statistical analysis was performed by using the SPSS statistical software (SPSS13.0, Chicago, USA). The differences between Foxp3 and $P16^{INK4a}$ expression in CINs and ISCCs among five groups were compared by Kruskal–Wallis test. Spearman correlation test was used for correlation between Foxp3 expression and $P16^{INK4a}$ immunoreactivity. Fisher's exact test was used to evaluate the association between Foxp3 and CK10 expression. P value of <0.05 was considered significant.

Results

Foxp3 expression is up-regulated during progression of CINs and ISCCs

To study effects of Foxp3 in progression of cervical cancer, immunohistochemical analysis was done in 20 normal cervical tissues, 80 paraffin-embedded CINs samples, and 40 ISCCs samples. Foxp3 staining was undetectable in normal cervical squamous epithelial cells (Fig. 1). The intensity of Foxp3 immunostaining was gradually increased from CIN I to CIN III (Fig. 1). Interestingly, Foxp3 immunoreactivity in CIN II/III was obviously stronger than the reactivity in normal cervical tissue and CIN I. The positive staining rates of Foxp3 in CIN I, CIN II, and CIN III were 15.6, 42.9, and 75.0 %, respectively (Table 1). The difference in Foxp3 immunostaining between normal cervical epithelium/CIN I and CIN II/III had statistical significance ($P < 0.05$). Of 40 ISCC specimens, 32 specimens were scored as positive (80.0 %) and Foxp3 staining was only present in nuclear (Fig. 1; Table 1). The results suggest that Foxp3 staining from normal cervical specimens, CINs to ISCCs was enhanced gradually.

To better study the role of Foxp3 in ISCCs, we analyzed the relationship between Foxp3 expression and clinicopathological factors. As shown in Table 2, tumor size had statistically significant correlation with Foxp3 expression ($P < 0.05$). However, there was no significant correlation between the level of Foxp3 expression and other pathologic features, including age, clinical stage, and lymph node metastasis. These results indicate that Foxp3 expression may contribute to tumor growth.

Foxp3 immunostaining is correlated with $P16^{INK4a}$

Taking into account the important role of Foxp3 in immune suppression, we assume that it may be correlated with HPV infection. $P16^{INK4a}$ is a key marker for the integration of high-risk HPV into host cells. Therefore, we detected expression of $P16^{INK4a}$ in these cervical specimens and demonstrated its relationship with Foxp3. Positive staining of $P16^{INK4a}$ was undetected in 10 ISCC specimens (Table 3). The positive rates of $P16^{INK4a}$ in CIN I, CIN II, CIN III, and ISCCs were 28.1, 50.0, 65.0, and 75.0 %, respectively. $P16^{INK4a}$ was gradually increased with progression of CINs. Moreover, as shown in Table 3, the positive rates of $P16^{INK4a}$ between normal cervical specimens and CINs, and between normal cervical specimens and ISCCs had significant difference ($P < 0.05$).

To further investigate the role of $P16^{INK4a}$ on Foxp3 expression, we use double staining for both $P16^{INK4a}$ and Foxp3. Similar to the results of single staining, immunoreactivity of $P16^{INK4a}$ and Foxp3 gradually increased from CIN I to CIN III (Fig. 1). Furthermore, this same staining pattern was found in ISCCs (Fig. 1). The results indicate that expression of Foxp3 is correlated with $P16^{INK4a}$ ($P < 0.001$; Table 4).

