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Induction of co-inhibitory molecule CTLA-4 by human papillomavirus E7 protein through downregulation of histone methyltransferase JHDM1B expression

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ABSTRACT

Human papillomavirus causes various skin diseases and even cancer. Unfortunately, the host immune system often fails to generate effective responses against HPV infection due to the ability of HPV to evade immune-mediated eradication, although the detailed mechanisms by which HPV inhibits host antiviral immunity are not fully understood. In this study, we reported a novel role of HPV E7 oncoprotein in inducing the expression of co-inhibitory molecule cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) in cells of epithelial origin. Mechanistically, HPV E7 protein downregulated the cellular abundance of Jumonji C histone demethylase 1B (JHDM1B), increasing the levels of H3K36 methylation within the promoter region of CTLA-4. Our findings expand the current understanding of HPV-mediated immune evasion mechanisms and may be helpful in developing optimal anti-HPV therapeutic strategies and relevant drugs.

1. Introduction

Human papillomavirus (HPV) infection is one of the most common sexually transmitted infections worldwide and is a causative factor of cancer, genital warts, and other epithelial lesions. Low-risk HPVs, such as types 6 and 11, primarily infect the skin and mucosal membranes of the external genitalia and its adjacent areas and can cause genital warts. More severely, persistent high-risk HPV infection can increase the risk of cancerous diseases, especially cervical cancer. Despite the mammalian immune system having immune defense functions against viral infection, HPV has evolved to escape host immunity using multiple mechanisms (Song et al., 2015; Steinbach and Riemer, 2018). For example, HPV infection disrupts the maturation of epidermal dendritic cells (DCs) (Iijima et al., 2013)or dampens their production of interferons (Crosbie et al., 2013). Moreover, HPV reduces the cytotoxicity of natural killer cells (Jimenez-Perez et al., 2012; Garcia-Iglesias et al., 2009). In terms of adaptive immunity, HPV infection increases the infiltration of regulatory T cells (Tregs) (de Vos van Steenwijk et al., 2008), upregulates Th2-associated cytokine production (e.g., IL-10), downregulates Th1-associated cytokine production (e.g., IFN-γ) (Bais et al., 2005; Scott et al., 2013), or reduces the expression of MHC-I molecules to avoid clearance by $CD8^+$ T cells (Piersma, 2011). The immune -evading nature of HPV is considered to be largely dependent on its expression of the HPV oncoproteins E5, E6 and E7, which can disrupt the expression or function of specific immune activators, such as TLR9, CXCL14, IRF1 or MHC-I molecules (Cicchini et al., 2016; Hasan et al., 2013; Park et al., 2000; Ashrafi et al., 2002). Despite these findings, the mechanisms by which HPV oncoproteins impair host immune surveillance are far from fully understood, greatly restraining the efficacy of immunotherapy against HPV.

Appropriate T cell responses are modulated by activating stimuli but also require proper negative signals to prevent tissue damage caused by excessive T cell activation. In this regard, co-inhibitory molecules, also known as immune checkpoint molecules, are necessary for terminating signaling activation in T cells. In addition to T cell-intrinsic mechanisms, T cell-extrinsic functions of specific checkpoint molecules have

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increasingly emerged as being important in recent years (Walker and Sansom, 2011). For example, CTLA-4 was reported to induce the production of immunoinhibitory mediators by antigen-presenting cells (APCs) (Grohmann et al., 2002; Fallarino et al., 2003; Chen et al., 1998) or to transendocytose CD80/CD86 co-stimulatory molecules in APCs (Qureshi et al., 2011), suggesting that the non-T cell-expressed CTLA-4 can also be immunosuppressive. Despite their crucial roles in maintaining tissue immune homeostasis, the abnormal expression of co-inhibitory molecules under certain pathological conditions contributes to the failure of defensive immunity. In the past decade, targeting immune checkpoint molecules, such as CTLA-4 and PD-1/PD-L1, has gained widespread clinical application in cancer immunotherapy (Pardoll, 2012; Callahan et al., 2016). Although considerable attention has been paid to the roles of immune checkpoints in tumor immunity, whether HPV can achieve immune evasion by modulating the expression of these molecules remains poorly understood. In our present study, we investigated the possible impact of HPV E7 oncoprotein on the expression of co-stimulatory and co-inhibitory proteins and identified HPV E7 as an inducer of CTLA-4 in epithelial cells. Further investigation showed that HPV E7 downregulated the expression of Jumonji C histone demethylase1B (JHDM1B), leading to an enhanced level of histone methylation that was associated with transcriptional activation at the CTLA-4 promoter region. Our findings provide a new mechanistic explanation for HPV-induced immune abnormalities.

2. Materials and methods

2.1. Cell lines and cultures

HaCaT keratinocyte cell line (ATCC, No.CRL-2309), SiHa (ATCC, No.HTB-35) and HeLa (ATCC, No.CCL2) cervical cancer cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and were maintained in RPMI-1640 medium containing 10% fetal bovine serum.

2.2. Microarray

HaCaT cells were transfected with HPV11E7-expressing or control lentiviruses for 48 h and then sorted by flow cytometry. HaCaT cells stably expressing HPV11E7-HaCaT and control cells were lysed using TRIzol (Thermo Fisher, 15596018). Microarray analysis was performed by Shanghai Biotechnology Corporation (Shanghai, China).

