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Original Article

The correlation between HLA-DRB1 and HLA-DQB1 gene polymorphisms and cytokines in HPV16 infected women with advanced cervical cancer

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Abstract: Objective: To analyze the distribution of HLA-DRB1 and HLA-DQB1 alleles and its correlation with IFN-γ, IL-2, IL-6, IL-10 in HPV16 infected women with advanced cervical carcinoma. Methods: We collected 137 blood samples of cervical carcinoma patients diagnosed by pathology as cervical cancer in stage IIb-IVb before the treatment, and we gathered 175 blood samples of healthy women living in the local. We determined the genetic subtypes of HLA-DRB1 and HLA-DQB1, and we measured the concentration of IFN-γ, IL-2, IL-6 and IL-10. We compared the difference of cytokines in patients with different clinical stages and the healthy in the control group. According to genetic subtypes of HLA-DRB1 and HLA-DQB1, we also compared the concentration of cytokine (CK) in different genetic subtypes. Results: Eight HLA-DRB1 alleles and four HLA-DQB1 alleles were found. There were not significant differences between each allele in the concentration of IFN-γ, IL-2, IL-6, and IL-10. Conclusion: HLA-DRB1*07, HLA-DQB1*02 and HLA-DQB1*03 were the differentially expressed gene in HPV16 infected patients with advanced cervical cancer. There may be correlations between the occurrence, development of cervical cancer and IFN-γ, IL-2, IL-6, IL-10.

Keywords: HLA-DRB1, HLA-DQB1, genetic polymorphism, cytokines, hpv16, cervical cancer

Introduction

Cervical cancer is the most common malignancy in female reproductive system, which follows breast cancer and becomes second-largest causes of death in women with malignant disease [1]. In China, the morbidity and mortality of cervical cancer accounts for about one-third of the world's morbidity and mortality [2-4].

Human leukocyte antigen (HLA) correlates with the occurrence of cervical cancer, especially for HLA II type DRB1 and DQB1 genes which closely related to the occurrence of cervical squamous cell carcinoma [5, 6]. It has been reported that cytokines play an important role in cancer occurrence and development [7-9].

There were differences in the distribution of HLA II gene in patients with cervical cancer between different races and different regions. A lot of literatures had reported that DRB1 and DQB1 of HLA II gene were closely related to the cervical cancer with HPV16 infection [10, 11]. Beskow et al. [10] had detected the HLA-DRB1 and HLA-DQB1 alleles in 440 cases of patients diagnosed with HPV16-infected cervical cancer and 476 cases of healthy individuals, and found that the frequency of HLA-DRB1*0801, HLA-DRB1*1501, HLA-DQB1*0402 and HLA-DQB1*0602 alleles increased, while the frequency of HLA-DRB1*0101, HLA-DRB1*1301, HLA-DQB1*0501 and HLA-DQB1*0603 alleles decreased; after correction, DQB1*0602 and DRB1*1501 were closely related to cervical cancer. Cuzick et al. [12] investigated the HLA genotyping in 116 cases of British women with HPV16-infected cervical cancer and found that HLA-DQB1*0301 (P = 0.02) and HLA-DRB1*0401 (P = 0.02) were the haploids susceptible to cervical cancer. Madeleine et al. [13] found that, HLA-DRB1*1501 and HLA-DQB1*0302 were the dominant genes. Guzalinuer et al. [14] suggested that, HLA-DRQB06 may be the sus-

Table 1. The distribution of HLA-DRB1 allele in HPV1 infected patients with advanced cervical cancer (2n = 274)

HLA-DRB1 gene	Number of alleles	Frequency (%)
DRB1*01	28	10.4
DRB1*03	28	10.4
DRB1*04	30	11.2
DRB1*07	119	44.2
DRB1*09	21	7.8
DRB1*11	21	7.8
DRB1*12	8	2.9
DRB1*13	14	5.2

ceptible gene of cervical cancer in Uygur women, which may increase the risk of cervical cancer in HPV16-positive women. The results of the study showed that, HLA-DRB1*07 was the dominant expressed gene, and its expression ratio was close to half. Madeleine et al. [13] found that HLA-DQB1*0301, HLA-DRB1*0301, and HLA-DQB1*0201 were associated with HPV16-positive squamous cell carcinoma (odds ratio was 0.5; 95% confidence interval 0.3~0.9).