Expression of Foxp3 is associated with differentiation of cervical cancer

To study the role of Foxp3 in differentiation of cervical cancer cells, we detected the relationship between Foxp3 and CK10. Positive rate of Foxp3 in well-differentiated squamous cell carcinoma was 96.0 % (24/25) (Fig. 2a) and was 72.7 % (8/11) in moderately differentiated samples. However, Foxp3 expression was negative in four poorly differentiated samples (Fig. 2b). Moreover, in all 40 ISCC specimens, Foxp3 was detectable in Tregs infiltrating in the tumor stroma (black arrow in Fig. 2a, b). CK10 staining was located in the cytoplasm of differentiated squamous cancer cells (Fig. 2c). CK10 was stained in 24 of 40 (60.0 %) and was detected in 18 of 25 (72.0 %) well-differentiated, 6 of 11 (54.5 %) moderately differentiated, and zero of four (0 %) poorly differentiated cervical cancer. In 40 ISCC samples, 22 cases were positive for both Foxp3 and CK10, whereas neither Foxp3 nor CK10 was expressed in 6 cases. In addition, 10 cases were Foxp3 positive but CK10 negative, whereas 2 cases were CK10 positive but Foxp3 negative. Therefore, occurrence of Foxp3 is associated with CK10 expression and differentiation of cervical cancer cells ($P = 0.042$).

Discussion

Regulatory T cells (Tregs) play a vital role in maintaining immunological self-tolerance and preventing autoimmune diseases [13, 14]. Foxp3 is the only definitive marker of $CD4^+CD25^+$ regulatory T cells (Tregs) and has been identified as a key regulator in the function of Tregs [15]. The prevalence of Tregs is increased in peripheral blood, lymph node, and tumor microenvironment of patients with a variety of different tumors. Moreover, high levels of Tregs in local lymph nodes are associated with a less favorable prognosis in patients with ovarian carcinoma [6].

Recently, some studies reported Foxp3 expression in tumor cells other than those of the T cell lineage. Foxp3 gene was reported to function as an X-linked tumor suppressor gene in breast and prostate cancers. Zuo et al. showed that Foxp3 expressed in normal breast epithelium but down-regulated in breast cancer. Furthermore, in ovarian cancer, Zhang et al. [16] found that up-regulation of Foxp3 inhibited cell proliferation, decreased cell migration, and reduced cell invasion. These findings indicate

