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*Original Article*

# Expression and clinicopathological significance of Foxp3 and VISTA in cervical cancer

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**Abstract:** Objective: To detect the expression differences of Foxp3 and VISTA in chronic cervical inflammation, cervical intraepithelial neoplasia, and cervical cancer, and to explore the role of Foxp3 and VISTA in the development of cervical cancer and the effect of Foxp3 and VISTA on the prognosis of cervical cancer, to provide a theoretical basis for clinical immunotherapy of cervical cancer. Methods: We collected 130 paraffin specimens of cervical tissue, which included 70 cases of cervical cancer tissue, 40 cases of cervical intraepithelial neoplasia tissues and 20 cases of chronic cervicitis. The expression of Foxp3 and VISTA in each group was detected, and the study was conducted based on the clinicopathological characteristics of the patients. The patients were followed up and the prognosis was statistically analyzed. Result: 1. The expression of Foxp3 and VISTA was statistically different between the cervical cancer group and other groups. 2. Expressions of Foxp3 and VISTA were significantly correlated. 3. In 70 cases of cervical cancer, the expression of Foxp3 and VISTA was related to the clinical stage. 4. The 3-year survival rate of 70 patients with cervical cancer was 72.9%, and there were no factors affecting 3-year OS found. The expression of Foxp3 and VISTA was significantly correlated with the prognosis of cervical cancer. Foxp3 and VISTA double positive expression group had the worst prognosis. Conclusion: 1. In cervical cancer, the expression of Foxp3 and VISTA was significantly higher than that of cervical intraepithelial neoplasia and chronic cervicitis, which suggested that they were closely related to the occurrence and growth of cervical cancer. 2. The expression of Foxp3 and VISTA was significantly related. 3. The positive expression of Foxp3 and VISTA could be used as independent prognostic factors for cervical cancer prognosis providing a strong basis for cervical cancer immunotherapy.

**Keywords:** Foxp3, VISTA, cervical cancer, Treg

## Introduction

Cervical cancer, a gynecological malignancy, is one of the most common tumors worldwide. The incidence ranks fourth in the globe [1]. Due to the rapid progress in surgery, radiotherapy, and chemotherapy, certain effects have been achieved in the treatment of cervical cancer [2]. However, the overall 5-year survival remains unsatisfactory. Orbegoso et al. [3] reported that overall, 5-year survival can reach about 68%, of which 40% of patients with advanced cervical cervix could develop disease soon. Therefore, predicting the development of the disease more accurately and evaluating the prognosis of the disease has become an important direction of clinical research. At present, the changes of many immune factors in clinical evaluation provide a feasible basis for tumor immunotherapy. However, tumor immunomodulatory therapy often works only in some patients, which may be due to successful immunotherapy which requires activation of the immune system. Therefore, in-depth study of immunosuppression mechanism of cervical cancer patients is of great necessity. Moreover, finding different tumor related immune checkpoints and reversing tumor immunosuppression may be the breakthrough of immunotherapy for cervical cancer and other solid tumors.

Treg can inhibit the immune response of the body and make the body immune tolerance to tumor cells, resulting in immune escape of cancer cells. Treg cells mainly express forkhead transcription factor (Foxp3) protein, Foxp3 is associated with the activation of CD4 + CD25 + Treg cells in some tumors, which also affects the development and function of Treg cells, thus affecting the proliferation of tumor [4]. In

**Table 1.** Clinical features of 70 cases of cervical cancer

| Clinicopathological parameters | | n (cases) | Percentage (%) |
|---|---|---|---|
| Age | < 45 | 26 | 37.1 |
| | ≥ 45 | 44 | 62.9 |
| Histological grade | Moderately differentiated | 44 | 62.9 |
| | Poorly differentiated | 26 | 37.1 |
| Pathological type | Squamous cell carcinoma | 58 | 82.9 |
| | Adenocarcinoma and adenosquamous carcinoma | 12 | 17.1 |
| FIGO staging | Phase I | 43 | 61.4 |
| | Phase II | 27 | 38.6 |
| Fibromuscular layer invasion range | > 1/2 | 27 | 38.6 |
| | < 1/2 | 43 | 61.4 |
| Lymph node metastasis | Negative | 56 | 80.0 |
| | Positive | 14 | 20.0 |
| Treatment method | Surgery | 16 | 22.9 |
| | Surgery + Radiotherapy | 12 | 17.1 |
| | Surgery + Chemotherapy | 9 | 12.9 |
| | Surgery + Radiotherapy + Chemotherapy | 33 | 47.1 |
| HPV | Negative | 37 | 52.9 |
| | Positive | 33 | 47.1 |
| Vessel | Negative | 48 | 68.6 |
| | Positive | 22 | 31.4 |

