RESEARCH HIGHLIGHTS

To Explore the Correlation between Tumor and Immune Markers and HPV Expression and Prognosis in Patients with Cervical Cancer

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Abstract: The aim of this study was to investigate the correlation between immune cells and tumor markers and HPV levels in patients with cervical cancer with high-risk human papilloma virus (HPV) rate and their prognosis. Firstly, 83 cases of cervical intraepithelial neoplasia were selected as CINI group, 72 cases of cervical carcinoma as cervical cancer group and 50 cases of chronic cervicitis as chronic cervicitis group. The different expression levels of immune cells (CD4+, CD8+, Treg, CD4+/CD8+, CD56+,) and the positive expression of tumor markers (K-ras, Ki-67) in patients with HPV were studied to explore the correlation between HPV levels and immune cells and tumor markers in cervical cancer patients with high-risk HPV infection, and that between positive expression of immune cells and tumor markers in patients with lymph node metastasis of cervical cancer with high-risk HPV infection, as well as that between the survival rate of patients and the immune cell levels and positive expression of tumor markers in patients who die of cervical cancer. The results showed that the levels of CD8 + and Treg in cervical cancer patients were higher than those in CINI group and chronic cervicitis group (P < 0.05). The levels of tumor markers were lower in those in CINI group and chronic cervicitis group (P < 0.05). The positive expression rates of K-ras and Ki-67 in the three groups were significantly different (P < 0.05). In cervical cancer group, CD4 + and CD56+ were negatively correlated with HPV-DNA levels, and CD8 + and Treg levels as well as k-RAS and KI-67 positive expression were positively correlated with HPV-DNA levels. The levels of immune markers in cervical cancer group were significantly lower than those in surviving patients (P < 0.01), while the levels of CD8 + and Treg, the proportion of K-RA and KI-67 were significantly higher than those in surviving patients (P < 0.01). Therefore, for patients with CINI, chronic cervicitis patients with high-risk HPV infection, and cervical cancer patients with reduced immune function and high-risk HPV infection, the expression of tumor markers K-ras and Ki-67 was increased. The detection of immune cells and tumor markers is helpful for the early prevention, diagnosis and prognosis evaluation of high-risk HPV infection in patients with cervical cancer.

Keywords: HPV diagnosis; Prognosis; Immune cells; Tumor markers

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1 Introduction

Cervical cancer is the second most common cancer in women. It is also one of the main causes of cancer-related death in women, which has attracted much attention in recent year due to the increasing incidence¹². Approximately 83% of cases occur in developing countries, particularly in South America, Asia and Africa, accounting for 15%³⁴ of all cancers among women. Human papilloma virus (HPV) is a common DNA virus that is transmitted
by sexual contact \[7-9\]. Low-risk HPV may usually cause genital condyloma acuminatum, while high-risk HPV may cause cervical cancer \[10-12\]. It has been reported that HPV infection in some people further develops into lesions with clinical signs after a certain incubation period, such as condyloma acuminatum, tumor and other diseases \[13\]. HPV persists in the mucocutaneous tissue for a long time after some people are infected with HPV, causing neither obvious clinical signs nor any discomfort \[14-16\]. HPV infection in some people is self-limited, and HPV infection may disappear gradually after a certain period of time, either automatically or spontaneously \[17-18\]. Studies have shown that 80% of women are infected with high-risk HPV at least once in their lifetime \[19\], and whether HPV can be converted to cervical cancer is also associated with various immune cells (e.g. T cells, B cells, neutrophils, phagocytes, mast cells, etc.) and immune factors (antibodies, complement, interleukin, etc.) \[20-21\]. If the constitution is poor and the immunity is low, the possibility of turning negative will be weak, and the probability of persistent infection will increase. CD4 cells are important immune cells in the human immune system. CD4 molecules are mainly expressed by helper T (Th) cells, which are the receptors for antigens recognized by the TCR of Th cells. They bind to non-polypeptide regions of MHC class II molecules, and participate in the process of antigen recognition by the TCR of Th cells \[22-23\]. CD8 molecules are a leukocyte differentiation antigen, a glycoprotein possessed on the surface of some T cells that assists T cell receptors (TCRs) to recognize antigens and participate in the transduction of T cell activation signaling, also known as TCR co-receptors. T cells expressing CD8 (CD8T cells) usually differentiate into cytotoxic T cells (CTLs) after activation and can specifically kill or damage target cells \[24\]. The amount of CD4+ and CD8+ markers has been found to be correlated with the differentiation of T cells, while CD56+ markers are correlated with the expression of natural killer (NK) cells in vivo \[25\]. T cells and NK cells are typical immune cells, and when their infiltration decreases, the immune function of the human body will be reduced, and long-term HPV infection will not be eliminated, leading to cervical cancer \[26\]. Some studies have reported that regulatory T cells (Treg) have some inhibitory effect on the immune function of the human body. This inhibitory effect will allow mutant cells to evade immune monitoring, which leads to the development of tumor \[27, 28\]. The positive expression of K-RAS and Ki-67 was related to cell proliferation, migration and angiogenesis \[29\]. Therefore, in the early stages of cervical lesions, detection of indicators that may lead to decreased immune function in patients and markers associated with cell proliferation (e.g. K-RAS and Ki-67) may contribute to early prevention and diagnosis of cervical cancer.