2.3. Lentiviral transduction

Cultured epithelial cells were incubated with appropriate lentiviral particles in the presence of 8 μg/ml polybrene (Sigma-Aldrich, H9268) for 24 h, after which the supernatant was replaced with fresh RPMI-1640 medium. Subsequently, the cells were cultured for another 48 h and then used for experiments.

2.4. Real-time PCR

Cellular RNA was extracted using TRIzol, and 1 μg RNA was reverse-transcribed into cDNA. Real-time PCR was performed using a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) with SYBR Green reagent (Roche, 04707516001). The PCR thermocycling parameters used were as follows: 50 °C for 2min, 95 °C for 10min, followed by 40 cycles of 95 °C for 15s and 60 °C for 35s. Gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method and was normalized to β-actin. The following primers were used: CTLA-4 forward (F): AGGTGACTGAAGTCTGTGCG, reverse (R): CATGAGCTCCACCTTGC AGA; PD-L1 (F): TGGCATTTGCTGAACGCATTT, (R): TGCAGCCAGGT CTAATTGTTTT; JHDM1B (F): GGGTTCCCCTGATATTTCGAGA, (R): GCTCCCCACTAGGAGTTTGAC; and KDM1B (F): GGCAAAACCGAACC TAGTCCC, (R): TGTGGAGTAAGCTGGATTTCCTT.

2.5. Chromatin immunoprecipitation (ChIP)

Total cell lysates immunoprecipitated using a SimpleChIP® Enzymatic Chromatin IP kit (Cell Signaling Technology, 9003) with H3K4me3 (Abcam, ab8580) or H3K36me2 (Abcam, ab9049) antibodies. The following sequences were used for PCR analysis of H3K4me3 or H3K36me2 enrichment at the CTLA-4 promoter: CTLA4 0 to -300 (F): CCACGGCTTCCTTTCTCGTA, (R): CATGTGCACACACAGA AGGC; CTLA4 -300 to -600 (F): GGCAGCTTCTTTTCCGCCTA, (R): CCTTGGGCTAATGGCAGGAT; CTLA4 -600 to -900 (F): TGGGTTGGCT TTTCTTTGGAC, (R): GCAGAAGGAAAGAATTCAGGTGT; and CTLA4 -900 to -1200 (F): AGACAAATGCAGGAAGGGGG, (R): TCCAGTCCGTC CATGGTTTC.

2.6. Immunohistochemistry

Clinical specimens were fixed in 4% paraformaldehyde, after which 5-μmthick paraffin-embedded sections were stained with anti-CTLA-4 (Abcam, ab237712) or anti-JHDM1B antibodies (Abcam, 234082).

2.7. Flow cytometry

Cells were digested with trypsin and washed twice with phosphate-buffered saline (PBS). The percentages of ZsGreen$^+$ cells were analyzed by flow cytometry using a FACSCalibur instrument. In some experiments, cells were stained with CTLA-4 antibody (Biolegend, 369603) or control IgG (Thermo Fisher, 12-4724-81).

2.8. Western blot

Total cell lysates were subjected to 10% SDS-PAGE, after which proteins were transferred to PVDF membranes that were probed with the appropriate antibodies.

2.9. Statistical analysis

Significant differences between two groups were determined using Student's*t*-test. All data are presented as the means ± SEM. Differences were considered to be significant when $P < 0.05$.

3. Results

3.1. Low-risk HPVE7 protein induces co-inhibitory molecule CTLA-4 expression in human keratinocytes

To investigate the possible role of HPVE7 protein in the cellular expression of co-stimulatory or co-inhibitory molecules, we first introduced low-risk HPV11E7 protein into HaCaT cells by lentiviral transduction. Flow cytometry analysis results demonstrated that the transduction efficiency was comparable between the HPV11E7 and control lentiviruses (Fig. 1a). Subsequently, we obtained the HaCaT cells stably expressing HPV11E7 protein by flow sorting and verified the successful overexpression of HPV11E7 protein in HaCaT cells by real-time PCR and Western blot analyses (Fig. 1b). Next, we evaluated HPV11E7-induced changes in the gene expression profile of HaCaT cells by microarray analysis. Among several candidate stimulatory or co-inhibitory molecules, the expression of CTLA-4 displayed a notable increase in HPV11E7-overexpressing HaCaT cells compared with control cells, whereas the levels of other genes were barely altered (Fig. 1c). The HPV11E7-mediated upregulation of CTLA-4 expression was also evidenced by the results of real-time PCR(RT-PCR) and Western blot(WB) analyses (Fig. 1d and e). Flow cytometry results confirmed an increased expression of CTLA-4 in HPV11E7-overexpressing HaCaT cells (Fig. 1f). These findings were further substantiated by clinical observations that there were more CTLA-4$^+$ cells in HPV-infected condyloma acuminatum tissues than in normal skin tissues