addition, Foxp3 overexpression is not only about low prognosis. Therefore, the monitoring of Foxp3 protein level in tumor tissue can help understand the changes of tumor immune microenvironment. VISTA could shut the response of T-cells to tumor antigen and inhibit the differentiation of T-cells into Treg cells, which has the function of immunosuppression and immunomodulation [5]. These results suggesed that the blocking of VISTA target or the combination with other immunosuppressants may be a new method for cancer immunotherapy.

At present, extensive researches have been reported on the application of tumor immune checkpoints and their inhibitors at home and abroad. However, there are relatively few experiments in the cervical cancer treatment. There are few novel points of view involving changes in the level of immune checkpoints after cervical cancer radiotherapy and further exploration of changes in the immune microenvironment of cervical cancer. Based on the past work experience and current experimental hot spots, starting from the immune microenvironment of cervical cancer, with immunohistochemical detection of cervicitis, cervical dysplasia, and cervical cancer tissues. This study analyzed the changes of immune checkpoints Foxp3 and VISTA in the occurrence and development of cervical cancer.

## Materials and methods

### *Cases collection*

Seventy cases of cervical cancer, 40 cases of cervical intraepithelial neoplasia, and 20 cases of chronic cervicitis who were admitted to the hospital where the author works from March 2014 to May 2017 (CIN I level 20 cases, CIN II-III level 20 cases) were included after obtaining the approval from our hospital ethic committee and written informed consent. Patients with cervical cancer were included in our study according to the following rules: i) Accessible clinical data with at least 3 years of follow-up; ii) There was no chemotherapy or radiotherapy before operation; iii) Cervical cancer was confirmed by clinical and histopathological diagnosis. All the cervical cancer patients had complete clinical and pathological data and had no other malignant tumor at the same time, did not receive chemotherapy before operation, had no autoimmune disease, and did not receive immunosuppressant use history. The general date of patients were shown in **Table 1**.

*Main reagent*

Foxp3 polyclonal antibody (Abcam); VISTA Polyclonal Antibody (CST); Universal immunohistochemical kit, 3, 3-diaminobenzidine, and Developer I anti diluent.

*Experimental method*

Immunohistochemical SP three-step method was used to detect the expression of Foxp3$^+$, VISTAprotein. ① Paraffin wax specimens of 70 cases with cervical cancer were sliced into 4 μm serial sections, dried, and prepared for later use. We placed the tissue section in a wet box, deparaffinized and hydrated it with conventional xylene and gradient ethanol, and added 50 μl of endogenous peroxidase blocking solution. After incubation at room temperature for 30 min, we washed it for 5 min with phosphate-buffered saline, repeated this 3 times to throw away the excess liquid, blocked the agent with 3% hydrogen peroxide with endogenous peroxidase, let it sit in room temperature for 15 min, washed it in PBS for 5 min, and repeated this 3 times; ② We placed the tissue section in the wet box again, added blocking solution, and blocked it at room temperature for 15 min; ③ We poured out the blocking liquid and added 1 drop (approximately 50 μl) of lantibody to two tissues with a sampler. They were Foxp3$^+$ antibody working solution, VISTA antibody working solution (must cover the entire tissue), placed in a refrigerator at 4°C overnight, used PBS instead of primary antibody as a negative control; ④ We took out the tissue section from the refrigerator at 4°C and rewarmed it at room temperature for 1 h, then washed it using PBS 3 times for 5 min each time to shake off excess liquid, added biotin-labeled anti-mouse/rabbit IgG II antibody to the sampler, which was incubated at room temperature, immersed in PBS for 5 min, repeated this 3 times, spun to dry, put the tissue section in the wet box again, added the appropriate amount of horseradish peroxidase-labeled streptavidin for 20 min at room temperature, immersed it in PBS for 5 min, and repeated 3 times; ⑤ We added the color developing agent DAB solution dropwise, observed and controlled the color development time under an optical microscope, which was rinsed with tap water after proper color development. After 3-5 min of hematoxylin restaining, there was differentiation with 1% hydrochloric acid alcohol, the stained glass slides were immersed in gradient ethanol for 5 min each for dehydration; ⑥ The slides were soaked in xylene I and II for 10 min to make the tissue transparent, put 1~2 drops of neutral gum on the slide tissue, and added a cover glass to let it dry naturally. Then, we carefully observed the staining results under a microscope, recorded, and took photos.

