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IL-37 attenuated HPV induced inflammation of oral epithelial cells via inhibiting PI3K/AKT/mTOR

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Abstract

Human papillomavirus (HPV) is the most prevalent sexually transmitted infection globally, with significant implications for various anogenital cancers, such as vulval, vaginal, anal, penile, head and neck cancers. HPV infections have been linked to the induction of inflammation. In contrast, Interleukin-37 (IL-37) is recognized as an anti-inflammatory cytokine. In this study, two distinct types of oral epithelial cells were employed to investigate the impact of HPV on inflammation. The experimental outcomes unequivocally demonstrated that human papillomavirus (HPV) elicited a pronounced and statistically significant induction of inflammatory responses within both varieties of oral epithelial cells under investigation. Interestingly, IL-37 exhibited a mitigating effect, attenuating the HPV-induced inflammation in oral epithelial cells. Further exploration into the molecular mechanisms involved revealed that knockdown (KD) of PI3K compromised the anti-inflammatory effects of IL-37 in response to HPV. Similarly, KD of AKT was found to compromise the regulatory effects of IL-37 on HPV-induced inflammation. Notably, KD of mTOR was identified as a key factor, compromising the anti-inflammatory effects of IL-37 in the context of HPV-induced inflammation. Additionally, the study uncovered that the mTOR inhibitor, rapamycin, could effectively compromise the effects of IL-37 on HPV-induced inflammation. These findings contribute valuable insights into the intricate pathogenesis of HPV-induced inflammation and may pave the way for the development of innovative treatments for this condition.

Keywords HPV, IL-37, Inflammation, Growth, Oral epithelial cells

Introduction

As a large family of small double-stranded DNA viruses, HPV is the most common sexually transmitted infection globally, with a prevalence in the United States and worldwide. The HPV genome comprises 8 kb of circular DNA containing 8 protein-coding genes (L1, L2, E1, E2, E4, E5, E6, and E7) involved in various cellular processes such as replication, transcription, and transformation. Additionally, a noncoding, regulatory long control region (LCR) is present. HPV exhibits a wide diversity, with more than 200 distinct types having been identified to date. These types can be classified into two principal categories, namely low-risk and high-risk variants, each possessing unique characteristics and implications in terms of human health and disease development. These

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viruses are nonenveloped double-stranded DNA viruses that primarily infect basal epithelial cells. The infection is mediated through interactions with cell surface receptors like integrin α6, which is abundant in basal cells and epithelial stem cells. Importantly, the majority of the HPV genome remains in the episomal state in infected cells and premalignant lesions. This information sets the stage for a deeper understanding of HPV pathogenesis and its implications for the development of novel therapeutic strategies.

Infection with specific types of human papillomavirus (HPV) is a recognized contributor to various anogenital cancers, encompassing vulval, vaginal, anal, penile, and head and neck cancers [3]. In addition to its association with cancer, HPV has been reported to elicit inflammatory responses in different tissues [1]. Inflammation is an intricate biological reaction orchestrated by the body's tissues in response to various stimuli, such as pathogens, irritants, or injured cells. This protective response involves the coordinated action of blood vessels, immune cells, and molecular mediators [4]. When the stimulus persists, acute inflammation can transition to a chronic state, and accumulating evidence suggests a strong association between chronic inflammation and cancer [4]. Cervical cancer patients infected with HPV exhibit elevated expression levels of IFN-γ mRNA, indicating a potential link between HPV infection and heightened inflammatory responses [4]. Persistent HPV infection has been reported to induce the production of interleukin-6 (IL-6), a cytokine associated with the progression of cervical cancer [4]. Furthermore, IL-6 has been shown to stimulate the oncogene signal transducer and activator of transcription 3 (STAT3), contributing to the establishment of chronic inflammation in cervical cancer [4]. Inflammatory markers, including IL-6 and IL-8, have been found to be increased in tumor tissues with HPV infection [5]. Given the intricate relationship between HPV and inflammation, there is a compelling interest in developing therapies to address HPV-induced inflammation. Exploring strategies to modulate inflammatory responses could open new avenues for preventing and treating the inflammatory aspects associated with HPV infections.

Interleukin 37 (IL-37), also known as IL1 family member 7 (IL1F7), was originally identified by Kumar et al., and it comprises five isoforms: IL1F7a to IL1F7e [6]. IL-37 demonstrates broad protective effects against inflammatory diseases, autoimmune conditions, and cancer [6]. It is constitutively expressed in various human tissues and cells, contributing to the maintenance of immune homeostasis [7]. Clinical studies have highlighted elevated IL-37 levels in the serum and skin tissue of atopic dermatitis patients compared to controls, suggesting its induction in response to skin barrier disruption [7]. IL-37 has been reported to mitigate inflammation through the regulation of the intracellular AMP-activated protein kinase (AMPK)-mammalian target of rapamycin (mTOR) signaling mechanism, a crucial regulator of autophagy [7]. This signaling pathway plays a key role in the cellular response to stress and nutrient availability, and its dysregulation has been implicated in various pathological conditions, including inflammatory diseases and cancer.

In this study, two types of oral epithelial cells were utilized to investigate the impact of human papillomavirus (HPV) on inflammation in these cells. The study also delved into the effects of interleukin 37 (IL-37) on HPV-induced inflammation in oral epithelial cells. Furthermore, the study sought to elucidate the involvement of the PI3K/AKT/mTOR signaling pathway in mediating the effects of IL-37 on inflammation induced by HPV in oral epithelial cells. These findings provide novel insights into the pathogenesis of HPV-induced inflammation and the anti-inflammatory actions of IL-37 on oral epithelial cells.

Materials and methods

Cell culture

Two types of oral cells, including HOEC and IPM-H026, were obtained from the Bio-resource Center of the Affiliated Hospital of Hebei University. These cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, catalog number: D6429-500ML, Sigma-Aldrich) supplemented with 10% Fetal Bovine Serum (FBS, catalog number: MFCD00132239, Sigma-Aldrich), 1% L-glutamine

(Invitrogen cat#2 5030–081), and 1% penicillin–streptomycin solution (PS, catalog number: V900929-100ML, Sigma-Aldrich) at 37 °C in a 5% CO2 incubator. Subculturing was performed when the cells reached 80–90% confluence. Regular testing for Mycoplasma using the MycoAlert Plus Kit (Lonza) was conducted to ensure that the cells were free from mycoplasma contamination.

