RESEARCH ARTICLE

TLR9 Expression in Uterine Cervical Lesions of Uyghur Women Correlate with Cervical Cancer Progression and Selective Silencing of Human Papillomavirus 16 E6 and E7 Oncoproteins *in Vitro*

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Abstract

Background: Cervical cancer is listed as one of high-incidence endemic diseases in Xinjiang. Our study aimed to evaluate the expression of TLR9 in uterine cervical tissues of Uyghur women and examine associations with clinicopathological variables. We further characterized the direct effects of TLR9 upon the selective silencing of human papillomavirus (HPV) E6 and E7 oncoprotein expression in HPV 16-positive human cervical carcinoma cells treated with siRNA *in vitro*. Materials and Methods: Immunohistochemistry was applied to evaluate TLR9 expression in 97 formalin-fixed paraffin-embedded cervical samples from Uyghur women; 32 diagnosed with cervical squamous cell carcinomas (CSCC) , 14 with low-grade cervical intraepithelial neoplasias (CINI), 10 medium-grade (CINII), 24 high-grade (CINIII), and 17 chronic cervicitis. BLOCK-iT™ U6 RNAi Entry Vector pENTR™/U6-E6 and E7 was constructed and transfected the entry clone directly into the mammalian cell line 293FT. Then the HPV 16-positive SiHa human cervical carcinoma cell line was infected with RNAi recombinant lentivirus. RT-PCR and Western blotting were used to determine the expression of TLR9 in both SiHa and HPV 16 E6 and E7 silenced SiHa cells. Results: Immunohistochemical staining showed that TLR9 expression was undetectable (88.2%) or weak (11.8%) in chronic cervicitis tissues. However, variable staining was observed in the basal layer of all normal endocervical glands. TLR9 expression, which was mainly observed as cytoplasmic staining, gradually increased in accordance with the histopathological grade in the following order: chronic cervicitis (2/17, 11.8%) <CINI (4/19, 28.6%) <CINII (3/10, 30.0%) <CINIII (12/24, 50.0%) <CSCC (17/32, 53.1%) (p<0.05), but not with tumor differentiation. RT-PCR and Western blotting showed that TLR9 expression was up-regulated in HPV16 E6 and E7 silenced SiHa cells at both mRNA and protein levels. Conclusions: TLR9 expression increases according to the histopathological grade of cervical pathological process. HPV E6 and E7 oncoprotein have negative effects on the expression and function of TLR9.

Keywords: TLR9 - cervical squamous cell carcinoma - human papillomavirus - Uyghur women - siRNA

Asian Pac J Cancer Prev, **15 (14)**, 5867-5872

Introduction

Cervical cancer is the second morbidity malignant tumor of women worldwide which ranks only second to breast cancer. It takes of nearly 27 million women's lives everyyear and has been severely threatening the health and life of women (Yang et al., 2004; Cohen et al., 2005; Ferlay et al., 2007). In south Xinjiang Uyghur women prevalence rate of cervical cancer (590/10 million) is four times of China's women's average prevalence (138/10 million), therefore cervical cancer was listed as one of high-incidence endemic diseases in Xinjiang, China (Suzuke et al., 2006; Zhang et al., 2006; Liu et al., 2006; Zhang et al., 2009).

The function of the Toll-like receptor (TLR) family members has been extensively studied in the recent decades. TLRs recognize microbial/viral-derived components that trigger innate immune response, but can also induce adaptive immune response involving in the defense against microbial infections (Akira et al., 2004; Akira et al., 2006). TLRs have also been found on epithelial and tumor cells. In some tumor types, activation of tumor cell TLRs not only promotes tumor cell proliferation and resistance to apoptosis, but also enhances tumor cell invasion and metastasis by regulating metalloproteinases and integrins (Matijevic et al., 2010). Thus, recent evidence shows that TLRs have a dual role: either promoting or inhibiting tumor progression and

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TLR signaling by tumor and immune cells is a double-edged sword (Huang et al., 2008). TLR9 can respond to bacterial hypomethylated CpG motifs and single-stranded RNA viruses (Lenert et al., 2010). High expression of TLR9 was recently detected in many cancer cell lines including gastric cancer, colorectal cancer, lung cancer, prostate cancer, breast cancer, hepatocellular carcinoma (IIvesaro et al., 2007; Krieg et al., 2008; Wang et al., 2013; Yang et al., 2014; Mukherjee et al., 2014). Lee JW (Lee et al., 2007) in Korea also proposed increased Toll-Like Receptor 9 expression was associated with disease progression in cervical neoplasia.

