Chinese Journal of Cancer

Original Article

Expressions of Toll-like receptors 3, 4, 7, and 9 in cervical lesions and their correlation with HPV16 infection in Uighur women

Ayshamgul Hasimu[1], Lin Ge[2], Qiao-Zhi Li[1], Rui-Ping Zhang[1] and Xia Guo[3]

Abstract

Recent findings show that Toll-like receptors (TLRs) expressed in immune cells play a crucial role in the innate immune response and the subsequent induction of adaptive immune responses against microbial infection on tissue injury. Furthermore, expression of TLRs in cancer cells is associated with tumor proliferation and invasion. To explore the role of TLRs expression in cervical carcinogenesis in Uighur women, we detected the expressions of TLR3, TLR4, TLR7, and TLR9 in 25 normal cervical tissues, 64 cervical intraepithelial neoplasia (CIN) tissues, and 63 cervical squamous cell carcinoma (CSCC) tissues using immunohistochemical staining, as well as human papillomavirus type 16 (HPV16) infection using PCR. All samples used in this study were from Xinjiang Uighur women. We found the expression levels of TLR4, TLR7, and TLR9 were significantly higher in CIN and CSCC than in normal controls ($P < 0.05$). Up-regulation of TLR4 and TLR7 were correlated with tumor differentiation but not FIGO stage or lymph node metastasis ($P > 0.05$). Up-regulation of TLR9 was correlated with lymph node metastasis ($P < 0.05$) but not tumor differentiation or FIGO stage ($P > 0.05$). We also analyzed the correlation between the expressions of TLRs and HPV16 infection and found that the expressions of TLR4 and TLR9 significantly correlated with HPV16 infection in CIN ($r = 7.434$, $P = 0.006$; $r = 7.123$, $P = 0.008$) and CSCC ($r = 6.423$, $P = 0.001$; $r = 8.478$, $P = 0.004$), whereas the expression of TLR3 was not significantly different in any of the three groups and had no significant correlation with HPV16 infection. Our results suggest that high expression of TLR4, TLR7, and TLR9 may play important roles in the development and progression of CIN and CSCC in Uighur women, and the expressions of TLR4 and TLR9 can be up-regulated by HPV16 infection.

Key words Cervical squamous cell carcinoma, Uighur women, Toll-like receptors, HPV16

In southern Xinjiang, China, the incidence of cervical cancer is high, notably occurring at a rate 3–4 times higher for Uighur women than for Han women in the same area. The mortality of cervical cancer in Uygur women ranks first among ethnic groups in China [1,2]. High-risk human papillomavirus (HPV) is the primary factor in cervical tumorigenesis. Studies have shown that approximately 40% of sexually active women have high-risk HPV infection, but in 1% of patients, the HPV infection cannot be cured and ultimately leads to cervical cancer [3]. These findings suggest that the immune system plays a critical role in the incidence of cervical cancer. Innate immunity is the first line of defense against infection, playing a role in infection resistance before the acquired immune response is activated. Cells in innate immune system can recognize pattern recognition receptors (PRRs) and pathogen-associated molecular patterns (PAMPs). Toll-like receptors (TLRs), as cellular transmembrane PRRs and PAMP receptors, play an important role in innate immunity. By recognizing different PAMPs, TLRs quickly and efficiently generate immune responses to clear pathogens[4]. Recent studies

Authors' Affiliations: [1]Department of Pathology, Xinjiang Medical University, Urumqi, Xinjiang 830011, P. R. China; [2]Department of Gynecology, Wujiaqu Hospital, Nongliushi, Xinjiang 831300, P. R. China; [3]Key Laboratory of Endemic Diseases, Xinjiang Medical University, Urumqi, Xinjiang 830011, P. R. China.