Transfection of HPV E6 on oral epithelial cells

Transfection of HPV E6 was following previous study [8]. Briefly, HPV-16 E6 plasmids were obtained from Addgene (Plasmid #8641). HOEC and IPM-H026 Cells were cultured at 37 °C in an atmosphere of 5% CO2. Cells were transfected with lipofectamine 3000 (Invitrogen, Carlsbad, CA) for plasmid transfection.

Western blotting

HOEC and IPM-H026 cells were lysed using RIPA Lysis Buffer (catalog number: P0013B, Beyotime Biotechnology, Shanghai, China). The samples were boiled at 95 °C with SDS/PAGE sample buffer (50 mM Tris–HCl [pH 6.8], 2% SDS, 0.1% bromophenol blue, 10% glycerol, and 1 mM dithiothreitol) for 10 min and then separated on SDS-PAGE. After transferring to PVDF membranes (Beyotime Biotechnology), the membranes were blocked with 5% non-fat milk in TBST buffer. Subsequently, they were incubated with primary antibodies (1:1000 dilution), including anti-Phospho-mTOR (2971, CST) and anti-beta-actin antibody (mAbcam 8226, Abcam), at 4 °C overnight. After incubation with horseradish-peroxidase-coupled secondary antibodies (1:5000 dilution, HRP-labeled goat anti-rabbit IgG [H + L, Catalog number: A0208, Beyotime Biotechnology]) at room temperature for 1 h, the immunoblots were visualized using BeyoECL Plus (catalog number: P0018S, Beyotime Biotechnology).

Cytokines detected by enzyme-linked immunosorbent assay (ELISA)

The concentrations of cytokines, including IL18, TNFα, and IL8, in cell supernatants was quantified using ELISA kits (Thermo Fisher, catalog number: IL18—KHC0181, TNFα—KHC3011, IL8—KHC0081). All assay procedures were performed following the manufacturer's instructions. Absorbance values of standards and samples were measured at 450 nm with a VICTOR Nivo Multimode Microplate Reader (reference wavelength 540 nm).

Table 1 Primers of qRT-PCR used in the present study

Gene	Primer	Sequence	Product length (nt)
IL18	Sense	CCGGGAACGAAAGAGAAGCTC	75
	Anti-sense	ACCGAAGGCGCTTGTGGAG	
IL8	Sense	AGTAGGCGACACTGTTCGTG	173
	Anti-sense	GCCTCCCATTCAATTGCCAC	
TNFα	Sense	GGCACCCAGTCTGAGAACAG	176
	Anti-sense	TGGCAACCCAGGTAACCCTTA	
GAPDH	Sense	AATGGGCAGCCGTTAGGAAA	168
	Anti-sense	GCGCCCAATACGACCAAATC	

RNA isolation and quantitative real time PCR (RT-qPCR)

TRIzol (Beyotime Biotechnology) was employed for total RNA isolation from both cell lines according to the manufacturer's protocol. The BeyoRT™ First Strand cDNA Synthesis Kit (catalog number: D7166, Beyotime Biotechnology) was utilized for cDNA synthesis from the isolated total RNA. RT-qPCR was conducted using BeyoFast™ SYBR Green qPCR Mix (2X) (catalog number: D7260-25 ml, Beyotime Biotechnology) on a 7500 Fast Real-time PCR System (Applied Biosystems). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as an internal control. The primers used in the study are listed in Table 1.

Statistical analysis

The data are presented as mean ± SEM. Statistical analysis of continuous variables was conducted using one-way ANOVA and Tukey's post hoc test, while categorical variables were analyzed using Fisher's exact tests. Prism GraphPad software (GraphPad Prism 5.0) was employed for statistical analysis. A significance level of $p < 0.05$ was considered to indicate statistically significant differences.

Results

HPV E6 significantly induces inflammation in oral epithelial cells

In order to comprehensively evaluate the influence exerted by human papillomavirus (HPV) infections on the inflammatory response within oral cells, human oral epithelial cells (HOEC) were subjected to a range of different concentrations of the HPV E6 protein. The experimental findings disclosed a significant

