vessels and whitish scales/diffusely distributed dotted vessels, respectively[3]. Obviously, further studies on larger groups of patients are needed to confirm our observations.

## CONFLICTS OF INTEREST

The authors have nothing to disclose.

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# Attenuated Nuclear Factor Kappa B Activity by E7 Protein of Human Papillomavirus Type 2 in Human Epidermal Keratinocytes

Yun Hee Ryu, Ju Hee Han, Ji Hyun Lee, Soon Young Choi[1], Young Min Park

*Department of Dermatology, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, [1]Department of Biological Sciences and Biotechnology, Hannam University, Daejeon, Korea*

Dear Editor:

Human papillomavirus (HPV) is a double stranded DNA virus that induces hyper-proliferative lesions of cutaneous and mucosal epithelium. HPV is composed of six early genes (E1, E2, E4, E5, E6, and E7) and two late genes (L1 and L2). Early genes are related to viral replication and transcription whereas late genes encode viral structural proteins[1,2]. The E6 and E7 genes are major oncoproteins which are associated with the tumor suppressor proteins, p53 and retinoblastoma protein. In addition, the E7 gene has been known to play a role in evading host immune responses[3].

Toll-like receptors (TLRs) are the first line of defense in host protection against invasion of microbial pathogens. TLR signaling pathways induce the transcription factor nuclear factor kappa B (NF-$\kappa$B)[4]. Among 10 human TLRs, TLR3 and TLR9 are known to be upregulated in wart lesions[5].

Common warts caused by type 2 HPV (HPV2) are clinically characterized by little inflammation. Thus, we used to adopt a kind of immunotherapeutic modalities including diphenylcyclopropenone and imiquimod, which can

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**Corresponding author:** Young Min Park, Department of Dermatology, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, 222 Banpo-daero, Seocho-gu, Seoul 06591, Korea. Tel: 82-2-2258-6223, Fax: 82-2-594-3255, E-mail: yymmpark6301@hotmail.com

