Human β-defensin-3 and nuclear factor-kappa B p65 synergistically promote the cell proliferation and invasion of oral squamous cell carcinoma

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Research Article

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Abstract

Background: Oral squamous cell carcinoma (OSCC) is a usual oral cancer; it's necessary to identify targets for its early diagnosis and treatment. This research aimed to investigate the roles of human β-defensin-3 (hBD-3) and nuclear factor-kappa B (NF-κB) p65 in the pathogenesis and progression of OSCC.

Methods: The connection between NF-κB p65 and the carcinogenesis of oral cancer was explored by immunohistochemical staining. The relative expressions of hBD-3 and NF-κB p65 in OSCC cells were evaluated by qRT-PCR and Western blot. Afterwards, hBD-3 was knocked down, and NF-κB p65 was overexpressed. The cell viability and invasion were tested via CCK-8 and Transwell experiment, and the expression of hBD-3, NF-κB p65 and its downstream molecules were evaluated by Western blot.

Results: The content of NF-κB p65 was increased with the aggravation of oral submucosal fibrosis. hBD-3 and NF-κB p65 were high-expressed in OSCC cells. The viability and invasion abilities of OSCC cells that knocking down hBD-3 were markedly decreased, while they were restored by co-overexpression of NF-κB p65. The expressions of NF-κB p65, c-myc were decreased and IκB, p21 were increased with the knockdown of hBD-3. After co-overexpression of NF-κB p65, the expression of hBD-3 and IκB didn't change significantly, while c-myc and p21 was dramatically changed.

Conclusion: hBD-3 and NF-κB p65 facilitate the proliferation and invasion of OSCC cells, and hBD-3 may promote this process by regulating the expression of NF-κB p65 and its downstream c-myc and p21.

1 Introduction

About 90% of oral cancers are OSCC, and its incidence is increasing year by year [1]. Surgical resection is currently the preferred treatment for OSCC, combined with radiotherapy and chemotherapy [2]. However, the long-term effect of these treatment is still not ideal because of the strong aggressiveness and high metastasis rate of OSCC [3]. Hence, seeking novel and efficient targets for the early detection and treatment of OSCC is urgent.

human β-defensin (hBD) is an important component of human innate immune system, mainly expressed in some epithelial tissues, and plays an essential role in inflammatory diseases, injury repair, and tumor formation and metastasis [4]. There are mainly 6 kinds of hBD (hBD-1~6), which are mainly distributed in skin, respiratory tract, tonsil epithelium, etc., while oral mucosa epithelium mainly produces hBD-1~3 [5]. hBD-3 was originally isolated from the skin lesions of psoriasis patients, and its antibacterial properties were broader and stronger than hBD-1 and hBD-2 [6]. As a part of the natural immune barrier of oral mucosal, hBD-3 is normally low expressed. When stimulated by external factors, the expression of hBD-3 in epithelial tissues would be remarkably increased, thus exerting its innate immune defense function [7]. Aberrant expression of hBD-3 has been reported in various cancer tissues, such as cervical cancer [8], colon cancer [9], head and neck cancer [10], and OSCC [11]. Overexpressed hBD-3 can affect the proliferation, invasion, cell cycle, apoptosis, migration and other processes of tumor cells, thus being

enrolled in the pathogenesis and development of tumors [12]. hBD-3 has been considered as a potential tumor biomarker, and it is associated with a variety of oral precancerous lesions or precancerous states, such as oral lichen planus and oral leukoplakia [13]. NF-κB is a crucial nuclear transcription factor that participate in cell proliferation, differentiation and apoptosis, which is tightly bound up with the progress of inflammatory tumors [14]. Recent study has shown that hBD-3 can promote the growth of cervical cancer cells by activating NF-κB signaling pathway [8]. However, whether hBD-3 can participate in the progression of OSCC by regulating NF-κB-mediated inflammatory response is still indistinct.

