

Clinical Correlation between Tumor-Associated Macrophages and High-Risk Human Papillomavirus Infection in Cervical Cancer: Postprint

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Abstract

Objective: To explore the correlation between tumor-associated macrophages and the occurrence and development of high-risk human papillomavirus (hr-HPV)-associated cervical cancer. **Methods:** 112 clinical cervical tissue samples were collected, comprising 16 normal cervical tissues, 55 cervical intraepithelial neoplasias, and 41 cervical squamous cell carcinomas. Immunohistochemistry was employed to detect the expression levels of CD163+ macrophages in cervical tissue sections, and statistical analysis was conducted between immunohistochemical findings and clinical data. **Results:** Immunohistochemical results demonstrated that the cell density of CD163+ macrophages increased with the degree of malignant transformation in cervical tissues, with correlation analysis showing a positive correlation between the two ($P=0.000$). Additionally, the cell density of CD163+ macrophages was significantly upregulated in hr-HPV-type cervical cancer ($P<0.05$). Statistical analysis of clinical data revealed significant correlations between CD163+ macrophages and lymph node metastasis as well as FIGO stage in cervical cancer ($P=0.005$, $P=0.004$). **Conclusion:** CD163+ macrophage expression is positively correlated with the degree of malignant transformation in cervical tissues, and hr-HPV infection shows significant correlation with CD163 expression levels in macrophages. CD163+ macrophages may serve as a prospective predictive factor for the occurrence and development of cervical cancer induced by hr-HPV infection.

Full Text

Preamble

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Abstract

Objective To investigate the correlation between tumor-associated macrophages (TAMs) and the development of high-risk human papillomavirus (hr-HPV)-related cervical cancer. **Methods** A total of 112 cervical tissue samples were collected, including 16 normal cervical tissues, 55 cervical intraepithelial neoplasia (CIN) tissues, and 41 squamous cervical cancer (SCC) tissues. The expression of CD163 macrophages in cervical tissues was detected by immunohistochemistry, and the results were statistically analyzed in relation to clinical data. **Results** Immunohistochemical analysis showed that the cell density of CD163 macrophages increased progressively with increasing tissue malignancy. Correlation analysis revealed a positive relationship between CD163 macrophage density and degree of malignancy ($P=0.000$). The density of CD163 macrophages was significantly upregulated in hr-HPV-positive SCC tissues ($P<0.05$). CD163 macrophage density was positively correlated with cervical lymph node metastasis ($P=0.005$) and FIGO stage ($P=0.004$). **Conclusion** The expression of CD163 macrophages is positively correlated with malignant transformation of cervical tissues, and hr-HPV infection is significantly correlated with CD163 expression levels in macrophages. CD163 macrophages may serve as prospective predictors for the occurrence and progression of cervical cancer caused by hr-HPV infection.

Keywords: squamous cervical cancer; high-risk human papillomavirus; tumor-associated macrophages; CD163

Introduction

Cervical cancer is one of the three major malignant tumors in gynecology, and infection with high-risk human papillomavirus (hr-HPV) is the primary etiological factor in the vast majority of cervical cancer cases. The tumor microenvironment (TME) plays a crucial role in the development and progression of malignant tumors. Inflammation caused by massive inflammatory cell infiltration in the TME has become recognized as the seventh hallmark of cancer. Tumor-associated macrophages (TAMs) are associated with cancer development, progression, and poor prognosis. TAMs can influence tumor proliferation, invasion, and metastasis through multiple mechanisms and serve as an objective indicator for malignant tumor prognosis assessment. TAMs express multiple

molecular markers, including CD68, CD163, and CD204. Studies have shown that CD163 serves as a specific marker for TAMs and can reflect functional clinical indicators of mononuclear macrophages. Research has found that the distribution of CD163 macrophages is significantly associated with the invasion pattern of cervical cancer cells, but no studies have yet investigated the correlation between CD163 macrophages and hr-HPV infection. This study collected clinical data from patients with normal cervical tissue, cervical intraepithelial neoplasia (CIN), and squamous cervical carcinoma (SCC), and used immunohistochemistry to detect CD163 macrophage expression levels in corresponding tissues to explore the correlation between TAMs and hr-HPV infection and its clinical significance.

