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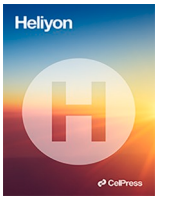

Research article

IL-37 attenuated HPV induced inflammation and growth of oral epithelial cells via regulating autophagy

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ABSTRACT

This study investigated the impact of Human Papillomavirus (HPV) on inflammation and growth in oral epithelial cells, with a focus on the role of Interleukin-37 (IL37). Oral epithelial cells, including HOEC and HSC-3 cells, were employed in the research. The results revealed that HPV significantly induced inflammation in both types of oral epithelial cells, concurrently promoting cell growth and inhibiting apoptosis. IL37, a cytokine, was found to mitigate HPV-induced inflammation in oral epithelial cells. Moreover, IL37 counteracted HPV's effects on apoptosis and cell viability in oral epithelial cells. The study also identified a reduction in autophagy in HPV-infected oral epithelial cells, a phenomenon alleviated by IL37. Furthermore, chemical inhibition of autophagy was observed to attenuate HPV-induced inflammation and growth in oral epithelial cells. These findings contribute valuable insights into the pathogenesis of inflammation in oral epithelial cells associated with HPV and oral cancers, offering potential avenues for novel therapeutic strategies.

1. Introduction

Human papillomavirus (HPV) is a small DNA virus with a particular affinity for squamous epithelia, posing a significant public health challenge (1). Identified by the International HPV Reference Center, there are currently 202 distinct HPV types, categorized into high- and low-risk groups based on their potential to cause malignant lesions in mucosal tissues [1]. The genomes of Papillomaviruses (PVs) consist of 8 kb double-stranded, circular DNA, encompassing eight protein-coding genes (L1, L2, E1, E2, E4, E5, E6, and E7) involved in various cellular processes, along with a noncoding regulatory long control region (LCR) [1]. While prophylactic vaccines against the most common oncogenic HPV types (16 and 18) are available, their uptake remains limited [2]. HPV is the causative agent of common dermatologic and sexually transmitted diseases, resulting in various benign lesions such as verrucas on the feet, common

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warts on the hands, or genital warts. HPV infects actively dividing basal epithelial cells, where its double-stranded DNA episomal genome enters the cell nuclei [3]. In the United States alone, 14 million individuals acquire HPV annually, with 79 million having prevalent infections [3]. Globally, the incidence of HPV infection is on the rise, particularly among young and sexually active individuals [3]. Despite the availability of effective vaccines, the challenges in achieving widespread vaccination coverage highlight the ongoing public health impact of HPV infections.

HPV infections have been implicated in the development of various diseases, with a significant role in carcinogenesis [3]. Following early HPV infection, this oncogenic virus integrates portions of its genome into the host cell's genome, notably incorporating two major HPV oncogenes, E6 and E7 [3]. The HPV E6 protein is known to induce the degradation of p53, resulting in the loss of p53 activity, a crucial factor in cancer prevention [3]. HPV has also been associated with alterations in vaginal microbiota among Chinese women [4]. The global impact of HPV infection is substantial, causing an estimated half a million incident cervical cancers and 60,000 incident non-cervical anogenital cancers annually worldwide [5]. Oral HPV infection is typically acquired through oral-genital contact and can persist, leading to various health implications [3]. In recent years, it has become evident that human papillomaviruses (HPV) are not solely responsible for genital and anal cancers but also play a role in other diseases and conditions. The multifaceted impact of HPV underscores the importance of understanding and addressing the diverse health consequences associated with this viral infection.

Accumulating evidence suggests a close association between HPV and inflammation. Studies have reported that HPV can elevate the expression levels of IFN-γ mRNA in oral cancer patients [6]. Additionally, HPV infection has been shown to increase the production of IL-6, potentially contributing to the progression of cervical cancer by activating the oncogene signal transducer and activator of transcription 3 (STAT3) [6]. Investigations have demonstrated that HPV infection leads to an increased count of macrophages in cervical epithelium, exacerbating inflammation and worsening the severity of HPV infection-induced lesions [7]. Interestingly, there are reports indicating that HPV infection might induce atheroma formation in infected patients, either by enhancing systemic inflammation or by directly targeting blood vessels through nucleic acids carried by extracellular vesicles such as exosomes [7]. Therefore, understanding the mechanisms through which HPV infections influence inflammation is crucial for unraveling the intricate relationship between HPV and various health outcomes.

The intricate interplay between the heightened inflammatory response and the augmented cell proliferation observed in human papillomavirus (HPV) infection of oral epithelial cells delineates a multifaceted landscape within the pathogenesis of HPV-associated oral diseases [8]. Upon HPV infection, the oral epithelium undergoes a cascade of immunological events, marked by the release of an array of pro-inflammatory cytokines, chemokines, and immune mediators [9]. This inflammatory microenvironment, orchestrated by the host immune system in response to viral invasion, not only facilitates viral replication and persistence but also fosters conditions conducive to aberrant cell proliferation [10]. The viral oncoproteins, particularly E6 and E7, wield remarkable influence over the intricate machinery of the host cell, disrupting key checkpoints in cell cycle regulation, promoting uncontrolled cell proliferation, and thwarting apoptotic pathways [10]. Concurrently, the persistent inflammatory milieu instigated by HPV infection perpetuates a cycle of cellular damage and repair, wherein inflammatory mediators fuel the proliferation of damaged cells [10]. This intricate interplay between inflammation and cell proliferation emerges as a hallmark feature of HPV-associated oral epithelial pathologies, unveiling potential avenues for targeted therapeutic interventions aimed at modulating the inflammatory microenvironment and restoring cellular homeostasis.

Accumulating literature suggests that HPV can infect the oral mucosa [11]. While tobacco has traditionally been considered a significant factor in the pathogenesis of oral squamous cell carcinoma (OSCC), recent research has identified HPV infections as a risk factor for OSCC [11]. The viral infection may lead to localized clinical, subclinical, or latent lesions [12]. Studies have indicated that the presence of anogenital HPV types 6/11 and 16/18 in the oral mucosa may indicate orogenital transmission, making this virus a potentially important cofactor in the development of oral cancer [12]. Therefore, investigating oral infections of HPV is of interest to develop effective strategies for treating viral infections.

As a new member of the IL1 gene family and a potent inhibitor of innate immune responses, IL37 has been extensively studied for its role in inflammation and various diseases [13]. Accumulating evidence suggests that IL-37 can reduce systemic and local inflammation and Th2 cytokines by inhibiting the production of pro-inflammatory mediators and cytokines in both innate and adaptive immunity [13]. In human THP-1 monocyte and A549 epithelial cell lines, IL37 has been shown to significantly decrease IL1β-induced expression of IL1α, IL8, and TNFα [14]. Additionally, transgenic mice expressing IL37 have demonstrated protective effects against lipopolysaccharide (LPS)-induced endotoxic shock [14]. In arthritis models and patients, IL37 has been found to inhibit the secretion of inflammatory cytokines, including IL1β, IL6, and IL17, in peripheral blood mononuclear cells (PBMCs) [15]. Therefore, IL37 represents an attractive target for investigating the pathogenesis of HPV infection in oral cells.