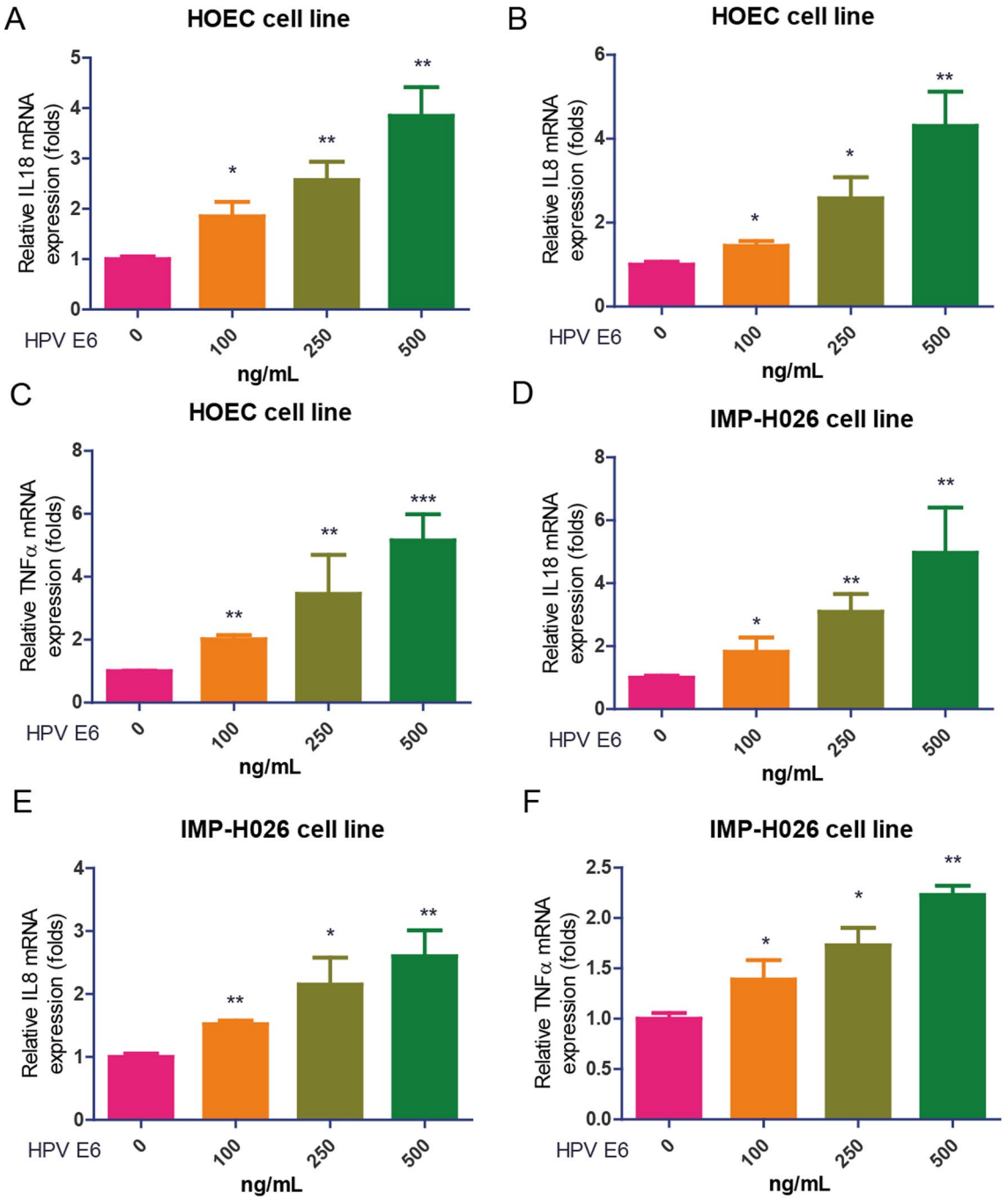

Fig. 1 HPV E6 significantly induces inflammation in oral epithelial cells. **A** HPV E6 significantly increased expression of IL18 on HOEC cells in dose dependent manner; **B** HPV E6 significantly increased expression of IL8 on HOEC cells in dose dependent manner; **C** HPV E6 significantly increased expression of TNFα on HOEC cells in dose dependent manner; **D** HPV E6 significantly increased expression of IL18 on IMP-H026 cells in dose dependent manner; **E** HPV E6 significantly increased expression of IL8 on IMP-H026 cells in dose dependent manner; **F** HPV E6 significantly increased expression of TNFα on IMP-H026 cells in dose dependent manner

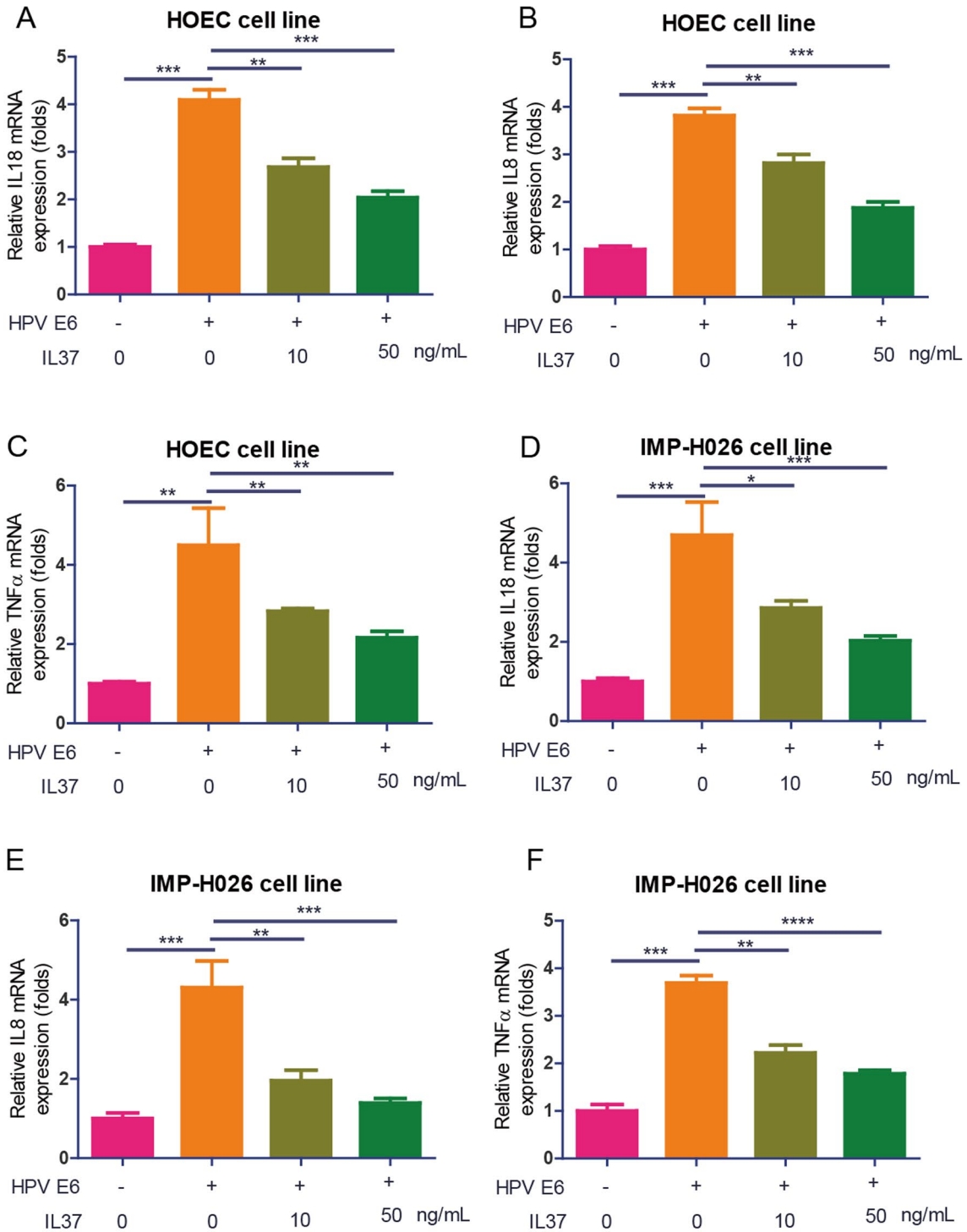

Fig. 2 IL37 attenuated HPV E6 induced inflammation on oral epithelial cells. **A** IL37 significantly attenuated HPV E6 induced increased of mRNA expression of IL18 on HOEC cells; **B** IL37 significantly attenuated HPV E6 induced increased of mRNA expression of IL8 on HOEC cells; **C** IL37 significantly attenuated HPV E6 induced increased of mRNA expression of TNFα on HOEC cells; **D** IL37 significantly attenuated HPV E6 induced increased of mRNA expression of IL18 on IMP-H026 cells; **E** IL37 significantly attenuated HPV E6 induced increased of mRNA expression of IL8 on IMP-H026 cells; **F** IL37 significantly attenuated HPV E6 induced increased of mRNA expression of TNFα on IMP-H026 cells