Materials and Methods

Patient Samples and Clinical Data

Cervical specimens were collected from 112 patients in the Department of Obstetrics and Gynecology at Nanfang Hospital of Southern Medical University between January 2011 and December 2013. These included 16 cases of normal cervical tissue from hysterectomy for uterine fibroids, 4 cervical biopsy tissues, 25 loop electrosurgical excision procedure (LEEP) specimens, 26 cold knife conization specimens, and 41 radical hysterectomy specimens for cervical cancer. The cohort comprised 16 normal cervical tissues, 29 CIN I tissues, 26 CIN II-III tissues, and 41 SCC tissues. Patients with adenocarcinoma, those who received preoperative radiotherapy or chemotherapy, pregnant women, and patients with acute pelvic infection were excluded. Clinicopathological data (including HPV infection status, age, FIGO stage, histological type, and lymph node metastasis) were obtained from the Department of Obstetrics and Gynecology. HPV infection was detected using either the Hybrid Capture 2 (HC2) assay or HPV genotyping tests. All pathological data were reviewed by chief physicians in the Pathology Department. Informed consent was obtained from all patients, and the study was approved by the hospital ethics committee.

Reagents

Mouse anti-human CD163 monoclonal antibody was purchased from Abcam (USA). Immunohistochemical detection kits and DAB chromogenic reagent kits were purchased from Zhongshan Jinqiao (China).

Immunohistochemistry

Tissue specimens were fixed in formalin, embedded in paraffin, and sectioned consecutively at 5 μ m thickness. Sections were deparaffinized and rehydrated, then incubated with 3% H₂O₂-methanol at room temperature for 30 minutes to block endogenous peroxidase activity. Antigen retrieval was performed by microwaving sections in citrate buffer (pH 6.0) for 15 minutes. Sections were blocked with 10% goat serum at 37°C for 1 hour, then incubated with mouse

anti-human CD163 monoclonal antibody (diluted 1:200) at 4°C overnight. After incubation with secondary antibody at 37°C for 30 minutes, DAB chromogenic reaction was performed for 1 minute, followed by hematoxylin counterstaining. Sections were dehydrated in graded alcohol, cleared in xylene, and mounted with neutral resin.

CD163 Macrophage Quantification and Scoring

Two experienced pathologists reviewed slides in a double-blind manner. CD163 expression on macrophage membranes appeared as brown-yellow granules. For each sample, the area with the highest density of brown-stained cells was selected under low magnification ($\times 100$). The average number of macrophages in five random high-power fields ($\times 400$) was calculated. CD163 macrophage expression was scored from 0 to 3 based on the proportion of brown-stained cells: 0 (<5%), 1 (5-20%), 2 (20-50%), and 3 (>50%). Scores of 0-1 were considered low expression, while 2-3 indicated high expression.

Statistical Analysis

Statistical analysis was performed using SPSS 19.0 software. Chi-square test was used for frequency table analysis, and Spearman's rank correlation test was used to evaluate correlations between categorical variables. Distribution tables are expressed as mean \pm standard deviation. All tests were two-tailed, and $P < 0.05$ was considered statistically significant.

Results

CD163 Macrophage Density Correlates with Cervical Malignant Transformation

CD163 macrophage density increased progressively with cervical tissue malignant progression (from normal tissue to SCC). The mean macrophage counts in normal cervical tissue, CIN I, CIN II-III, and SCC were 5.4, 39.1, 69.4, and 132.1, respectively. CD163 expression in SCC was significantly higher than in all CIN stages ($P < 0.05$), and expression in CIN tissues was higher than in normal cervical tissues ($P < 0.05$). Additionally, the difference in CD163 expression between CIN I and CIN II-III was statistically significant ($P < 0.05$). CD163 macrophage density was positively correlated with cervical cancer development (Spearman's $\rho = 0.700$, $P = 0.000$) [Figure 1: see original paper].

CD163 Macrophage Expression Associates with hr-HPV Infection

Among the 112 patients, hr-HPV infection rates were 12.5% (2/16) in normal patients, 68.9% (20/29) in CIN I patients, 92.3% (24/26) in CIN II-III patients, and 100% (41/41) in SCC patients. CD163 macrophage density was significantly higher in hr-HPV-positive patients than in hr-HPV-negative patients ($P < 0.05$) [Figure 1: see original paper]. As shown in , CD163 macrophage

expression scores were significantly correlated with hr-HPV infection and cervical tissue malignancy. The proportion of high CD163 macrophage expression scores was higher in hr-HPV-positive patients (81/88, 92.0%) compared to hr-HPV-negative patients (6/24, 25.0%). Among hr-HPV-positive patients, the proportions with high expression scores were higher in CIN I (58.6%), CIN II-III (84.6%), and SCC (97.5%) compared to normal cervical tissue (12.5%), and also higher than corresponding hr-HPV-negative groups.