Corresponding Author: Ayshamgul Hasimu, Department of Pathology, Xinjiang Medical University, Urumqi, Xinjiang 830011, P. R. China. Tel: +86-991-4361012; Fax: +86-991-4327997; Email: axiangu@yahoo.com.cn.

have shown that TLRs are also expressed on the surface of a variety of tumor cells and partly activated by corresponding ligands. After activation, TLRs promote tumor cell growth and cytokine secretion, leading to the escape of tumor cells from immune surveillance [5]. At present, members of the human TLR family, including TLR2, TLR3, TLR4, TLR7, TLR8, and TLR9, have been identified to recognize viruses. Among them, TLR9 can recognize CpG dinucleotides of bacteria and viruses and react with synthesized unmethylated dinucleotide CpG DNA [6]. When the stomach infected with *Helicobacter pylori* (*H. pylori*), the liver infected with Hepatitis B virus (HBV), or the uterus cervix infected with HPV, TLRs initiate classic PI3K/Akt signaling and produce cytokines, resulting in formation of the tumor microenvironment, promotion of tumor development, and strengthened resistance to cancer chemotherapy [7,8]. Because HPV16 infection is a major factor in cervical cancer tumorigenesis in Xinjiang Uighur women[9], we measured the expression levels of TLR3, TLR4, TLR7, and TLR9 in cervical intraepithelial neoplasia (CIN) and cervical cancer tissues of Uighur women to explore their association with HPV16 infection and their role in cervical cancer incidence in Uighur women.

Materials and Methods

Case information and specimen source

A total of 127 paraffin-embedded tissues, including 64 CIN specimens and 63 cervical cancer specimens, as well as 25 normal cervical tissues from resected uterine leiomyoma specimens, which were used as controls, were collected from the First Affiliated Hospital of Xinjiang Medical University and the People's Hospital of Xinjiang Uighur Autonomous Region between December 2007 and May 2009. Of 64 CIN, 31 were at stage I and 33 at stage II–III. Of 63 cervical cancer cases, 32 were well differentiated and 31 were poorly differentiated.

Reagents and instruments

The QIAamp DNA FFPE Tissue kit was used for DNA extraction (Qiagen Company, Germany). The streptavidin/peroxidase (SP) kit and DAB reagent were purchased from Beijing Zhongshan Golden Bridge Biological Technology Co., Ltd. Mouse anti-human TLR3 antibody, rabbit anti-human TLR4 antibody, rabbit anti–human TLR7 antibody, and mouse anti–human TLR9 antibody were purchased from Beijing Jingtian Company. The high-speed cold centrifuge was purchased from Beckman; spectrophotometer (Gene Quant II) from GE; and PCR machine (iCycler) and nucleic acid gel imaging system (GelDoc XR) from Bio-Rad.

DNA sample preparation and detection of HPV genes

DNA was extracted from paraffin-embedded cervical cancer tissues using the QIAamp DNA FFPE Tissue kit. DNA concentration and purity were determined by the absorbance at 260 nm using a spectrophotometer. These DNA samples were used as templates to amplify HPV16-specific primers. The primers used included the forward primer, 5'-TCAAAAGCCACTGTGTCCTG-3', and the reverse primer, 5'-CGTGTTCTTGATGATCTGCA-3'. The PCR reaction system included 2.5 μL of 10× PCR buffer, 0.5 μmol/L primers, 1.2 μL dNTP, and 0.5 U *Taq* DNA polymerase, with distilled water added to a final volume of 25 μL. Reaction condition were as follows: denaturation at 95℃ for 4 min, followed by 35 cycles of denaturation at 94℃ for 45 s, annealing at 52.4℃ for 45 s, and extension at 72℃ for 45 s, and further extension at 72℃ for 7 min. PCR product (5 μL) was separated by 2% agarose gel electrophoresis (voltage = 120 V). After 30 min, results were observed and pictures were taken using the nucleic acid gel imaging system.

Immunohistochemistry

The SP immunohistochemical assay was performed on formalin-fixed and paraffin-embedded samples. Slides were conventionally dewaxed by xylene and hydrated with gradient alcohol, and antigen was then retrieved using a microwave. Endogenous peroxidase was blocked with 3% H_2O_2. Slides were incubated with primary antibody at 4℃ overnight. PBS was used instead of primary antibody as a negative control. In the following day, secondary antibody was added, followed by DAB detection, hematoxylin staining, and conventional dehydration. Finally, the slides were clearly mounted. Slides were washed using PBS for 10 min between two steps.