Cervical cancer development is linked to the persistent infection by high-risk mucosal human papillomaviruses (HPVs) types in which HPV16 is the most prevalent type in premalignant and malignant cervical lesions (Clifford et al., 2003; Tezcan et al., 2014). The E6 and E7 major oncoproteins of this dsDNA virus play a key role in the deregulation of the cell cycle, apoptosis, and adaptive immune surveillance (Tommasino et al., 2001; Wu et al., 2014). They can promote the transforma- tion of the infected cell by altering the regulation of fundamental cellular events, such as apoptosis and the cell cycle. The E6 and E7 oncoproteins exert their functions by inducing the degradation of two important tumor suppressors, p53 and retino-blastoma (pRb), respectively, and cooperates with each other to immortalize primary keratinocytes (Stanley et al., 2007). However, Hasan UA (Hasan et al., 2007) found that TLR9 expression and function also was abolished by the cervical cancer-associated human papillomavirus type 16.

In this study, we the first time investigated that the expression of TLR9 in Uyghur women of uterine cervical tissues, examined the association between TLR9 expression and clinicopathological variables. We further characterized the direct effects of TLR9 upon the selective silencing of high-risk HPV16 E6 and E7 oncoprotein expression in HPV 16-positive human cervical carcinoma cells treated with siRNA *in vitro* with the purpose of revealing the carcinogenic mechanism of HPV.

Materials and Methods

Collection of uterine cervical samples

A total of 97 paraffin-embedded, formalin-fixed tissue specimens of Uyghur women were used in this study. These included 32 diagnosed cervical squamous cell carcinomas (CSCC) tissues, 14 low-grade cervical intraepithelial neoplasias (CINI), 10 medium-grade (CINII), 24 high-grade (CINIII) in addition to 17 chronic cervicitis specimens as shown in Tab 2. Tissue samples and patients tumor specimens were obtained from patients admitted for diagnosis and treatment to the Department of Gynecology of the Affiliated Cancer Hospital in Xinjiang Medical University. The stage of cervical cancer was established according to the International Federation of Gynecology and Obstetrics (FIGO) criteria: 2, 2, 1, and 1 and cancers were classified as FIGO stage Ib, IIa, IIb, and IIIb, respectively. Informed consent was obtained from each patient. As normal controls, samples were obtained from patients with benign gynecologic disease chronic cervicitis. Fresh surgical samples from patients was fixed with formalin, embedded with paraffin wax and kept at RT.

Immunochemistry

Paraffin-embedded specimens were cutted into 4 um sections and baked at 65°C for 30 minutes. The sections were deparaffinized with xylenes and rehydrated. Sections were submerged into EDTA (pH=8.0) and autoclaved for antigen retrieval, then treated with 3% hydrogen peroxide, followed by incubation with 1% FBS. Anti-TLR9 (ab12121, Abcam, USA, 1:100 dilutions) was added and incubated overnight at 4°C. Horseradish peroxidase (HRP) labeled secondary antibody in the HRP-Polymer antimouse IHC kit (Zhongshan GoldenBridge, Beijing, China) was applied and incubated for 30 mins at room temperature, followed by 5 minutes incubation at room temperature with DAB provided in the kit for color development. The sections were finally counterstained with haematoxylin and mounted with Permount Medium (BIOS, Beijing, China). Results were visualized and photographed under a light microscope. The degree of immunostaining of sections was viewed and scored separately by two independent investigators, the scores were determined by combining the proportion of positively stained cells and the intensity of staining. The proportion of positively stained cells was graded as follows: 0 (≤5% positive stained cells), 1 (>5% ~ 25% positive stained cells), 2 (>25% ~ 75% positive stained cells) and 3 (>75% positive stained cells). The intensity of staining were recorded on a scale of 0 (no staining), 1 (weak staining, light yellow), 2 (moderate staining, yellowish brown) and 3 (strong staining, brown). The staining index was calculated as follows: staining index = (intensity of staining × proportion of positively stained cells) /2. The sum both scores was used to identify four categories of expression: 0~1 was negative expression (-), 2~4 was weak expression (+), 5~8 was moderate expression (++); ≥9 was highlevel expression (+++).