In the present study, the focus was on examining the impact of IL37 on HPV-induced inflammation in two types of oral cells, namely the HOEC cell line and HSC-3 cell line. The study delved into dysfunctions such as inflammation and apoptosis triggered by HPV infections in oral cells. Furthermore, the investigation aimed to understand the effects of IL37 on inflammation and apoptosis induced by HPV infections. Mechanistic insights into how IL37 regulates inflammation and apoptosis induced by HPV infections were also explored.

2. Materials and methods

2.1. Transfection of HPV E6 on oral epithelial cells

HPV-16 E6 plasmids were obtained from Addgene (Plasmid #8641). HOEC and HOK Cells were cultured at 37 °C in an atmosphere of 5 % CO2. Cells were transfected with lipofectamine 3000 (Invitrogen, Carlsbad, CA) for plasmid transfection.

2.2. Cell culture

Two types of oral cells, including HOEC and HSC-3, were obtained from the Bio-resource Center of The Fourth Hospital of Hebei Medical University. These cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Sigma-Aldrich, catalog number: D6429-500 ML) supplemented with 10 % Fetal Bovine Serum (FBS, Sigma-Aldrich, catalog number: MFCD00132239), 1 % L-glutamine (Invitrogen, catalog number: 25030-081), and 1 % penicillin-streptomycin solution (PS, Sigma-Aldrich, catalog number: V900929-100 ML) at 37 °C in a 5 % CO2 incubator. Sub-culturing was performed when the cells reached 80–90 % confluence. Regular testing for Mycoplasma using the MycoAlert Plus Kit (Lonza) ensured that the cells were free from mycoplasma contamination.

2.3. Western blotting

HOEC and HSC-3 cells were lysed using RIPA Lysis Buffer (Beyotime Biotechnology, Shanghai, China, catalog number: P0013B). The obtained samples were boiled at 95 °C with SDS/PAGE sample buffer (containing 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. Following the separation, PVDF membranes (Beyotime Biotechnology) were blocked using 5 % non-fat milk in TBST buffer. Subsequently, the membranes were incubated with primary antibodies (diluted at 1:1000) including anti-ATG8 (Abcam, catalog number: ab4753), anti-LC3A/B (Cell Signaling Technology, catalog number: #4108), and anti-beta-actin antibody (Abcam, catalog number: mAbcam 8226) **at 4°C overnight.** Afterward, the immunoblots were visualized using horseradish-peroxidase-coupled secondary antibodies (diluted at 1:5000, HRP-labeled goat anti-rabbit IgG [H + L], Beyotime Biotechnology, catalog number: A0208) at room temperature for 1 h, and the signal was developed using BeyoECL Plus (Beyotime Biotechnology, catalog number: P0018S).

2.4. Flow cytometry analysis of Annexin V/Propidium iodide staining

HOEC and HSC-3 cells were plated in 6-well plates (Costar; 150,000 cells/well) and allowed to reach 60–70 % confluence. Subsequently, the cells were treated with HPV E6 and IL37 for 24 h. After the treatment period, cells were detached using trypsin/EDTA and stained using the FITC Annexin V Apoptosis Detection Kit I (BD Pharmingen #556547). Flow cytometry analysis was performed using a FACSCalibur instrument (BD) to assess the results.

2.5. Cytokines detected by enzyme-linked immunosorbent assay (ELISA)

The concentrations of cytokines, including IL-6, IL-1β, and IL-10, in cell culture supernatants were quantified using ELISA kits (Thermo Fisher, catalog numbers: IL6 - EH2IL6, IL1β - BMS224HS, IL10 - BMS215-2). All assay procedures were conducted in accordance with the manufacturer's instructions. Absorbance values of standards and samples were measured at 450 nm (with a reference wavelength of 540 nm) using a VICTOR Nivo Multimode Microplate Reader.

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

Total RNA isolation from both cell lines was carried out using TRIzol (Beyotime Biotechnology) following the manufacturer's protocol. The BeyoRT™ First Strand cDNA Synthesis Kit (catalog number: D7166, Beyotime Biotechnology) was employed for cDNA synthesis from the isolated total RNA. RT-qPCR was performed 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

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

Gene	Primer	Sequence	Product length (nt)
IL6	Sense	CCGGGAACGAAAGAGAAGCTC	75
	Anti-sense	ACCGAAGGCGCTTGTGGAG	
IFNb1	Sense	AGTAGGCGACACTGTTCGTG	173
	Anti-sense	GCCTCCCATTCAATTGCCAC	
IL10	Sense	GGCACCCAGTCTGAGAACAG	176
	Anti-sense	TGGCAACCCAGGTAACCCTTA	
GAPDH	Sense	AATGGGCAGCCGTTAGGAAA	168
	Anti-sense	GCGCCCAATACGACCAAATC	
CASP9	Sense	AGGCCCCATATGATCGAGGA	193
	Anti-sense	TCGACAACTTTGCTGCTTGC	
CASP7	Sense	GAGCAGGGGGTTGAGGATTC	241
	Anti-sense	TTCCGTTTCGAACGCCCATA	
CASP3	Sense	TGCTATTGTGAGGCGGTTGT	112
	Anti-sense	TCACGGCCTGGGATTTCAAG	
ATG8	Sense	CCACTCGCTGGAACACAGAT	75
	Anti-sense	ACAATCACCGGAACCCTGTC	
P62	Sense	GAGCGAGTGCTGATACCTGT	105
	Anti-sense	ACCAGTCTCTTCCTGCAGTC	

dehydrogenase (GAPDH) was utilized as an internal control. The primers employed in the study are listed in Table 1.

