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HPV11E6/E7 induces nasal epithelial hyperplasia through JAK2/STAT3 signaling pathway

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Abstract

Objectives Nasal mucosal epithelial hyperplasia can cause nasal hyperplastic diseases, more studies have confirmed that different subtypes of HPV infection play a significant role in nasal proliferative diseases, especially nasal inverted papilloma (NIP). This study aims to elucidate the role and mechanism of the HPV11 subtype in regulating nasal epithelial hyperplasia.

Methods In our previous study, the expression of HPV infection in NIP was analyzed by Flow-through hybridization and gene chip (HybridMax), with the highest expression rate observed for the HPV11 subtype. Therefore, we aimed to overexpress HPV11E6/E7 in nasal mucosal epithelial cells (HNEpC) to verify the regulatory role and mechanism of HPV11 in nasal epithelial hyperplasia at the cellular level. In this manuscript, we constructed a lentiviral vector overexpressing HPV11E6/E7 and transfected it into HNEpC. We used HNEpC as the control group and HPV11E6/E7-overexpressing cells as the experimental group. Cell proliferation was assessed using CCK-8, EdU, and colony formation assays. Cell migration ability was evaluated by wound healing and Transwell assays. Protein expression levels related to apoptosis, epithelial–mesenchymal transition (EMT), and the JAK2/STAT3 pathway were analyzed by western blot.

Results The results showed that overexpression of HPV11E6/E7 significantly increased the proliferation and migration of nasal epithelial cells, promoted the progression of EMT, and inhibited cell apoptosis. Further verification showed that the overexpression of HPV11E6/E7 significantly promoted the activation of the JAK2/STAT3 signaling pathway.

Conclusions In summary, we found that low-risk subtype HPV11 promotes nasal mucosal epithelial hyperplasia and malignant progression by increasing activation of the JAK2/STAT3 pathway. The JAK2/STAT3 pathway has been prioritized due to its established role in promoting cell proliferation and EMT in HPV-related diseases.

Keywords Nasal hyperplastic diseases, HPV11 E6/E7, JAK2/STAT3, Nasal inverted papilloma, EMT

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Introduction

Nasal mucosal epithelial hyperplasia can result in hyperplastic nasal disorders, encompassing hyperplastic rhinitis, nasal polyps, and nasal tumors. Nasal inverted papilloma (NIP) is a common epithelial tumor in the clinical setting, and it has the highest incidence among benign tumors of the nasal sinuses. The mean age of onset is 50–60 years, with a male-to-female ratio of 3:1 [1, 2]. Although histologically benign, NIP exhibits distinct biological behavior characterized by three clinically significant features that differentiate it from other sinonasal tumors: locally aggressive growth patterns, high recurrence rates, and potential for malignant transformation [3, 4]. The etiology and pathogenesis of NIP remain incompletely understood, although potential risk factors include infection, smoking, alcohol consumption, rhinitis, occupational exposure, and DNA alterations [5]. The occurrence and progression of NIP, along with the diverse causes of recurrence, have presented challenges in clinical treatment. Hence, it is of considerable significance to study the mechanism underlying the development and progression of NIP. Our previous studies have demonstrated a close association between HPV11 infection and NIP, thereby underscoring the importance of further exploring the role of HPV11 and NIP [6].

Human papillomavirus (HPV) is a small double-stranded DNA virus with high species and tissue specificity, which can infect human skin and mucosal tissue, causing benign lesions or malignant tumors, and its viral particles are composed of nucleic acid and capsid protein [7, 8]. HPV infection of the skin and mucous membrane is a long-term process, and the virus parasitizes the squamous epithelium and columnar epithelium. Once the body's immunity weakens, the latent virus can reactivate and cause benign and malignant lesions at the infection site [9, 10]. The integration of HPV–DNA into host DNA leads to high expression of the E6/E7 gene in the early gene region of HPV, which is the main coding protein gene for HPV disease and has been shown to regulate a variety of cellular proteins to induce cell proliferation, avoid immune surveillance and promote cell survival [11]. HPV infection is closely related to the occurrence and development of NIP and NP, and more and more scholars have joined the research in recent years. However, scholars have not reached consistent conclusions on HPV with NIP studies; moreover, the detection rate of HPV in NIP varies greatly among different scholars. Consequently, it is important to explore different subtypes of HPV infection to guide clinical behavior.

The Janus Kinase (JAK) - Signal transducer and activator of transcription (STAT) signaling pathway is a crucial mediator of a broad spectrum of vital biological signaling pathways, which assumes a significant role in cellular proliferation, migration, immune response, and concomitantly impacts tumorigenesis and development [12]. The JAK/STAT signaling pathway has been demonstrated to be implicated in a plethora of malignancies, and the specific mode of action of JAK/STAT signaling in HPV infection-associated nasal hyperplastic disorders remains elusive. Therefore, we can further investigate whether HPV11 facilitates nasal mucosal epithelial hyperplasia and contributes to disease progression by activating the JAK2/STAT3 pathway.