siRNA and Transfection

Design and synthesize two complementary single-stranded DNA oligonucleotides targeted The HPV-encoded early proteins, the E6 and E7 oncoproteins, with one encoding the shRNA of interest. Then synthesized by Takara Biotechnology (Dalian, China). The sequences of the siRNAs are shown in Table 1. To enable directional cloning, the forward PCR primer must contain the sequence, CACC, at the 5´end of the primer, and the reverse primer must contain the sequence, AAAAAAA, at the 5´end of the primer. Anneal the single-stranded oligonucleotides to generate a double-stranded oligo (ds oligo). Clone the ds oligo into the linearized pENTR™ / U6 vector. Transform the ligation reaction into One Shot® TOP10 chemically competent E. coli and select for kanamycin-resistant transformants. Co-transfect the pLenti-based expression vector and the ViraPower™ Packaging Mix into the 293FT Cell Line to produce a lentiviral stock. On the day before transfection, 293FT cells were plated out at a density of 6 x 106 cells in a 25 cm^2 plate with Opti-MEM® I Medium containing serum and not antibiotics. 3 μg of pLenti expression plasmid

Table 1. The Sequences of The HPV16-E6 and E7-siRNA Oligo

	Sequences (5'-3')	length (bp)
HPV16-E6-siRNA sense	CACCGAAGAGGTATATGACTTTGC TTTTTTCTCCATATACTGAAACGAATTT	51
HPV16-E6-siRNA antisense	AAAAAAATTCGTTTCAGTATATGGA GAAAAAGCAAAGTCATATACCTCTTC	51
HPV16-E7-siRNA sense	CACCGAAAGGAGGATGAAATAGAT GGCGAACCATCTATTTCATCCTCCTTT	51
HPV16-E7-siRNA antisense	AAAAAAAGGAGGATGAAATAGAT GGTTCGCCATCTATTTCATCCTCCTTTC	51

DNA and 9 μg of the ViraPower™ Packaging Mix was diluted to 1.5 ml of Opti-MEM® I Medium without serum, to which 80 ul of Opti-MEM I reduced-serum media (Invitrogen) was added. Oligofectamine reagent (Invitrogen) (36 ul) was added to 1.5 ml of Opti-MEM I Medium without serum, and the mixture was incubated at room temperature for 5 minutes. After incubation, combine the diluted DNA with the diluted Lipofectamine™ 2000. Mix gently. Incubate for 20 minutes at room temperature to allow the DNA-Lipofectamine™ 2000 complexes to form. The solution may appear cloudy. Transfection complexes were added to to the plate of 293FT cells. Mix gently by rocking the plate back and forth and incubated the cells overnight at 37°C in a humidified 5% CO_2 incubator. After incubation, transfection complexes were removed and replaced with complete culture medium without antibiotics. Post-transfection (Day 5 or 6), harvest virus-containing supernatants by removing and transferring the medium into a 15 ml sterile, capped, conical tube and then centrifuge.

Infection of human cervical cancer Cells SiHa with RNAi Lentivirus

The human cervical cancer cell lines SiHa HPV 16 were first obtained from ATCC and maintained in our Laboratory. Cervical cancer cells were grown in RPMI 1640 (Gibco, Gaithersburg, MD, USA) supplemented with 10% FBS (Gibco, Gaithersburg, MD, USA), 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C with 5% CO2 in humidified air. Transduce the different dilutions of lentivirus in the presence of the polycation Polybrene® into a mammalian cell line SiHa. Select for stably transduced cells using 8ug Blasticidin. After 14-16 days, collect and count the number of Blasticidin-resistant cell.