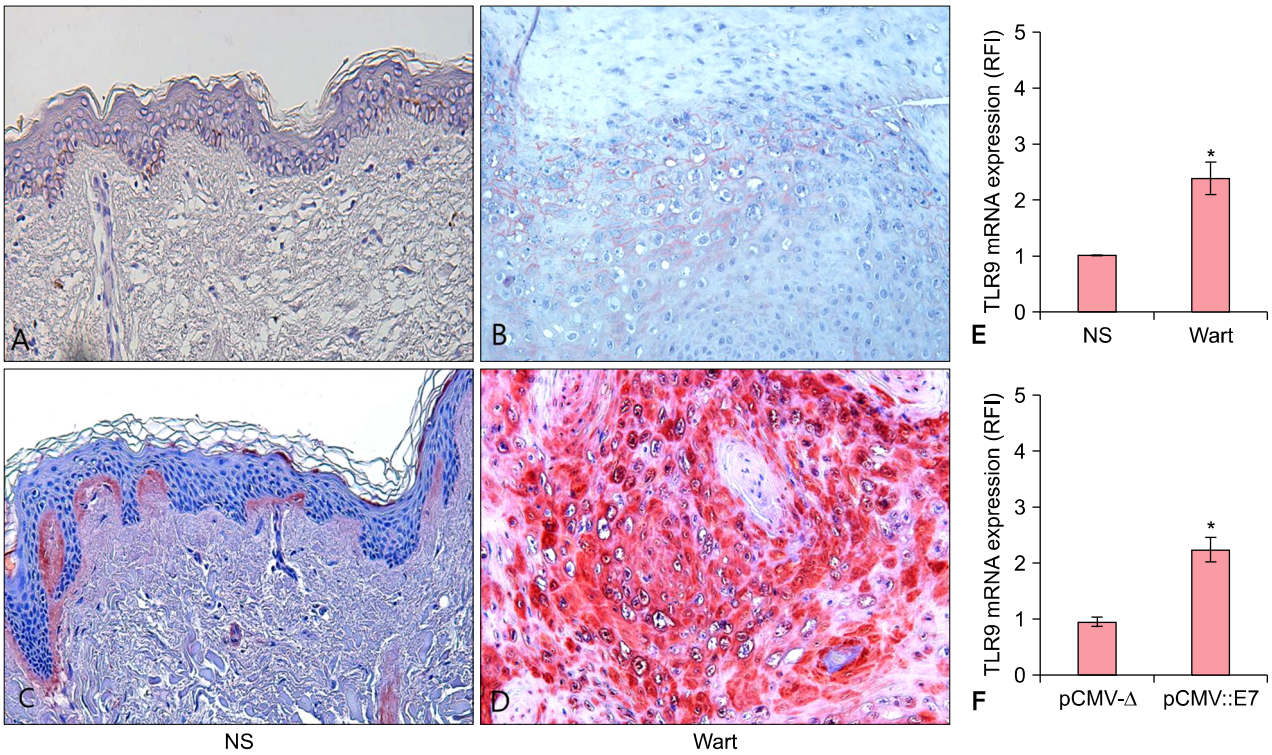


**Fig. 1.** Human papillomavirus type 2 (HPV2) E7 (A, B) and toll-like receptor 9 (TLR9) (C, D) protein expression in normal human skin and wart lesions. TLR9 mRNA expression in wart lesions (E) and normal human keratinocytes (NHKs) transfected with HPV2 E7 (F). TLR9 protein and mRNA expressions were assessed by immunohistochemical staining and quantitative real time polymerase chain reaction, respectively. The specific antibodies for HPV2 E7 and TLR9 were diluted with phosphate-buffered saline (1:100). Original magnification: ×200. TLR9 specific primer was used and normalized to 18s rRNA in the quantitative real time polymerase chain reaction. Each bar indicates the mean±standard error. *$p<0.05$. NS: normal human skin, RFI: relative fold increase, pCMV-$\Delta$: NHKs transfected with empty vector, pCMV::E7: NHKs transfected with HPV2 E7.

provoke inflammation, for the treatment of recalcitrant warts[6,7]. The little inflammation might be presumed to be associated with HPV2 E7 and NF-κB signaling. Although there have been some studies on the role of E7 in other types of HPV in regulating NF-κB signaling[3,8], the role of HPV2 E7 is not clearly understood. In this study, we investigated whether HPV2 E7 can attenuate the NF-κB activity in normal human keratinocytes (NHKs). Our Study is approved by the Ethics Committee of Seoul St. Mary's Hospital, The Catholic University of Korea (KC16SASE0748). Twelve wart samples were taken from six patients with common warts and three normal skin ones were obtained from the patients who underwent excisional surgery for benign cutaneous tumors. All of them were used for immunohistochemical staining and quantitative real time polymerase chain reaction. HPV E7 was transfected into cultured NHKs for luciferase assay and immunofluorescence staining. As a negative control, we used empty vector transfected keratinocytes. Experiments were repeated at least twice. Data was evaluated by Student's t-test and $p<0.05$ was as significant.

We first examined the HPV2 E7 and TLR9 expressions in both wart lesions and normal human skin using immunohistochemical staining and quantitative real time polymerase chain reaction. HPV2 E7, which was not expressed in the epithelial layers of the normal human skin, was focally expressed in the corneal and granular layer of wart lesions (Fig. 1A, B). TLR9 expression was diffusely increased in the corneal and granular layers of wart lesions, compared to the normal human skin (Fig. 1C, D). Similarly TLR9 mRNA expression was significantly increased in the wart lesions, compared to the normal human skin ($p=0.01$, Fig. 1E). The TLR9 mRNA expression was also increased in the NHKs transfected with HPV2 E7, compared to the NHKs transfected with the empty vector ($p=0.01$, Fig. 1F).

Next, to investigate the role of HPV2 E7 on the NF-κB activity of the NHKs, a luciferase assay was performed. To induce an inflammation, NHKs were stimulated with tumor necrosis factor-α (50 ng/ml) for 3 hours. The NF-κB activity was significantly decreased in the NHKs transfected with HPV2 E7, compared to the NHKs transfected