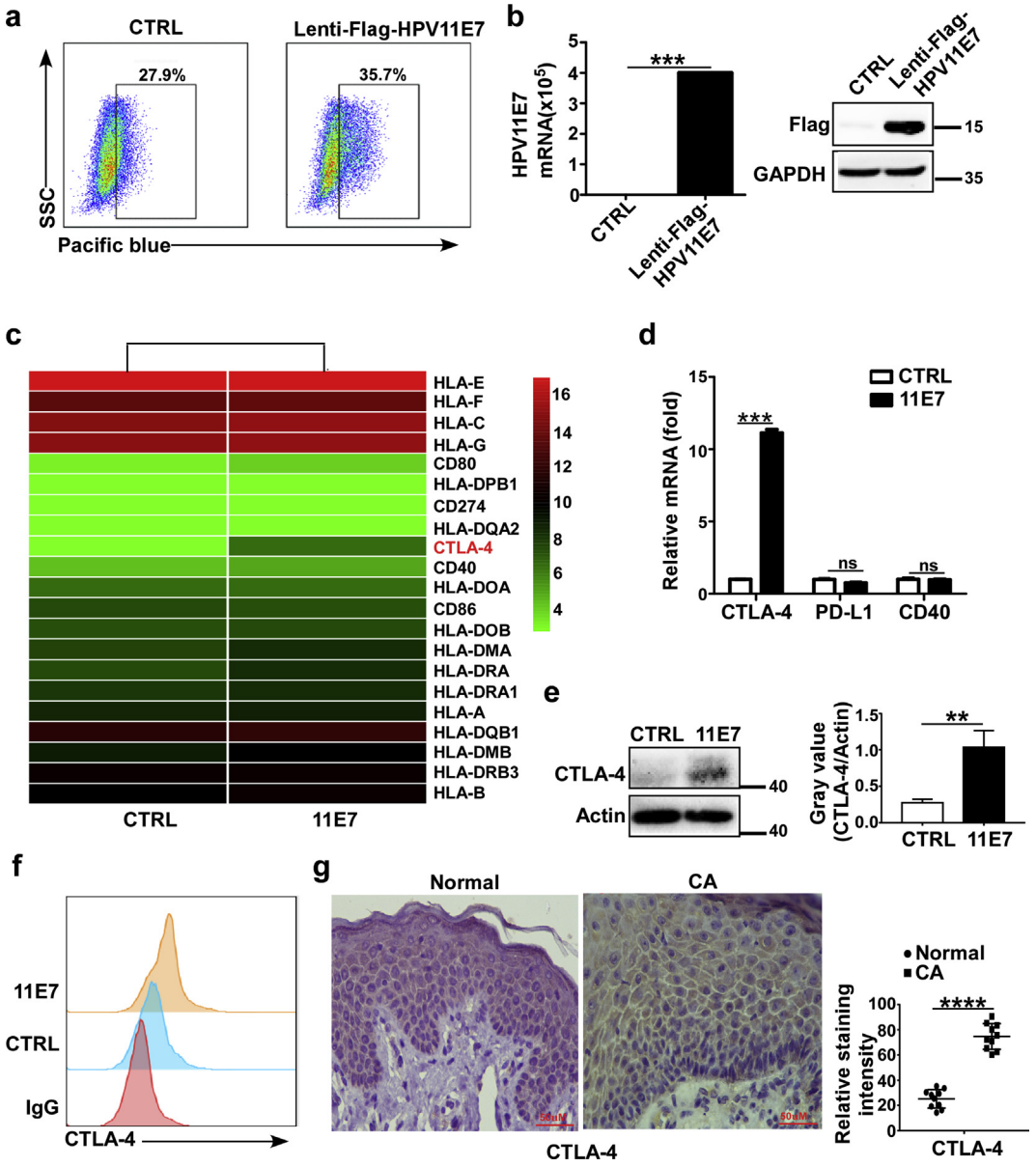

Fig. 1. Low-risk HPVE7 protein induces co-inhibitory molecule CTLA-4 expression in human keratinocytes. (a) HaCaT cells were untransfected or transfected with HPV11E7-expressing or control lentiviruses, and the transfection efficiency was validated by flow cytometry. (b) The expression of HPV11E7 in HaCaT cells was examined by real-time PCR and Western blot analyses. (c) HPV11E7 protein was overexpressed in HaCaT cells by lentiviral transfection, and the abundances of the indicated co-stimulatory/co-inhibitory molecules were examined by microarray analysis. (d) The mRNA levels of CTLA-4, PD-L1 and CD40 were determined by qPCR. (e) The protein level of CTLA-4 was detected by Western blot analysis. (f) Surface expression of CTLA-4 was determined by flow cytometry. (g) Immunohistochemical examination of CTLA-4 expression in condyloma acuminatum tissues or normal skin tissues, scale bar = 50 μm. ***, $P < 0.001$.

(Fig. 1g). Our results demonstrated the ability of low-risk HPVE7 protein to induce CTLA-4 expression of in human keratinocytes.

3.2. *High-risk HPVE7 proteins induce CTLA-4 expression in human cervical epithelial cells*

To further investigate whether the CTLA-4-inducing function of HPV11E7 protein could be recapitulated in cancer-associated, high-risk HPVE7 protein, we introduced the HPV16 and HPV18E7 protein into SiHa or HeLa cells, respectively. We observed that the overexpression of either HPV16 or HPV18E7 proteins significantly enhanced CTLA-4 expression in SiHa or HeLa cells by RT-PCR (Fig. 2a). Flow cytometry results showed that CTLA-4 expression was significantly increased in HPV16E7-overexpressing SiHa cells or HPV18E7-overexpressing HeLa cells compared that observed in with control cells (Fig. 2b). Furthermore, Western blot results revealed that HPV16E7-overexpressing SiHa cells or HPV18E7-overexpressing HeLa cells expressed higher levels of CTLA-4 than the control cells (Fig. 2c and d).To further confirm whetherHPV16 or HPV18E7 regulate the expression of CTLA-4, we knocked downHPVE7 in HeLa or SiHa cells and observed that CTLA-4 expression was compromised (Fig. 2e). Moreover, the results of Western blot and flow cytometry analyses showed that CTLA-4 expression was decreased in HPV16E7-silenced SiHa cells or HPV18E7-silenced HeLa cells (Fig. 2f–h). Consistent with these *in vitro* data, we observed higher CTLA-4 expression in HPV^+ cervical cancer tissues than in normal cervical epithelium by immunohistochemistry assays (Fig. 2i). Furthermore, the GEO data(GSE7410) showed that the expression of CTLA-4 was significantly higher in cervical cancer than in normal tissues (Fig. 2j). Taken together, our results demonstrated that high-risk HPVE7 proteins can function as CTLA-4 inducers in cervical