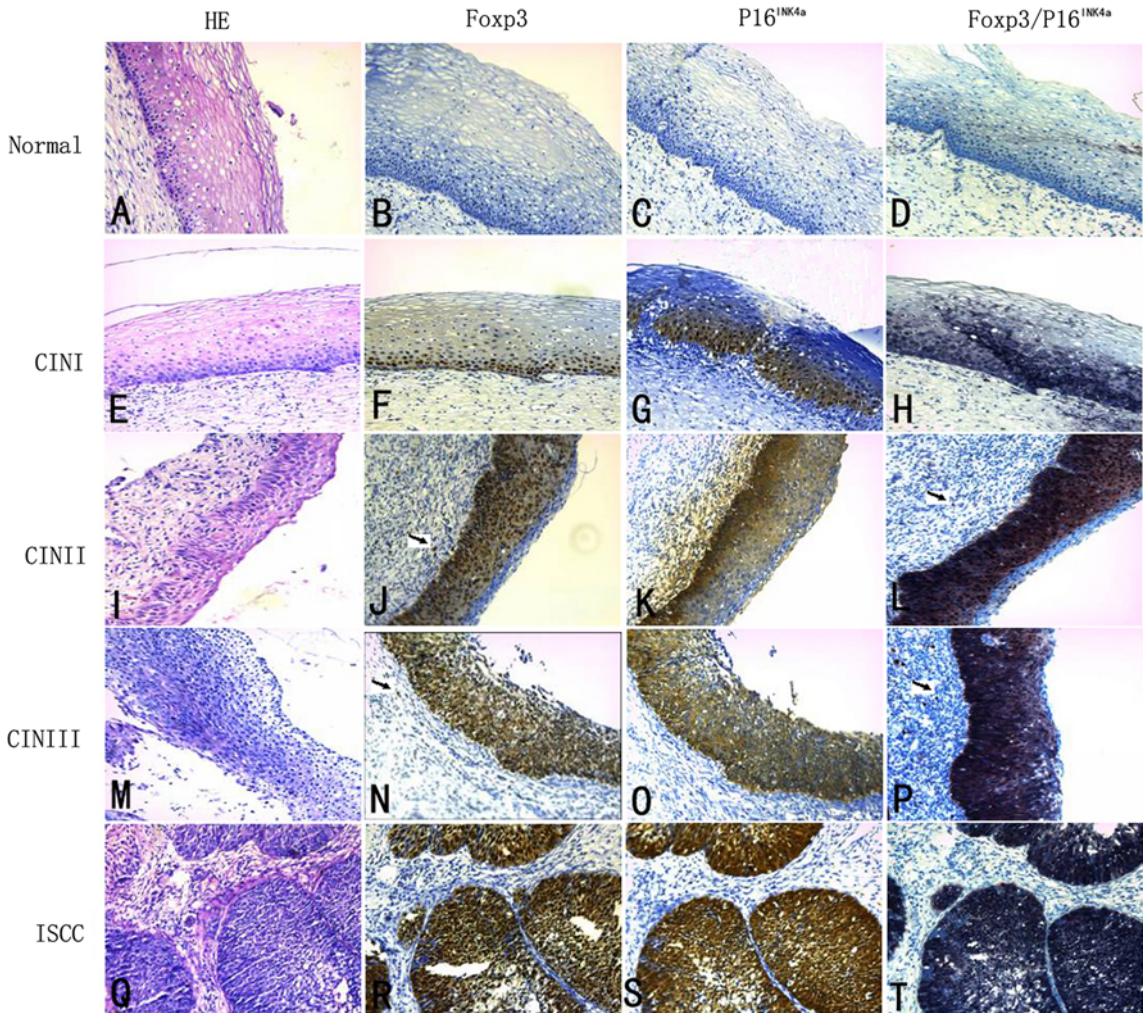

Fig. 1 Immunohistochemical analysis of Foxp3 and $P16^{INK4a}$ proteins. Normal cervical squamous epithelium (**a**), CIN I (**e**), CIN II (**i**), and CIN III (**m**) were stained by hematoxylin and eosin (HE). Cancer cells invaded into muscle tissue in ISCC (**q**). No Foxp3 staining was detected in the normal cervical squamous epithelium (**b**). Immunoreactivity of Foxp3 gradually increased from CIN I (**f**), CIN II (**j**), and CIN III (**n**). Strong Foxp3 staining was observed in tumor cells of ISCC (**r**). In (**j**), (**n**), (**l**), and (**p**), many Foxp3-positive lymphocytes are tumor-infiltrating Tregs (*black arrow*). No $P16^{INK4a}$ immunostaining was observed in the normal cervical squamous epithelium (**c**). Immunoreactivity of $P16^{INK4a}$ increased from CIN I (**g**), CIN II (**k**), CIN III (**o**), and ISCC (**s**). Double immunostaining of Foxp3 and $P16^{INK4a}$ in normal cervical squamous epithelium (**d**), CIN I (**h**), CIN II (**l**), CIN III (**p**), and ISCC (**t**)

Table 1 Expression of Foxp3 in CINs and ISCCs

Variables	Degree of immunoreactivity (%)			
	−	+	++	+++
Normal[a]	20/20 (100.0)	0/20 (0.0)	0/20 (0.0)	0/20 (0.0)
CIN I[b]	27/32 (84.4)	3/32 (9.4)	2/32 (6.3)	0/32 (0.0)
CIN II[c]	16/28 (57.1)	5/28 (17.9)	5/28 (17.9)	2/28 (7.1)
CIN III[d]	5/20 (25.0)	7/20 (35.0)	5/20 (25.0)	3/20 (15.0)
ISCCs[e]	8/40 (20.0)	13/40 (32.5)	11/40 (27.5)	8/40 (20.0)