*Result judgment*

All slides were double-blinded and read independently by two pathologists. After immunohistochemical staining, 3 sections were randomly selected from each specimen. The sections were first observed under a low-power microscope (×40), and the areas with the highest density of infiltrating lymphocytes in the nests and stroma were selected. Then the positive staining cell count was carried out in 5 different fields randomly which were selected at high magnification (×400). The positive expression of tumor cells was analyzed by cell count. The result was judged by semi-quantitative integral evaluation method [6]: According to the degree of cell staining, no staining was 0, light yellow or light black was 1 point, brown yellow or brown black was 2 points, and brown or black was 3 points. According to the number of staining cells, 1 point for stained cells in one field of view < 5%; 5%~25% was 2 points; 26%~75% was 3 points; > 75% was 4 points. The multiplication of the two scores was the semi-quantitative test score result. Qualitative analysis was divided into positive expression and negative expression. The two product scores ≥ 3 were divided into positive expression <, and 0~2 were divided into negative expression.

*Follow-up*

Telephone follow-up was mainly used. The initial follow-up time was the operation time of cervical cancer. The follow-up time was 36-54 months. The median follow-up time was 40 months. Total survival was calculated starting from the surgery to the date of death or the last follow-up.

*Statistical treatment*

Foxp3 and VISTA semi-quantitative data were expressed as mean ± standard deviation ($\bar{x}$ ±

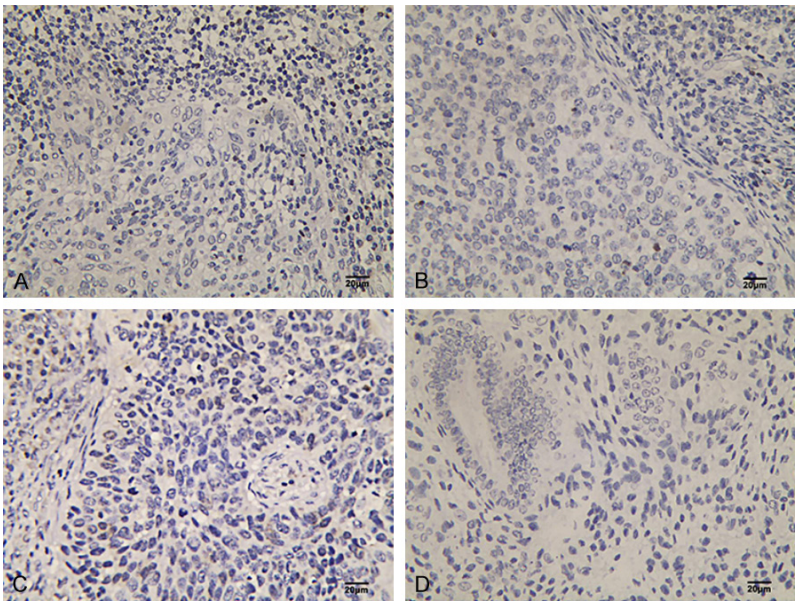


**Figure 1.** Expression of Foxp3 protein in cervical cancer. The staining grade was (A) strong, (B) moderate or (C) weak, and (D) was the negative control of chronic cervicitis.

S). The comparison of Foxp3 and VISTA expression levels among different pathological types and the relationship between each clinicopathological indicator and Foxp3 and VISTA expression levels were analyzed by one-way ANOVA. Foxp3 and VISTA were calculated into qualitative data. After the normal distribution test, the spearman test in correlation analysis was used for correlation analysis. A multivariable Cox proportional hazard model was used to assess the relationship between Foxp3 and VISTA expression and clinicopathological characteristics and overall survival. For all tests, significance was claimed at $P < 0.05$. All analyses were performed using SPSS V21.0 software (IBM Corp, Armonk, NY, USA).