Methods

Cell culture and cell transfection

We purchased human nasal epithelial cells (HNEpC) from Shanghai Fito Biotechnology Company. The cells were passed through DMEM/F-12 (BasalMedia, Shanghai, China) containing 10% FBS (BIOEXPLORER Fetal Bovine Serum collected in South America) and 1% antibiotic penicillin/streptomycin. The cells were cultured in a humid environment at 37 °C with a continuous supply of 5% CO_2.

We verified the effect of HPV11 in vitro by constructing lentivirus-stable HPV11E6/E7-HNEpC cells overexpressing HPV11E6/E7.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated by Fastagen kit (RNAfast200), and the concentration of total RNA was measured. Complementary DNA (cDNA) was synthesized using a cDNA synthesis kit (Takara, Otsu, Japan). Then, real-time PCR amplification was performed by a real-time fluorescence PCR amplification instrument. The HPV11E6/E7 and β-actin primers used in this study are as follows:

HPV11 E6/E7: Forward: 5′-GGAAGGGTCGTTGCT TACACT-3′;

Reverse: 5′-TGTCCACCTCATCTTCTGAGC-3′;

β-actin: Forward: 5′-GGCTGTGCTATCCCTGTA CG-3′;

Reverse: 5′-CTTGATCTTCATTGTGCTGGGTG-3′.

β-actin was an endogenous control, while $2^{\Delta\Delta CT}$ was used to estimate relative gene expression. The thermal cycle conditions of PCR were 0.5 min at 95 ℃, 10 s at 95 ℃, 30 s at 60 ℃, and 40 cycles.

Cell counting kit-8 (CCK-8) assay

Monitor relative cell viability at 24, 48, and 72 h after transfection using Cell Counting Kit-8 (CCK-8, Yeasen, Shanghai, China) as specified by the manufacturer. The cells were inoculated into 96-well plates with 3500 cells per well, 100 μl of complete medium was added to each well, and three repeat wells were set up. After 0, 24, 48, and 72 h, add 10 μl of CCK-8 reagent per well and

incubate the 96-well plate for 1 h in a 37 °C cell culture incubator protected from light. Monitor the absorbance at 450 nm ($OD_{450\ nm}$) for each sample with a microplate reader (Bio-Rad).

5-Ethynyl-2′-deoxyuridine (EdU) assay

According to the manufacturer's instructions, cell proliferation was assessed using the ethynyl deoxyuridine (EdU) test kit (US EVERBRIGHT, Suzhou, China). HNEpC-NC and HPV11E6/E7-HNEpC cells at the logarithmic growth phase were inoculated into 24-well plates with 5×10^4 cells per well and cultured overnight. On the second day, EdU working solution was added and incubated for 2 h, then 4% paraformaldehyde was used to fix the cells, 2 mg/ml glycine neutralization solution was added, 0.5%Triton X-100 osmotic promoting agent was added, EdU and DAPI staining solution were added, respectively. EdU-positive cells (red fluorescence) and DAPI (blue fluorescence) in each field of vision were observed and photographed under a fluorescence microscope. Finally, the number of cells was counted by Image J software.

Colony formation assays

HNEpC-NC and HPV11E6/E7-HNEpC cells at the logarithmic growth phase were inoculated into 6-well plates with 1500 cells per well and cultured in incubators for 7–14 days. When cell colonies were formed, cell fixation and staining were carried out, fixed with 4% paraformaldehyde for 30 min, and then stained with 0.5% crystal violet for 20 min. The cell colonies were counted using Image J software.

Wound healing assay

HNEpC-NC and HPV11E6/E7-HNEpC cells at the logarithmic growth phase were inoculated into 6-well plates with 5×10^5 cells per well. When cells are full or more than 95% full, prepare a sterile 20 μl gun tip and, using a ruler, make two straight scratches in each hole of the six-well plate. The cells were washed 3 times with PBS and then replaced with fresh serum-free medium. Set the timepoint at 0, 24, 48, and 72 h after the scratch, observe and take photos under the microscope, and analyze the scratch area with Image J software.

Transwell assay

HNEpC-NC and HPV11E6/E7-HNEpC cells at the logarithmic growth phase were inoculated into the Transwell plate. A total of 2×10^4 cells were inoculated into the upper chamber of the Transwell plate after being resuspended in a serum-free medium. The lower chamber was added with 15% FBS medium 500 μl as inducer and then cultured in a 37 ℃ cell incubator for 24 h. On the second day, the cells were fixed and stained, observed, and photographed under a microscope, and the number of migrations was counted by Image J software.