Expression of HPV E6 and E7 Protein By Western Blot Analysis

Cell extracts were prepared in RIPA lysis buffer (150 mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate, 1%Nonidet *p*-40, and 50 mM Tris, pH 8.0), with the addition of 2 mM phenylmethylsulfonyl fluoride and 1 ul/ml protease inhibitory cocktail (Merck, Calbiochem, DE). Lysis buffer was freshly prepared and added to transfected cells in wells of six-well plates (100 ul/well) on ice, which were then incubated for 10 minutes. Protein concentrations were determined by protein assay kit (Merck, Calbiochem, DE). Cell extracts were boiled for 10 min in loading buffer before being separated on 8 to 15% SDS-PAGE gels. Separated proteins were transferred to polyvinylidene difluoride membranes at 100 V for 1 h before membrane blocking in 5% skim milk powder in TBS with 0.1% Tween 20. Primary and secondary antibodies were respectively diluted 1:400 and 1:1000 in TBS with 0.1% Tween 20 and incubated for 60 min at room temperature (or 2 days at 4°C for HPV16 E6 and E7) with three washes between each step. Protein bands were visualized by enhanced chemiluminescence.

TLR9 Expression Analysis by RT-PCR and Western Blotting

Total RNA was extracted from cervical cancer cell lines SiHa and HPV 16 E6 and E7 silenced SiHa using Trizol reagent (Invitrogen, USA), 2 ug RNA was used as template for cDNA Synthesis, cDNA was synthesized with First-Strand Synthesis System for RT-PCR Kit (Catalog No.A3500 Promega). RT-PCR analyses were performed using the following primer sets, human TLR9 (sense: 5'-CCGTGACAATTACCTGGCCTTC-3', antisense: 5'-CAGGGCCTTCAGCTGGTTTC-3', productsize:97bp), GAPDH (sense:5'-GCACCGTCAAGGCTGAGAAC-3', antisense: 5'-TGGTGAAGACGCCAGTGGA-3', product size:138 bp). GAPDH was used as housekeeping gene control. Reactions were carried out in a Gradient Thermal Cycler (BioRad, Hercules, CA) for 30 cycles which consisted of 94.0°C 30s, 59°C 30s, 72°C 30s. The reaction took place in a total volume of 25 ul. Products were analyzed by 2% agarose gel electrophoresis and visualized Under the UV transilluminator. All primers were synthezed by Takara Biotechnology (Dalian, China). The expression of TLR9 in SiHa and HPV 16 E6 and E7 silenced SiHa were further confirmed by Western Blotting analysis.

Statistical Analysis

Statistical calculations were carried out with SPSS statistical software for Windows version 17.0 (SPSS, Inc., Chicago, Illinois, USA). The Kruskal-Wallis H test was used to correlate cumulative TLR9 expression with cancer progression grading, and for comparisons between groups, $p<0.05$ was defined as significance.

Results

Correlation between expression of TLR9 and procession of cervical lesion

To determine whether the expression level of TLR9 protein is associated with the histological characteristics of cervical lesion, cervical tissue were examined by immunohistochemical staining with an antibody against human TLR9. As shown in Figure 1 and Table 2, TLR9, mainly as cytoplasmic staining was found to be expressed in CSCC, no strong immunoreactivity was detected in non-neoplastic cervical tissues. TLR9 expression was undetectable or weak in benign leision chronic cervicitis epithelial tissue (11.8% were weakly positive). In fact, no strong immunoreactivity was detected in squamous epithelium of chronic cervicitis, CINI and CINII. However, in CINIII group intermediate expression (6/24, 25%) and strong expression (1/10, 10%) cases significant increased, and were more than in CINI and CINII. The strong expression rate (5/32, 15.6%) of TLR9 was the