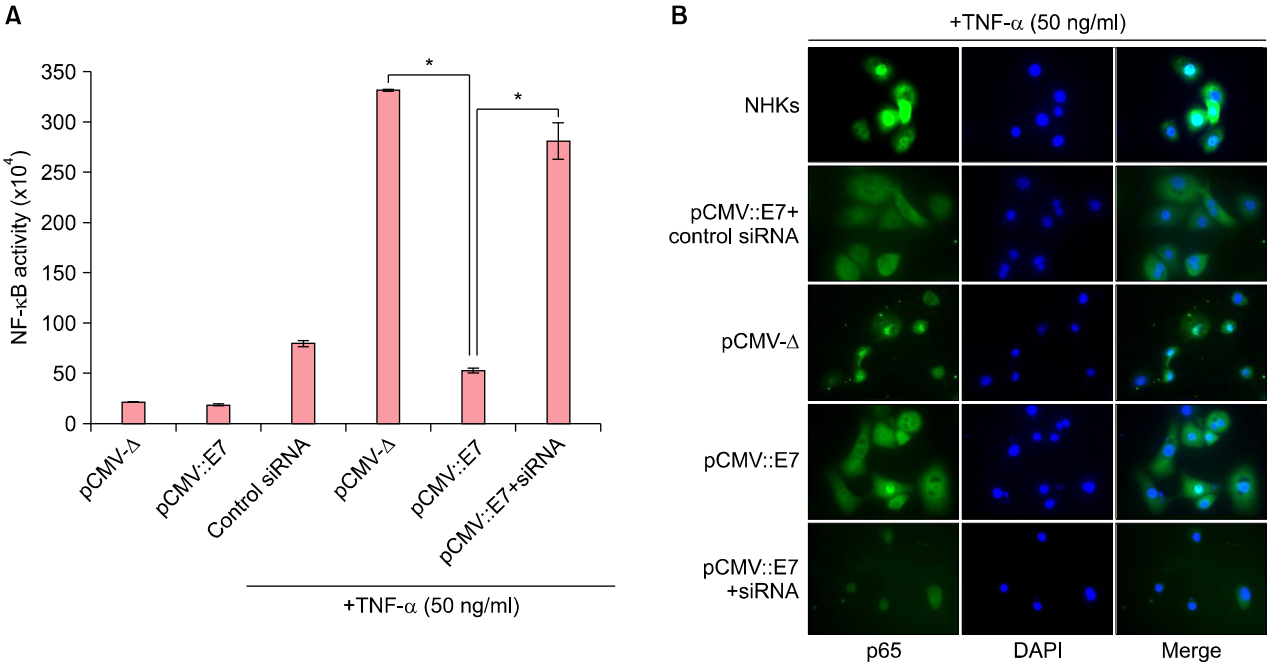


**Fig. 2.** (A) Effect of human papillomavirus type 2 (HPV2) E7 on the nuclear factor kappa B (NF-κB) activity of the normal human keratinocytes (NHKs). NHKs were pretreated with tumor necrosis factor-α (TNF-α) (50 ng/ml) for 3 hours before HPV2 E7 transfection. A luciferase assay using the NF-κB dependent firefly luciferase reporter vector was performed. Values of NF-κB activity were normalized to β-galactosidase activity. Each bar indicates the mean±standard error. *$p<0.05$. (B) The nuclear translocation of p65 was investigated by immunofluorescence staining. Nuclei were stained with 4, 6-diamidino-2-phenylindole. Original magnification: ×400. pCMV-Δ: NHKs transfected with empty vector, pCMV::E7: NHKs transfected with HPV2 E7, DAPI: 4',6-diamidino-2-phenylindole.

with the empty vector ($p=0.02$). When the expression of HPV2 E7 was knocked-down by its corresponding small interfering RNA (siRNA), the NF-κB activity of the NHKs transfected with E7 was significantly upregulated ($p=0.006$, Fig. 2A). To investigate the inhibitory effect of NF-κB in NHKs transfected with E7 gene, we used immunofluorescence staining of p65, a subunit of NF-κB complex, which plays a crucial role in inflammatory conditions. The NHKs transfected with HPV2 E7 did not show p65 translocation. However, cells transfected with the empty vector showed p65 translocation into the nucleus. In contrast, when we used siRNA, the NHKs transfected with HPV2 E7 showed p65 translocation (Fig. 2B).

In the present study, we demonstrated that TLR9 expression was diffusely increased in the corneal and granular layers of wart lesions in which HPV2 E7 was focally expressed, and that TLR9 mRNA expression was also significantly upregulated in the NHKs transfected with HPV2 E7, compared to the NHKs transfected with the empty vector. These results indicated that TLR9 is the receptor that is involved in the first-line defense mechanism against HPV2 infection.

Some viruses are known to evade the immune response by modulating the NF-κB pathway in a cell type dependent manner[9,10], and common wart lesions are clinically characterized by little inflammation. Our results demonstrated that NF-κB activity was suppressed in the NHKs transfected with HPV2 E7. These results were confirmed by immunofluorescence staining of p65. Furthermore, attenuated NF-κB activity was restored by siRNA specific to HPV2 E7.

Taken together, although the number of samples in this study is relatively small, these results indicate that HPV2 E7 can attenuate the NF-κB activity in NHKs, and suggest that suppressed E7 might be a potential therapeutic target for the clearance of cutaneous warts.

## ACKNOWLEDGMENT

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## CONFLICTS OF INTEREST

The authors have nothing to disclose.

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