Immunohistochemical results were observed independently by two pathologists. The expressions of TLR3, TLR7, and TLR9 were defined as light or dark brown granules in cytoplasm, whereas the expression of TLR4 was defined as light or dark brown granules in cytoplasm or on cell membrane. Immunohistochemical staining was scored according to the percentage of positive cells and staining degree: 0, ⩽10% positive; 1, 11% to 25% positive; 2, 26% to 50% positive; 3, 51% to 75% positive; and 4, ⩾ 76% positive; light yellow for score 1, yellow for score 2, and brown for score 3. The two scores were multiplied and the final scores ranged from 0 to 12 [10,11]. Then, a medium score was calculated. The tissues with scores equal to or more than median were defined as the high

expression group, and those with scores less than median were defined as the low expression group.

Statistical analysis

The Chi-square test was used to determine whether the clinicopathologic variables are associated with the expression levels of TLR3, TLR4, TLR7, and TLR9. The Fisher's exact test was used for multiple comparisons between each two of the control, CIN, and CSCC groups. Correlation between the TLR3, TLR4, TLR7, and TLR9 expression and HPV16 infection were analyzed with the Pearson correlation test. All the *P* values presented in this study were two-sided and the significance level was set to less than 0.05. All of the statistical analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, Ill, USA).

Results

Expression levels of TLR3, TLR4, TLR7, and TLR9 in CIN and cervical cancer tissues

Immunohistochemistry results showed that the expression levels of TLR4, TLR7, and TLR9 were higher in CIN and cervical cancer tissues than in the control group. In the control group, staining for TLR4, TLR7, and TLR9 was mainly observed in epithelial glands and immune cells. In contrast, the cytoplasm of epithelial basal cells and mesenchymal immune cells were stained in CIN tissues; tumor cells, stromal fibroblast-like cells, and mononuclear cells were stained in cervical cancer tissues. TLR staining was stronger in cervical cancer tissues than in the control group (Figure 1). The positive rates of TLR4 and TLR7 increased significantly with tumor grade and were higher in poorly differentiated carcinoma than in well differentiated carcinoma ($P < 0.05$), as shown in Table 1. Expression levels of TLR3 were higher in CIN and cervical cancer than in the control group, but the difference was not significant ($P > 0.05$).

Association between the expressions of TLR4, TLR7, and TLR9 and the clinicopathologic features of cervical cancer patients

Among all clinicopathologic features of cervical cancer patients listed in Table 1, tumor differentiation was significantly correlated with the expressions of TLR4 and TLR7 ($P < 0.05$), whereas there was no significant correlation between TLR4 and TLR7 expression and FIGO stage or lymph node metastasis ($P > 0.05$). TLR9 expression was not significantly associated with FIGO stage or tumor differentiation, but its expression levels were significantly higher in cervical cancer tissues without lymph node metastasis than in those with lymph node metastasis ($P < 0.05$, Table 1).

