Chronic Diseases Prevention Review
21 (2022) 1-10
Available at http:// www.cancercellresearch.org
ISSN 2161-2609

The role of PKC ι and HPV in the immune microenvironment of cervical cancer and its clinical significance

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Abstract: Objective: To explore the role and clinical significance of $PKC_ι$ and human papillomavirus (HPV) in the immune microenvironment of cervical cancer.

Methods: RT-PCR, Immunohistochemistry and Flow cytometry assays were used to detect the high-risk HPV infection rate, $PKC_ι$/ YAP1 level, and CD3+, CD4+, CD8+ levels, respectively.

Results: The HPV infection rate was 53.89%. The levels of PKCand YAP1 in CINI, CINII, CINIII and cervical squamous cell carcinoma tissues were higher than normal cervical tissues (P<0.05). Moreover, the higher the CIN grade, the higher the levels of $PKC_ι$ and YAP1. The CD3+ and CD4+ levels in CINI, CINII, CINIII and cervical squamous cell carcinoma tissues were lower, while CD8+ levels were higher than normal cervical tissue (P<0.05; P<0.05). The positive rate of PKC/YAP1 in patients with high-risk HPV infection was negatively correlated with CD3+ and CD4+ levels (P<0.05), while positively correlated with the CD8+ level (P<0.05).

Conclusions: Cervical cancer patients are often accompanied by abnormal expression levels of $PKC_ι$/YAP1 and CD3+, CD4+ and CD8+, and there is a close relationship between the two, which can regulate the immune microcirculation of patients with cervical cancer.

Keywords: Cervical cancer; $PKC_ι$; Human papillomavirus; immune microenvironment; Clinical significance; Real-time fluorescent PCR; immunohistochemistry

Received 06 October 2022, Revised 011 October 2022, Accepted 15 October 2022

1. Introduction

Cervical cancer is a malignant tumor with a higher incidence in women, ranking second in female malignant tumors, second only to breast cancer, and ranking first in genital malignant tumors in incidence. It affects the health of women in China, and the incidence of diseases is showing a younger trend [1]. Previous studies have shown [2] that Human papillomavirus (HPV) infection, especially in patients with high-risk HPV persistent infection, is the main cause of cervical precancerous lesions and cervical cancer, especially cervical squamous cell carcinoma. The data survey results show that [3] 90.0% of women may be infected with HPV in their lifetime, while 10.0% of women have persistent infections, and only 1% of them become cervical cancer. However, domestic and foreign scholars believe that simple HPV infection may not cause cervical cancer, and there may be synergistic factors such as immunosuppression, smoking and estrogen. The immune system includes various organs, tissues and cells involved in the immune response, and immunosuppression refers to the suppression of the immune response, which can increase infections such as bacteria, viruses, and fungi, and is an important cause of cervical cancer [4-5] .

Protein Kinase C (PKC), as a signal molecule, belongs to a family of serine/threonine proteases that are dependent on calcium, phospholipid and diglyceride activation [6]. PKC is widely present in body tissues and cells, and plays an important role in the process of transmembrane signal transmission. The aPKC subtype family has domain characteristics and functional characteristics that are different from other subtypes of this family, with PKC-λ/ι and PKC-ζ [7]. PKCι is widely involved in the regulation of tumor cell resistance, anoikis resistance, cell senescence resistance and other malignant biological behaviors [8]. Therefore, this study focused on normal cervical tissues, CINI, CINII, CINIII and cervical squamous cell carcinoma tissues to explore the role and clinical significance of PKCι and HPV in the immune microenvironment of cervical cancer. The reports are as follows.

2. Materials and methods

2.1 Clinical data

Normal cervical tissue, CINI, CINII, CINIII and cervical squamous cell carcinoma from February 2010 to February 2020 were selected as the objects. 30 cases of normal cervical tissues, age (34-61) years, average (46.98±5.45) years; 80 cases of CINI, age (33-62) years, average (47.11±5.51) years; duration of disease (1-8) months, average (4.31±0.69) months; 80 cases of CINII, age (32-63) years, average (47.04±5.55) years;

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21 (2022) 1-10
ISSN 2161-2609

duration of disease (1-9) months, average (4.36±0.73) months; 80 cases of CIN III, age (32-63) years old, average (46.08±5.54) years old; disease course (1-9) months, average (4.43±0.72) months; 80 cases of cervical squamous cell carcinoma, age (34-66) years old, average (48.15) ±5.61) years old; duration of illness (1-10) months, average (4.43±0.76) months. This study was approved by the ethics committee, and the patients/family members signed the consent form.

2.2 Inclusion and exclusion criteria

Inclusion criteria: (1) Meet the diagnostic criteria for cervical disease and cervical cancer [9], and both are diagnosed by surgical tissue examination (2) All are planned to undergo surgical treatment, and the lesion tissue is taken during the operation, and the patient can tolerate it (3) Both can complete PKCι/YAP1, immune microenvironment determination.

Exclusion criteria: (1) Patients with mental abnormalities, blood system diseases or autoimmune system diseases (2) Patients with coagulation abnormalities, malignant tumors in other parts or cognitive dysfunction (3) Chemotherapy, radiotherapy, and biological immunotherapy.