2.7. Measurement of cytotoxicity using cell counting Kit-8 (CCK8) assay

The Cell Counting Kit-8 (Cat NO: C0037, Beyotime Technology, Beijing, China) was employed to measure cytotoxicity in both cell lines following the manufacturer's instructions. Briefly, the HSC-6 cell line and CAL-27 cell line were seeded into 96-well plates at a cell density of 5 × 104/mL overnight. After 24 h, the cell culture medium was replaced with the indicated concentrations of chemicals, and

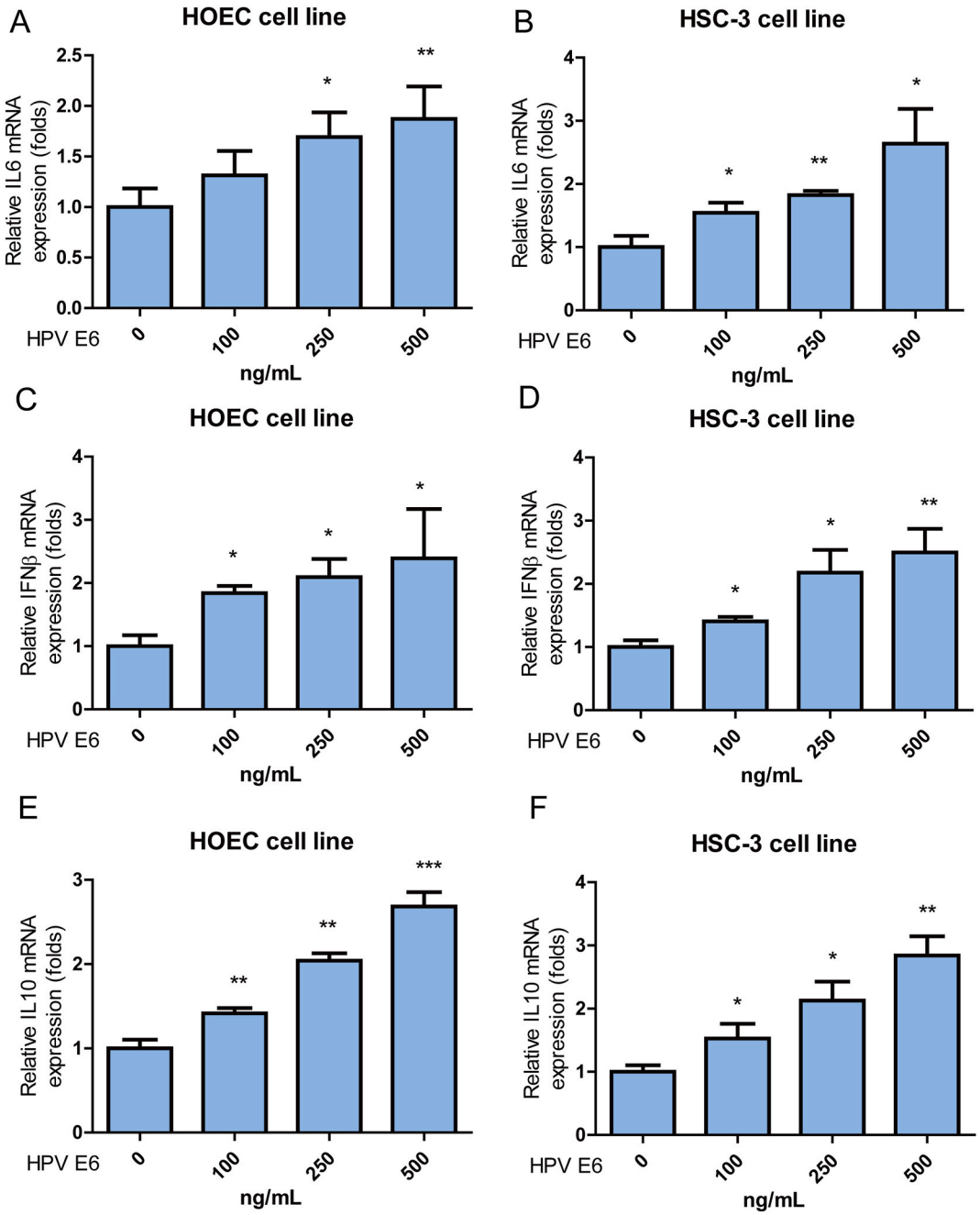

Fig. 1. HPV E6 significantly induces inflammation in oral cells. (A) HPV E6 significantly increased expression of IL6 in HOEC cells in dose dependent manner; (B) HPV E6 significantly increased expression of IFNβ in HOEC cells in dose dependent manner; (C) HPV E6 significantly increased expression of IL10 in HOEC cells in dose dependent manner; (D) HPV E6 significantly increased expression of IL6 in HSC-3 cells in dose dependent manner; (E) HPV E6 significantly increased expression of IFNβ in HSC-3 cells in dose dependent manner; (F) HPV E6 significantly increased expression of IL10 in HSC-3 cells in dose dependent manner.