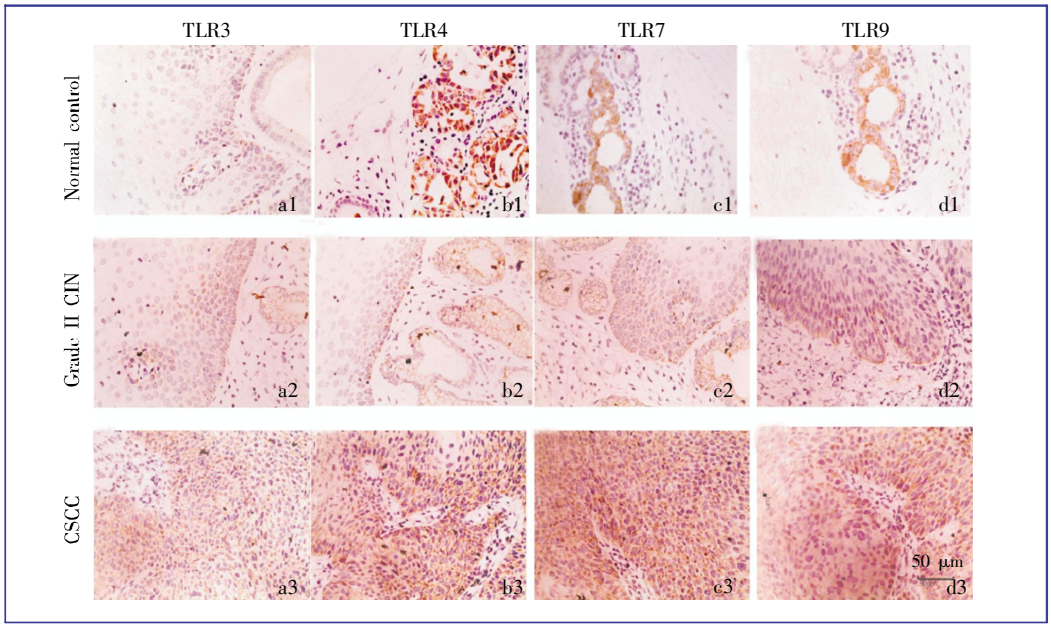

Figure 1. Typical immunohistochemical (IHC) staining patterns of TLR3, TLR4, TLR7, and TLR9 in cervical tissues in Uighur women. TLR3, TLR4, TLR7, and TLR9 express in cytoplasm, whereas TLR3 expresses both in cytoplasm and on cell membrane. The staining intensity of TLR3, TLR4, TLR7, and TLR9 gradually increased from normal control (a1–d1), grade II cervical intraepithelial neoplasia (CIN) (a2–d2) to cervical squamous cell carcinoma (CSCC) (a3–d3).

Table 1. Correlation between TLR expression and the clinicopathological features of cervical tissues

Clinicopathologic feature	No. of cases	TLR3 expression		TLR4 expression		TLR7 expression		TLR9 expression	
		High	Low	High	Low	High	Low	High	Low
Normal	25	13(52.0)	12(48.0)	9(36.0)	16(64.0)	7(28.0)	18(72.0)	6(24.0)	19(76.0)
CIN grade	64	37(57.8)	27(42.2)	38(59.4)	26(40.1)	36(56.3)	28(43.7)	36(56.3)	28(43.7)
I	31	16(51.6)	15(48.4)	16(51.6)	15(48.4)	16(51.6)	15(48.4)	17(54.8)	14(45.2)
II–III[a]	33	21(63.6)	12(36.4)	22(66.7)	11(33.3)	20(60.6)	13(39.4)	19(57.6)	14(42.4)
CSCC	63	45(71.4)	18(28.6)	49(77.8)	14(22.2)	46(73.0)	17(27.0)	50(79.4)	13(20.6)
Well differentiated	32	20(62.5)	12(37.5)	21(65.6)	11(34.4)	19(59.4)	13(40.6)	25(78.1)	7(21.9)
Moderately to poorly diferentiated[b]	31	25(80.6)	6(19.4)	28(90.3)	3(9.7)	27(87.1)	4(12.9)	25(80.6)	6(19.4)
FIGO stage									
Ia–Ib	21	11(52.4)	10(47.6)	15(71.4)	6(28.6)	11(52.4)	10(47.6)	16(76.2)	5(23.8)
IIa–IIb	23	18(78.3)	5(21.7)	18(78.3)	5(21.7)	19(82.6)	4(17.4)	18(78.3)	5(21.7)
IIIa–IIIb	19	16(84.2)	3(15.8)	16(84.2)	3(15.8)	16(84.2)	3(15.8)	16(84.2)	3(15.8)
LN status									
Negative	38	25(65.8)	13(34.2)	30(78.9)	8(21.1)	29(76.3)	9(23.7)	35(92.1)	3(7.9)
Positive[c]	25	23(92.0)	2(8.0)	20(80.0)	5(20.0)	17(68.0)	8(32.0)	15(60.0)	10(40.0)

All values are presented as number of cases with percentage in parentheses.