In this study we analyzed the distribution of HLA-DRB1 and HLA-DQB1 alleles in HPV16 infected women with advanced cervical carcinoma, compared the levels of cytokines in people with different clinical stages and the healthy, and discussed the correlation between distribution of HLA-DRB1 and HLA-DQB1 allele and IFN-γ, IL-2, IL-6, IL-10.

Materials and methods

General information

137 cases of HPV16 infected patients with advanced cervical cancer (cervical cancer) in Binzhou People's Hospital from January 2010 to March 2014 were enrolled in this study. The age ranged from 38 to 70, with a median age of 54 years. Cervical cancer stage (according to FIGO staging): 63 cases were at IIb stage, 60 cases were at IIIb stage, 3 case was at IIIA stage, 7 cases were at IVA stage. 4 case was at IVb stage. 175 cases of healthy women in the local were enrolled in the control group.

Methods

Whole blood genomic DNA extraction: 5 mL of Cubital vein blood were collected from the cervical cancer patients before treatment and from the healthy controls (EDTA anticoagulant). Whole blood genomic DNA extraction kit was purchase form BaiTektronix Biotechnology Co., Ltd. (Beijing, China). The operation was in strict accordance with the instructions for DNA extraction. UV spectrophotometer was used for measuring the concentration and purity, and its A260/280 ratios were 1.8 to 1.9. Adjust the final concentration at 0.3~0.5 μg/μL and placed them at -20°C refrigerator for detection.

Table 2. The distribution of HLA-DQB1 allele in HPV16 infected patients with advanced cervical cancer (2n = 274)

HLA-DQB1-1 gene	Number of alleles	Frequency (%)
DQB1*02	156	56.93
DQB1*03	94	34.31
DQB1*05	12	8.76
DQB1*06	9	6.57

HLA-DRB1 and HLA-DQB1 gene subtype classification

Polymerase chain reaction/sequence specific primer method (PCR-SSP) was used. Specific steps refer to the reference [15].

Detection of cytokines

Plasma from blood samples were obtained by centrifuge and stored at -80°C refrigerator. IFN-γ, IL-2, IL-6, IL-10 levels were detected by ELISA method according to Li's protocol [15].

Statistical analyses

Spss17.0 statistical software (Chicago, CA, USA) was used for analyzing the distribution of HLA-DRB1 and HLA-DQB1 allele; *t* test was used for cytokine concentrations in the two groups. Univariate analysis of variance was used for cytokine concentrations between HLA-DRB1 and HLA-DQB1 alleles. $P < 0.05$ was considered statistical significance.

Results

HLA-DRB1 expression in patients with cervical cancer

Eight HLA-DRB1 alleles were detected. The number of detected HLA-DRB1 alleles and composition ratio were showed in **Table 1**.

Table 3. Comparison of CK concentration between each allele of HLA-DRB1 (pg/mL, Mean ± SD)

CK	HLA-DRB1*01 (n = 28)	HLA-DRB1*03 (n = 28)	HLA-DRB1*04 (n = 30)	HLA-DRB1*07 (n = 119)	HLA-DRB1*09 (n = 21)	HLA-DRB1*11 (n = 21)	HLA-DRB1*12 (n = 8)	HLA-DRB1*13 (n = 14)
IFN-γ	114.1 ± 48.3	124.4 ± 57.6	103.3 ± 68.4	125.4 ± 78.1	128.5 ± 65.1	121.6 ± 50.3	120.1 ± 60.1	124.5 ± 50.1
IL-2	23.4 ± 12.3	24.8 ± 5.9	20.3 ± 10.4	18.1 ± 8.7	19.0 ± 9.8	21.6 ± 10.6	22.0 ± 9.1	21.4 ± 10.3
IL-6	98.9 ± 7.5	100.4 ± 28.5	107.1 ± 23.8	115.1 ± 19.0	120.3 ± 18.5	111.9 ± 18.3	122.3 ± 18.0	116.3 ± 18.2
IL-10	105.4 ± 18.7	104.5 ± 17.5	113.8 ± 19.0	121.3 ± 18.4	113.5 ± 15.4	111.8 ± 17.3	119.3 ± 15.1	114.2 ± 17.2

Table 4. Comparison of CK concentration between each allele of HLA-DQB1 (pg/mL, Mean ± SD)