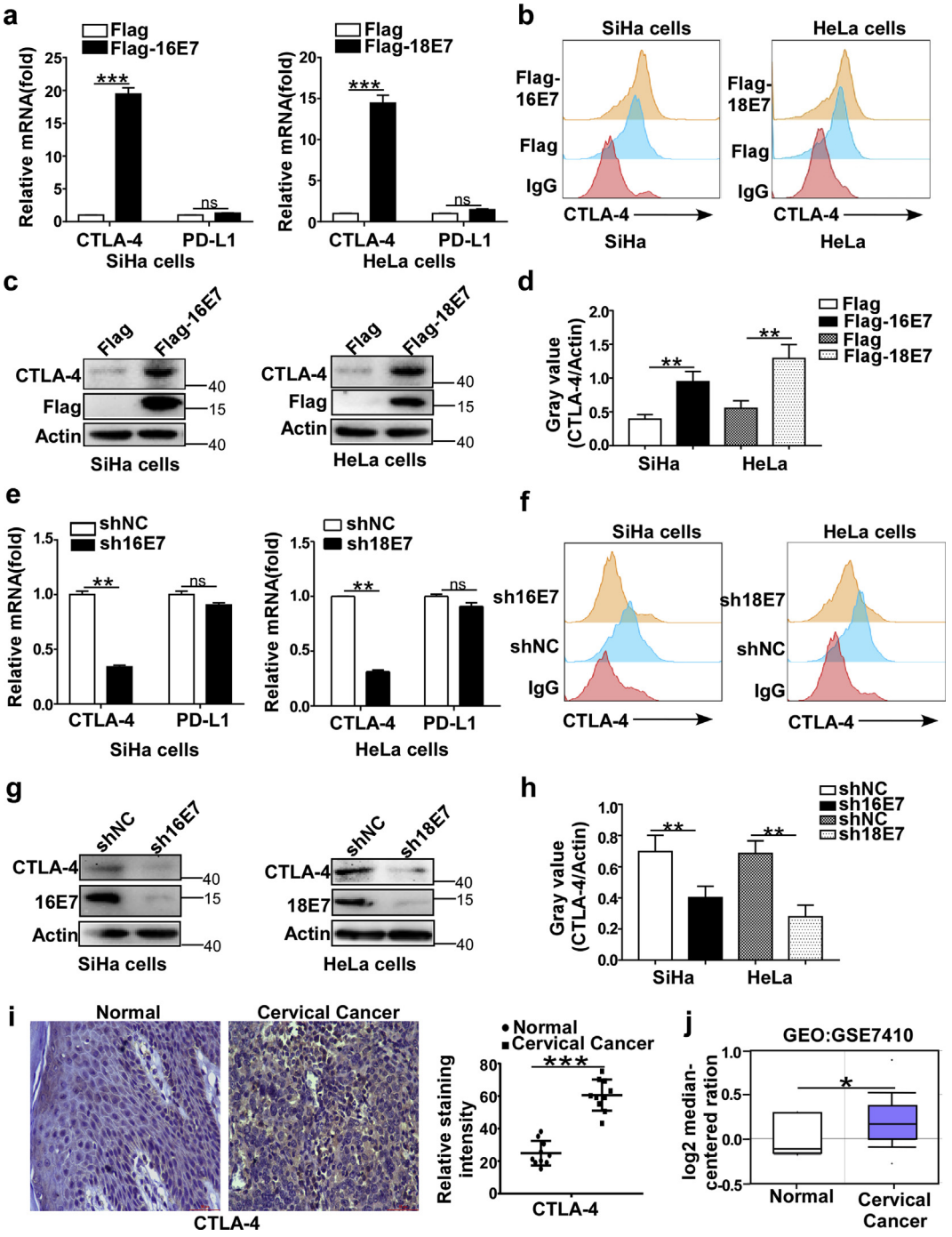

Fig. 2. High-risk HPVE7 proteins induce CTLA-4 expression in human cervical epithelial cells. (a–d) HPV16 or HPV18E7 protein was overexpressed in SiHa or HeLa cells, and the mRNA levels of CTLA-4 and PD-L1 were evaluated by real-time PCR (a); surface expression of CTLA-4 was determined by flow cytometry (b); the protein level of CTLA-4 was detected by Western blot analysis (c); and the gray value of CTLA-4/actin was analyzed using ImageJ (d). (e–h) HeLa or SiHa cells were transfected with sh18E7 or sh16E7, and the mRNA levels of CTLA-4 and PD-L1 were evaluated by real-time PCR (e); the surface expression of CTLA-4 was determined by flow cytometry (f); the protein level of CTLA-4 was detected by Western blot analysis (g); and the gray value of CTLA-4/actin was analyzed using ImageJ (h). (i) Immunohistochemical examination of CTLA-4 expression in HPV^+ cervical cancer tissues (n = 9) and normal cervical epithelium (n = 9), scale bar = 50 μm.(j)GEO data (GSE7410) analysis CTLA-4 expression in cervical cancer tissues (n = 19) and normal cervical epithelium (n = 5).*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

epithelial cells.