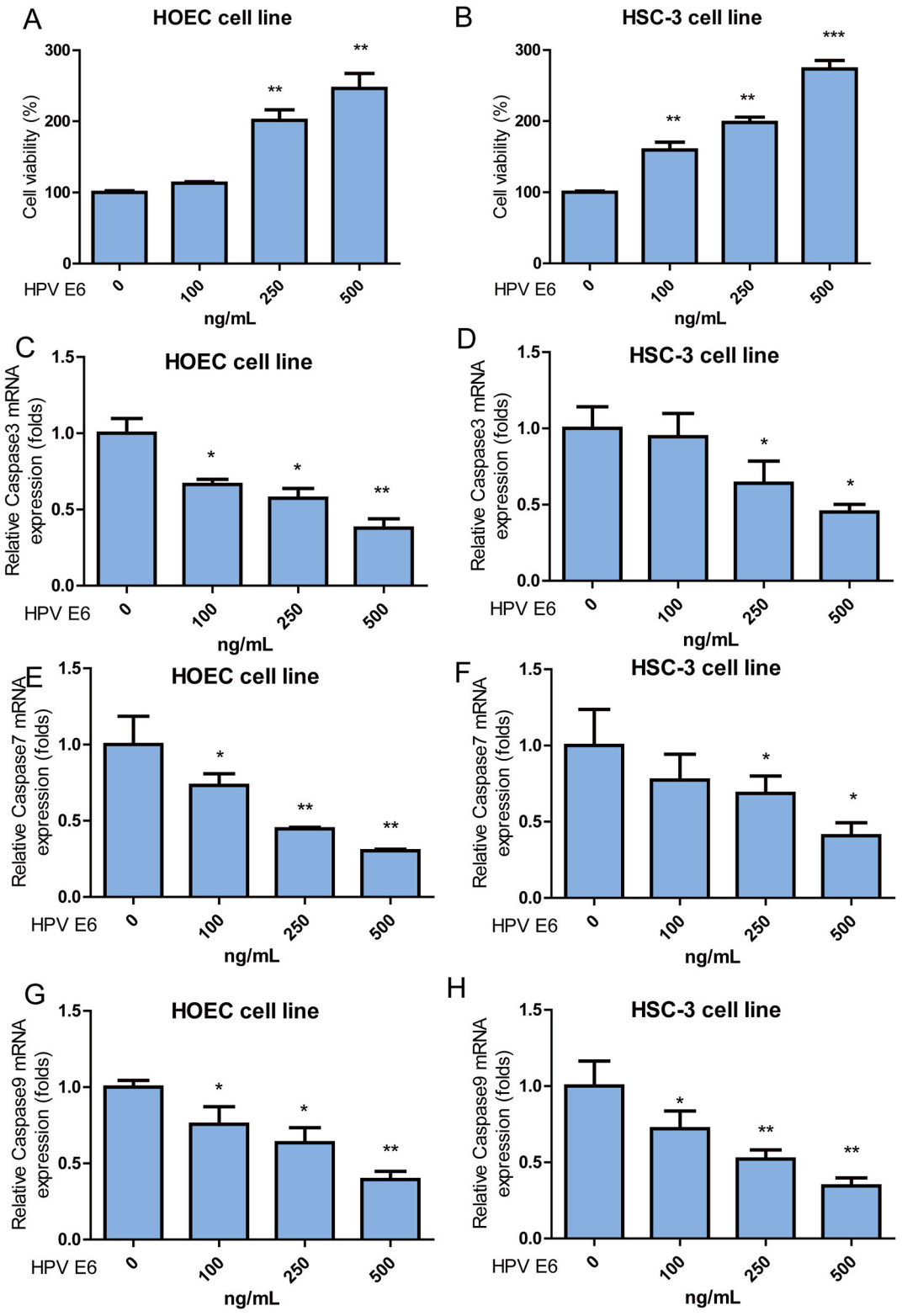

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Fig. 2. HPV E6 significantly promoted growth and decreased apoptosis of oral epithelial cells. (A) HPV E6 significantly increased cell viability of HOEC cells in a dose dependent manner; (B) HPV E6 significantly increased cell viability of HSC-3 cells in a dose dependent manner; (C) HPV E6 significantly decreased expression level of caspase 3 on HOEC cells in a dose dependent manner; (D) HPV E6 significantly decreased expression level of caspase 3 on HSC-3 cells in a dose dependent manner; (E) HPV E6 significantly decreased expression level of caspase 7 on HOEC cells in a dose dependent manner; (F) HPV E6 significantly decreased expression level of caspase 7 on HOEC cells in a dose dependent manner; (G) HPV E6 significantly decreased expression level of caspase 9 on HOEC cells in a dose dependent manner; (H) HPV E6 significantly decreased expression level of caspase 9 on HOEC cells in a dose dependent manner.

the treatment was continued for 48 h. Subsequently, 100 μL of CCK8 solution (0.5 mg/mL) was added to each well, incubated for 3 h, and the optical density (OD) values were detected at 450 nm using the Infinite M200 PRO Multimode Microplate Reader (Tecan, Männedorf, Switzerland). The viability of living cells was calculated as a percentage of the control.

2.8. Statistical analysis

All data are presented as mean ± SEM. Statistical analysis of continuous variables was conducted using one-way ANOVA followed by Tukey's post hoc test. For categorical variables, Fisher's exact tests were employed. The statistical analysis was performed with Prism GraphPad software (GraphPad Prism 5.0). A p-value less than 0.05 was considered to indicate statistically significant differences.

3. Results

3.1. HPV E6 significantly induces inflammation in oral cells

To assess the impact of HPV infections on oral cells, HOEC cells were exposed to varying concentrations of HPV E6. The results revealed a substantial upregulation of inflammation-related genes, namely IL6 (Fig. 1A), IFNβ (Fig. 1C), and IL10 (Fig. 1E), in a dose-dependent manner in HOEC cells. Similarly, the treatment with HPV E6 led to a significant elevation in the expression of inflammation genes, including IL6 (Fig. 1B), IFNβ (Fig. 1D), and IL10 (Fig. 1F), in HSC-3 cells in a dose-dependent manner. Collectively, these findings demonstrate that HPV E6 significantly induces inflammation in oral cells.

3.2. HPV E6 significantly promoted growth and decreased apoptosis of oral epithelial cells

To assess the impact of HPV infections on the apoptosis of oral cells, HOEC cells were treated with various concentrations of HPV E6. The results indicated a significant increase in cell viability in a dose-dependent manner (Fig. 2A). Similarly, treatment with HPV E6 led to a dose-dependent increase in cell viability in HSC-3 cells (Fig. 2B). Further exploration of the effects of HPV E6 on apoptosis markers, including caspase 3, caspase 7, and caspase 9, revealed a significant decrease in the expression levels of caspase 3 (Fig. 2C and D), caspase 7 (Fig. 2E and F), and caspase 9 (Fig. 2G and H) in both HOEC and HSC-3 cells. These findings collectively demonstrate that HPV E6 significantly induces apoptosis in oral cells.

3.3. IL37 attenuated HPV induced inflammation on oral epithelial cells

To investigate the effects of IL37 on HPV-induced inflammation in oral epithelial cells, both HOEC and HSC-3 cells were co-treated with HPV E6 and IL37. The results showed that IL37 at concentrations of 5 and 20 ng/mL significantly attenuated the HPV E6-induced increase in IL17 protein levels in both HOEC (Fig. 3A) and HSC-3 (Fig. 3B) cells, although IL37 per se did influence protein level of IL17 in HOEC and HCS cells (Fig. 3A and B). Additionally, IL37 at the same concentrations significantly mitigated the HPV E6-induced elevation of IL6 protein levels in HOEC (Fig. 3C) and HSC-3 (Fig. 3D) cells, although IL37 per se did influence protein level of IL17 in HOEC and HCS cells (Fig. 3C and D). Similarly, IL37 (5 and 20 ng/mL) significantly attenuated the increase in TNFα protein levels induced by HPV E6 in both HOEC (Fig. 3E) and HSC-3 (Fig. 3F) cells, although IL37 per se did influence protein level of TNFα in HOEC and HCS cells (Fig. 3C and D). Collectively, these findings demonstrate that IL37 effectively reduces HPV-induced inflammation in oral epithelial cells.

3.4. IL37 attenuated HPV promoted growth and decreased apoptosis on oral tumor cells

To investigate the effects of IL37 on HPV-induced apoptosis in oral epithelial cells, both HOEC and HSC-3 cells were co-treated with HPV E6 and IL37. The results showed that IL37 at concentrations of 5 and 20 ng/mL significantly attenuated the HPV E6-induced decrease in caspase 3/7 activity in both HOEC (Fig. 4A) and HSC-3 (Fig. 4B) cells, although IL37 per se did influence caspase 3/7 activity in HOEC and HCS cells (Fig. 4A and B). Furthermore, IL37 (5 and 20 ng/mL) significantly mitigated the HPV E6-induced increase in apoptosis in HOEC (Fig. 4C) and HSC-3 (Fig. 4D) cells, although IL37 per se did influence apoptosis in HOEC and HCS cells (Fig. 4C and D). To further confirm the effects of IL37 on HPV E6-induced apoptosis, fluorescence-activated cell sorting (FACS) was employed to evaluate apoptosis in a mixed culture of HOEC and HSC-3 cells. It was found that IL37 (20 ng/mL) significantly attenuated the HPV E6-induced decrease in apoptosis in the mixed culture of HOEC and HSC-3 cells (Fig. 4E). Overall, these results demonstrate that IL37 effectively reduces HPV-induced apoptosis in oral epithelial cells.