CK	HLA-DRB1*02 (n = 156)	HLA-DRB1*03 (n = 94)	DQB1*05 (n = 12)	DQB1*06 (n = 9)
IFN-γ	124.5 ± 18.5	118.4 ± 17.6	119.2 ± 11.8	112.1 ± 17.4
IL-2	13.4 ± 8.7	15.8 ± 8.9	13.0 ± 8.9	14.2 ± 8.1
IL-6	108.5 ± 48.8	113.9 ± 38.4	113.5 ± 42.1	115.4 ± 44.1
IL-10	135.6 ± 22.5	128.3 ± 27.4	132.3 ± 21.4	129.5 ± 23.2

HLA-DQB1 expression in patients with cervical cancer

Four HLA-DQB1 alleles were detected in 137 cases of patients in the whole group. The number of detected HLA-DQB1 alleles and composition ratio were showed in **Table 2**.

Comparisons of CK concentrations in HLA-DRB1 alleles

Excluding small sample size of HLA-DRB1*12 and HLA-DRB1*13 allele groups, the CK levels in HLA-DRB1*01, HLA-DRB1*03, HLA-DRB1 *04, HLA-DRB1*07, HLA-DRB1*09, and HLA-DRB1*11 allele groups were compared; ANOVA analysis showed that: There were no significant differences among alleles in IFN-γ, IL-2, IL-6, and IL-10 (**Table 3**).

Comparisons of CK concentrations in HLA-DQB1 alleles

Excluding small sample size of HLA-DQB1*05 and HLA-DQB1*06 groups, the CK levels in HLA-DQB1*02 and HLA-DQB1*03 groups were compared by ANOVA analysis, and it showed that: There were no significant differences between the alleles in IFN-γ, IL-2, IL-6, and IL-10, as shown in **Table 4**.

Discussion

Cytokine is the intercellular communication tool, and a component of the host's immune response to foreign pathogens. Interferon-γ (IFN-γ) is the only member of the family of type II interferon, mainly generated by T lymphocytes and NK cells, having antiviral, antitumor and immunomodulatory effects. Studies have shown that in malignant melanoma, gastric cancer, lung cancer, glioblastoma, nasopharyngeal cancer, colorectal cancer, cervical cancer and head and neck cancer, IFN-γ levels decreased [16]. Soong et al. [17] found that IFN-γ receptor agonists may improve the immune response and enhance the anti-tumor effects. Wang et al. [18] reported that due to lymph node metastases in patients with advanced cervical cancer, immune microenvironment was destroyed, resulting in immune escape; the study observed that IL-6 and IL-10 levels were higher than the normal level, and IFN-γ level was lower than normal level. The results of the study showed that in patients with different clinical stages of cervical cancer, the IFN-γ levels were significantly lower than that in the control group, consistent with the findings of Lippitz et al. [19] and Wang et al. [18]. IL-2 cytokine is mainly produced by T lymphocytes, playing a regulatory role in immune response, which can enhance immune activity. It can induce the differentiation of activated killer cells (LAK), natural killer cells (NK) and cytotoxic T cell (CTL) to play anti-tumor effects. Paradkar et al. [20] reported that, in peripheral blood of women with HPV infection, IL-2 levels in lymphocytes decreased compared with the normal group. Valle-Mendiola et al. [21] reported that high doses of IL-2 could inhibit the proliferation of cervical cancer cells.

The results of the study showed that IL-2 in cervical cancer group was lower than that in the healthy control group ($P < 0.05$), which was consistent with the literature [20, 21]. IL-6 is a typical cytokine with multiple biological functions; IL-6 could be generated by B cells, T cells, monocytes, fibroblasts, endothelial cells, astrocytes and microglia, which plays an important role in cell immunity, inflammation and hematopoietic regulation. The clinical studies of Guo et al. [22] showed that in patients with advanced multiple myeloma, non-small cell lung cancer, colorectal cancer, renal cell carcinoma, prostate cancer, breast cancer and ovarian cancer, serum IL-6 increased, so blocking IL-6 signaling has become as a potential cancer therapeutic strategy. Wang et al. [18] found that IL-6 was not expressed or lowly expressed in normal cervical tissue, and highly-expressed in cervical squamous cell carcinoma; IL-6 expression rate in patients with cervical squamous cell carcinoma was significantly higher than that in normal group ($P < 0.01$). This study showed that in IIb and IIIb cervical cancer group, serum levels were significantly higher than those in the healthy control group (all $P < 0.05$), which was