dose-dependent increase in the expression of inflammation-related genes, including IL18 (Fig. 1A), IL8 (Fig. 1B), and TNFα (Fig. 1C) in HOEC cells. Similarly, HPV E6 treatment led to a dose-dependent upregulation of inflammation genes including IL18 (Fig. 1D), IL8 (Fig. 1E), and TNFα (Fig. 1F) in IMP-H026 cells. In summary, the research findings unequivocally suggest that the human papillomavirus E6 protein plays a substantial role in provoking an inflammatory response within oral cells.

IL37 attenuated HPV E6 induced inflammation on oral epithelial cells

Considering the known anti-inflammatory effects of IL37, its impact on HPV-induced inflammation in oral epithelial cells was investigated. HOEC cells were co-treated with HPV E6 and different concentrations (10 and 50 ng/mL) of IL37. The results revealed that IL37 significantly mitigated the HPV E6-induced increase in mRNA expression of IL18 (Fig. 2A), IL8 (Fig. 2B), and TNFα (Fig. 2C) in HOEC cells. Similarly, IL37 was demonstrated to significantly attenuate HPV E6-induced upregulation of mRNA expression of IL18 (Fig. 2D), IL8 (Fig. 2E), and TNFα (Fig. 2F) in IMP-H026 cells. In summary, the research findings presented herein offer compelling and substantive evidence to support the notion that Interleukin-37 (IL-37) functions as a potent modulator, effectively mitigating the inflammation instigated by human papillomavirus E6 (HPV E6) within oral epithelial cells.

PI3K is involved in that IL37 attenuated HPV induced inflammation on oral epithelial cells

Given the reported involvement of PI3K/AKT/mTOR signaling in the activity of IL37, we sought to investigate how PI3K is implicated in the effects of IL37 on HPV-induced inflammation in oral epithelial cells [9]. IL37 was found to dose-dependently reduce the expression of PI3K in both HOEC cells (Fig. 3A) and IMP-H026 cells (Fig. 3B). Intriguingly, PI3K knockdown (KD) was observed to compromise the effects of IL37 on the increase in IL18 (Fig. 3C), IL8 (Fig. 3D), and TNFα (Fig. 3E) induced by HPV in the HOEC cell line. Similarly, PI3K KD compromised the effects of IL37 on the increase in IL18 (Fig. 3F), IL8 (Fig. 3G), and TNFα (Fig. 3H) induced by HPV in the IMP-H026 cell line. In summary, the research findings presented herein strongly imply a critical role for phosphatidylinositol 3-kinase (PI3K) in mediating the capacity of Interleukin-37 (IL-37) to temper the inflammation triggered by human papillomavirus (HPV) within oral epithelial cells.

AKT is involved in that IL37 attenuated HPV induced inflammation on oral epithelial cells

In order to conduct a more in-depth exploration into the far-reaching implications of the PI3K/AKT/mTOR signaling cascade on the functionality and efficacy of Interleukin-37 (IL-37), we undertook a comprehensive study wherein we meticulously scrutinized the influence that IL-37 exerts on the expression levels of AKT. IL37 was found to dose-dependently reduce the expression of AKT in both HOEC cells (Fig. 4A) and IMP-H026 cells (Fig. 4B). Notably, AKT KD compromised the effects of IL37 on the increase in IL18 (Fig. 4C), IL8 (Fig. 4D), and TNFα (Fig. 4E) induced by HPV in the HOEC cell line. Similarly, AKT KD compromised the effects of IL37 on the increase in IL18 (Fig. 4F), IL8 (Fig. 4G), and TNFα (Fig. 4H) induced by HPV in the IMP-H026 cell line. In summary, these findings suggest that AKT is involved in the ability of IL37 to attenuate HPV-induced inflammation in oral epithelial cells.

mTOR is involved in that IL37 attenuated HPV induced inflammation on oral epithelial cells

In an endeavor to conduct a more profound and exhaustive exploration into the manifold effects that the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/mechanistic target of rapamycin (mTOR) signaling axis exerts on the functional potency and activity of Interleukin-37 (IL-37), we meticulously designed and executed a comprehensive research initiative to explore effects of IL-37 on mTOR. IL37 was found to dose-dependently reduce the expression of mTOR in both HOEC cells (Fig. 5A) and IMP-H026 cells (Fig. 5B). Importantly, mTOR KD compromised the effects of IL37 on the increase in IL18 (Fig. 5C), IL8 (Fig. 5D), and TNFα (Fig. 5E) induced by HPV in the HOEC cell line.