3.3. HPVE7 proteins downregulate cellular Jumonji C histone demethylase1B (JHDM1B) expression

HPVE7 protein has been reported to induce the expression of histone demethylases KDM6A and KDM6B in human foreskin keratinocytes (McLaughlin-Drubin et al., 2011). This finding prompted us to examine the possible involvement of HPVE7 protein in altering the expression of specific histone demethylase(s). Microarray results demonstrated that HPV11E7 overexpression caused a marked downregulation in the level of JHDM1B in HaCaT cells, as well as that of KDM7A to a lesser extent (Fig. 3a). The levels of other selected histone demethylases were minimally altered by HPV11E7 overexpression. Consistent with the microarray data, HPV11E7 overexpression decreased both the mRNA and protein levels of JHDM1B in HaCaTcells

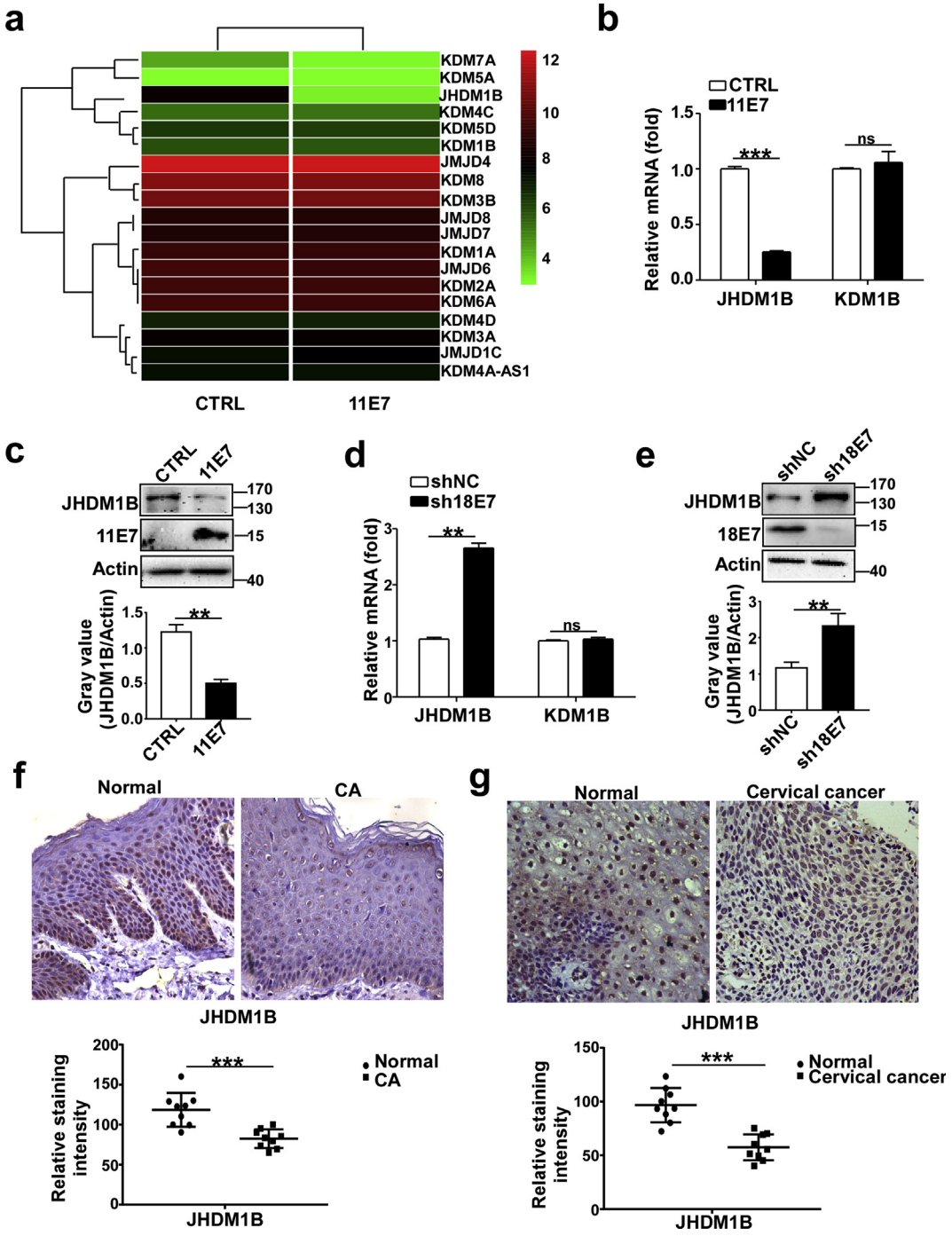

Fig. 3. HPVE7 proteins downregulate the cellular expression of Jumonji C histone demethylase1B (JHDM1B). (a) HaCaT cells were transfected with an HPV11E7expression construct or empty vector, and the cellular abundance of the indicated histone demethylases was examined by microarray analysis. (b, c) HaCaT cells were transfected with HPV11E7 or mock-transfected, after which the mRNA levels of JHDM1B and KDM1B were evaluated by real-time PCR (b), and the protein level of JHDM1B was detected by Western blot analysis (c). (d, e) HeLa cells were transfected with sh18E7 or scramble shRNA, after which the mRNA levels of JHDM1B and JHDM1B were evaluated by real-time PCR (d), and the protein level of JHDM1B was detected by Western blot (e).(f,g) Immunohistochemical examination of JHDM1B expression in normal skin (n = 9) and condyloma acuminatum tissues (n = 9) (f) or in cervicitis (n = 9)and HPV + cervical cancer tissues (n = 9)(g), scale bar = 50 μm. **, $P < 0.01$; ***, $P < 0.001$.