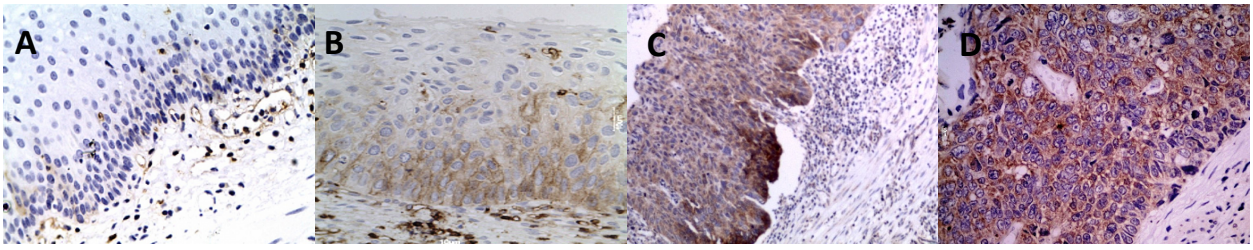


Figure 1. Immunohistochemical Staining of TLR9 Protein in different Cervical Leisions. (A) Benign leision chronic cervicitis epithelium showing no immunostaining, few expressed in the vessels epithelium cell. (B) Medium-grade cervical intraepithelial neoplasia (CINII) showing weak staining. (C) High-grade (CINIII) showing intermediate staining. (D) Cervical squamous cell carcinoma (CSCC) showing strong staining. (Original magnifcation, ×400)

Table 2. Correlation Between Expression of TLR9 and Malignancy of Uterine Cervical

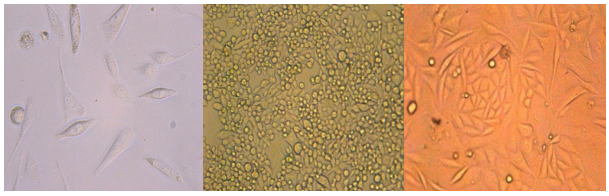
Cervical specimen	Case	Degree of immunoreactivity (%)				H	*P*
		0	1	2	3		
chronic cervicitis[a]	17	15/17 (88.2)	2/17 (11.8)	0/17 (0.0)	0/17 (0.0)		
CINI[b]	14	10/14 (71.4)	4/14 (28.6)	0/14 (0.0)	0/14 (0.0)		
CINII[c]	10	7/10 (70.0)	3/10 (30.0)	0/10 (0.0)	0/10 (0.0)		
CINIII[d]	24	12/24 (50.0)	5/24 (20.8)	6/24 (25)	1/10 (10)		
CSCC[e]	32	15/32 (46.9)	4/32 (12.5)	8/32 (25)	5/32 (15.6)	8.86	0.031

*[a,d]p=0.007; [a,c]p=0.003; [b,e]p=0.001; [a,b,c,d,e]p=0.031

Table 3. Relationship Between The Expression of TLR9 Protein and The Differentiation of CSCC

Differentiation	Case	Degree of immunoreactivity (%)			
		-	+	++	+++
High	12	7/12 (58.3)	2/12 (16.7)	2/12 (16.7)	0/12 (0)
Moderate	11	5/11 (45.5)	2/11 (18.2)	2/11 (18.2)	2/11 (18.2)
Poor	9	3/9 (33.3)	0/9 (0)	4/9 (44.4)	3/9 (33.3)

*H=3.748, P=0.154



A. SiHa **B. siRNA E6** **C. siRNA E7**

Figure 2. Morphological Observation of SiHa Cells after HPV16 E6 and E7 RNA Interference. Compared with SiHa cells, the growth speed of HPV 16 E6 and E7 silenced SiHa cell decreased obviously, and the appearance of cells were from fusiform into a pleomorphic or spherical. After subculturing, the shape of cells remained unchanged. (Original magnifcation, ×100)