CIN, cervical intraepithelial neoplasia; CSCC, cervical squamous cell carcinoma; LN, lymph node. The Chi-square test was used to evaluate associations between TLR3, TLR4, TLR7, and TLR9 expression and clinicopathologic parameters, whereas the Fisher's exact test was used to compare each two groups among the control group, CIN, and CSCC groups. [a]$P < 0.05$, vs. grade I CIN for expressions of TLR4, TLR7, and TLR9; [b]$P < 0.05$, vs. well differentiated CSCC for expressions of TLR4 and TLR7; [c]$P < 0.05$, vs. LN-negative cases for TLR9 expression.

HPV16 infection in cervical lesions and its association with the expressions of TLR3, TLR4, TLR7, and TLR9

The expression level of HPV16 increased gradually among the control, CIN, and cervical cancer groups (Figure 2). The expression level of HPV16 was significantly associated with clinical stage and tumor differentiation ($P < 0.05$, Table 2). The expression levels of TLR4 and TLR9 were correlated with HPV16 infection in CIN tissues ($r = 7.434$, $P = 0.006$ and $r = 7.123$, $P = 0.008$, respectively) and in cervical cancer tissues ($r = 6.428$, $P = 0.001$ and $r = 8.478$, $P = 0.004$, respectively), whereas the expression levels of TLR3 and TLR7 had no significant correlation with HPV16 infection in cervical tissues.

Discussion

In the present study, we found that the expression levels of TLR4, TLR7, and TLR9 were significantly higher in CIN and CSCC tissues than in the control group in Uighur women. The expression levels gradually increased along with the grade of CIN and malignancy of cervical cancer. The expression levels were mainly very low in CIN but high in cervical cancer, suggesting that expressions of TLR4, TLR7, and TLR9 are early events in the development of cervical cancer in Uighur women. TLR4 and TLR7 up-regulation was shown to be a significant predictor of cervical cancer progression because they were significantly correlated with CSCC differentiation. The expression level of TLR9 was higher in CSCC without lymph node metastasis than in CSCC with lymph node metastasis, suggesting that TLR9 may be related to a low tumor metastasis rate. In addition, the expression levels of TLR4 and TLR9 in CIN and CSCC tissues in Uighur women were significantly correlated with HPV16 infection, suggesting that high expression of TLR4 and TLR9 could be associated with HPV16 infection and that TLR4 and TLR9 may serve as receptors for HPV16.

Recent studies have demonstrated that recognition of PAMP by TLR3, TLR4, TLR7, and TLR9 can promote immune response against viruses and deteriorate virus-induced diseases[12]. After the TLR-mediated signaling pathway is activated, pro-inflammatory cytokines are produced, antigen-presenting cells are activated, and innate immunity and acquired immune response is initiated. Yang *et al.*[13] found that HPV activates TLR7 through the MyD88 signaling pathway and stimulates dendritic cells to produce Th1-type immune response against HPV16 infection. On the other hand, TLRs are activated by their corresponding ligands and then activate specific downstream signaling pathways. Alterations in oncogenes, tumor suppressor genes, or

Table 2. Correlation between expression of TLRs and HPV16 infection in cervical intraepithelial neoplasia and cervical squamous cell carcinoma

TLRs	Expression	HPV16 infection in CIN		HPV16 infection in CSCC	
		Negative	Positive	Negative	Positive
TLR3	Low	10(15.6)	17(26.6)	4(6.3)	14(22.3)
	High	11(17.2)	26(30.6)	10(15.9)	35(55.6)
TLR4	Low	13(20.3)	13(20.3)	10(15.9)	4(6.3)
	High	8(12.5)	30(46.9)	4(6.3)	45(71.4)
TLR7	Low	10(15.6)	18(28.1)	6(9.5)	11(17.5)
	High	11(17.2)	25(39.1)	8(12.7)	38(60.3)
TLR9	Low	13(20.3)	15(23.4)	8(12.7)	5(7.9)
	High	8(12.5)	28(43.8)	6(9.5)	44(69.8)