## Results

*Expression of Foxp3 and VISTA in cervical cancer tissues*

The immunohistochemical results were observed under the microscope. Foxp3 protein was mainly expressed in the stroma of tumor cells, and occasionally in the nucleus and cell membrane (**Figure 1**). Foxp3 are differently expressed in cervical cancer, CIN II-III, CIN I, and chronic cervicitis. The cervical cancer possessed the highest expression, followed by CIN II-III, CIN I, and chronic cervicitis. Vista was only expressed in cervical cancer and CIN II-III, and it was expressed highest in cervical cancer. There was statistical significance in each group ($P=0.035 < 0.05$) (**Table 2**). By comparison, it found that there was a significant difference between cervical cancer and chronic cervicitis ($P < 0.05$). Expression of Foxp3 protein in cervical cancer was shown in **Figure 1**.

The expression of VISA protein was mainly located in the cytoplasm and membrane of tumor cells (**Figure 2**). Between CIN and chronic cervicitis, there was an obvious difference, CIN I/CIN II/III ($P > 0.05$). Expression of Vista in cervical cancer was shown in **Figure 2**: In 35/70 cases, VISTA positive tumor cells (50%) were observed.

*Correlation analysis of Foxp3 and VISTA expression in paraffin section of cervical cancer*

In 70 cases of cervical cancer, the average level of Foxp3 was 2.30±2.799. The average expression level of VIST was 2.81±2.409. K-S (Kolmogorov Smirnov) test was performed, and showed that the expression of Foxp3 and VISTA in cervical cancer tissue did not conform to the bivariate normal distribution (P=0.000). Therefore, the spearman test was used for correlation analysis.

*Relationship between Foxp3 expression and clinical features of cervical cancer*

As shown in **Table 3**, Foxp3 expression was higher in stage II patients. In addition, Foxp3 expression was related to vascular invasion. Although none of the other 6 clinicopathological parameters reached any statistical significance in the Foxp3 expression. However, there was a trend that Foxp3 positive expression was higher in patients with age ≥ 45 years, poorly differentiated, squamous cell carcinoma, fibromuscular layer invasion > 1/2, and HPV infection. The relationship between Foxp3-expression and clinicopathological characteristics in

**Table 2.** Different expressions of Foxp3 and VISTA in different pathological types

| Pathological type | n (cases) | Expression of Foxp3 $\bar{x} \pm S$ | F | P | Expression of Vista $\bar{x} \pm S$ | F | P |
|---|---|---|---|---|---|---|---|
| Cervical cancer | 70 | 2.30±2.799 | 2.954 | 0.035 | 2.81±2.409 | 16.609 | 0.00 |
| CIN II-III | 20 | 1.25±2.314 | | | 1.25±2.314 | | |
| CIN I | 20 | 1.25±2.245 | | | No expression | | |
| Chronic cervicitis | 20 | 0.60±2.037 | | | No expression | | |

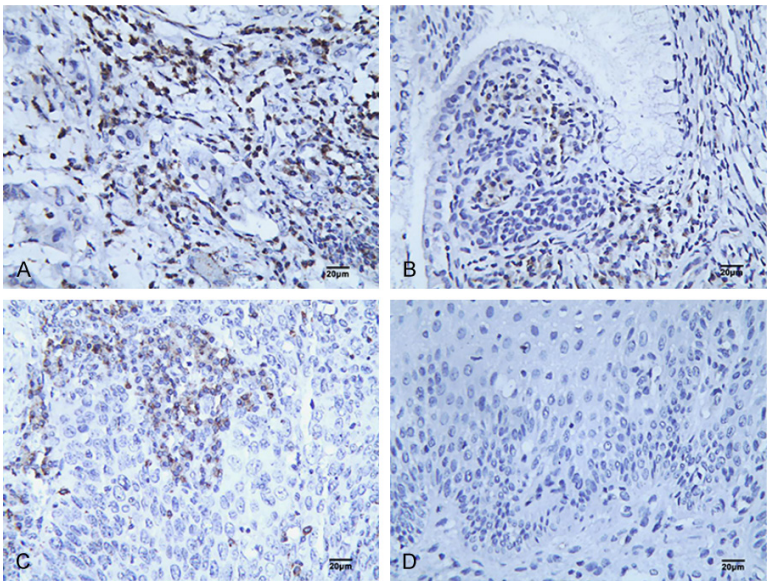


**Figure 2.** Expression of Vista in cervical cancer. In 35/70 cases, VISTA positive tumor cells (50%) were observed, the dyeing grade was (A) strong, (B) moderate or (C) weak, and (D) was a negative control of chronic cervicitis tissue.