Western blot (WB)

Total Protein was isolated using RIPA lysate with PMSF, and concentration was measured using a Protein assay kit. The protein sample (15 μg) was electrophoretic in SDS–PAGE gel and then transferred to the PVDF membrane. Next, it was enclosed with skim milk powder (5%) for 1 h and incubated with primary antibody overnight in a 4 °C shaker. Corresponding primary antibody: anti-Bax (1:5000, Proteintech, Wuhan, China), anti-Bcl-2 (1:5000, Proteintech, Wuhan, China), anti-E-cadherin (1:5000, Proteintech, Wuhan, China), anti-N-cadherin (1:5000, Proteintech, Wuhan, China), anti-JAK2 (1:1000, Affinity Biosciences, Jiangsu, China), anti-p-JAK2 (1:1000, Affinity Biosciences, Jiangsu, China), anti-STAT3 (1:1000, Zenbio, Chengdu, China), anti-p-STAT3 (1:1000, Zenbio, Chengdu, China) and anti-β-actin (1:10000, Proteintech, Wuhan, China). β-actin was used as the internal reference. On the second day, the PVDF membrane was cleaned three times, and the secondary antibody was incubated. Then, the protein bands were developed, and the gray values were analyzed by Image J software.

Statistical analysis

Differences between groups were evaluated using an unpaired *T* test, and the data were expressed as mean ± SEM. We considered with $p<0.05$ considered statistically significant. All statistical analyses were performed using GraphPad Prism 8.0.

Results

Overexpression of HPV11E6/E7 was verified in cells

The nasal mucosa epithelial cell line HNEpC was established, and the lentiviral vector model with HPV11E6/E7 protein gene overexpression was constructed (labeled with green fluorescent protein) and stably transfected into HNEpC. One day after transfection culture, the transfection was observed and photographed under a fluorescence microscope (Fig. 1A). The results showed that HNEpC cells overexpressed HPV11E6/E7 showed green fluorescence, but Negative Control (NC) did not. qRT-PCR results showed that compared with the control group, the expression level of HPV11E6/E7 in the overexpression group was significantly up-regulated ($^{**}P<0.01$, Fig. 1B). The results indicated that the HPV11E6/E7 cell line was successfully constructed.

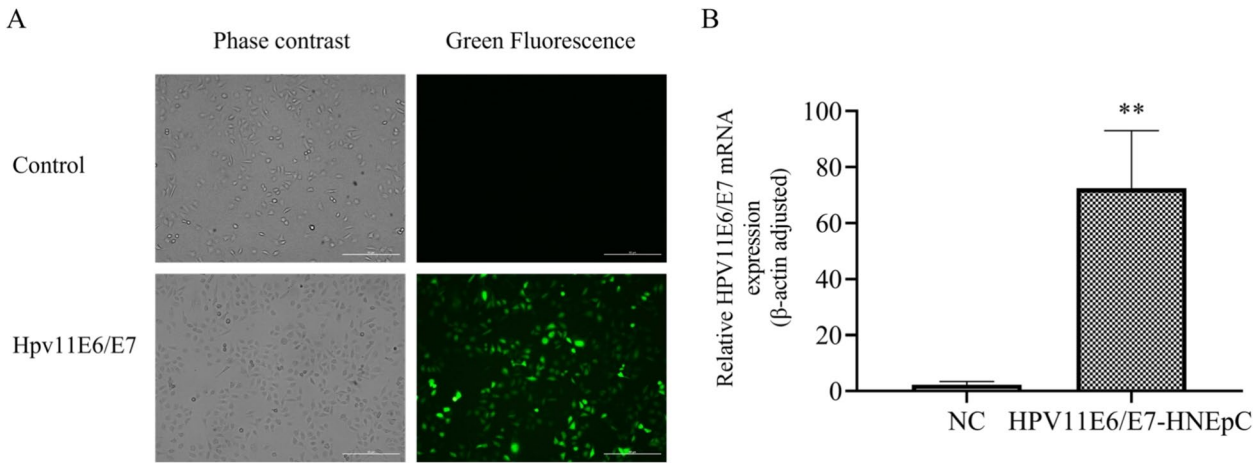

Fig. 1 Overexpression of HPV11E6/E7 was verified in cells. **A** Fluorescence observation of HPV11E6/E7-HNEpC cells transfected with lentivirus. **B** Expression of HPV11E6/E7 in HNEpC cells was verified by qRT-PCR. Data are presented as mean ± SD; $^{**}P < 0.01$ vs. NC

Overexpression of HPV11E6/E7 promotes the proliferation of nasal mucosal epithelium

CCK-8 assay (Fig. 2A), plate cloning assay (Fig. 2B, C), and EdU assay (Fig. 2D, E) showed that overexpression of HPV11E6/E7 promoted the proliferation of nasal mucosal epithelial cells. The cell proliferation activity of the NC group and the overexpressed HPV11E6/E7 group was measured by CCK-8 method. The results showed that the mean $OD_{450\ nm}$ value of the overexpressed HPV11E6/E7 group was significantly increased at 72 h (1.757) compared with the mean $OD_{450\ nm}$ value of the NC group (1.307) ($^{**}P < 0.01$). Compared with the