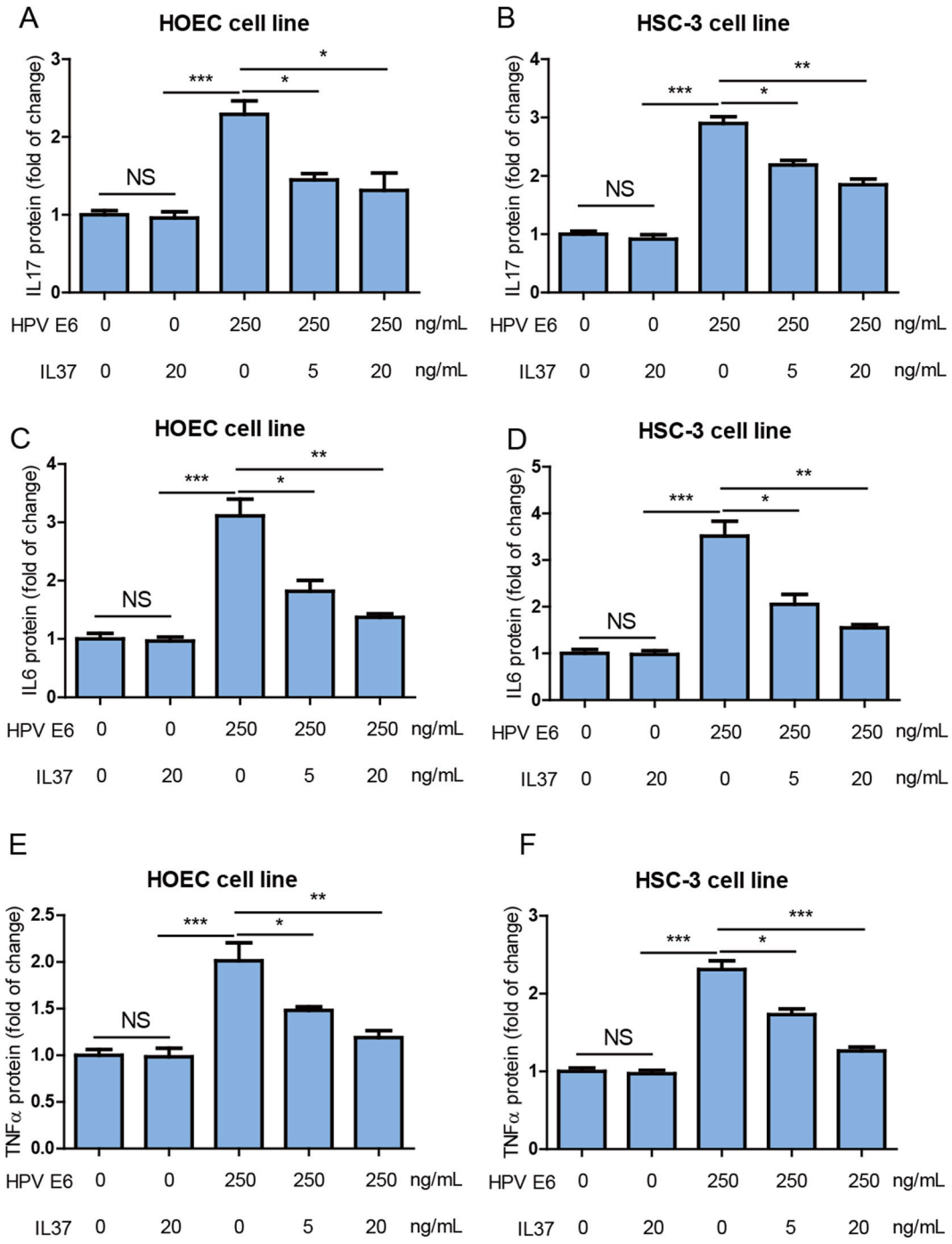

Fig. 3. IL37 attenuated HPV induced inflammation on oral epithelial cells. (A)
IL37 (5 and 20 ng/mL) significantly attenuated HPV E6 induced increase of IL17 protein level on HOEC cells; (B) IL37 (5 and 20 ng/mL) significantly attenuated HPV E6 induced increase of IL17 protein level on HSC-3 cells. (C) IL37 (5 and 20 ng/mL) significantly attenuated HPV E6 induced increase of IL6 protein level on HOEC cells; (D) IL37 (5 and 20 ng/mL) significantly attenuated HPV E6 induced increase of IL6 protein level on HSC-3 cells; (E) IL37 (5 and 20 ng/mL) significantly attenuated HPV E6 induced increase of TNFα protein level on HOEC cells (F) IL37 (5 and 20 ng/mL) significantly attenuated HPV E6 induced increase of TNFα protein level on HSC-3 cells.