In this study, immune cells and tumor markers were detected in patients with different cervical diseases, and they were found to be useful for the early prevention and diagnosis of cervical cancer.

2 Information and Test Methods

2.1 Experimental Equipment

Flowcytometry (FCM) was performed with BD Biosciences Accuri C6 flow cytometer in the United States. The HPV-DNA type detection instruments were purchased from Shenzhen Kerunda Biotech Co., Ltd.

2.2 Experiment Reagent

Treg, K-ras and Ki-67 antibodies labeled with Fluorescein isothiocyanate (FITC) were purchased from BD Bioscience Company in the United States. Phycoerythrin (PE)-labeled mouse anti-human CD4, CD8, CD56 antibodies, trypan blue staining solution, red blood cell lysate and phosphate buffered solution (PBS) were purchased from Shanghai YuanMu Biological Technology Co. Ltd.

2.3 The Object of the Experiment

Seventy-two patients with HPV-infected cervical cancer, 83 patients with CIN1 and 50 patients with chronic cervicitis who visited Hebei PetroChina Central Hospital from February 2014 to October 2017 were selected. Among them, the age of 72 patients with cervical cancer was (26–64 years old) and the average age was (42.6±5.7 years old). Clinical stages: 12 cases of stage 1, 28 cases of stage 2, 20 cases of stage 3 and 12 cases of stage 4; among them, 28 cases had lymph node metastasis and 44 cases had no lymph node metastasis. Eighty-three CIN patients included (CIN group) aged 25–66 years old, with a mean age of (41.5±4.9 years old); among these cases, 23 had CIN grade 1, 27 had grade 2, 33 had grade 3. The age of 50 included patients with chronic cervical cancer was 23-64 years, with a mean age of 40.8±5.2 years old. Exclusion criteria: Immunity of disease to the epidemic; Pregnant and lactating women; History of immunotherapy, chemoradiotherapy or other pharmacotherapy in the last 3 months.

On the first day after admission, 2mL of fasting venous blood was drawn from all patients. The levels of CD4+, CD8+, CD56+ and Treg in the peripheral blood of pa-
Patients were detected by FCM, and the expression of tumor markers (K-RAS and KI-67) in the diseased tissues was detected by immunohistochemistry (IHC). The positive expressions of CD4+, CD8+, CD4+/CD8+, CD56+ and Treg, as well as k-RAS and KI-67 in the three groups were compared and analyzed. FCM detection steps: After extracting peripheral blood from patients in each group, anticoagulant were added and stored in a refrigerator at 4°C for detection within 1 hour. The lymphocyte concentration was adjusted to about 5×10^6/L, and > 85% viable cells were determined with trypanosome blue staining, ensuring the accuracy of the test. Add 100 µL whole blood to the measuring tube, dilute it to 200 µL with PBS, then add 20 µL of FCM antibody and mix. Incubation was performed in a refrigerator at 4°C for 30 minutes in the dark environment; Red blood cell lysis buffer was added to lyse red cells. After washing with PBS for 3 times, the cells were fixed with 0.1% formaldehyde solution and the cells were detected on the machine within 2 hours.

SPSS 19.0 statistical software was used to collate and analyze the data. The measurement data were expressed by Mean ± SE, and the comparison between the two groups was performed by independent sample t test. χ2 test was used for comparison of enumeration data, and Spearman correlation analysis were used for analyzing the correlation. P < 0.05 indicated that the difference was statistically significant.