CD163 Macrophages Correlate with FIGO Stage and Lymph Node Metastasis

When tissue section results were correlated with clinical data from all 112 patients, CD163 macrophage density showed significant correlation with lymph node metastasis ($P=0.005$) and FIGO stage ($P=0.004$). However, CD163 macrophage density showed no significant correlation with patient age or histological type ($P=0.179$ and $P=0.571$, respectively).

Discussion

hr-HPV infection is recognized as a critical factor in cervical cancer pathogenesis. The progression from initial hr-HPV infection to precancerous lesions and ultimately to invasive cervical cancer requires at least 10-15 years, suggesting that hr-HPV infection is merely an initiating factor. Tumor growth depends not only on genetic alterations in malignant cells but also on changes in the TME, including stroma, vasculature, and infiltrating inflammatory cells. As an important component of the TME, TAMs participate in tumor development and progression. Studies have shown that viral infections are closely related to tumorigenesis and can promote recruitment of inflammatory cells (such as TAMs) and inflammatory mediators (such as cytokines and chemokines) into the TME, participating in malignant transformation. Extensive research demonstrates that TAM accumulation in the TME is closely associated with viral infection, and virus-induced chronic inflammation can activate CD163 macrophage function. Our finding that CD163 macrophages are more highly expressed in hr-HPV-positive patients suggests that TAMs may participate in the progression of cervical malignant lesions caused by hr-HPV infection.

We further found a significant correlation between CD163 macrophages and cervical malignant transformation (Spearman's $\rho=0.700$, $P=0.000$), with expression increasing alongside malignant progression. These results suggest that increased macrophage infiltration and the accompanying inflammatory microenvironment may contribute to cervical malignant lesion development. TAMs influence tumors through multiple mechanisms, including promoting lymphangiogenesis via vascular endothelial growth factor C (VEGF-C), enhancing tumor cell proliferation, migration, and invasion, and interacting with cancer-associated fibroblasts (CAFs) and cytokines in the TME to suppress immune surveillance and tumor clearance. These findings collectively indicate that CD163 macrophages play a critical role in cervical cancer development.

In studying the correlation between CD163 macrophages and clinicopathological parameters in cervical cancer patients, we found significant associations with lymph node metastasis and FIGO stage. Similar findings have been reported in other malignancies, where tumor-infiltrating TAMs produce various tumor growth-promoting cytokines and release matrix-degrading enzymes that facilitate tumor invasion and metastasis. In cervical cancer, locally activated macrophages express abundant VEGF-C, which is associated with peritumoral lymphatic vessel formation, suggesting that TAMs secrete multiple factors promoting lymphangiogenesis and thereby facilitating lymph node metastasis. These results align with our findings.

Cervical cancer stage progression and lymph node metastasis are primary causes of treatment failure, recurrence, and mortality. Numerous studies have shown that TAM infiltration in tumor tissues correlates with poor prognosis. However, whether TAMs can serve as prognostic predictors for cervical cancer remains controversial. Some researchers have reported that CD163 macrophages are significantly associated with survival after cervical cancer treatment, and increased TAMs can elevate lymphatic vessel density, thereby promoting lymph node metastasis and reducing survival time. Conversely, other studies have found no significant association between CD68 macrophages and tumor recurrence. Due to limited sample size and insufficient follow-up data, we could not further analyze the relationship between CD163 macrophages and prognosis or survival time in cervical cancer treatment. Larger sample sizes and more comprehensive follow-up data are needed to verify whether CD163 macrophages can serve as prognostic factors for cervical cancer treatment.

This study evaluated the correlation between CD163 macrophages and hr-HPV-infected cervical cancer using immunohistochemistry and clinical data. We found that CD163 macrophage expression is positively correlated with the degree of cervical tissue malignant transformation and significantly associated with hr-HPV infection, suggesting that CD163 macrophages may serve as prospective predictors for cervical cancer caused by hr-HPV infection. However, the specific interaction mechanisms between CD163 macrophages and hr-HPV in cervical cancer development require further investigation.

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