70 cases with cervical cancer was shown in **Table 3**.

*Relationship between VISTA expression and clinical features of cervical cancer*

Among 70 cases with cervical cancer, cases with age ≥ 45 years, poorly differentiated, squamous cell carcinoma, fibromuscular layer invasion > 1/2, HPV infection, and vascular infiltration had higher VISTA expression, but there was no statistical significance. This means that the expression of VISTA had no obvious relationship with age, pathological differentiation, pathological type, fibromuscular layer invasion range, lymph node metastasis, HPV virus infection, and vascular infiltration. However, the difference in the expression of VISTA between stage II and stage I in pathological staging was statistically significant, compared to Phase I. The expression of VISTA was higher in patients with stage II cervical cancer (**Table 4**). The relationship between the expression of VISTA and clinicopathological characteristics in 70 cases with cervical cancer was shown in **Table 4**.

*The relationship between Foxp3, VISTA expression, and postoperative survival time of cervical cancer*

In this study, the 3-year survival rate of cervical cancer was 72.9%. **Table 5** listed the correlation between OS and 9 clinicopathological parameters and treatment methods in patients with cervical cancer (P > 0.05). The results showed that there were no factors affecting the 3-year OS in univariate analysis of clinicopathological parameters. Univariate analysis of clinicopathological parameters for 3-year survival rate of cervical cancer was shown in **Table 5**.

The positive expression rate of Foxp3 was 32.9% (23/70). The average survival time of Foxp3 positive expression group was (34.087± 3.761) months. The 3-year survival rate was 43.5% (13/23). The average survival time of patients in the Foxp3 negative expression group was (49.426±1.783) months. The 3-year survival-rate was 87.2% (41/47). The Kaplan-Meier curve (Log-rank test) analysis showed that the survival time of cervical cancer patients with negative Foxp3 expression was longer than that of cervical cancer patients with positive Foxp3 expression ($\chi^2$=25.952, P=0.000). The death risk of Foxp3 positive expression

**Table 3.** The relationship between Foxp3 expression and clinicopathological characteristics in 70 cases with cervical cancer

| Clinicopathological parameters | n (cases) | Expression of Foxp3 Mean ± SD | F | P |
|---|---|---|---|---|
| Age | | | | |
| < 45 | 26 | 1.73±2.920 | 1.728 | 0.193 |
| ≥ 45 | 44 | 2.64±2.703 | | |
| Histological grade | | | | |
| Moderately differentiated | 44 | 1.17±1.329 | 1.922 | 0.499 |
| Poorly differentiated | 26 | 2.65±3.298 | | |
| Pathological type | | | | |
| Squamous cell carcinoma | 58 | 2.50±2.867 | 1.746 | 0.191 |
| Adenocarcinoma and adenosquamous carcinoma | 12 | 1.33±2.309 | | |
| FIGO staging | | | | |
| I | 48 | 1.44±2.383 | 12.173 | 0.001* |
| II | 22 | 3.67±2.909 | | |
| Fibromuscular invasion | | | | |
| > 1/2 | 27 | 1.70±2.667 | 2.024 | 0.159 |
| < 1/2 | 43 | 2.67±3.053 | | |
| Lymph node metastasis | | | | |
| Negative | 56 | 2.34±2.772 | 0.054 | 0.816 |
| Positive | 14 | 2.14±3.009 | | |
| HPV virus | | | | |
| Negative | 37 | 2.65±3.298 | 1.922 | 0.108 |
| Positive | 33 | 2.65±3.298 | | |
| Vessel | | | | |
| Negative | 48 | 2.85±3.094 | 6.459 | 0.013* |
| Positive | 22 | 1.09±1.444 | | |

*P < 0.05, Staging and vascular infiltration have a significant impact on Foxp3 expression.

was 2.656 times that of negative expression (**Figure 3**).