[a,b] >0.05, [a,c] <0.05, [a,d] <0.05, [a,e] <0.05, [b,c] <0.05, [b,d] <0.05, [b,e] <0.05, [c,d] < 0.05, [c,e] <0.05, [d,e] >0.05

that Foxp3 is a potential cancer suppressor gene. On the other hand, Cunha et al. [17] demonstrated Foxp3 expression in differentiated thyroid carcinoma cells and found evidence that this expression may exert an important influence on tumor aggressiveness. Similarly, Wang et al. [18] found that up-regulation of Foxp3 had a close

Table 2 Association between Foxp3 expression and the clinicopathological features of ISCCs

Variable	*N*	Foxp3 expression		*P*
		−	+∼+++	
Age (year)				
≤40	15	2	13	
>40	25	6	19	0.686
Tumor size				
≤4 cm	12	5	7	
>4 cm	28	3	25	0.039
Clinical stage				
I–IIa	30	4	26	
IIb–III	10	4	6	0.089
Lymph node metastasis				
Absent	27	7	20	
Present	13	1	12	0.236

Table 3 Expression of $P16^{INK4a}$ in CINs and ISCCs

Variables	Degree of immunoreactivity (%)			
	−	+	++	+++
Normal[a]	20/20(100.0)	0/20 (0.0)	0/20 (0.0)	0/20 (0.0)
CIN I[b]	23/32 (71.9)	4/32 (12.5)	4/32 (12.5)	1/32 (3.1)
CIN II[c]	14/28 (50.0)	5/28 (17.9)	6/28 (21.4)	3/28 (10.7)
CIN III[d]	7/20 (35.0)	7/20 (35.0)	3/20 (15.0)	3/20 (15.0)
ISCCs[e]	10/40 (25.0)	15/40 (37.5)	7/40 (17.5)	8/40 (20.0)

^{a,b} <0.05, ^{a,c} <0.05, ^{a,d} <0.05, ^{a,e} <0.05, ^{b,c} >0.05, ^{b,d} <0.05, ^{b,e} <0.05, ^{c,d} >0.05, ^{c,e} >0.05, ^{d,e} >0.05

Table 4 Correlation between Foxp3 and $P16^{INK4a}$ expression

Foxp3 expression	$P16^{INK4a}$ expression				Total
	−	+	++	+++	
−	45	19	7	5	76
+	16	4	5	3	28
++	8	5	6	4	23
+++	5	3	2	3	13
Total	74	31	20	15	140

140 samples that include 20 normals, 80 CINs, and 40 ISCCs

Correlation of Foxp3 and $P16^{INK4a}$ expression: $rs = 0.322$, $P < 0.001$

relationship with lymph node metastasis of gastric cancer. Furthermore, Niu et al. [19] reported Foxp3 expression correlated with the Treg-like suppressive activity on T cells, and they concluded Foxp3 expression in melanoma cells as a possible mechanism of resistance to immune destruction.

Although Foxp3 expression has been examined in various types of cancers [20, 21], it has not been described in cervical cancer. In the present study, 32/40 (80.0 %) of cervical cancer specimens were scored positive for Foxp3 expression in cervical cancer cells. Our results were similar with previous observations in pancreatic carcinoma. In addition, in the present study, Foxp3 staining was detected predominantly in the nuclear of cancer cells. However, subcellular staining of Foxp3 ranged from mostly cytoplasmic to both cytoplasmic and nuclear in pancreatic carcinoma and breast cancer tissues, and this difference might be explained by discrepancy in posttranslational modification and types of cancer. To our knowledge, this study detected for the first time the up-regulation of Foxp3 in CINs and ISCCs. In addition, by analyzing the correlation between Foxp3 expression and clinicopathological parameters of cervical cancer, we found expression of Foxp3 was significantly correlated with the tumor size and suggest that Foxp3 may play an important role in tumor growth.