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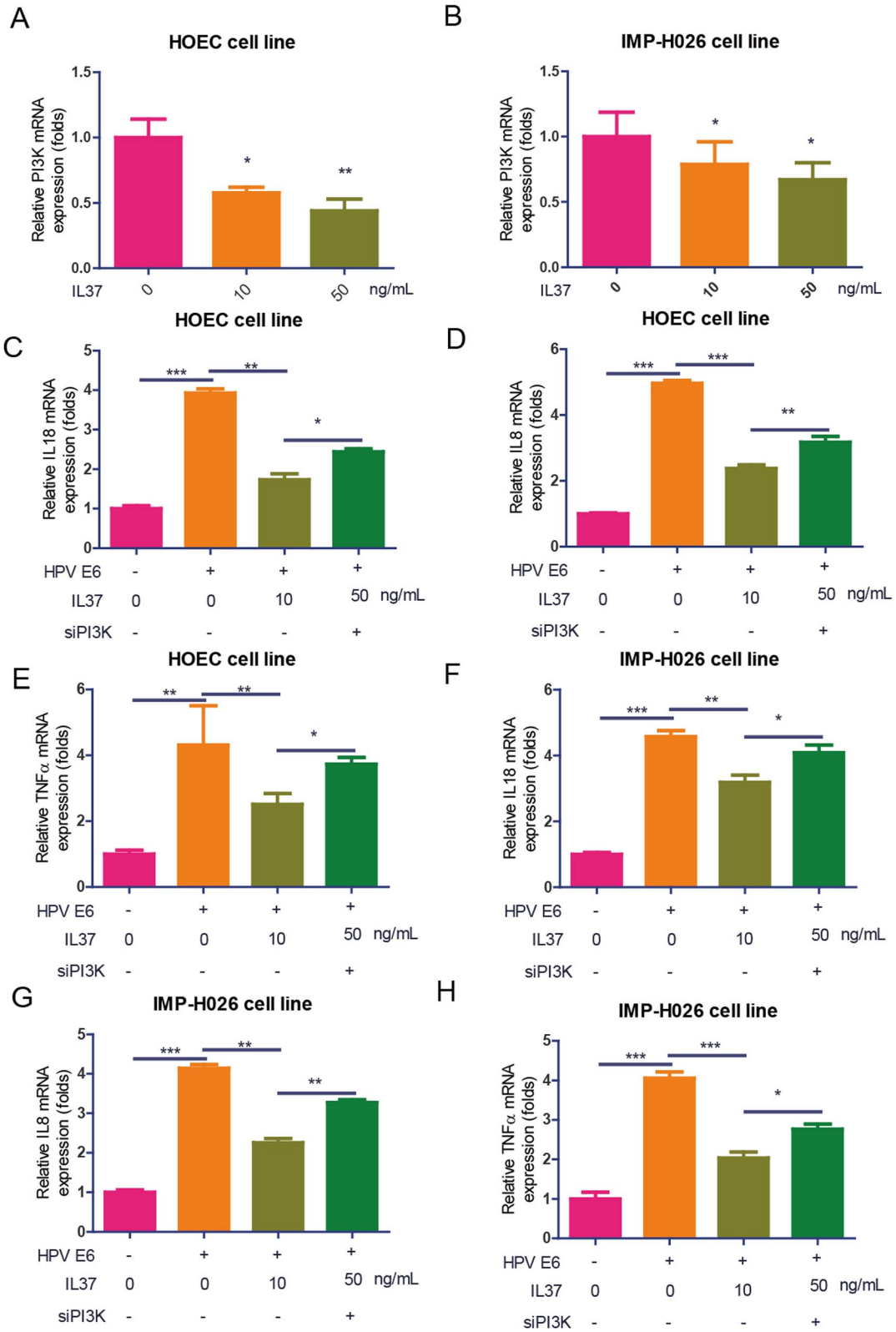
Fig. 3 PI3K is involved in that IL37 attenuated HPV induced inflammation on oral epithelial cells. **A** IL37 dose dependently reduced expression of PI3K on HOEC cells; **B** IL37 dose dependently reduced expression of PI3K on IMP-H026 cells; **C** PI3K KD compromised effects of IL37 on increase of IL18 induced by HPV on HOEC cell line; **D** PI3K KD compromised effects of IL37 on increase of IL8 induced by HPV on HOEC cell line; **E** PI3K KD compromised effects of IL37 on increase of TNFα induced by HPV on HOEC cell line; **F** PI3K KD compromised effects of IL37 on increase of IL18 induced by HPV on IMP-H026 cell line; **G** PI3K KD compromised effects of IL37 on increase of IL8 induced by HPV on IMP-H026 cell line; **H** PI3K KD compromised effects of IL37 on increase of TNFα induced by HPV on IMP-H026 cell line



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Similarly, mTOR KD compromised the effects of IL37 on the increase in IL18 (Fig. 5F), IL8 (Fig. 5G), and TNFα (Fig. 5H) induced by HPV in the IMP-H026 cell line. In summary, the research findings presented herein offer persuasive and substantial evidence suggesting that the mTOR plays an integral role in mediating the capacity of IL-37 to effectively dampen and modulate the inflammation instigated by HPV within the context of oral epithelial cells.

TOR inhibitor, Rapamycin compromises effects of IL37 on HPV induced inflammation on oral epithelial cells

To further investigate the effects of mTOR on the activity of IL37 in the context of HPV-induced inflammation in oral epithelial cells, we utilized a mTOR inhibitor, rapamycin. Treatment with rapamycin effectively inhibited the phosphorylation of mTOR in both HOEC cells (Fig. 6A) and IMP-H026 cells (Fig. 6B). Significantly, rapamycin compromised the ability of IL37 to attenuate the increase in IL18 (Fig. 6C) and TNFα (Fig. 6D) induced by HPV in the HOEC cell line. Similarly, rapamycin compromised the effects of IL37 on the increase in IL18 (Fig. 6E) and TNFα (Fig. 6F) induced by HPV in the IMP-H026 cell line. In summary, these results suggest that rapamycin compromises the effects of IL37 on HPV-induced inflammation in oral epithelial cells, further supporting the involvement of mTOR in this regulatory mechanism.

Discussion

Human papillomavirus (HPV) stands out as the most prevalent viral infection affecting reproductive tracts, with the vast majority of sexually active individuals acquiring the virus at some point in their lives [10]. Notably, HPV-associated oral diseases are on the rise, occurring in individuals with both intact and compromised immune systems [11]. HPVs employ diverse strategies to target immune signaling pathways [11]. In our study, we observed a potent induction of inflammation by HPV in oral epithelial cells. Importantly, IL37 demonstrated a significant ability to attenuate HPV-induced inflammation in oral epithelial cells. Mechanistically, our findings highlight the critical involvement of the PI3K/AKT/mTOR signaling pathway in mediating the effects of IL37 on HPV-induced inflammation in oral epithelial cells.

It has been documented that HPV 16 induces varying degrees of chronic inflammation, ranging from mild to severe inflammation in oropharyngeal squamous cell carcinoma (OPSCC) [11]. HPV E5, E6, and E7 have been implicated in the onset of HPV-induced inflammation by upregulating the expression of cyclooxygenase (COX)−2 and prostaglandin (PG) E2, subsequently activating the COX-PG pathway [12]. Studies have reported that HPVs can enhance inflammatory cell infiltration (macrophages and neutrophils), elevate cytokine levels (IL-6, TNF-α, and IL-1β), chemokine levels (MCP-1), and levels of cell adhesion molecules (ICAM-1 and VCAM-1) in the lung [13]. Additionally, HPV oncogenes E5, E6, and E7 have been associated with the development of chronic inflammation through various mechanisms [14]. Similarly, in our present study, we observed that HPV E6 significantly increased the expression of cytokines, including IL18, IL8, and TNFα, in two types of oral epithelial cells (Fig. 1). This suggests that HPV may contribute to cancer development by modulating inflammation.