(Fig. 3b and c). In contrast, HPV18E7 knockdown led to an increased expression of JHDM1B in HPV^+ HeLa cells (Fig. 3d and e). In line with our *in vitro* findings, JHDM1B showed decreased expression in HPV-infected condyloma acuminatum lesions than that observed in normal skin tissues (Fig. 3f). Furthermore, we observed lower JHDM1B expression in HPV^+ cervical cancer tissues compared with that observed in cervicitis tissues (Fig. 3g). These data indicated that HPV E7 protein negatively regulates the expression of JHDM1B in epithelial cells.

3.4. JHDM1B inhibits CTLA-4 expression by reducing H3K36me2 levels at the CTLA-4 promoter

We next investigated the impact of JHDM1B in modulating CTLA-4 expression in epithelial cells. Knockdown of JHDM1B enhanced CTLA-4 expression in HeLa cells, whereas it had no effect on PD-L1 expression (Fig. 4a–c). In contrast, JHDM1B overexpression reduced that of CTLA-4 in HeLa cells (Fig. 4d–f). Since JHDM1B is a histone demethylase with

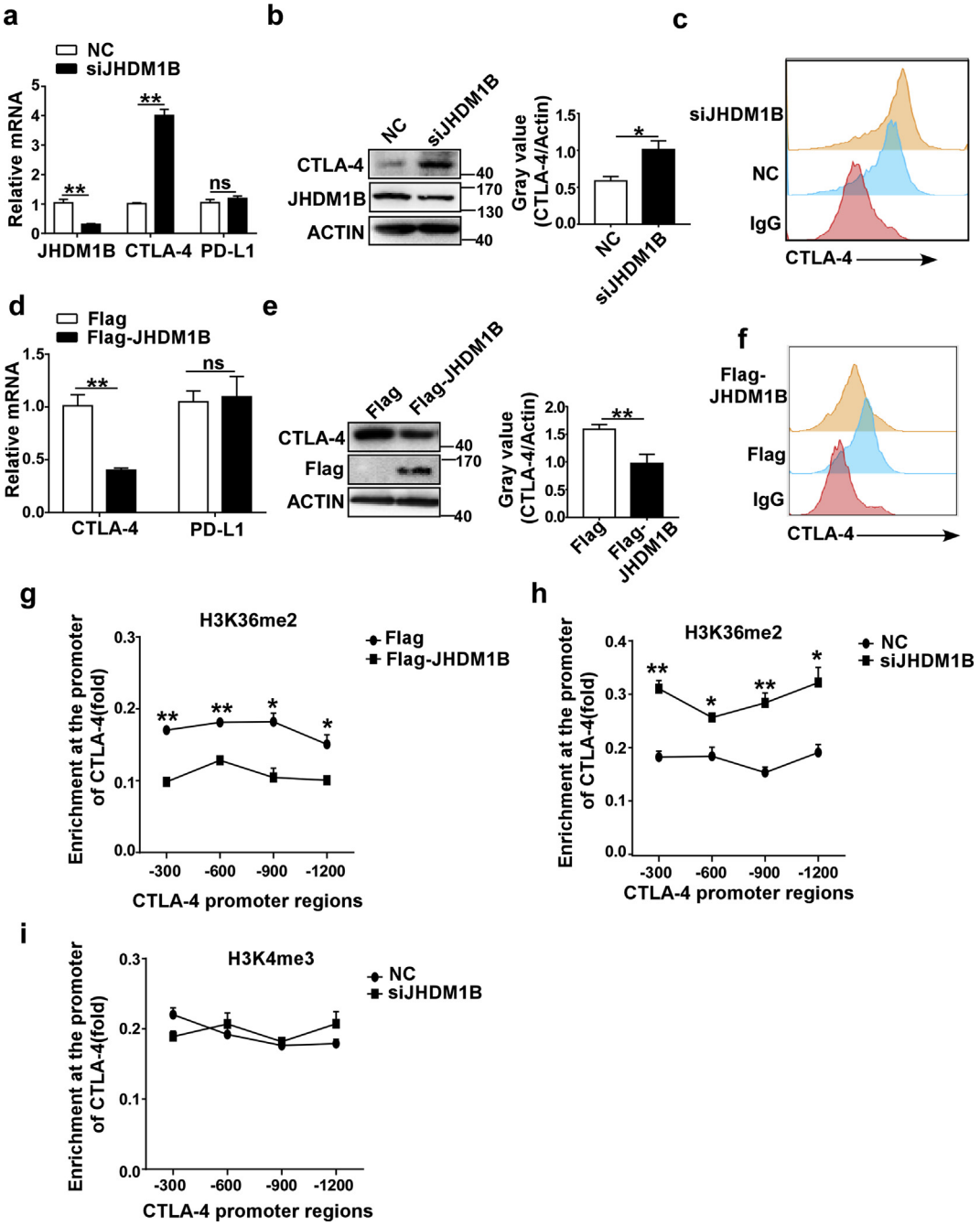

Fig. 4. JHDM1B inhibits CTLA-4 expression by reducing H3K36me2 levels at the CTLA-4 promoter. (a–c) HeLa cells were transfected with siJHDM1B or scrambled siRNA, after which the mRNA levels of CTLA-4 and PD-L1 were evaluated by real-time PCR (a); the protein level of CTLA-4 was detected by Western blot analysis (b), and the surface expression of CTLA-4 was determined by flow cytometry (c). (d–f) JHDM1B was overexpressed in HeLa cells, the mRNA levels of CTLA-4 and PD-L1 were evaluated by real-time PCR (d); the protein level of CTLA-4 was detected by Western blot analysis (e), and the surface expression of CTLA-4 was determined by flow cytometry (f). (g–i) HeLa cells were transfected with the JHDM1B overexpression vector (g) or siJHDM1B (h, i), and the enrichment of H3K36me2 (g, h) and H3K4me3 (i) at the CTLA-4 promoter was evaluated by ChIP assay. *, $P < 0.05$; **, $P < 0.01$.