The correlation between the expressions of TLR3, TLR4, TLR7, or TLR9 and HPV16 infection was examined by Pearson correlation test. In both CIN and CSCC, HPV16 infection was positively correlated with TLR4 expression (r = 7.434, P = 0.006; r = 6.428, P = 0.001, respectively) and TLR9 expression (r = 7.123, P = 0.008; r = 8.478, P = 0.004, respectively).
Other footnotes as in Table 1.

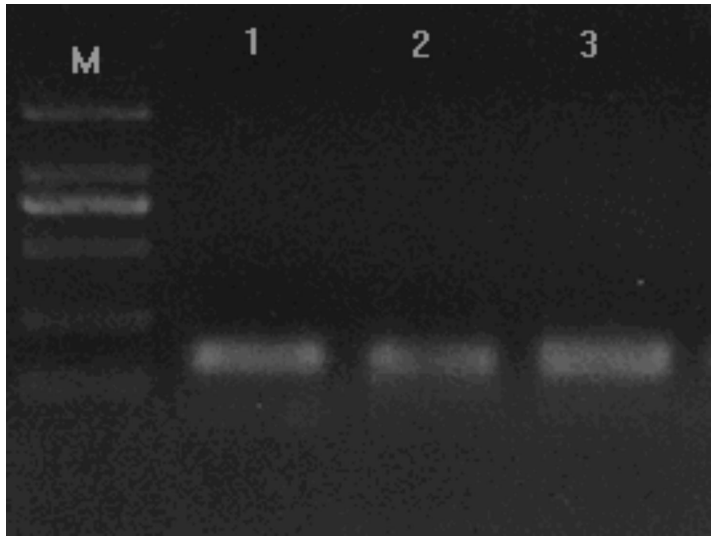

Figure 2. DNA expression pattern of the HPV16 E6 gene in cervical tissues from Uighur women. M denotes the 100–2000 bp marker; lanes 1–3 show the expected 120 bp PCR product of HPV16 E6 DNA detected in normal control (lane 1), CIN (lane 2), and CSCC (lane 3).

abnormal cell adhesion molecules in this TLR-mediated signaling pathway lead to inappropriate activation and carcinogenesis. Studies have demonstrated that TLR4, TLR7, and TLR9 are highly expressed in a variety of solid tumors, such as gastric cancer[14], colon cancer[15], ovarian cancer[16], and prostate cancer[17], and can promote tumor development. Moreover, TLR4, TLR7, and TLR9 are expressed in stromal cells as well as cancer cells. Thus, in addition to their function of activating immune response, TLRs establish a suitable microenvironment for tumor cell growth, which allows tumor cells to evade immune cells, infiltrate and metastasize, and undergo malignant progression[18]. Furthermore, TLRs promote tumor cell proliferation and malignant biological behavior by inducing anti-apoptotic protein expression through NF-κB activation as well as COX-2 and PGE2 up-regulation[19]. He *et al.*[20] found that after TLR4 was activated by its ligands, it induced immunosuppressive factors TGF-β, VEGF, and IL-8 production, which resulted in tumor cell resistance to TNF-α induced apoptosis and immune evasion of cancer cells.