Accumulating evidence indicates that IL-37 is a distinctive member of the IL-1 family of cytokines, acting as a natural suppressor of inflammatory and immune responses [15]. Notably, IL-37 has been reported to suppress pro-inflammatory cytokines (IL-1 and IL-6) and lung inflammation induced by COVID-19 [16]. Furthermore, IL-37 is recognized as a potent anti-inflammatory cytokine with anti-tumor activity against hepatocellular carcinoma (HCC) in patients infected with hepatitis B virus (HBV) [17]. In our present study, we observed that IL37 significantly mitigated HPV-induced inflammation in oral epithelial cells (Fig. 2). This aligns with findings from Wang et al., who reported that IL-37 inhibited STAT3 expression at both mRNA and protein levels [18]. Consequently, IL37 is likely to play a crucial role in the context of HPV-induced inflammation.

The phosphatidylinositol-3-kinase (PI3K)/Akt and mammalian target of rapamycin (mTOR) (PI3K/AKT/mTOR) signaling pathways play crucial roles in various cellular activities [19]. Additionally, this signaling pathway has been implicated in inflammation [20]. Li et al. reported that IL-37 induced autophagy in hepatocellular

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Fig. 4 AKT is involved in that IL37 attenuated HPV induced inflammation on oral epithelial cells. **A** IL37 dose dependently reduced expression of AKT on HOEC cells; **B** IL37 dose dependently reduced expression of AKT on IMP-H026 cells; **C** AKT KD compromised effects of IL37 on increase of IL18 induced by HPV on HOEC cell line; **D** AKT KD compromised effects of IL37 on increase of IL8 induced by HPV on HOEC cell line; **E** AKT KD compromised effects of IL37 on increase of TNFα induced by HPV on HOEC cell line; **F** AKT KD compromised effects of IL37 on increase of IL18 induced by HPV on IMP-H026 cell line; **G** AKT KD compromised effects of IL37 on increase of IL8 induced by HPV on IMP-H026 cell line; **H** AKT KD compromised effects of IL37 on increase of TNFα induced by HPV on IMP-H026 cell line

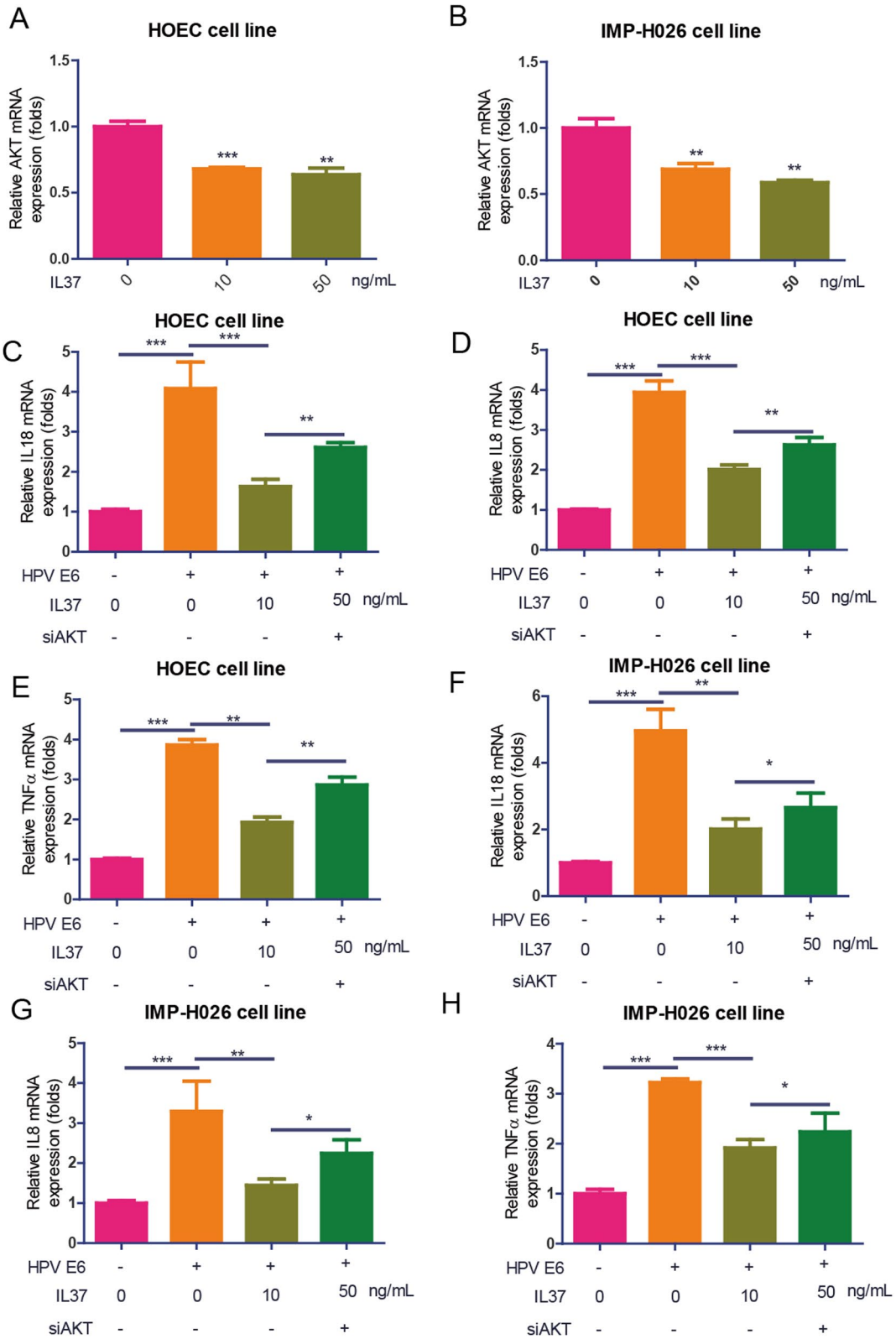

Fig. 4 (See legend on previous page.)