It is generally known that Foxp3 is essential for the differentiation of T cells into regulatory T cells [22]. Therefore, we assume that Foxp3 participates in differentiation of cervical cancer cells. Subsequently, we

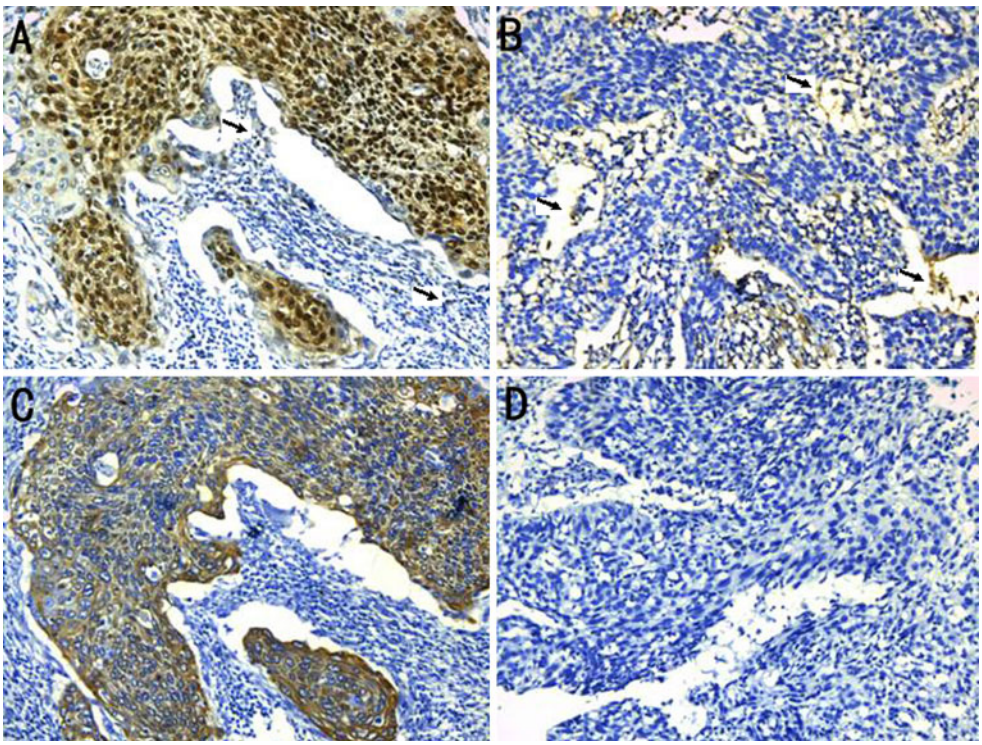

Fig. 2 Relationship between Foxp3 and CK10 expression. Positive (**a**) and negative (**b**) Foxp3 expression. Positive (**c**) and negative (**d**) CK10 expression. **a**, **c** Well-differentiated squamous cell carcinoma with positive staining for Foxp3 and CK10. **b**, **d** Poorly differentiated squamous cell carcinoma negative for Foxp3 and CK10. Foxp3 expressed in Tregs infiltrating in the tumor stroma (*black arrow*)

investigate the role of Foxp3 in differentiation of cervical cancer cells by testing expressions of Foxp3 and CK10. CK10 is a differentiation marker of squamous epithelium [23]. In this study, we found a significant correlation exists between nuclear Foxp3 and CK10. Furthermore, each of nuclear Foxp3 and CK10 expression also significantly correlated with tumor differentiation. Figure 2 showed that nuclear Foxp3 was stained stronger in well-differentiated squamous cancer cells than in poorly differentiated cells. Consistent with our result, Wang et al. [24] also reported that Foxp3 was mainly expressed in the nucleus in well-differentiated hepatocellular carcinoma tissues. Taken together, our data indicate that nuclear Foxp3 is involved in differentiation of cervical cancer cells.