The positive expression rate of VISTA was 50% (35/70). The average survival period of the VISTA positive expression group was (35.114±2.828) months. The 3-year survival rate was 54.3% (19/35). The average survival time of patients in the VISTA negative expression group was (51.486±1.403) months, and the 3-year survival-rate was 91.4% (32/35). The results of the log-rank test showed that patients with cervical cancer with negative expression of VISTA survive longer than those with positive expression of VISTA ($\chi^2$=13.16, P=0.000). The risk of death with positive expression of VISTA was 3.184 times that of negative expression (**Figure 4**). Survival curves of patients with different VISTA expression levels were shown in **Figure 4**.

According to expression of Foxp3 and VISTA, patients were divided into double negative expression groups of Foxp3 and VISTA, Foxp3 single positive expression, VISTA single positive expression, and double positive expression of FoxP3 and VISTA. There were 28 cases, 7 cases, 19 cases, and 16 cases, respectively. The **Figure 5** showed that the Foxp3 and VISTA double negative expression group had the best prognosis. The average survival period can reach 52.929±1.052 months. Foxp3 single positive expression and VISTA single positive expression followed, while the prognosis of FoxP3 and VISTA double positive expression group was the worst. Its average survival time was 26.813±3.584. A survival curve of patients with different Foxp3 and VISTA expression levels was shown in **Figure 5**.

The treatment methods and fibromuscular layer with higher *P* value were excluded from

**Table 4.** The relationship between the expression of VISTA and clinicopathological characteristics in 70 cases with cervical cancer

| Clinicopathological parameters | n (cases) | Expression of VISTA Mean ± SD | F | P |
|---|---|---|---|---|
| Age | | | | |
| < 45 | 26 | 2.38±2.351 | 1.321 | 0.254 |
| ≥ 45 | 44 | 3.07±2.434 | | |
| Histological grade | | | | |
| Moderately differentiated | 44 | 2.17±1.835 | 0.819 | 0.445 |
| Poorly differentiated | 26 | 3.27±2.616 | | |
| Pathological type | | | | |
| Squamous cell carcinoma | 58 | 2.95±2.358 | 1.047 | 0.310 |
| Adenocarcinoma and adenosquamous carcinoma | 12 | 2.17±2.657 | | |
| FIGO staging | | | | |
| I | 43 | 1.79±2.122 | 27.988 | 0.000 |
| II | 27 | 4.44±1.908 | | |
| Fibromuscular invasion | | | | |
| > 1/2 | 27 | 2.33±2.402 | 1.771 | 0.188 |
| < 1/2 | 43 | 3.12±2.393 | | |
| Lymph node metastasis | | | | |
| Negative | 56 | 2.82±2.383 | 0.002 | 0.961 |
| Positive | 14 | 2.79±2.607 | | |
| HPV virus | | | | |
| Negative | 37 | 2.92±2.732 | 0.146 | 0.703 |
| Positive | 33 | 2.70±2.023 | | |
| Vessel | | | | |
| Negative | 48 | 3.17±2.263 | 3.379 | 0.070 |
| Positive | 22 | 2.05±2.591 | | |

**Table 5.** Univariate analysis of clinicopathological parameters for 3-year survival rate of cervical cancer

| Clinicopathological parameters | $\chi^2$ | P |
|---|---|---|
| Age | 1.153 | 0.283 |
| Histological grade | 2.792 | 0.095 |
| Pathological type | 0.551 | 0.458 |
| FIGO staging | 2.209 | 0.137 |
| Fibromuscular invasion | 0.045 | 0.831 |
| Lymph node metastasis | 2.962 | 0.085 |
| HPV virus | 1.162 | 0.281 |
| Vessel | 1.356 | 0.244 |
| Treatment | 0.925 | 0.819 |

univariate analysis. The Cox regression model was used in multivariate analysis of the remaining nine clinicopathological factors and Foxp3 and VISTA levels. The results showed that Foxp3 (relative risk, HR=0.144, P=0.001) and VISTA (HR=0.214, P=0.031) might play an independent role in predicting the adverse prognosis of cervical cancer.