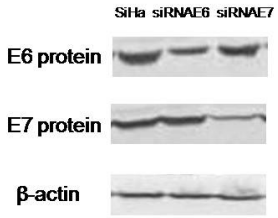


Figure 3. Western Blot Analysis of E6 and E7 in SiHa and HPV 16 E6 and E7 silenced SiHa to Validation Interference Efficiency

highest in CSCC. As summary in Table 2, when the five specimen grades (i.e, chronic cervicitis, CINI, CINII, CINIII and CSCC) were compared with regard to the frequency of positive staining, TLR9 expression was found to gradually increase with the histopathological grade in the following order: chronic cervicitis (2/17, 11.8%) <CINI (4/19, 28.6%) <CINII (3/10, 30.0%) <CINIII (12/24, 50.0%) <CSCC (17/32, 53.1%) (p=0.031, p<0.05) (Table 1, Figure 2). As shown in Table 2, the differentiation degree of TLR9 positive cervical cancer was not significantly lower than that of TLR9 negative cervical cancer.

TLR9 is up-regulated in HPV16 E6/E7 silenced SiHa cell

A recombinant lentiviral siRNA expression vector was constructed using a HPV16-E7 oncogene specific siRNA fragment, and an RNAi cell model stably expressing the HPV16E7-siRNA was established by transfection of 293FT virus-packiging cells with the viral vector followed by infection of HPV16-positive SiHa carcinoma cells with recombinant virus, antibiotica selection and molecular biological characterization. Before and after intervention, morphological change of was observed under invertphase contrast microscope. Compared with SiHa cells, the growth speed of HPV 16 E6 and E7 silenced SiHa cell decreased obviously, and the appearance of cells were from fusiform into a pleomorphic or spherical. After subculturing, the shape of cells remained unchanged (Figure 2).

After collecting the cells, at first step, we confirmed the expression of E6 and E7 in SiHa and HPV 16 E6 and E7 silenced SiHa by Western Blot analysis. As shown in Figure 3, E6 and E7 were silenced to some extent at level of protein. In addition, next we analyzed the mRNA and protein expression levels of TLR9 in three cell lines

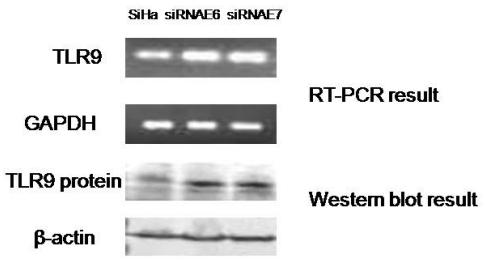


Figure 4. RT-PCR and Western Blotting Analysis of The TLR9 mRNA and Protein Expression Levels in SiHa, HPV16 E6 and E7 Silenced SiHa Cells Respectively

derived from SiHa and HPV16 E6 and E7 silenced SiHa cells respectively by RT-PCR and Western Blotting analysis. The first line SiHa is positive for HPV16 and contain integrated HPV16 DNA and the last two lines was the HPV16 E6 or E7 silenced SiHa by siRNA. RT-PCR and Western Blot analysis showed that TLR9 is weakly expressed in SiHa and was more pronounced in HPV16 E6 and E7 silenced SiHa cells. In particular, TLR9 mRNA and protein were clearly up-regulated in HPV16 E6/E7 silenced SiHa cell (Figure 4).

Discussion

Recent findings show that functional TLRs are expressed not only on immune cells but also on cancer cells. TLRs play an active role in carcinogenesis and tumor progression. Cancer cells in which TLR ligands activate TLRs expressed release cytokines and chemokines in result that an aberrant cytokine profile associated with immune tolerance, cancer progression and propagation of the tumor microenviron-ment during cancer progression in the setting of chronic inflammation (Sato et al., 2009). Among the TLR family, TLR9 recognizes the ODN with CpG motif. After binding with the ligand, TLR9 signal pathway leading to subsequent downstream activation of the NFκB, and MAPK signaling pathways (Takeshita et al., 2004), which may responsible for the proinflammatory or progrowth microenvironment of tumor.