So far, the expression of $P16^{INK4a}$ is related to cervical cancer and development of CIN lesion, and it may be used as a biomarker for diagnosis of high-risk HPV infection and cervical cancer [25]. In cervical intraepithelial neoplasia, HPV DNA integrated into the host genome results in overexpression of viral oncoprotein and induces $P16^{INK4a}$ expression [26]. In the present study, we found expression of Foxp3 is significantly correlated to the expression of $P16^{INK4a}$, and then we conclude that the up-regulation of Foxp3 detected in this study is likely the consequence of HPV persistent infection and it could accelerate the malignant transformation of cervical epithelial cells. Therefore, we hypothesize that the up-regulation of Foxp3 may be a vital mechanism utilized by HPV to suppress the host immune response.

In summary, the present study demonstrates that up-regulation of Foxp3 is correlated with tumor growth and participates in progression of cervical cancer. Moreover, the degree of differentiation of cervical cancer is correlated with nuclear Foxp3 expression, and Foxp3 maybe involves in differentiation of cervical cancer cells. Although further studies are needed to clarify the role and mechanism of Foxp3 up-regulation in the progression of cervical cancer, the current study will provide new insights into the carcinogenesis of cervical cancers.

Acknowledgments This work was supported by Initial Doctoral Funding of Guang Dong Medical College (B2011002).

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Parkin D, Bray F (2006) Chapter 2: The burden of HPV-related cancers. Vaccine 24:S11–S25