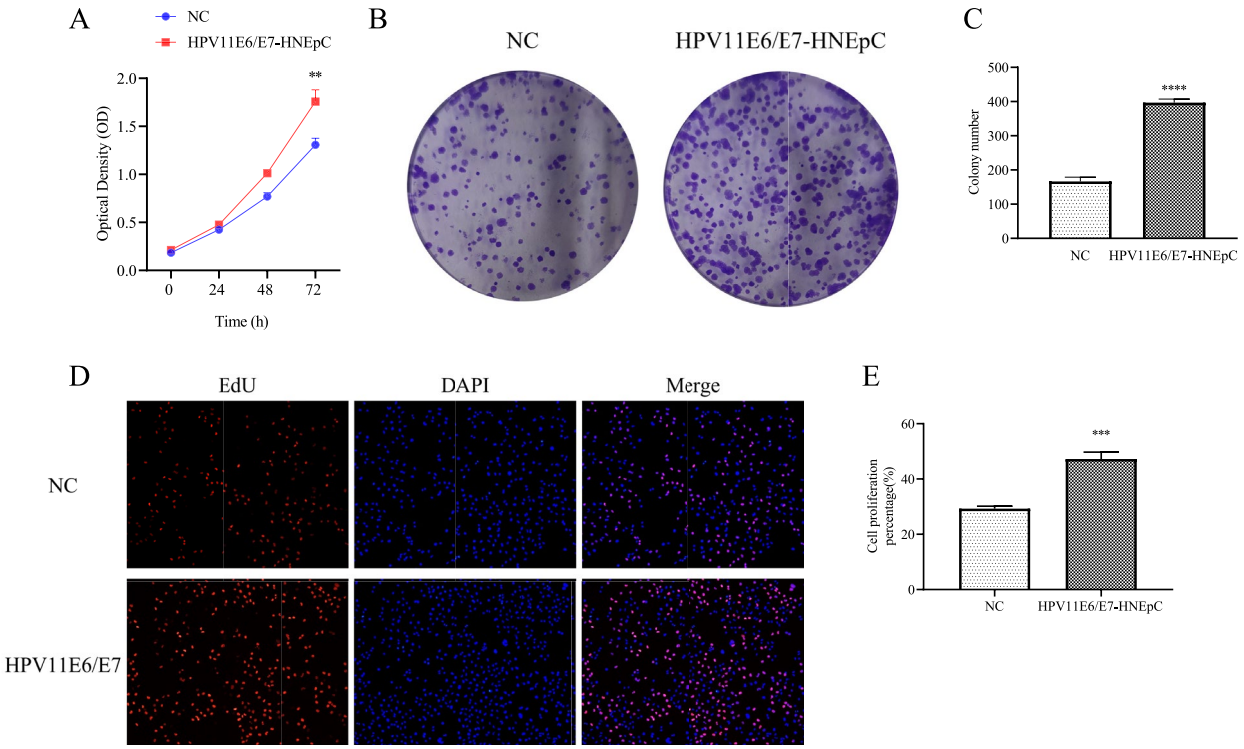

Fig. 2 Overexpression of HPV11E6/E7 promotes the proliferation of nasal mucosal epithelium. **A** CCK-8 assays were used to evaluate cell proliferation. Data are presented as mean ± SD; $^{**}P < 0.01$ vs. NC. **B**, **C** Colony formation assay showing proliferation in cells. Data are presented as mean ± SD; $^{****}P < 0.0001$ vs. NC. **D**, **E** EdU assays were used to evaluate cell proliferation. Data are presented as mean ± SD; $^{***}P < 0.001$ vs. NC

NC group (166.3), the number of clonal spots in the overexpression group (396.7) was significantly increased ($^{****}P<0.0001$). The DNA replication ability of the NC group and overexpressed HPV11E6/E7 group was determined by EdU test. The EdU/DAPI ratio was the cell proliferation percentage, which was significantly higher in the overexpressed group (26.97%) than in the NC group (18.43%) ($^{***}P<0.001$).

Overexpression of HPV11E6/E7 promoted nasal epithelial migration and EMT progression

Wound healing assay (Fig. 3A, B) and Transwell assay (Fig. 3C, D) showed that overexpression of HPV11E6/E7 promoted the migration of nasal mucosal epithelial cells and the progression of EMT. Compared with the mean wound healing rate (25.33%) in the NC group at 72 h, the wound healing rate in the overexpression group (49%) was significantly higher ($^{***}P<0.001$). Transwell results showed that the number of cells migrated per field was significantly higher in the overexpression group (966.3) than in the control group (323.7) on average ($^{***}P<0.001$). The expression of migration-related proteins in the cells of the two groups was detected by Western blot (Fig. 3E, F). Compared with the control group, the overexpression group had lower expression of migration-inhibiting protein E-cadherin ($^{**}P<0.01$) and higher expression of migration-promoting protein N-cadherin ($^{**}P<0.01$). The results showed that overexpression of HPV11E6/E7 increased the migration of HNEpC cells.

Overexpression of HPV11E6/E7 inhibited apoptosis of nasal epithelial cells

The expressions of apoptosis-related proteins in the two groups were detected by Western-blot assay (Fig. 4A, B). The results showed that compared with the NC group, the overexpression group had lower expression of apoptosis-related protein Bax ($^{****}P<0.0001$) and higher expression of anti-apoptosis-related protein Bcl-2 ($^{**}P<0.01$). The results indicated that overexpression of HPV11E6/E7 inhibited apoptosis of nasal epithelial cells.