carcinoma cells by inhibiting the PI3K/AKT/mTOR pathway [9]. Consistent with this, our study also revealed that IL-37 could inhibit the PI3K/AKT/mTOR pathway (Fig. 3, 4, 5, and 6). Furthermore, the PI3K/AKT/mTOR signaling pathway has been closely associated with HPV-driven head and neck carcinogenesis [21]. It was also found to regulate the virus/host cell crosstalk in HPV-positive cervical cancer cells [22]. Enhancing our understanding of how the PI3K/Akt/mTOR signaling pathway contributes to the immortalization and carcinogenesis of HPV-transduced cells is crucial for developing novel strategies to prevent and treat HPV-induced cancers [23]. In our study, we demonstrated that KD of PI3K, AKT, or mTOR compromised the suppressive effect of IL37 on HPV-induced cytokine release (Fig. 3, 4, 5, and 6). Consistently, in our previous study, it was demonstrated that HPV significantly induced inflammation in both types of oral epithelial cells, and IL-37 attenuated HPV induced inflammation of oral epithelial cells via regulating autophagy [8]. Thus, our findings support the notion that IL37 suppresses HPV-induced inflammation by inhibiting the PI3K/Akt/mTOR signaling pathway.

The limitations of your work and outline future perspectives

Although the present study demonstrated that IL-37 attenuated HPV induced inflammation of oral epithelial cells via inhibiting PI3K/AKT/mTOR. Some limitations are wanted to be solved in the future studies. For example, two specific types of oral epithelial cells may not fully represent the heterogeneity of oral epithelial cells in vivo. Different subsets of oral epithelial cells, such as those from distinct anatomical locations within the oral cavity (e.g., buccal mucosa, gingiva, tongue epithelium), could potentially exhibit varying responses to HPV infection and IL-37 modulation. All investigations were conducted in vitro, which, despite allowing for precise control of variables and molecular manipulations, lacks the systemic and physiological context of the human body. Thus, in the future, exploration should aim to validate the in vitro findings in animal models. The development of small molecule agonists or enhancers of IL-37 function could be explored, along with the use of targeted inhibitors of the PI3K/AKT/mTOR pathway to optimize the cytokine's anti-inflammatory effects in the context of HPV infection.

Conclusion

In conclusion, our study revealed that HPV significantly induced inflammation in both oral epithelial cells. IL37 demonstrated an inhibitory effect on HPV-induced inflammation in oral epithelial cells. KD of PI3K compromised the anti-inflammatory effects of IL37, and similar compromising effects were observed with KD of AKT and mTOR. Notably, the mTOR inhibitor rapamycin effectively compromised the effects of IL37 on HPV-induced inflammation. These findings contribute valuable insights into a deeper understanding of the pathogenesis of HPV-induced inflammation and may

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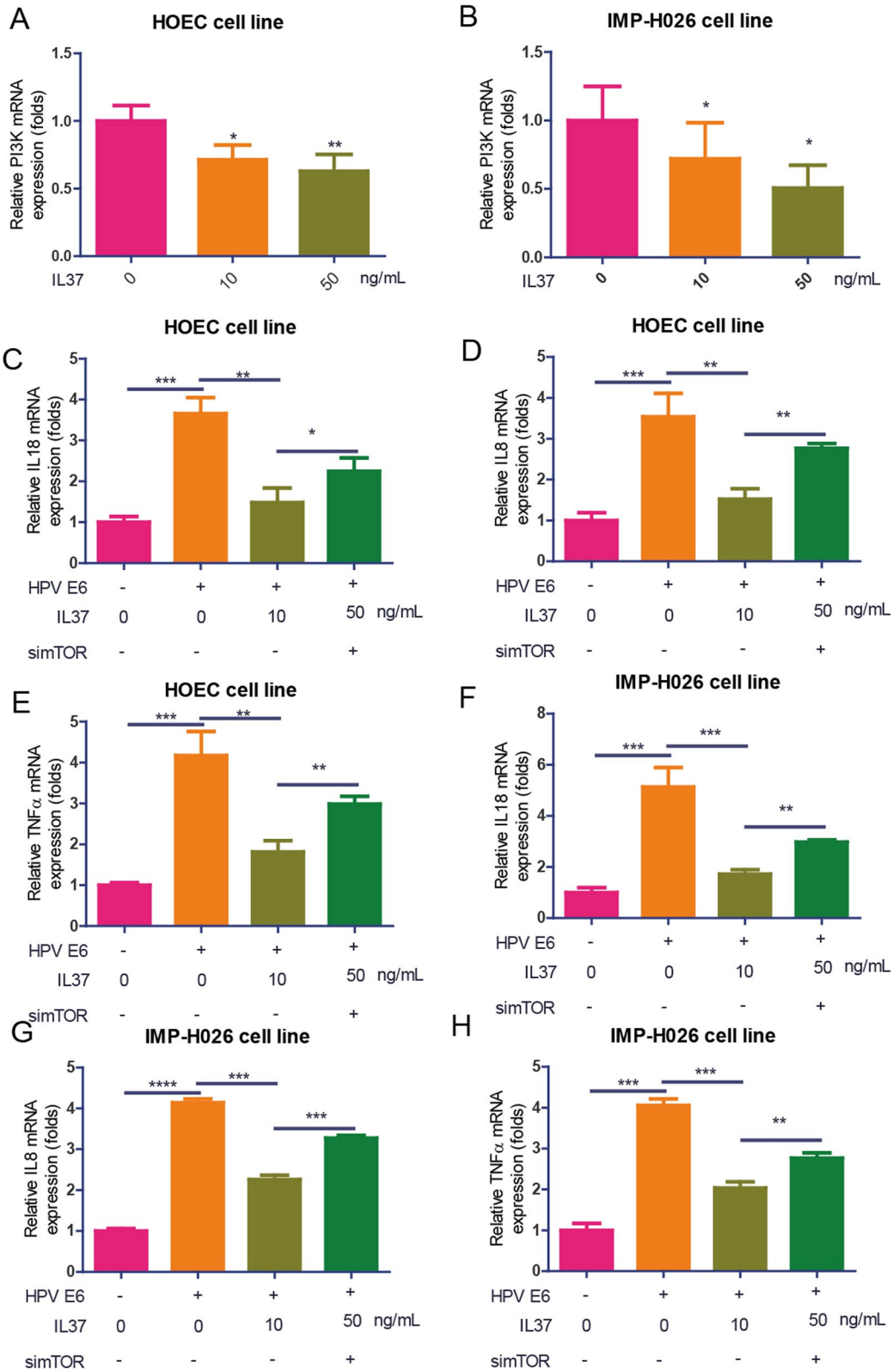
Fig. 5 mTOR is involved in that IL37 attenuated HPV induced inflammation on oral epithelial cells. **A** IL37 dose dependently reduced expression of mTOR on HOEC cells; **B** IL37 dose dependently reduced expression of mTOR on IMP-H026 cells; **C** mTOR KD compromised effects of IL37 on increase of IL18 induced by HPV on HOEC cell line; **D** mTOR KD compromised effects of IL37 on increase of IL8 induced by HPV on HOEC cell line; **E** mTOR KD compromised effects of IL37 on increase of TNFα induced by HPV on HOEC cell line; **F** mTOR KD compromised effects of IL37 on increase of IL18 induced by HPV on IMP-H026 cell line; **G** mTOR KD compromised effects of IL37 on increase of IL8 induced by HPV on IMP-H026 cell line; **H** mTOR KD compromised effects of IL37 on increase of TNFα induced by HPV on IMP-H026 cell line