## Discussion

Marcy found that the genital tract HPV infection rate of 8581 women was as high as 33.06% [7]. Two years after HPV infection, most of the patients were immune cleared. Only about 5.13% of the infected people could not clear the virus because of the immune system disorder. They were progressed to cervical intraepithelial neoplasia and even cervical cancer. The immune system is regulated by both co-stimulating signal molecules and inhibitory molecules called immune checkpoints. Among them, immunosuppression checkpoint, such as CTLA-4 and PD-1-PD-L1 have been found to play a vital role in many parts of the tumor body [8-10]. Immunosuppressive checkpoints can lower the killing activation of T-cells against tumor cells, block these inhibitory signals to

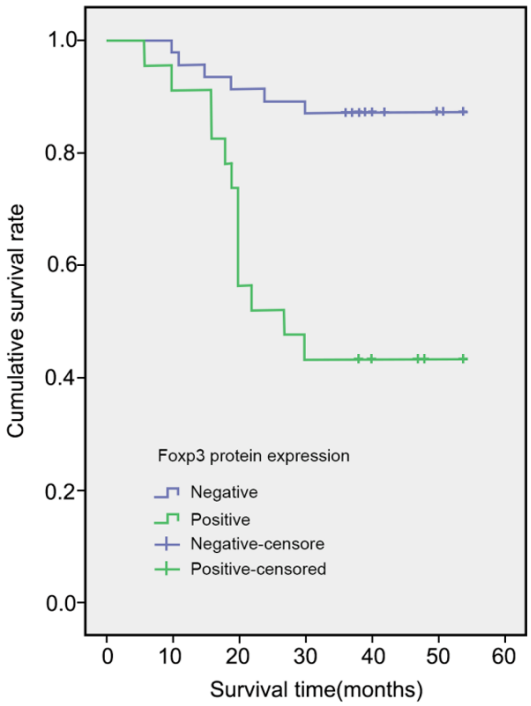


**Figure 3.** Survival curve of patients with different Foxp3 expression levels.

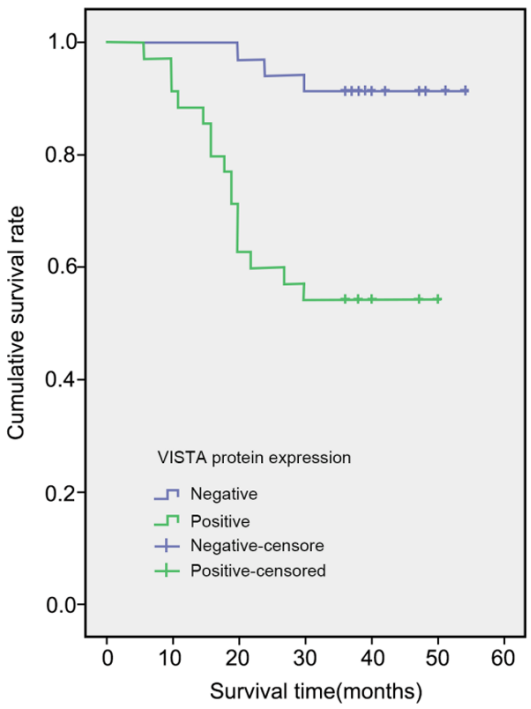


**Figure 4.** Survival curves of patients with different VISTA expression levels.

improve anti-cancer immunity, and ultimately eliminate tumors [11, 12]. When the body's immunosuppressive effect is strong, the virus cannot be effectively eliminated. Researchers analyzed the relationship in HPV and the expression of PDL1 at the immunosuppressive checkpoint in neck squamous cell carcinoma and anal-cancer associated with human HPV and observed that PD-L1 expression was up-regulated on the cell membrane [13, 14]. An in-depth analysis of the key links in the local immune microenvironment dialogue network of cervical cancer can provide new ideas for the l cancer prevention and the cervical cancer treatment with immunosuppressant.