Current research found two TLRs (TLR5 and TLR9) might contribute to cervical carcinogenesis and correlated with progression of cervical lesions (Lee et al., 2007; Kim et al., 2008). Our immunohistochemical result was accordance with previous studies regard to TLR9 expression in cervical cancer. This study demonstrated, for the first time In south Xinjiang uyghur women as high-risk groups, that TLR9 expression increases according to the histopathological grade of cervical lesions including chronic cervicitis, CIN and cervical cancer. TLR9 expression was undetectable or weak in squamous epithelial tissue of chronic cervicitis; however, its expression gradually increased from low-grade CIN to high-grade CIN and in particular in CSCC. The expression of TLR9 was significantly higher in CINIII and CSCC than in normal controls. However, the differentiation degree of TLR9 positive cervical cancer was not significantly lower than that of TLR9 negative cervical cancer. It suggested that expression of TLR9 had nothing to do with the differentiation degree of cervical cancer.

HPV infection is the initial factor of the development of low or high-grade CIN, which may regress or progress to invasive carcinoma. However, the majority of HPV infections do not lead to cytological anomalies or canceration in result that they are cleared by the immune system in a relatively short time (6-18 months) (Ostor et al., 1993). Therefore, as the failure of the immunological surveillance and clearance is a critical factor for the development of cervical cancer, preventing viral cellular transformation. Several studies have already shown that E6 and E7 from HPV16 and 18 are able to efficiently subvert the immune response by binding and blocking IFN regulatory factor -3 and -1 regulatory signaling pathways, and then eliminateing the induction of the antiviral IFN response (Frisch et al., 2000). HPV16 has the ability to interfere with the first response to infectious agents via TLRs. In this study, we show that TLR9 was expressed in both cervical carcinoma tissue and HPV 16-positive human cervical carcinoma cell SiHa, but was upregulated upon the selective silencing of high-risk HPV16 E6 and E7 oncoprotein expression in SiHa treated with siRNA *in vitro*. Previous research also found that infection of human primary keratinocytes with HPV16 E6 and E7 recombinant retroviruses inhibits TLR9 transcription and hence functional loss of TLR9-regulated pathways. We suggests that TLR9-signaling may play an double-edged role in tumor and normal cells. In carcinogenesis, HPV virus might exploit TLR9-signaling regulating and accelerating tumor growth under some conditions. Ibrahim et al found that HPV 16 infections that cleared were significantly associated with an increase in expression of the four viral nucleic acid-sensing TLRs including TLR9 (Daud et al., 2011). This study suggests that dampened TLR expression in the cervical mucosa is a type-specific mechanism by which HPV 16 interferes with innate immune responses, contributing to viral persistence, and that TLR9 upregulation and resultant cytokine induction is important in subsequent viral clearance. Similarly to what we have discussed for HPV16, it is becoming clearly understand that many other viruses have adopted ways to escape TLR recognition. Chronic hepatitis C infection leads to expression and functional impairment of TLR2 (Horsmans et al., 2005; Dolganiuc et al., 2006). Respiratory syncytial and measles viruses are also able to block IFN-mediated responses induced via TLR-dependent and independent mechanisms (Groskreutz et al., 2006). In addition, many researcher clarified the role of NF-κB induced TLR9 down-regulation, in the context of HPV16-induced cancers. Takeshita et al identified NF-κB as being one of the suppressive transcription factors associated with TLR9 down-regulation (Takeshita et al., 2004). And it has been reported that NF-κB levels are up-regulated in HPV16E6/E7 infected keratinocytes (Nees et al., 2001) as well as HPV16-positive patients with oral cancer (Mishra et al., 2006).

In conclusion, we show for the first time that the increased expression of TLR9 protein during the progression of of cervical cancer in in Uyghur women as high-risk groups. HPV 16 E6 and E7 oncoprotein have subduction effects on the expression and function

of TLR9 leading to the innate immunity failure of the immunological surveillance and clearance. Further investigations are being aimed to dissect out the mechanism (s) involved in active human papillomavirus replication/transcription resulting in immortalization in keratinocytes.

Acknowledgements

This work was supported by Natural Science Foundation of China (81260380) and Open subject of Xinjiang Key Laboratory of Molecular Biology and Endemic Diseases (XJDX0208-2008-03).

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