Overexpression of HPV11E6/E7 promoted the activation of JAK2/STAT3 signaling pathway in nasal mucosal epithelial cells

Western blot was used to detect the expression of JAK2/STAT3 pathway-related proteins in the two groups of cells (Fig. 5A, B). Compared with the control group, the protein expressions of p-JAK2 ($^{****}P<0.0001$) and p-STAT3 ($^{**}P<0.01$) were significantly up-regulated after HPV11E6/E7 overexpression, while the protein

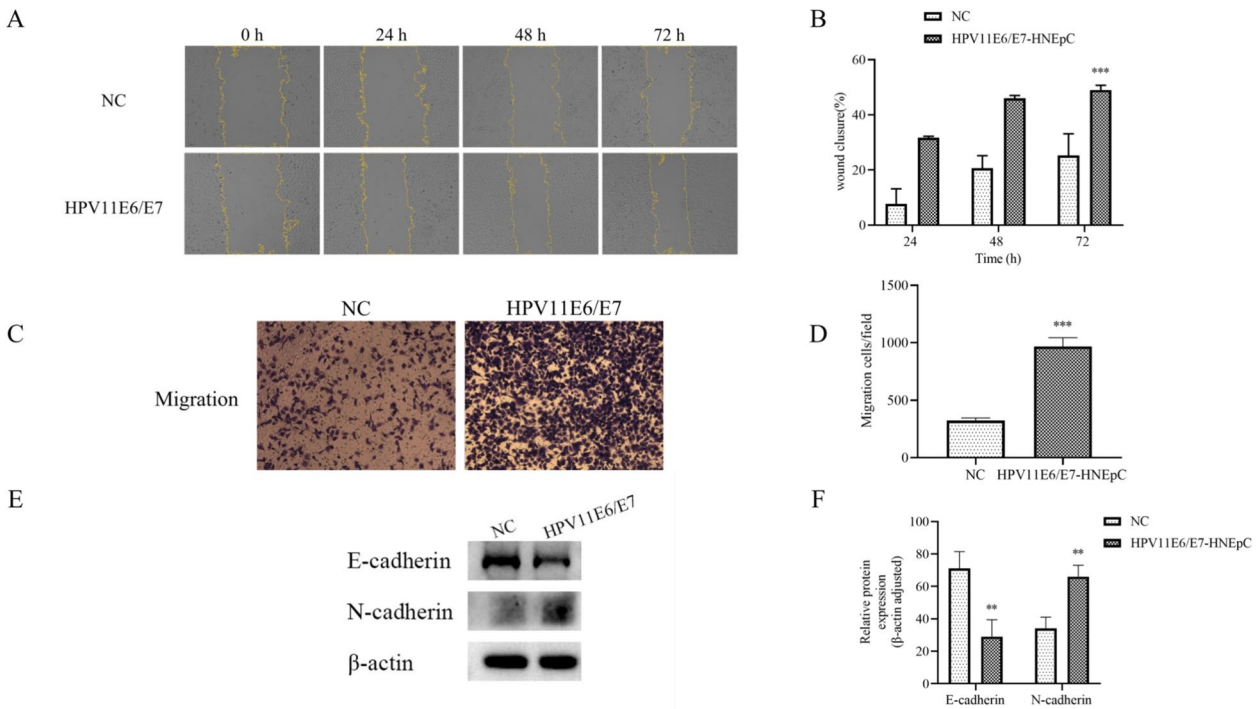

Fig. 3 Overexpression of HPV11E6/E7 promoted nasal epithelial migration and EMT progression. **A**, **B** Cell migration ability was detected by wound healing assay. Data are presented as mean ± SD; $^{***}P<0.001$ vs. NC. **C**, **D** Transwell assay was used to detect cell migration ability. Data are presented as mean ± SD; $^{***}P<0.001$ vs. NC. **E**, **F** Relative expression of E-cadherin and N-cadherin in HNEpC cells was detected by Western blot. The expression of N-cadherin was increased and the expression of E-cadherin was decreased. Data are presented as mean ± SD; $^{**}P<0.01$, $^{**}P<0.01$ vs. NC