similar to the results of Wang et al. [18]. IL-10 is a cytokine with immunosuppressive activity, which can inhibit the generation of IL-2, INF-γ and other factors; literature [23] reported that IL-10 interacted with HPV by inhibiting immune function to promote the formation and development of cervical cancer. Related studies showed that IL-10 was overexpressed in many human tumors, such as renal cancer, gastrointestinal cancer and melanoma. Therefore, inhibition of IL-10 overexpression may play an antitumor effect [24]. The results of the study showed that IL-10 levels in IIb and IIIb groups were significantly higher than that in the control group, and the differences were statistically significant. This was consistent with the literature [23, 24]. Comprehensive analysis of the results showed that, compared with the healthy control group, IFN-γ and IL-2 in women with HPV16-infected IIb and IIIb cervical cancer were lower than the normal level, while IL-6 and IL-10 were higher than the normal level, and the differences were statistically significant; this means that these cytokines may play an important role in the occurrence and development of cervical cancer.

We hypothesized that there are differences between HLA-DRB1 and HLA-DQB1 alleles in cytokines, and compared the CK concentrations of HLA-DRB1*01, HLA-DRB1*03, HLA-DRB1*04, HLA-DRB1*07, HLA-DRB1*09 and HLA-DRB1*11; one-way ANOVA analysis showed that there were no significant differences among the alleles. The CK concentrations of HLA-DQB1*02 and HLA-DQB1*03 alleles were compared by one-way ANOVA analysis, which showed no significant difference in CK concentration between the alleles. Comprehensive analysis showed it may be related to the small sample size or that there may be no difference in CK itself between HLA-DRB1 and HLA-DQB1 alleles.

In summary, HLA-DRB1*07, HLA-DQB1*02 and HLA-DQB1*03 may be the superior genes in HPV16 infected women with middle or advanced cervical cancer; there were statistically significant differences in IFN-γ, IL-2, IL-6 and IL-10 levels between patients with cervical cancer and healthy individuals; but the relevance between alleles (such as HLA-DRB1 and HLA-DQB1) in advanced cervical cancer with HPV16 infection and cytokine levels, such as IFN-, IL-2, IL-6 and IL-10, still need to be further studied by expanding the sample size.

Disclosure of conflict of interest

None.

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References

[1] Liu Z, Shi Y, Meng W, Liu Y, Yang K, Wu S, Peng Z. Expression and localization of maspin in cervical cancer and its role in tumor progression and lymphangiogenesis. Arch Gynecol Obstet 2014; 289: 373-82.

[2] Xu QX, Zhang ZY. High-risk Human Papillomavirus Genotypes in Cervical Lesions and Vaccination Challenges in China. Asian Pac J Cancer Prev 2015; 16: 2193-7.

[3] Xu F, Cao M, Shi Q, Chen H, Wang Y, Li X. Integration of the full-length HPV16 genome in cervical cancer and Caski and Siha cell lines and the possible ways of HPV integration. Virus Genes 2015; 50: 210-20.

[4] Chen W, Zheng R, Zeng H, Zhang S, He J. Annual report on status of cancer in China, 2011. Chin J Cancer Res 2015; 27: 2-12.

[5] Chen D, Gyllensten U. A cis-eQTL of HLA-DRB1 and a frameshift mutation of MICA contribute to the pattern of association of HLA alleles with cervical cancer. Cancer Med 2014; 3: 445-52.

[6] Ben Othmane Y, Ghazouani E, Mezlini A, Lagha A, Raïs M, Kochkar R, Zidi S, Afrit M, Mota-Vieira L, Yacoubi Loueslati B. HLA class II susceptibility to cervical cancer among Tunisian women. Bull Cancer 2012; 99: 81-6.

[7] Ding AP, Zhang Y, Wei H, Luo QS, Zhang SL. Correlation study of HPV-16 existential status with Th17/Treg cytokines. Zhonghua Yi Xue Za Zhi 2013; 93: 2957-60.

[8] Sasagawa T, Takagi H, Makinoda S. Immune responses against human papillomavirus (HPV) infection and evasion of host defense in cervical cancer. J Infect Chemother 2012; 18: 807-15.