specificity for H3K4 and H3K36, we performed chromatin immunoprecipitation (ChIP) assays to further assess whether JHDM1B can affect the levels of H3K4 and H3K36 methylation at the CTLA-4 promoter region. The results showed that JHDM1B overexpression significantly promoted the enrichment of H3K36me2 at the CTLA-4 promoter in HeLa cells (Fig. 4g), whereas JHDM1Bknockdown had the opposite effect (Fig. 4h). Unexpectedly, knockdown of JHDM1B failed to augment H3K4me3 levels at the CTLA-4 promoter (Fig. 4i). These data indicated that JHDM1B inhibits CTLA-4 expression by removing transcriptional activation-associated H3K36me2 modifications at its promoter.

3.5. HPVE7 proteins induce CTLA-4 expression by downregulating JHDM1B

Finally, we assessed the role of JHDM1B-mediated H3K36 demethylation on HPVE7 protein-induced changes in CTLA-4 expression. ChIP results showed that HPV11E7 overexpression in HaCaT cells increased the level of H3K36me2 (Fig. 5a), whereas knockdown of HPV18E7 in HeLa cells reduced H3K36me2 levels (Fig. 5b).

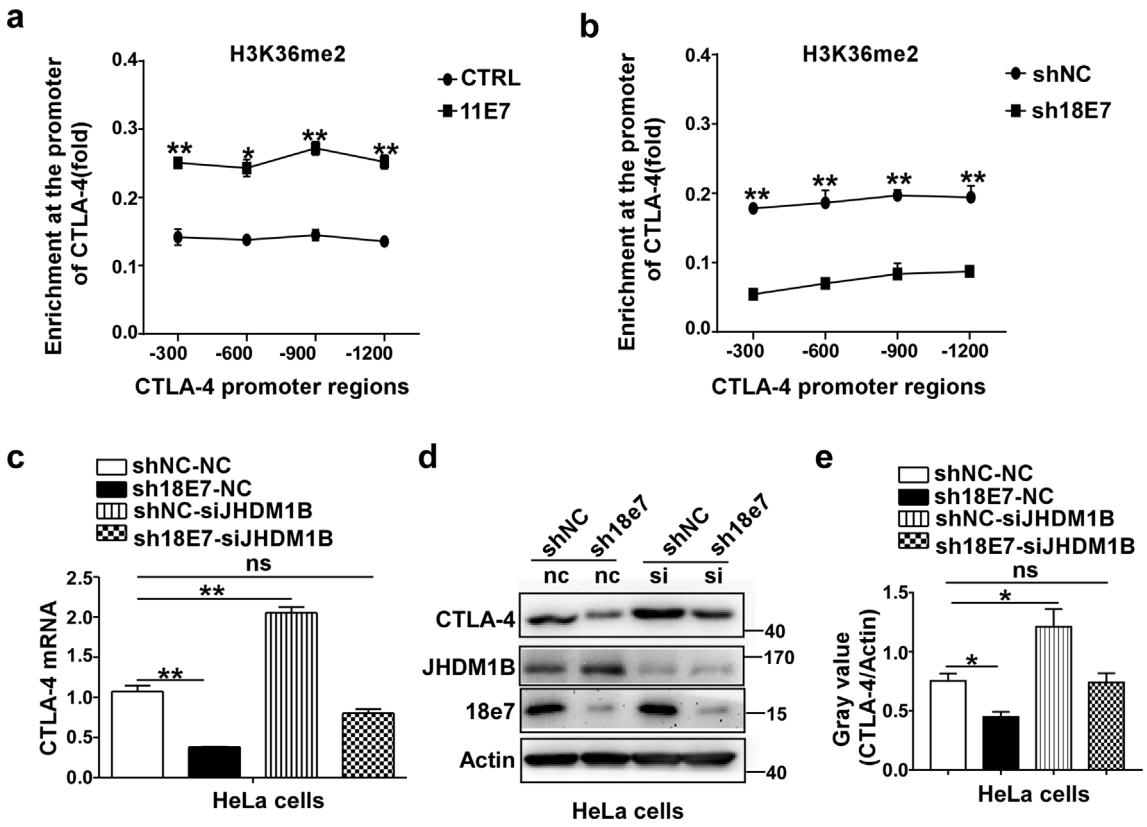

Fig. 5. HPVE7 proteins induce CTLA-4 expression by downregulating JHDM1B. (a) HPV11E7 protein was overexpressed in HaCaT cells, and H3K36me2 enrichment at the CTLA-4 promoter was evaluated by ChIP assay. (b) HeLa cells were transfected with sh18E7, andH3K36me2 enrichment at the CTLA-4 promoter was evaluated by ChIP assay. (c–e) HeLa cells were transfected with the indicated shRNA or siRNA, and the expression of CTLA-4 was evaluated by real-time qPCR (c) and Western blot analyses (d). The gray value of CTLA-4/actin was analyzed using ImageJ (e) *, $P < 0.05$; **, $P < 0.01$.