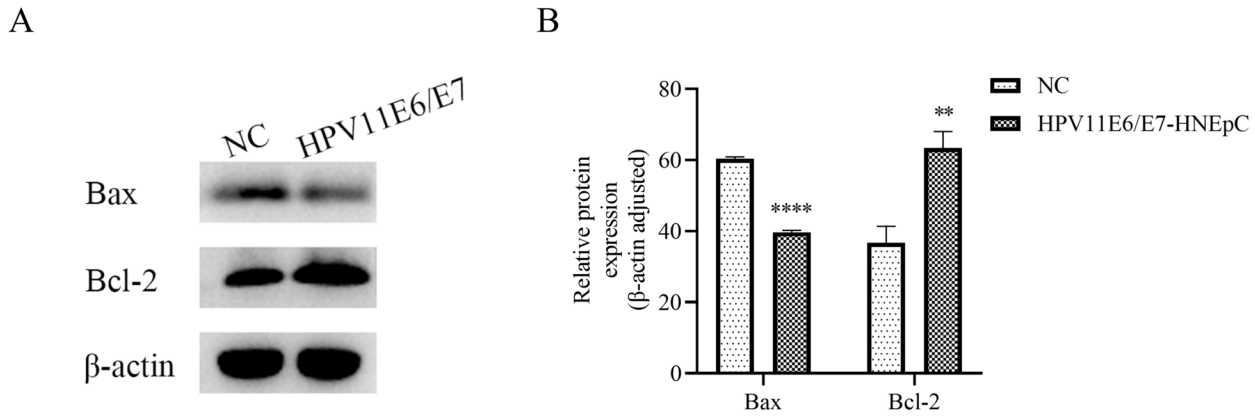

Fig. 4 Overexpression of HPV11E6/E7 inhibited apoptosis of nasal epithelial cells. **A**, **B** Relative expression of Bax and Bcl-2 in HNEpC cells was detected by Western blot. The expression of Bcl-2 was increased and the expression of Bax was decreased. Data are presented as mean ± SD; $^{**}P < 0.01$, $^{****}P < 0.0001$ vs. NC

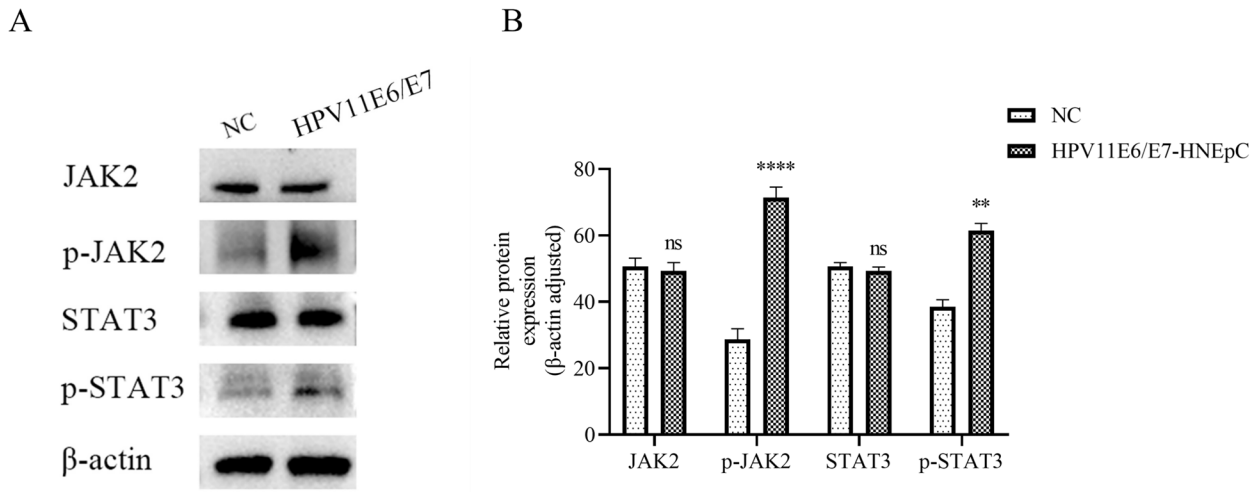

Fig. 5 Overexpression of HPV11E6/E7 promoted the activation of JAK2/STAT3 signaling pathway in nasal mucosal epithelial cells. **A** Expression of JAK2, p-JAK2, STAT3, and p-STAT3 proteins in cells after overexpression of HPV11E6/E7 in HNEpC cells. **B** Expression of JAK2 and STAT3 proteins was not obvious changed. Data are presented as mean ± SD; $P > 0.05$ vs. NC. While the expression of p-JAK2 and p-STAT3 proteins was significantly upregulated. Data are presented as mean ± SD; $^{****}P < 0.0001$, $^{**}P < 0.01$ vs. NC

expressions of JAK2 and STAT3 were not significantly changed ($P > 0.05$). These results indicated that overexpression of HPV11E6/E7 could promote nasal epithelial hyperplasia by promoting the expression of JAK2/STAT3 signaling pathway-related proteins, thus leading to malignant progression of nasal hyperplastic diseases.

Discussion

The recurrence of NIP is significantly associated with the location of the lesion. NIP frequently affects multiple regions within the nasal cavity or sinus, predominantly the lateral wall of the nasal cavity and the middle nasal passage, particularly near the ethmoid bone, maxillary sinus opening, and medial wall. It can occasionally involve the frontal sinus, sphenoid sinus, nasal septum, lower nostril, and in rare cases, extend into intracranial, orbital, or pterygopalatine fossa after bone injury [13, 14]. Analysis of recurrence rates at different focal sites shows that frontal sinus lesions usually have a high recurrence rate because of incomplete removal by endoscopic sinus surgery. This incomplete resection leaves a residual tumor base, which is a primary site for recurrence [15–17]. Fatima Alghamdi et al. [18] found nasal recurrence, middle ear involvement and transformation into squamous cell carcinoma 20 months after NIP treatment. Daniela Parrino et al. [19] found that NIP progressed to

basal-like squamous cell carcinoma. Tiago Fraga Vieira et al. [20] found that the NIP invaded both temporal bones and metastasized to the lungs after malignant transformation. Among the risk factors for NIP, HPV plays an important role in the pathogenesis of NIP, and the positive status of HPV infection is an independent factor related to the relapse of NIP.