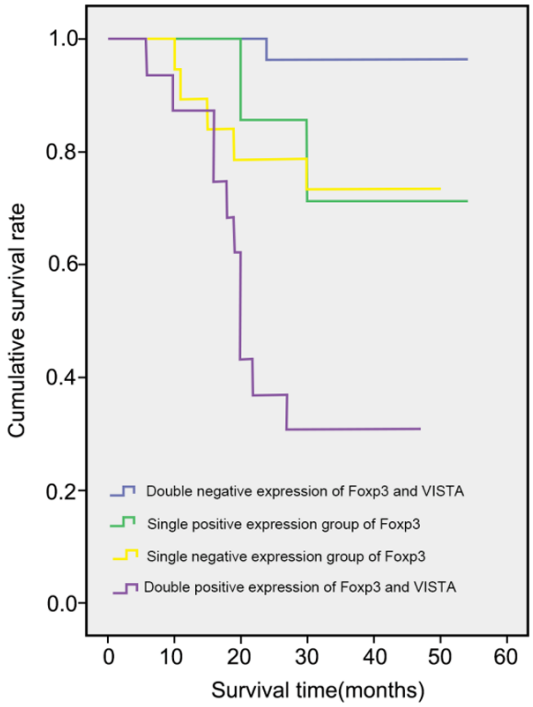


**Figure 5.** Survival curves of patients with different Foxp3 and VISTA expression levels.

In recent years, immunotherapy for immune checkpoints has made rapid progress, such as drugs developed for CTLA4 targets and anti-PD-1 drugs, which has achieved good results in clinical trials and applications. However, the effective rates of the above two immunosuppressants were limited and high drug resistance occurred. Many studies have found that due to tumor heterogeneity, the immune escape mechanism of solid tumors may involve the abnormal expression of multiple immune checkpoint molecules. The search for new immunosuppressive checkpoints or combined immunosuppressive therapy has become a hot topic.

Foxp3 is a member of the family of transcriptional regulatory factors, and a specific marker of T-regulatory cells [15, 16]. In normal tissues, it is involved in local immune response, but when overexpressed in local tumor microenvironment, it helps tumor cells evade immune system surveillance and promotes tumor growth. The positive high expression of Foxp3 was found in lung-cancer, esophageal-cancer, colon-cancer, bladder-cancer, breast-cancer, melanoma, lung-cancer, and other tumors. Schneider [17] found the Foxp3 + Treg expression was abnormally increased in cervical cancer tissues. Shimizu [18] found the number of Foxp3 + Treg infiltration was related to recurrence and survival.

VISTA is like the B7 Ig superfamily containing PD-L1 [19]. VISTA may be an important mediator for controlling autoimmune development and immune response to cancer. Le Mercier [20] pointed out that the VISTA expression in microenvironment of tumor lymph nodes was increased; expression of closed VISTA inhibited differentiation of natural regulatory T cells and tumor-specific induced T cells, which could block the growth of tumors. Some studies have found that the overexpression of VISTA on tumor cells in the mouse cancer model can induce the immune protection of tumor cell growth. However, the use of anti-VISTA monoclonal antibody therapy can control the tumor growth [21].

Previous studies have shown that the positive expression of Foxp3 is closely related to tumor progression and prognosis [22-26]. In our study, 23 of 70 cervical cancer patients were positive for Foxp3 expression. Further analysis showed that the mean survival time (34.087± 3.761 months) and 3-year survival rate (43.5%, 13/23) in Foxp3 positive group were lower than those in Foxp3 negative expression group (49.426±1.783 months) and 3-year survival rate (87.2%, 41/47). The results of the Kaplan Meier curve (Log-rank test) showed that the survival time of patients with Foxp3 negative expression was longer than that of a patient with positive Foxp3, and the difference was statistically significant ($\chi^2$=25.952, P=0.000). Kondo reported that the use of VISTA blockers could significantly improve the antitumor T cell response to inhibit tumor growth and improving survival rate. The risk of death with positive expression of VISTA was 3.184 times higher than that of negative expression. We also found that the double negative expression group of Foxp3 and VISTA had the best prognosis with an average survival time of 52.929±1.052 months. Foxp3 single positive expression and VISTA single positive expression followed, and the prognosis of Foxp3 and VISTA double positive expression group was the worst. Based on the above results, it is indicated that Foxp3 and VISTA can be used as auxiliary indicators for cervical cancer screening, preoperative disease severity evaluation, and postoperative treatment effect monitoring.

At present, the results of preclinical and clinical trials for immune checkpoints (including PD-L1, CTLA-4, and VISTA, etc.) are exciting. However, since it is impossible to classify which cervical cancer patients are more suitable for immunotherapy and which targets are more effective in cervical cancer immunotherapy, the application of cervical cancer immunotherapy is still challenged. Foxp3 and VISTA can be used as biomarkers of cervical cancer to formulate effective individualized treatment plans.

## Disclosure of conflict of interest

None.

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