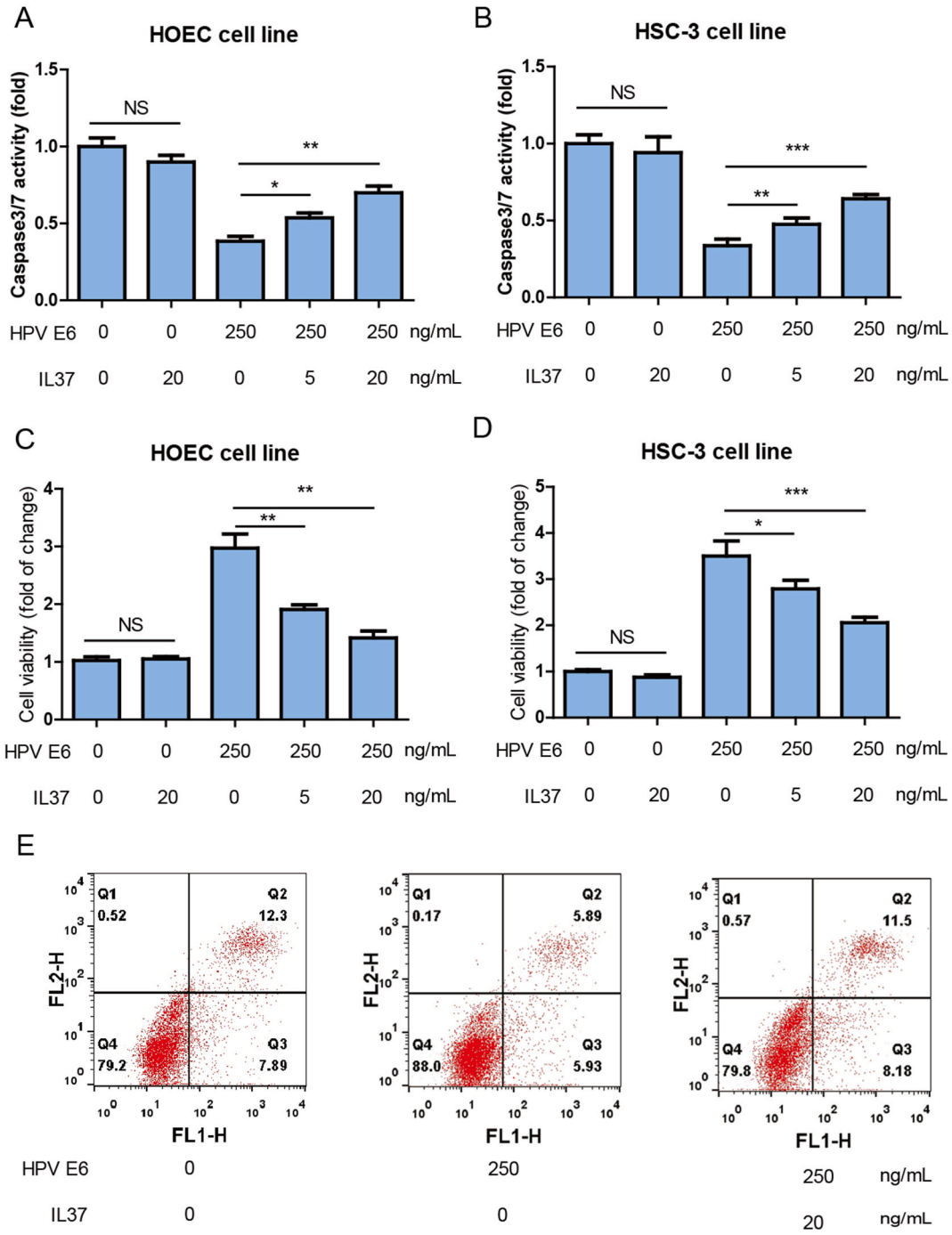

Fig. 4. IL37 attenuated HPV promoted growth and decreased apoptosis on oral tumor cells. (A) IL37 (5 and 20 ng/mL) significantly attenuated HPV E6 induced decrease of casepase 3/7 activity on HOEC cells; (B) IL37 (5 and 20 ng/mL) significantly attenuated HPV E6 induced decrease of casepase 3/7 activity on HSC-3 cells; (C) IL37 (5 and 20 ng/mL) significantly attenuated HPV E6 induced increase of apoptosis on HOEC cells. (D) IL37 (5 and 20 ng/mL) significantly attenuated HPV E6 induced increase of apoptosis on HSC-3 cells; (E) IL37 (20 ng/mL) significantly attenuated HPV E6 induced decrease of apoptosis on HOEC and HSC-3 mixed cells evaluated by FACS.

3.5. IL37 attenuated HPV diminished autophay on oral epithelial cells

Given the tight regulation between autophagy and apoptosis [16], we investigated the effects of HPV on autophagy and the modulatory effects of IL37. It was observed that HPV E6 significantly decreased the expression of autophagy markers, including ATG8 (Fig. 5A and B) and P62 (Fig. 5C and D), in both HOEC and HSC-3 cells, although IL37 per se did influence expression level of ATG8

(Fig. 5A and B) and P62 (Fig. 5C and D) in both HOEC and HCS cells. Notably, IL37 (5 and 20 ng/mL) significantly attenuated the HPV E6-induced reduction in ATG8 mRNA expression levels in HOEC (Fig. 5A) and HSC-3 (Fig. 5B) cells. Furthermore, IL37 (5 and 20 ng/mL) significantly mitigated the HPV E6-induced decrease in P62 mRNA expression levels in HOEC (Fig. 5C) and HSC-3 (Fig. 5D) cells. To further validate the effects of IL37 on HPV E6-induced autophagy, Western blot analysis was conducted to assess the impact of IL37 on HPV E6-induced autophagy. It was found that IL37 significantly attenuated the decrease in ATG8 protein levels induced by HPV E6 in both HOEC cells (Fig. 5E) and HSC-3 cells (Fig. 5F). Overall, these findings demonstrate that IL37 diminishes HPV-induced autophagy in oral epithelial cells.

3.6. Increase of inflammation and decrease of apoptosis induced by HPV was through authopahgy

Since we have demonstrated that HPV could induce authopahgy, which provoked us Having established that HPV induces autophagy, we further investigated whether the effects of HPV on inflammation and apoptosis in oral epithelial cells were mediated through autophagy. Autophagy inhibitor 3-Methyladenine (3-MA) was employed, and it was found that 3-MA significantly inhibited the HPV E6-induced increase in the expression of IL17A on HOEC cells (Fig. 6A) and HSC-3 cells (Fig. 6B). Similarly, 3-MA significantly inhibited the HPV E6-induced increase in the expression of IL6 on HOEC cells (Fig. 6C) and HSC-3 cells (Fig. 6D). Furthermore, 3-MA significantly inhibited the HPV E6-induced increase in the expression of TNFα on HOEC cells (Fig. 6E) and HSC-3 cells (Fig. 6F). Additionally, 3-MA significantly inhibited the HPV E6-induced decrease in caspase3/7 activity on HOEC cells (Fig. 6G) and HSC-3 cells (Fig. 6H). Collectively, these findings demonstrate that the increase in inflammation and decrease in apoptosis induced by HPV are