2.3 Materials

Rabbit anti-PKC ι antibody (Lot No. BJ07176986), rabbit anti-YAP1 antibody (lot No. AI08203707), rabbit anti-CD4 antibody (lot no. AI01301602) and rabbit anti-CD8 antibody (lot No. BJ05096373) were purchased from Beijing Boausen Biotechnology Co., LTD. Zeiss metallographic microscope -Axio Imager 2 was used.

2.4 Method

(1) HPV mRNA measurement. The real-time fluorescent PCR method was used to determine the infection rate of high-risk HPV in the 5 groups of tissues. The specific method is as follows. ① Prepare a positive standard solution. Take the positive standard solution (106copies/mL) and complete the 10-fold dilution to obtain the solution concentration of 105copies/mL. Dilute in sequence to obtain 104copies/mL, 103copies/mL, 102copies/mL, and 10copies/mL. ② PCR mixture preparation. Complete the configuration of the mixed solution according to the kit instructions. ③ Add samples. After the above operations, each tube of 18uL is placed in the corresponding test tube, 2uL of DNA solution is added to the reaction tube containing the reaction mother liquor with the help of a pipette. ④ Measurement on the computer. Set PCR parameters: 10 min at 30°C; 30 min at 42°C; 5 min at 99°C; 5 min at 5°C, complete 55 consecutive cycles, extend 10 min at 72°C, put the final product into 1.5% agarose gel electrophoresis, β-actin is the internal control. ⑤ Interpretation of results. For cervical and ureteral skin cells, it is 0, indicating that the sample is unqualified, for HPV measurement value>1.0E+2copies, it indicates infection [10]. (2) Determination of PKCι/YAP1 level. A. Detection method. Immunohistochemistry (IHC) was used to detect the level of PKCι/YAP1 in the 5 groups of tissues. The sample tissues obtained above were taken, after routine deparaffinization and washing, sealed with a concentration of 0.3% H_2O_2, and a microwave method was used to restore the antigen. Serum blocking solution was given to seal, goat anti-human PKCι/YAP1 monoclonal antibody and PKCι/YAP1 secondary antibody were added to the obtained tissues, and then stained with hematoxylin after DBA color development. After the above operations, the slides were mounted [11]. B. Judgment criteria. Interpret the results of immunohistochemistry under an inverted microscope. 0 points have no positive staining cells. 1 point: positive staining cells, but the staining site is light yellow. 2 points: positive staining cells, but the staining site is brown. 3 points: positive staining cells, but the staining site is brown [12]. (3) Immunological microenvironment determination. For all surgical patients, the peripheral fasting blood was taken on the next day, centrifuged for 35 minutes at a speed of 3000 rpm, and the serum was separated and placed in a -80 ℃ refrigerator for use. Flow cytometry was used to determine the expression levels of CD3+, CD4+ and CD8+ in different tissues [13]. (4) Correlation analysis. SPSS Pearson correlation analysis software was used to analyze the correlation between the high-risk HPV infection rate and the expression levels of PKCι/YAP1, CD3+, CD4+ and CD8+.

Table 1. Comparison of PKCι/YAP1 positive rates in different tissues [n(%)]

Tissue types	Cases	PKCιpositive rate	YAP1positive rate
CINI	80	9（11.25）	11（13.75）
CIN II	80	22（27.50）	27（33.75）
CIN III	80	31（38.75）	35（43.75）
Cervical squamous cell carcinoma	80	54（67.50）	58（72.50）
Normal cervical tissue	30	2（6.67）	3（10.00）
F	/	6.491	5.138
P	/	0.030	0.037

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21 (2022) 1-10
ISSN 2161-2609

2.5 Statistical analysis

The SPSS18.0 software was used to process the count data and the $\chi 2$ test was performed using n (%), and the measurement data was performed by t test using ($\overline{x} \pm s$). The difference was statistically significant at P<0.05.

3. Results

3.1 Comparison of HPV infection rate and PKCι/YAP1 positive rate in different tissues

HPV mRNA was detected in 360 tissues. The results showed that among those, 194 HPV infections, the infection rate was 53.89%. The positive rates of PKCι and YAP1 in CINI, CINII, CINIII and cervical squamous cell carcinoma tissues were higher than normal cervical tissue (P<0.05), and the higher the CIN grade, the higher the positive rate of PKCι and YAP1, as shown in Table 1.

2.2. Comparison of immune microenvironment in different patients

CD3+ and CD4+ levels in the immune microenvironment of patients with CINI, CINII, CINIII and cervical squamous cell carcinoma were lower than normal cervical tissue (P<0.05). CD8+ levels were higher than normal cervical tissue (P<0.05). CINI, CINII, CINIII CD3+, CD4+, and CD8+ levels in different patients with cervical squamous cell carcinoma were significantly different (P<0.05) (Table 2).