2. Dehn D, Torkko KC, Shroyer KR (2007) Human Papillomavirus testing and molecular markers of cervical dysplasia and carcinoma. Cancer 111:1–14
3. Coffer PJ, Burgering BM (2004) Forkhead-box transcription factors and their role in the immune system. Nat Rev Immunol 4:889–899
4. Fontenot JD, Rasmussen JP, Williams LM, Dooley JL, Farr AG, Rudensky AY (2005) Regulatory T cell lineage specification by the forkhead transcription factor foxp3. Immunity 22:329–341
5. Hori S, Nomura T, Sakaguchi S (2003) Control of regulatory T cell development by the transcription factor Foxp3. Science 299:1057–1061
6. Liyanage UK, Moore TT, Joo HG, Tanaka Y, Herrmann V, Doherty G, Drebin JA, Strasberg SM, Eberlein TJ, Goedegebuure PS, Linehan DC (2002) Prevalence of regulatory T cells is increased in peripheral blood and tumor microenvironment of patients with pancreas or breast adenocarcinoma. J Immunol 169:2756–2761
7. Hiraoka N, Onozato K, Kosuge T, Hirohashi S (2006) Prevalence of $FOXP3^+$ regulatory T cells increases during the progression of pancreatic ductal adenocarcinoma and its premalignant lesions. Clin Cancer Res 12:5423–5434
8. Sasaki A, Tanaka F, Mimori K, Inoue H, Kai S, Shibata K, Ohta M, Kitano S, Mori M (2008) Prognostic value of tumor-infiltrating $FOXP3^+$ regulatory T cells in patients with hepatocellular carcinoma. Eur J Surg Oncol 34:173–179
9. Hinz S, Pagerols-Raluy L, Oberg HH, Ammerpohl O, Grüssel S, Sipos B, Grützmann R, Pilarsky C, Ungefroren H, Saeger HD, Klöppel G, Kabelitz D, Kalthoff H (2007) Foxp3 expression in pancreatic carcinoma cells as a novel mechanism of immune evasion in cancer. Cancer Res 67:8344–8350
10. Merlo A, Casalini P, Carcangiu ML, Malventano C, Triulzi T, Mènard S, Tagliabue E, Balsari A (2009) FOXP3 expression and overall survival in breast cancer. J Clin Oncol 27:1746–1752
11. Zuo T, Liu R, Zhang H, Chang X, Liu Y, Wang L, Zheng P, Liu Y (2007) FOXP3 is a novel transcriptional repressor for the breast cancer oncogene SKP2. J Clin Invest 117:3765–3773
12. Wang L, Liu R, Li W, Chen C, Katoh H, Chen GY, McNally B, Lin L, Zhou P, Zuo T, Cooney KA, Liu Y, Zheng P (2009) Somatic single hits inactivate the X-linked tumor suppressor FOXP3 in the prostate. Cancer Cell 16:336–346
13. Zheng Y, Rudensky AY (2007) Foxp3 in control of the regulatory T cell lineage. Nat Immunol 8:457–462
14. Sakaguchi S (2005) Naturally arising Foxp3-expressing $CD25^+CD4^+$ regulatory T cells in immunological tolerance to self and non-self. Nat Immunol 6:345–352
15. Sakaguchi S (2004) Naturally arising $CD4^+$ regulatory t cells for immunologic self-tolerance and negative control of immune responses. Annu Rev Immunol 22:531–562
16. Zhang HY, Sun H (2010) Up-regulation of Foxp3 inhibits cell proliferation, migration and invasion in epithelial ovarian cancer. Cancer Lett 28:791–797
17. Cunha LL, Morari EC, Nonogaki S, Soares FA, Vassallo J, Ward LS (2012) Foxp3 expression is associated with aggressiveness in differentiated thyroid carcinomas. Clinics 67:483–488
18. Wang LH, Lin S, Wang JT (2010) Correlation between elevated FOXP3 expression and increase lymph node metastasis of gastric cancer. Chin Med J 123:3545–3549
19. Niu J, Jiang C, Li C, Liu L, Li K, Jian Z, Gao T (2011) Foxp3 expression in melanoma cells as a possible mechanism of resistance to immune destruction. Cancer Immunol Immunother 60:1109–1118
20. Tao H, Mimura Y, Aoe K, Kobayashi S, Yamamoto H, Matsuda E, Okabe K, Matsumoto T, Sugi K, Ueoka H (2012) Prognostic potential of FOXP3 expression in non-small cell lung cancer cells combined with tumor-infiltrating regulatory T cells. Lung Cancer 75:95–101
21. Karube K, Aoki R, Sugita Y, Yoshida S, Nomura Y, Shimizu K, Kimura Y, Hashikawa K, Takeshita M, Suzumiya J, Utsunomiya A, Kikuchi M, Ohshima K (2008) The relationship of FOXP3 expression and clinicopathological characteristics in adult T-cell leukemia/lymphoma. Mod Pathol 21:617–625
22. Bolzer K, Käser T, Saalmüller A, Hammer SE (2009) Molecular characterisation of porcine Forkhead-box p3 (Foxp3). Vet Immunol Immunopathol 132:275–281
23. Boisvieux-Ulrich E, Le Pechon-Vallée C, Million K, Baeza-Squiban A, Houcine O, Guennou C, Reichert U, Marano F (2000) Differential effects of several retinoid receptor-selective ligands on squamous differentiation and apoptosis in airway epithelial cells. Cell Tissue Res 300:67–81
24. Wang WH, Jiang CL, Yan W, Zhang YH, Yang JT, Zhang C, Yan B, Zhang W, Han W, Wang JZ, Zhang YQ (2010) FOXP3 expression and clinical characteristics of hepatocellular carcinoma. World J Gastroenterol 16:5502–5509
25. Halloush RA, Akpolat I, Jim Zhai Q, Schwartz MR, Mody DR (2008) Comparison of ProEx C with p16INK4a and Ki-67 immunohistochemical staining of cell blocks prepared from residual liquid-based cervicovaginal material: a pilot study. Cancer 114:474–480
26. Klaes R, Benner A, Friedrich T, Ridder R, Herrington S, Jenkins D, Kurman RJ, Schmidt D, Stoler M, von Knebel Doeberitz M (2002) p16 immunohistochemistry improves interobserver agreement in the diagnosis of cervical intraepithelial neoplasia. Am J Surg Pathol 26:1389–1399