In this study, we found that TLR4 and TLR7 were expressed not only in cervical cancer cells but also in mesenchymal immune cells. These cells can release a

variety of cytokines, growth factors, and extracellular matrix and signal transduction-related proteins. It could be speculated that the expressions of TLR4 and TLR7 in different mesenchymal cells can promote tumor cell growth and establish an anti-apoptotic microenvironment, thereby increasing tumor cell infiltration, metastasis, and progression. TLR9 is also expressed in the interstitial fibroblast-like cells, suggesting that it could be related to low metastasis rate. Thus, the expression levels of TLRs may correlate with tumor prognosis and tumor invasion. Hasan *et al.*[21] found that CpG sequences of the E6 and E7 oncogenes, which are from HPV, can specifically bind to TLR9 and reduce the viral clearance capability of the host, contributing to persistent infection. TLR9 can also promote tumor cells to secrete a variety of cytokines and cell adhesion molecules, increase tumor cell adhesion and invasion, promote tumor cell growth, and inhibit tumor cell apoptosis. Moreover, TLR9 can induce immune inhibitor production and inhibit the attack of cytotoxic lymphocytes on tumor cells, thus leading to immune escape [22]. Fahey *et al.* [23] found that TLR7 inhibitors provide benefits on therapeutics and HPV infection reversal. HPV16 infection is one of the major factors for cervical cancer incidence in Uighur women[6]. Our results showed that the expression levels of TLR4 and TLR9 were significantly correlated to HPV16 infection. Therefore, the next questions, which warrant further study, are how TLR4 and TLR9 play a role in immune escape of tumor cells and whether TLR4 and TLR9 are receptors for HPV16.

Lee *et al.*[11] detected the expression levels of TLR9 in CIN and cervical cancer tissues in Korean women and found its expression in CIN and cervical cancer increased, which is consistent with our results. The expression levels of TLR4 in CIN and cervical cancer tissue in Han women are lower than that in the chronic cervicitis group[24], which are opposite to our results in Uighur women. Such ethnic differences could be related to the different genetic background of ethnic groups and tumor pathogenesis. In our study, there was no difference in the expression levels of TLR3 between CIN and cervical cancer tissues in Uighur women, nor was TLR3 expression related to HPV16 infection, suggesting that TLR3 may not be involved in the pathogenesis of cervical cancer. However, the expressions of TLR4, TLR7, and TLR9 were correlated with CIN grade and cervical cancer differentiation, indicating that they could promote cervical cancer progression in Uighur women. In addition, expression of TLR9 in tumor interstitial fibroblast-like cells may be related to the low rate of tumor metastasis. Therefore, further studying the differences between distinct populations and looking for the function of TLRs in cervical carcinoma in Uighur women will provide a basis for cancer-specific gene and immune therapy.

Acknowledgement

This study was supported by a grant from National Natural Science Foundation of China (No. 81060164).

Received: 2010-09-26; revised: 2011-01-06;
accepted: 2011-01-31.

References

[1] Guzalnur, Peng ZL, Guo Y. Different expression of HPV and its subtype in cervical cancer tissue between Sichuan Han and Xinjiang Uighur women [J]. Chin J Microbiol Immunol, 2004,24 (5):406. [in Chinese]
[2] Lalai S, Peng YH, Zhou K, et al. The analysis of pathogenetic tendency of cervical cancer in various ethnic women in Xinjiang [J]. J Xinjiang Med Univ, 2006,29(7):569–571. [in Chinese]
[3] Maclag PC, Schlcht NF, Souza PS, et al. Major histocompatibility complex and human papillomavirus infection in Brazilian women [J]. Cancer Epidemiol Biomakers Prev, 2000,9(11):1183–1191.
[4] Akira S, Hemmi H. Recognition of pathogen-associated molecular patens by TLR family [J]. Immunol Lett, 2003,85(2): 85–95.
[5] Huang B, Zhao J, Li H, et al. Toll-like receptors on tumor cells facilitates evasion of immune surveillance [J]. Cancer Res, 2005,65(12):5009–5014.
[6] Wang H, Rayburn E, Zhang R. Synthetic oligodeoxynucleotides containing deoxycytidyl-deoxyguanosine dinucleotides (CpG ODNs) and modified analogs as novel anticancer therapeutics [J]. Curr Pharm Des, 2005,11(22):2889–2907.
[7] Chen R, Alvero AB, Silasi DA, et al. Inflammation, cancer and chemoresistance: taking advantage of the toll-like receptor signaling pathway [J]. Am J Reprod Immunol, 2007,57(2):93–107.
[8] Li H, Han Y, Guo Q, et al. Cancer-expanded myeloid-derived suppressor cells induce anergy of NK cells through membrane-bound TGF-beta1 [J]. J Immunol, 2009,182(1):240–249.
[9] Lalai S, Amy E, Aili M, et al. Detection of human papilloma virus DNA in cervical squamous cell carcinoma in Xinjiang Uighur women [J]. Chin J Obstet Gynecol, 1997,32(7):405–408. [in Chinese]
[10] Gokdeniz R,Ozen S, Mizrak B,et al. GnRH agonist decreases endothelial nitric oxide synthase (eNOS) expression in leiomyoma [J]. Int J Gynaecol Obstet, 2000,70 (3):347–352.
[11] Lee JW, Choi JJ, Seo ES, et al. Increased toll-like receptor 9 expression in cervical neoplasia [J]. Mol Carcinogen, 2007,46 (11):941–947.
[12] Thompson JM, Iwasaki A. Toll-like receptors regulation of viral infection and disease [J]. Adv Drug Deliv Rev, 2008,60(7): 786–794.
[13] Yang R, Murillo FM, Cui H, et al. Papilloma virus HPV1-like