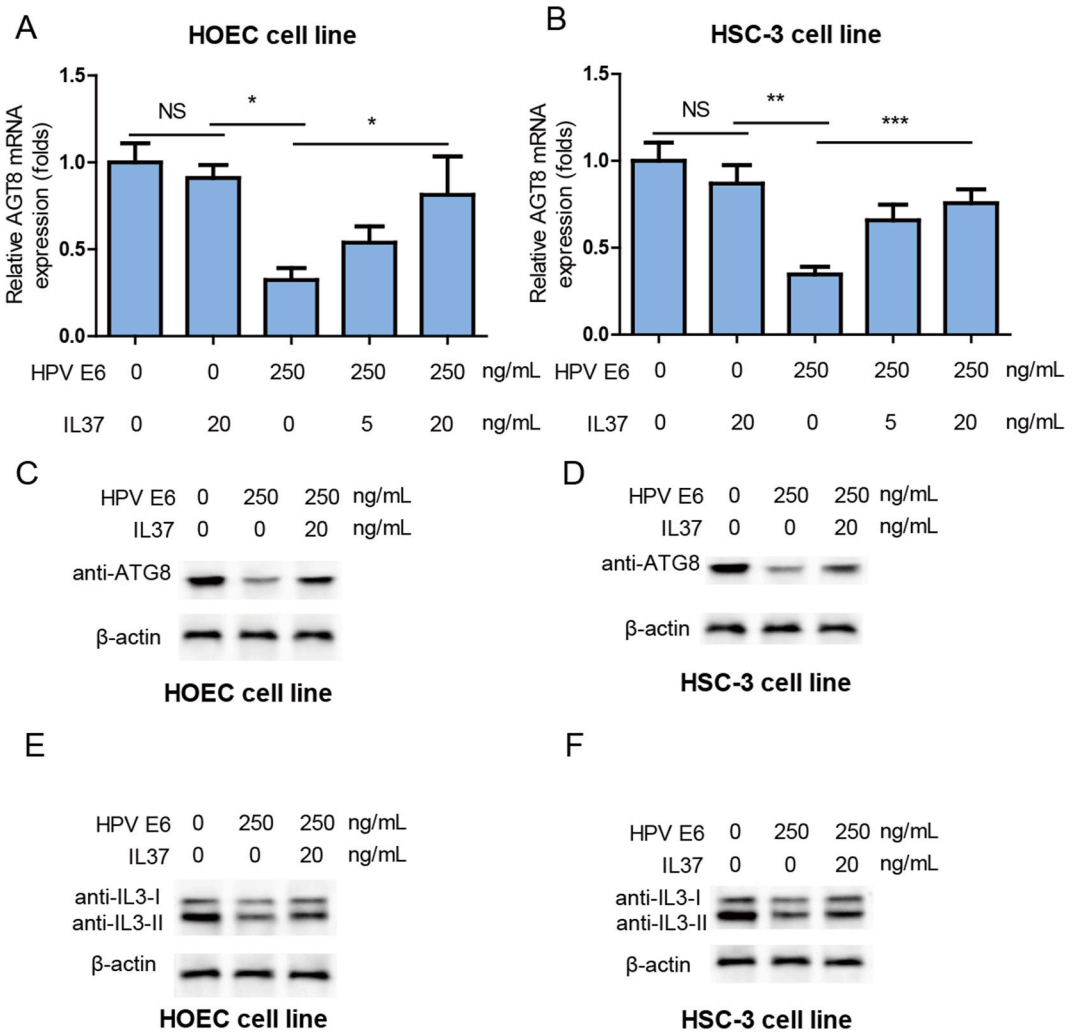

Fig. 5. IL37 attenuated HPV diminished autophay on oral epithelial cells. (A) HPV E6 significantly decreased expression of ATG8 on HOEC cells; (B) HPV E6 significantly decreased expression of P62 on HOEC cells; (C) HPV E6 significantly decreased expression of ATG8 on HSC-6 cells; (D) HPV E6 significantly decreased expression of P62 on HSC-3 cells; (E) HPV E6 significantly decreased protein level of ATG8 on HOEC cells; (F) HPV E6 significantly decreased protein level of ATG8 on HSC-3 cells.