[9] Bolpetti A, Silva JS, Villa LL, Lepique AP. Interleukin-10 production by tumor infiltrating macrophages plays a role in Human Papillomavirus 16 tumor growth. BMC Immunol 2010; 11: 27.

[10] Beskow AH, Gyllensten UB. Host genetic control of HPV 16 titer in carcinoma in situ of the cervix uteri. Int J Cancer 2002; 101: 526-31.

[11] Hoa BK, Hang NT, Kashiwase K, Ohashi J, Lien LT, Horie T, Shojima J, Hijikata M, Sakurada S, Satake M, Tokunaga K, Sasazuki T, Keicho N. HLA-A, -B, -C, -DRB1 and -DQB1 alleles and haplotypes in the Kinh population in Vietnam. Tissue Antigens 2008; 71: 127-34.

[12] Cuzick J, Terry G, Ho L, Monaghan J, Lopes A, Clarkson P, Duncan I. Association between high-risk HPV types, HLA DRB1* and DQB1* alleles and cervical cancer in British women. Br J Cancer 2000; 82: 1348-52.
[13] Madeleine MM, Johnson LG, Smith AG, Hansen JA, Nisperos BB, Li S, Zhao LP, Daling JR, Schwartz SM, Galloway DA. Comprehensive analysis of HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 loci and squamous cell cervical cancer risk. Cancer Res 2008; 68: 3532-9.
[14] Guzalinuer A, Mihrinsa A, Zhang SQ, Li H, Gulishare N, Zhang GQ. Association between HPV infection and HLA-DQB1 alleles polymorphism in the cervical carcinogenesis in Uyghur women in southern Xinjiang. Zhonghua Zhong Liu Za Zhi 2010; 32: 492-6.
[15] Li YW, Gulina K, Tan Y, Wang RZ. The correlation between Human leukocyte antigen and cytokines in HPV16 infected Xinjiang Uyghur women in advanced cervical cancer. J Xinjiang Med Univ 2015; 138: 30-35.
[16] Young MR, Wright MA, Lozano Y, Matthews JP, Benefield J, Prechel MM. Mechanisms of immune suppression in patients with head and neck cancer: influence on the immune infiltrate of the cancer. Int J Cancer 1996; 67: 333-338.
[17] Soong RS, Song L, Trieu J, Knoff J, He L, Tsai YC, Huh W, Chang YN, Cheng WF, Roden RB, Wu TC, Trimble CL, Hung CF. Toll-like receptor agonist imiquimod facilitates antigen-specific CD8+ T-cell accumulation in the genital tract leading to tumor control through IFNγ. Clin Cancer Res 2014; 20: 5456-67.
[18] Wang HL, Xu H, Lu WH, Zhu L, Yu YH, Hong FZ. In vitro and in vivo evaluations of human papillomavirus type 16 (HPV16)-derived peptide-loaded dendritic cells (DCs) with a CpG oligodeoxynucleotide (CpG-ODN) adjuvant as tumor vaccines for immunotherapy of cervical cancer. Arch Gynecol Obstet 2014; 289: 155-62.
[19] Lippitz BE. Cytokine patterns in patients with cancer: a systematic review. Lancet Oncol 2013; 14: e218-28.
[20] Paradkar PH, Joshi JV, Mertia PN, Agashe SV, Vaidya RA. Role of cytokines in genesis, progression and prognosis of cervical cancer. Asian Pac J Cancer Prev 2014; 15: 3851-64.
[21] Valle-Mendiola A, Weiss-Steider B, Rocha-Zavaleta L, Soto-Cruz I. IL-2 enhances cervical cancer cells proliferation and JAK3/STAT5 phosphorylation at low doses, while at high doses IL-2 has opposite effects. Cancer Invest 2014; 32: 115-25.
[22] Guo Y, Xu F, Lu T, Duan Z, Zhang Z. Interleukin-6 signaling pathway in targeted therapy for cancer. Cancer Treat Rev 2012; 38: 904-10.
[23] Saraiva M, O'Garra A. The regulation of IL-10 production by immune cells. Nat Rev Immunol 2010; 10: 170-81.
[24] Song S, Wang Y, Wang J, Lian W, Liu S, Zhang Z, Liu F, Wei L. Tumour-derived IL-10 within tumour microenvironment represses the antitumour immunity of Socs1-silenced and sustained antigen expressing DCs. Eur J Cancer 2012; 48: 2252-9.