3 Results and Discussion

Table 1 shows that levels of CD4+, CD56+ and CD4+/CD8+ in patients with cervical cancer were lower than those in CIN and chronic cervicitis groups, but the levels of CD8+ and Treg were higher than those in CIN and chronic cervicitis groups, and the difference was statistically significant (P < 0.05). The levels of CD4+/CD8+ in CIN group was lower than that in chronic cervicitis group, while the level of Treg in CIN group was higher than that in chronic cervicitis group (P < 0.05), and the difference was statistically significant. In cervical cancer group, the levels of Treg and CD8+ and the positive expression of K-RAS and KI-67 in peripheral blood were positively correlated with the levels of hpV-DNA (r=0.567, 0.431, 0.631, 0.753, P < 0.05). However, the levels of CD4+ and CD56+ were negatively correlated with HPV-DNA (r=-0.488, -0.452, P < 0.05). The levels of CD4+, CD56+ and CD4+/CD8+ in patients with lymph node metastasis in the cervical cancer group were significantly lower than those in patients without lymph node metastasis, and the difference was statistically significant (2=6.771, 3.391, 5.541, P < 0.01). However, the levels of CD8+ and Treg were significantly higher than those in patients without lymph node metastasis (Table 3). Table 4 shows that the percentages of K-ras (++) and Ki-67 (++) in patients with lymph node metastasis in the cervical cancer group were 46.4% (13/28) and 50% (14/28), 18.2%, respectively, which was higher than those in patients without lymph node metastasis (8/44), 25.0% (11/44), and the difference was statistical learning mean (chi-square = 6.60).

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<th>Table 1. Comparison of immune cell levels in peripheral blood in patients of the three groups</th>
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<td>CD4/CD8*</td>
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<td>CD56 (%)</td>
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<td>Treg (%)</td>
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Note: * Compared with chronic cervicitis group, P < 0.05; † Compared with CIN group, P < 0.05

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<th>Table 2. Positive expression of tumor markers in patients of the three group [n (%)]</th>
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Note: * Composition ratio is rounded value

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<th>Table 3. Immune cell levels in patients with or without lymph node metastasis in the cervical cancer group</th>
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<th>Table 4. Immune cell levels in dead and surviving patients with cervical cancer</th>
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The results of this study showed that the levels of CD4+, CD56+ and CD4+/CD8+ in cervical cancer group were lower than those in CIN group and chronic cervical...
spondylitis group (P < 0.05). It is suggested that the decrease of immunity in patients with cervical cancer may be caused by long-term immunodeficiency and HPV infection leading to the occurrence of cervical cancer. The levels of CD8+ and Treg in cervical cancer group were higher than those in CIN group and chronic cervicitis group (P < 0.05), indicating that the immune function of patients with cervical cancer was inhibited, which may be caused by the escape of tumor cells, and therefore leading to the occurrence of cervical cancer. The accumulation of tumor cells and gradual deterioration of the disease. The positive rates of k-RAS and KI-67 in three groups were compared, and the difference was statistically significant (P < 0.05), among which the positive rates of K-RAS and KI-67 is highest in the cervical cancer group, indicating that the activity ability of cell proliferation and cell migration in the cervical cancer was higher than those in other two groups (P < 0.05). With the progression of disease, the corresponding levels of inhibitory T cells and the positive expression of K-RAS and KI-67 tended to increase in patients. Helper T cells decreased.

The levels of Treg and CD8+ and the positive expression of K-ras and Ki-67 in the peripheral blood of the patients in the cervical cancer group were positively correlated with HPV-DNA levels, while the levels of CD4+ and CD56+ were negatively correlated with HPV-DNA levels. At the same time, the levels of CD4+, CD56+ and CD4+/CD8+ in patients with lymph node metastasis in cervical cancer group were significantly lower than those in patients without lymph node metastasis (P < 0.01); The levels of CD8+, Treg and the proportions of K-ras (++) and Ki-67 (++) in patients with cervical cancer were significantly higher than those in patients without lymph node metastasis (P < 0.05); The levels of the above helper T lymphocytes in patients who died of cervical cancer were significantly lower than those in patients who survived (P < 0.01), and the levels of the above suppressor T cells and the proportion of positive tumor markers were higher than those in patients who survived (P < 0.01), further demonstrating that with the progress of the disease, the immunity of patients decreased, which promotes the proliferation and metastasis of tumor cells. In addition to being used as indicators for immune function, K-ras and Ki-67 can also be used as auxiliary indicators for tumor detection. The combined detection of immune cells and tumor markers in patients is not only of great significance for the early diagnosis of cervical cancer, but also of certain significance in the mechanism of tumor occurrence and development, which provides some reference for the early prevention, diagnosis and treatment of cervical cancer. Due to the limitation of time and technology, this study has not been further studied in depth on the pathogenesis of tumor and needs to be further improved.

4 Conclusion

In short, immunodeficiency and immune escape play important roles in the evolution of HPV-infected cervical cancer. The detection of immune cells and tumor markers is helpful for the early prevention, diagnosis and prognosis evaluation of HPV infection in cervical cancer.

References

[10] Ewaisha R, Panicker G, Maranian P. Serum immune profil-