Table 2. Comparison of immune microenvironment in different patients

Organization Type	Cases	CD3+	CD4+	CD8+
CINI	80	63.29±5.63	60.16±4.59	23.08±2.64
CIN II	80	55.41±5.15	52.43±4.32	25.69±3.28
CIN III	80	47.51±4.39	46.78±4.15	31.86±4.53
Cervical squamous cell carcinoma	80	41.29±4.14	39.61±3.69	34.61±4.56
Normal cervical tissue	30	66.54±6.49	64.14±5.32	21.87±5.09
F	/	6.392	8.325	7.125
P	/	0.000	0.000	0.000

3.3 The correlation between PKCι/YAP1 and CD3+, CD4+ and CD8+ in patients with high-risk HPV infection

The results of SPSS Pearson correlation analysis showed that the positive rate of PKCι/YAP1 in patients with high-risk HPV infection was negatively correlated with CD3+ and CD4+ levels (P<0.05). CD8+ levels were positively correlated (P<0.05), as shown in Table 3.

4. Discussion

Cervical cancer is a malignant tumor with a high clinical incidence, and it is generally believed to be related to HPV infection. However, not all patients with HPV infection can cause cervical cancer, and it can also be related to immunosuppression, estrogen, and smoking [14]. For normal people, the immune system mainly includes various organs, tissues and cells that participate in the immune response, and common ones include: lymph nodes, thymus, tonsils, etc. And immunosuppression refers to the ability to suppress the immune response, and continued low immunity will increase the rate of bacterial, fungal and viral infections. Previous studies have shown [15] that HPV can be concealed for a long time and replicate in large quantities after entering the human body, and it is related to the suppression of the patient's immune system, causing the body's immune function to decline. In this study, the HPV infection rate of 360 tissues was 53.89%. CD3+ and CD4+ levels in the immune microenvironment of patients with CINI, CINII, CINIII and cervical squamous cell carcinoma are lower than normal cervical tissue (P<0.05). CD8+ levels are higher than normal cervical tissue (P<0.05). CINI, CINII, CINIII CD3+, CD4+, and CD8+ levels in different patients with cervical squamous cell carcinoma are significantly different (P<0.05), indicating that the detection rate of HPV infection in patients with cervical cancer is relatively high, and the patients are often accompanied by abnormal immune microenvironment, which played an important role in development and occurrence. Studies by foreign scholars have shown [16] that there are three different stages of immune cell infiltration, immune modification and immune escape in the tumor immune microenvironment, and immune modification also includes clearance, balance and escape. Therefore, how to take effective measures to intervene in the above three different stages and improve the patient's immune level can provide a new method for the treatment of cervical cancer.

PKCι is a member of the aPKC subfamily, and is complementary to PKC-ζ. It is widely involved in and regulated tumor cell resistance, anoikis resistance, epithelial-mesenchymal transition, and cell senescence resistance and other malignant organisms [17]. Clinical studies have shown [18] that PKCι can directly participate in the malignant transformation of cells, and is related to tumors such as chronic myeloid leukemia

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21 (2022) 1-10
ISSN 2161-2609

Table 3. Correlation between PKCι/YAP1 and CD3+, CD4+ and CD8+ in patients with high-risk HPV infection (r, P)

Correlation analysis	CD3+	CD4+	CD8+
PKC ι	-0.761（0.000）	-0.779（0.000）	0.832（0.000）
YAP1	-0.803（0.000）	-0.817（0.000）	0.715（0.000）

and non-small cell lung cancer. At present, it has been clinically confirmed that PKCι can directly participate in the differentiation of Th2 cells and play an important role in the regulation of T cells [19]. In this study, the positive rates of PKCι and YAP1 in CINI, CINII, CINIII and cervical squamous cell carcinoma tissues were higher than normal cervical tissues (P<0.05), and the higher the CIN grade, the higher the PKCι. The higher the positive rate of YAP1, the higher the positive rate of PKCι and YAP1 in cervical cancer tissues, which can reflect the severity of the patient's disease and can guide clinical treatment. Studies by domestic scholars have shown [20] that overexpression of PKCι can shape the tumor immunosuppressive microenvironment, cause the overexpression of pro-inflammatory factors such as S100, IL-6/Ib, and reduce NK cell infiltration. In order to further analyze the relationship between the positive rate of PKCι/YAP1 and the immune microenvironment in patients with high-risk HPV infection, SPSS Pearson correlation analysis was carried out in this study. The results showed that the positive rate of PKCι/YAP1 in patients with high-risk HPV infection and the level of CD3+, CD4+ were negatively correlated (P<0.05), while with CD8+ level was positive (P<0.05). Therefore, clinically high-risk HPV infection and cervical cancer patients should strengthen their PKCι/YAP1 determination, assess and predict the tumor microenvironment, and guide clinical treatment.

5.Conclusion

Cervical cancer patients are often accompanied by abnormal expression levels of PKCι/YAP1 and CD3+, CD4+ and CD8+, and the two are closely related, which can regulate the immune microcirculation of patients with cervical cancer and provide new targets for clinical immunotherapy.

Acknowledgements

This work was supported by the Hainan Province Health and Family Planning Industry Research Project (Grants No. 19A200097)

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