Recent studies have emphasized the relationship between HPV and papilloma. Shuyi Wang et al. [21] conducted in situ hybridization analysis of HPV in 10 patients with papilloma of the external auditory canal, and found that 7/10 cases were positive for low-risk HPV subtypes (11,16), while no cases showed that high-risk HPV subtypes were detected (31, 33, 35, 39, 51, 52, 56, 58, 66, 18 and 29). These results indicate that papilloma of the external auditory canal is related to HPV low-risk subtype infection. Farrel J Buchinsky et al. [22] showed that recurrent respiratory papillomatosis was characterized by repeated formation of papilloma in the respiratory tract, caused by HPV type 6 and 11, and patients can generate humoral responses to HPV6 and HPV11. There has long been controversy regarding whether high-risk or low-risk HPV subtypes drive malignant progression in NIP [23]. Our preliminary study found a 64.36% HPV positivity rate in NIP tissue specimens, predominantly low-risk subtype HPV11. Elucidating the distribution of HPV subtypes in NIP has certain guiding significance for predicting the clinical behavior of tumors [6].

Previous studies have shown that HPVE6 cancer protein interacts with a variety of cellular proteins, thereby activating a variety of carcinogenic pathways, resulting in cell apoptosis blocked, transcriptional mechanism changes, interference with cell–cell interaction and cell immortalization [24]. In oral cancer, low-risk subtype HPV11E6 promotes cell proliferation and inhibits p53 induction, thus enhancing anti-apoptotic effect [25]. E6 protein can induce p53 degradation, and E7 protein can increase the rate of DNA synthesis in keratinocytes, suggesting that E6 and E7 oncoproteins are essential for cancer development and play a crucial role in cancer occurrence [26, 27]. Studies have shown that in addition to the two important suppressor genes p53 and pRb, E6 and E7 also bind to other proteins, such as the pro-apoptotic proteins Bax and Bak, which may play an important role in increasing their carcinogenic potential [28]. Bax expression was evaluated by staining and immunocytochemistry in 120 cervical samples, indicating that the weak expression of Bax was not associated with cervical intraepithelial development [28]. Yi Xu et al. [29] found that HPV infection may be a special factor in patients with lung squamous cell carcinoma, in which the expression level of Bcl-2 in HPV-positive patients was significantly higher than that in HPV-negative patients.

Epithelial–mesenchymal transition (EMT) is critical in tumor metastasis. EMT procedure can enhance tumor metastasis, chemical resistance and tumor dryness, which is characterized by altered expression of cell adhesion molecules (e.g., E-cadherin and N-cadherin). E-cadherin is a potential marker for malignancy [30, 31]. The decrease or loss of E-cadherin expression can lead to the decrease of adhesion between cancer cells, make cancer cells easy to shed and metastasize, and promote the occurrence, invasion and metastasis of upper dermal tumors [32, 33]. N-cadherin is a transmembrane adhesion molecule associated with advanced cancer progression and poor prognosis. Increased expression of N-cadherin is a marker of EMT and promotes tumor cell survival, migration, and invasion [34, 35]. YuFen Lu et al. [36] showed that HPV16 E6 could promote the potential cell proliferation, migration and invasion of human cervical cancer cells by regulating EMT and cell dryness. Jing Jiang et al. [37] found that in patients with cervical intraepithelial neoplasia, HPV16 infection was negatively correlated with the expression of E-cadherin and positively correlated with the expression of N-cadherin. At present, the role of HPV11E6/E7 in EMT in NIP remains unexplored. Therefore, the expression of EMT-related proteins was detected in this study, and we found low expression of E-cadherin and high expression of N-cadherin in cells after transfection of HPV11E6/E7, which indicated that transfection with HPV11E6/E7 promoted the progression of EMT and increased the migration ability of cells, thus promoting the malignant progression of the disease.

The role of HPV infection in the JAK/STAT signaling pathway has been demonstrated in cervical cancer but has not been studied in NIP. Therefore, we explored the role of HPV infection in nasal proliferative diseases through the JAK2/STAT3 signaling pathway. In the previous study of our group, we found a high positive rate of HPV in NIP, collected samples of NIP for HPV screening of 37 subtypes, and found that the highest detection rate was low-risk subtype HPV11. Thus, lentivirus overexpression HPV11E6/E7 plasmids were established. After the transfection of HPV11E6/E7, the cell proliferation experiment was conducted. It was found that transfection of HPV11E6/E7 promoted cell proliferation and increased DNA replication. The cell migration experiment showed that transfection of HPV11E6/E7 promoted cell migration and increased cell mobility. Western blot analysis showed that transfection of HPV11E6/E7 inhibited apoptosis, promoted EMT progression, and promoted the activation of the JAK2/STAT3 pathway. These results indicate that HPV11E6/E7 can promote nasal epithelial hyperplasia by activating the JAK2/STAT3 signaling pathway, potentially driving the malignant