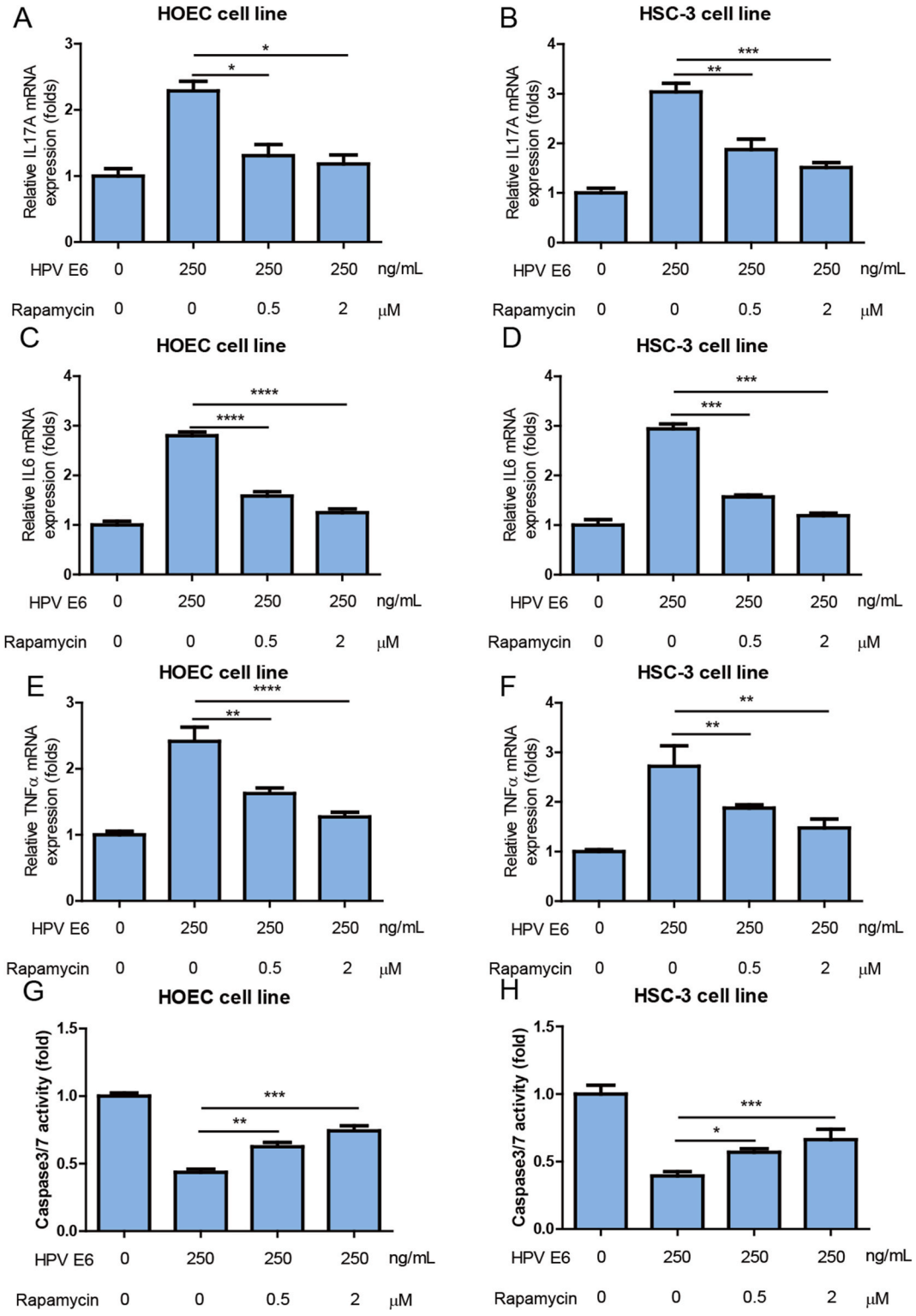

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Fig. 6. Increase of inflammation and decrease of apoptosis induced by HPV was through authopahgy. (A) Autophagy inhibitor 3-Methyladenine (3-MA) significantly inhibited HPV E6 induced increase of expression level IL17A on HOEC cells; (B) Autophagy inhibitor 3-Methyladenine (3-MA) significantly inhibited HPV E6 induced increase of expression level IL17A on HSC-3 cells; (C) Autophagy inhibitor 3-Methyladenine (3-MA) significantly inhibited HPV E6 induced increase of expression level IL6 on HOEC cells; (D) Autophagy inhibitor 3-Methyladenine (3-MA) significantly inhibited HPV E6 induced increase of expression level IL6 on HSC-3 cells; (E) Autophagy inhibitor 3-Methyladenine (3-MA) significantly inhibited HPV E6 induced increase of expression level TNFα on HOEC cells; (F) Autophagy inhibitor 3-Methyladenine (3-MA) significantly inhibited HPV E6 induced increase of expression level TNFα on HSC-3 cells; (G) Autophagy inhibitor 3-Methyladenine (3-MA) significantly inhibited HPV E6 induced decrease of caspase3/7 activity on HOEC cells; (H) Autophagy inhibitor 3-Methyladenine (3-MA) significantly inhibited HPV E6 induced decrease of caspase3/7 activity on HSC-3 cells.

mediated through autophagy.

4. Discussion

Human papillomavirus (HPV)-induced cancers are anticipated to pose a significant global health challenge for several decades [17]. Chronic inflammation is closely linked to recurrent tissue damage and the emergence of mutations in crucial tumor suppressor genes, thereby contributing to regulated tumorigenesis [17]. Exploring the impact of HPV on inflammation and tumor growth becomes particularly interesting. In the current study, it was demonstrated that HPV E6 significantly induced inflammation in oral cells while also decreasing apoptosis and promoting the growth of oral epithelial cells. Intriguingly, IL37 was found to mitigate HPV-induced inflammation, counteract the promotion of growth, and alleviate the reduction in apoptosis in oral epithelial cells. Furthermore, IL37 was observed to attenuate HPV-induced autophagy in oral epithelial cells, suggesting that the increase in inflammation and decrease in apoptosis induced by HPV are mediated through autophagy. Therefore, the findings of the present study may offer valuable insights into understanding the oncogenic mechanisms of HPV and aid in the development of novel therapies for oral cancers.

HPV infections are considered to be closely linked to various tumors, including cervical cancers, head and neck cancers (HNCs), and oropharyngeal cancer [18]. A substantial portion of the population has been identified as HPV positive, and HPV-positive cancers affecting the oral cavity and oropharynx are recognized as distinct pathological conditions necessitating specific management approaches, including the application of appropriate treatment regimens and protocols [19]. Schäfer et al. found that HPV DNA was present in approximately 9 % of patients with esophageal squamous cell carcinoma (OSCC), primarily involving the oncogenic type HPV18. In this context, several inflammatory markers, such as IL6 and IL8, were found to be elevated in HPV-infected OSCC [20]. In cervical cancer, it has been observed that HPV infections induce a local Th2 inflammation at an early stage, involving antibody-forming cells, and create an immunosuppressive microenvironment that supports tumor progression [21]. Similarly, in the present study, it was noted that HPV induced inflammation in oral epithelial cells (Fig. 1). Mounting evidence suggests that inflammation is often associated with the development and progression of cancer [22]. Intriguingly, our findings revealed that HPV increased the cell viability of oral epithelial cells (Fig. 2), indicating that HPV might contribute to the tumorigenesis of oral cancer by inducing inflammation.

IL-37 is a distinctive member of the IL-1 family of cytokines, acting as a natural suppressor of inflammatory and immune responses [23]. Notably, IL-37 has been identified as a mitigator of inflammation induced by coronavirus-19 (COVID-19) [24]. Sun et al. demonstrated that IL-37 reduces coxsackievirus B3-induced viral myocarditis by inhibiting NLRP3 inflammasome-mediated pyroptosis [25]. In the present study, our findings revealed that IL-37 mitigated HPV-induced inflammation in oral epithelial cells (Fig. 3). Furthermore, it was observed that IL-37 attenuated the HPV-induced growth of oral epithelial cells (Fig. 4).

Autophagy plays a crucial role in both viral infections and cancers [26]. Antonioli et al. demonstrated that HPV promotes the growth of oropharyngeal squamous cell carcinoma (OPSCC) cells by suppressing autophagy through E7-mediated degradation of AMBRA1 [27]. Additionally, Nie et al. found that HPV E6 enhances cell proliferation in cervical cancer cells by accelerating the accumulation of RBM15, which is dependent on suppressing autophagy [28]. In line with these findings, our study revealed that HPV E6 significantly inhibited autophagy in oral epithelial cells (Fig. 5). Furthermore, IL37 was found to attenuate HPV-induced suppression of autophagy in oral epithelial cells (Fig. 5). Additionally, chemical inhibition of autophagy effectively mitigated HPV-induced inflammation and promoted the growth of oral epithelial cells (Fig. 6), suggesting that the effects of HPV on inflammation and growth of oral epithelial cells might be mediated through the suppression of autophagy.

In summary, our study demonstrated that HPV significantly induced inflammation in oral epithelial cells, promoting cell growth and suppressing apoptosis. The cytokine IL37 effectively attenuated HPV-induced inflammation and mitigated the reduction in apoptosis, counteracting the promotion of cell viability induced by HPV. Moreover, HPV was found to inhibit autophagy in oral epithelial cells, and IL37 was effective in attenuating this autophagy suppression. Importantly, the chemical inhibition of autophagy demonstrated efficacy in mitigating HPV-induced inflammation and promoting the growth of oral epithelial cells. These findings provide valuable insights for a deeper understanding of the pathogenesis of oral cancer and may contribute to the development of novel therapeutic strategies for the disease.

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Ethics approval and consent to participate

Not Applicable.

Patient consent for publication

Not applicable.

Data availability statement

Raw data were generated at [The Fourth Hospital of Hebei Medical University]. Derived data supporting the findings of this study are available from the corresponding author [N.L.] on request.

CRediT authorship contribution statement

Yahong Shi: Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Wenjing Wang:** Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yunfang Bai:** Methodology, Investigation, Conceptualization. **Xiaoying Liu:** Data curation, Conceptualization. **Liwei Wu:** Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ning Liu:** Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no competing financial interests.

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35131.

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