particles stimulate murine bone marrow-derived dendritic cells to produce alpha interferon and TH1 immune responses via MyD88 [J]. Virol, 2004,78(20):11152–11160.
[14] Rakoff-Nahoum S, Medzhitov R. Toll-like receptors and cancer [J]. Nat Rev Cancer, 2009,9(1):57–63.
[15] Fukata M, Chen A, Vamadevan AS, et al. Toll-like receptor-4 promotes the development of colitis-associated colorectal tumors [J]. Gastroenterology, 2007,133(6):1869–1881.
[16] Zhou M, McFarland-Mancini MM, Funk HM, et al. Toll-like receptor expression in normal ovary and ovarian tumors [J]. Cancer Immunol Immunother, 2009,58(9):1375–1385.
[17] Kundu SD, Lee C, Billips BK, et al. The toll-like receptor pathway: an ovel mechanism of infection-induced carcinogenesis of prostate epithelial cells [J]. Prostate, 2008,68 (2):223–229.
[18] Li H, Han Y, Guo Q, et al. Cancer-expanded myeloid-derived suppressor cells induce anergy of NK cells through membrane-bound TGF-beta1 [J]. J Immunol, 2009,182(1):240–249.
[19] Chochi K, Ichikura T, Kinoshita M, et al. Helicobacter pylori augments growth of gastric cancers via the lipopolysaccharide-toll-like receptor 4 pathway where as its lipopolysaccharide attenuates antitumor activities of human mononuclear cells [J]. Clin Cancer Res, 2008,14(10):2909–2917.
[20] He W, Liu Q, Wang L, et al. TLR4 signaling promotes immune escape of human lung cancer cells by inducing immunosuppressive cytokines and apoptosis resistance [J]. Mol Immunol, 2007,44(11):2850–2859.
[21] Hasan UA, Bates E, Takeshita F, et al. TLR9 expression and function is abolished by the cervical cancer-associated human papillomavirus type 16 [J]. J Immunol, 2007,178 (5):3186 – 3197.
[22] Huang B, Zhao J, Li H, et al. Toll-like receptors on tumor cells facilitate evasion of immune surveillance [J]. Cancer Res, 2005,65(12):5009–5014.
[23] Fahey L, Raff A, Da Silva, et al. Reversal of human papillomavirus-specific T cell immune suppression through TLR7 agonist treatment of Langerhans cells exposed to human papillomavirus type 16 [J]. Immunol, 2009,182(5):2919–2928.
[24] Yu L, Wang L, Li M, et al. Expression of toll-like receptor 4 is down-regulated during progression of cervical neoplasia [J]. Cancer Immunol Immunother, 2010,59(7):1021–1028.