Importantly, the HPV18E7 shRNA-induced downregulation of CTLA-4 expression was significantly abrogated by the concomitant knockdown of JHDM1B in HeLa cells. However, siJHDM1B failed to fully restore CTLA-4 expression to the level observed in mock-transfected cells, indicating that factors other than JHDM1B were also involved in the HPV18E7-mediated regulation of CTLA-4 (Fig. 5c). These results were further supported at the protein level by the results of Western blot assays (Fig. 5d and e). Taken together, our data suggested that HPVE7 protein disrupts the expression of histone demethylase JHDM1B, causing an upregulation of CTLA-4 expression in human epithelial cells.

4. Discussion

HPV infection often causes abnormalities in host innate immunity and adaptive immunity functions. For instance, the percentage of plasmacytoid dendritic cells is significantly reduced in the peripheral blood of HPV-infected populations (Strickler et al., 2014). Iijima et al.reported that high-risk HPV E6 blocks the differentiation of Langerhans cells from monocyte precursors (Iijima et al., 2013). The cytotoxity of natural killer cells was also reported to be compromised by HPV infection (Jimenez-Perez et al., 2012; Garcia-Iglesias et al., 2009). In terms of T cell responses, cells infected by HPV showed a decreased level of MHC-I molecules and were more resistant to $CD8^+$ T cell-mediated killing (Piersma, 2011). In addition to the aforementioned mechanisms, although co-inhibitory molecules and their ligands play pivotal roles in the negative regulation of T cell immunity, their involvement in mediating HPV-induced immune suppression has not been investigated to date. In this study, we revealed that HPV E7 oncoprotein can trigger the expression of CTLA-4 in cells of epithelial origin, whereas other checkpoint molecules, such as PD-L1, were minimally affected by E7 overexpression.

CTLA-4, also known as CD152, is a co-inhibitory molecule that is highly expressed on activated effector T cells, regulatory T cells or tumor cells. CTLA-4 has long been believed to primarily exerts its immunosuppressive functions through competitive inhibition of CD28 signaling via binding with CD80/CD86 on antigen-presenting cells. In recent years, emerging evidence has revealed the T cell-extrinsic functions of CTLA-4. For instance, CTLA-4 triggers reversed signals in APCs and induces the secretion of tryptophan-degrading enzyme indoleamine 2,3-dioxygenase, leading to the suppression of T cell proliferation (Grohmann et al., 2002; Fallarino et al., 2003). CTLA-4 can also promote the production of regulatory cytokine TGF-β to inhibit local immune responses (Chen et al., 1998). Furthermore, Qureshi et al. reported that CTLA-4 can remove CD80/CD86 present on APCs via transendocytosis, reducing the availability of these co-stimulatory molecules (Qureshi et al., 2011).In this study, we showed that HPV E7 protein strongly induces CTLA-4 expression in epithelial cells. The inductive effect of HPV E7 protein on CTLA-4 expression may impair the antigen-presenting capacity of APCs or create an immunosuppressive cytokine milieu, which is typically seen at HPV-infected sites. The precise impact of HPV-induced CTLA4 expression on the local immune microenvironment requires further exploration.

Histone modification is a principal epigenetic mechanism used to modulate gene expression. The impact of histone methylation on transcription is determined by the lysine position at which methyl groups are added, which is different from histone acetylation in that these modification ways repressest transcriptional activation. Margaret et al. reported that HPV16 E7 protein can induce the expression of histone demethylases KDM6A and KDM6B, promoting the demethylation of the repressive trimethyl mark H3K27me3 and leading to an enhanced level of the cervical cancer biomarker $p16^{INK4A}$ in primary human epithelial cells (McLaughlin-Drubin et al., 2011). Another study

showed that HPV E6/E7 protein downregulated toll-like receptor 9 (TLR9) expression by recruiting histone deacetylase 1 (HDAC1) and lysine-specific demethylase 5B (JARID1B) to the promoter region of TLR9 (Hasan et al., 2013; Hasan, 2014). These results suggest the involvement of HPV in modulating the epigenetic reprogramming events in infected cells. In our study, we showed that HPV E7 overexpression induced elevated levels of transcriptional activation-associated H3K36me2 within the CTLA-4 promoter region in epithelial cells. In contrast, the level of H3K27me3 was either unchanged or decreased by HPV E7 overexpression. Mechanistically, HPV E7 protein suppressed the expression of histone demethylase JHDM1B in infected cells but had a milder or negligible impact on other histone demethylases. The detailed mechanisms for the selective downregulation of JHDM1B by HPV E7 remain unknown and require further investigation. Collectively, our results suggest the possibility that targeting CTLA-4 may be an optional strategy for preventing HPV-mediated immune escape.

5. Conclusions

Our study demonstrates that human papillomavirus E7 protein can induce the expression of co-inhibitory molecule CTLA-4 by through downregulation of histone methyltransferase JHDM1B expression and provides a new insight into developing optimal anti-HPV therapeutic strategies and relevant drugs.

Declaration of competing interest

The authors declare no conflicts of interest.

Acknowledgments

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