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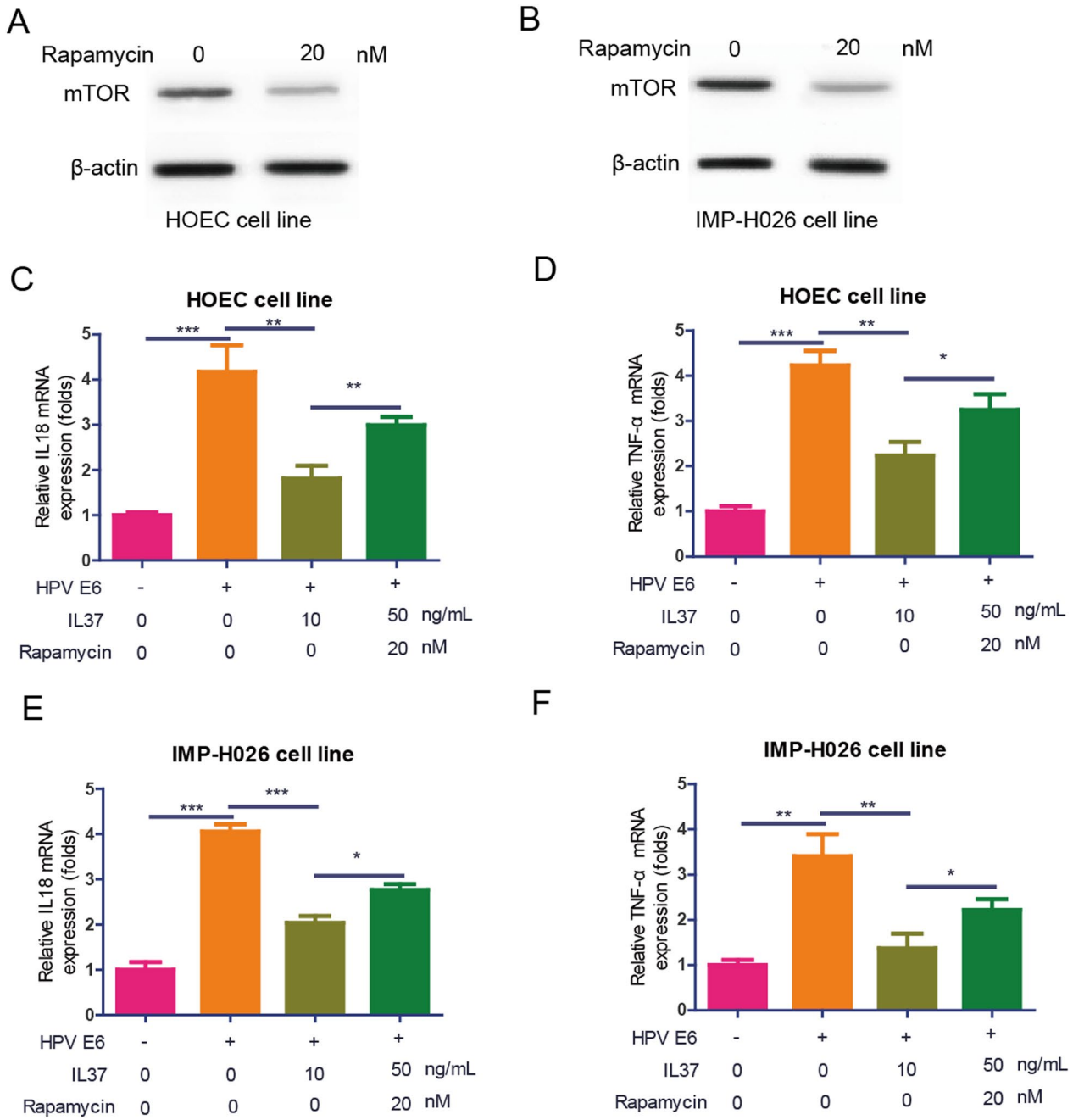

Fig. 6 mTOR inhibitor, Rapamycin compromises effects of IL37 on HPV induced inflammation on oral epithelial cells. **A** rapamycin could inhibit phosphorylated mTOR on HOEC cells; **B** Rapamycin could inhibit phosphorylated mTOR on IMP-H026 cells; **C** rapamycin compromised effects of IL37 on increase of IL18 induced by HPV on HOEC cell line; **D** rapamycin compromised effects of IL37 on increase of TNFα induced by HPV on HOEC cell line; **E** rapamycin compromised effects of IL37 on increase of IL18 induced by HPV on IMP-H026 cell line; **F** rapamycin compromised effects of IL37 on increase of TNFα induced by HPV on IMP-H026 cell line

pave the way for the development of innovative treatments for this condition.

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Author contributions
YS., NL., YB., KL., CL., and YH. performed experiments; YS., NL., and YH. designed the research; HY. And YH. wrote the manuscript; and YH. supervised the project.

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Data Availability Statement
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Competing interests
The authors declare no competing interests.

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