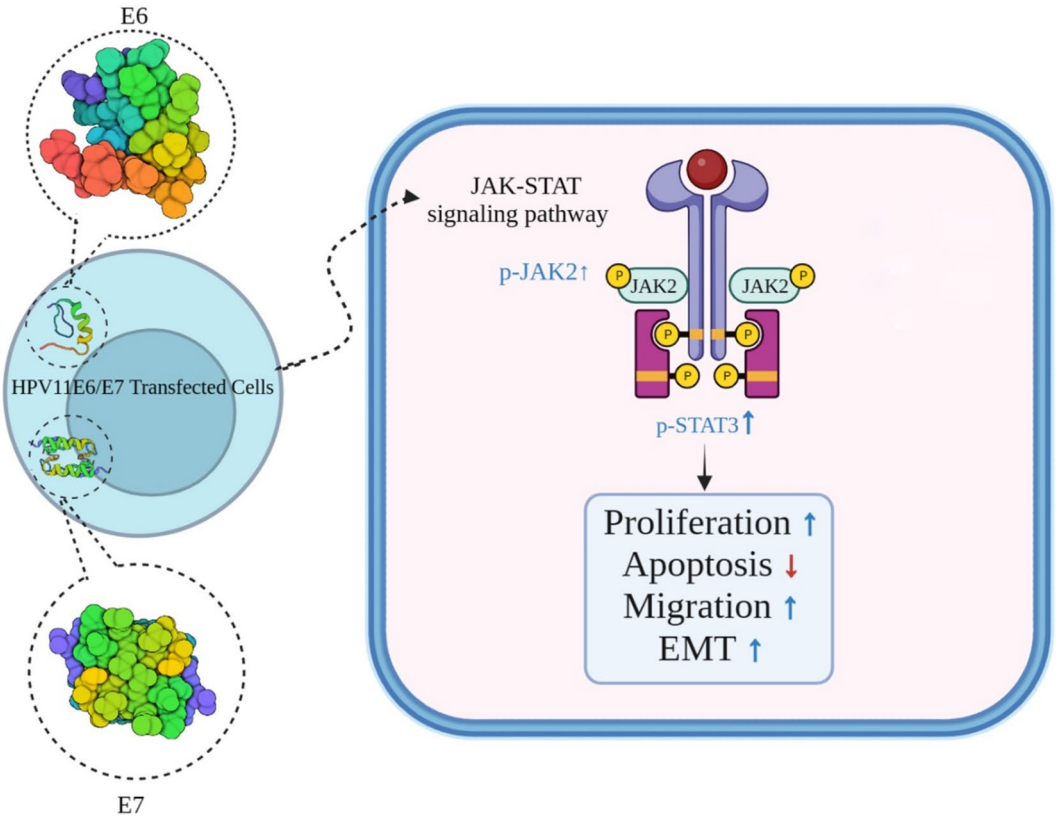

Fig. 6 HPV11E6/E7 induces nasal epithelial hyperplasia through JAK2/STAT3 signaling pathway

progression of nasal hyperplastic diseases. This mechanistic insight suggests that pharmacological inhibition of JAK2/STAT3 could represent a targeted therapeutic strategy for HPV11-associated nasal lesions, particularly in recurrent or premalignant cases.

Conclusion

In summary, HPV infection of low-risk subtypes is also closely related to nasal epithelial hyperplasia. Overexpression of HPV11E6/E7 promoted epithelial proliferation and migration in the nasal mucosa through the JAK2/STAT3 signaling pathway while inhibiting apoptosis (Fig. 6). Epithelial hyperplasia of nasal mucosa leads to malignant progression of the disease, so it is important to pay attention to the infection of different subtypes of HPV early.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40001-025-02496-5.

Supplementary Material 1.

Supplementary Material 2.

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None.

Author contributions
Yi Zhang†, Kaisai Tian†, and Liying Zheng†: The main contributions to this manuscript were the design of the experimental scheme, the verification of the research content, the analysis of experimental data, and the writing of the article. Gaohan Zhu and Runyu Zhao: The main contributions to this manuscript were the collection of experimental data, the visualization of pictures, and the review of the article content. Enhui Zhou and Xiaocheng Xue: The main contributions in this manuscript were the analysis of experimental data and the review of the content of the article. Shuixian Huang: The main contributions to this manuscript were the formal analysis of the research scheme and the review of the article content. Xiaoping Chen: is the co-corresponding author of this manuscript, and the main contributions to this manuscript were the supervision of the implementation of the overall plan and the review of the content. Baoji Hu: is the co-corresponding author of this manuscript, and the main contributions to this manuscript were the data analysis and manuscript revision. Wenhao Yao: is the corresponding author of this manuscript, mainly contributed to the overall conception, experimental design, and project management of the research project, and made great contributions to the writing.

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Data availability
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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