In the present report, we assessed the predictability of NF-κB p65 in the carcinogenesis of oral cancer, then we examined the expression of hBD-3 and NF-κB p65 in oral mucosal epithelial cells and OSCC cell lines, and explored the roles and correlation of hBD-3 and NF-κB p65 in the occurrence and progression of OSCC, so as to provide reference for the control and treatment of OSCC.

2 Materials And Methods

2.1 Tissue samples

A total of 40 wax blocks of patients with oral submucosal fibrosis (OSF) confirmed by pathology were selected from the Pathology Department of Haikou People's Hospital from January 2017 to December 2020, including 15 cases in the early stage, 13 cases in the middle stage and 12 cases in the late stage. None of the them underwent any preoperative treatment and were not associated with other systemic diseases or mucosal diseases. This research program has been permitted by the ethics Committee of Haikou People's Hospital.

2.2 Immunohistochemical (IHC) staining

The two-step IHC method was applied to assess the expression of NF-κB p65 and PDE4 in OSF (early, middle and late stage) tissue sections. After the dewaxing and rehydrating of paraffin sections, they were cleaned twice with PBS. Then the tissue antigen repair was performed, and the sections were blocked with a few drops of 3% H_2O_2. Furthermore, the sections were reacted with primary antibodies and secondary antibody successively, and DAB solution (Aike Reagent, China) was dropped for color-developing. All the antibodies used were purchased from Abcam, including: anti-NF-κB p65 (ab16502), anti-PDE4 (ab14613) and goat anti-rabbit IgG (ab97051). The slices were then washed with PBS, restained with hematoxylin (J&K Scientific, China), dehydrated and sealed for microscopic examination.

2.3 Cell culture

The OSCC cell lines CAL27 and HN30 were acquired from GuangZhou Jennio Biotech (China), and oral mucosal epithelial cells (OMES) were provided by Shanghai Baiyi Biotechnology Center (China). These two kinds of OSCC cells were all cultured in DMEM (Gibco, US) that containing 10% fetal bovine serum (Hyclone, US) and 1% double antibodies (Absin, China). The cell culture environment is constant 37°C with 5% CO_2.

2.4 qRT-PCR

Total RNA of CAL27 and HN30 cells was collected using Trizol (Keybio, China). Then the cDNA was obtained using the reverse transcription kit (Biopike, China). The following were sequences of hBD-3 and NF-κB p65 primers: hBD-3 F: 5′-TAGCCTAACGTAATCGACTG-3′, R: 5′-GACTAATGACCTACGTTCGAC-3′; NF-κB p65 F: 5′-GAGCTACATTGCAACTAGAC-3′, R: 5′-CTATGACCTACGACTGATCC-3′. The qPCR reaction system was constructed using 2× SYBR Green qPCR kit (Dingguo Changsheng, China), and the reaction was conducted and analyzed by Gentier 96E PCR analysis system (Tianlong, China). The tests were repeated independently for three times.

2.5 Western blot

CAL27 and HN30 cells were digested and lysed, and the protein of cell lysate was quantified using the BCA kit (Absin, China). Then 15 μg of total protein was injected into the sample holes for SDS-PAGE. Furthermore, the dividual proteins in the gel were transferred to the PVDF membrane (Absin, China). After blocking with 1% bovine serum albumin, the membrane was incubated overnight with diverse primary antibodies at 4°C. The antibodies were all provided by Abcam (UK): anti-hBD-3 (ab172703), anti-NF-κB p65 (ab288751), anti-IκB (ab97783), anti-c-Myc (ab152146), anti-p21 (ab227443), anti-GAPDH (ab9484). The second day, after incubating with secondary antibodies for 1 h, the protein bands could be visualized by developing.

2.6 Cell transfection

Oligonucleotide si-hBD-3 and si-NC was synthesized by Guangzhou Ribobio (China), si-hBD-3 F: 5′-UUCAUCGACCAUUACAGCUTT-3′, R: 5′-CAGUCACGUUCAGUGACAATT-3′, si-NC F: 5′-UGCAAGUUCAUGACUGATT-3′, R: 5′-GAUUACCGAUGACUAACGTT-3′. si-hBD-3 or si-NC was transfected into CAL27 and HN30 cells following the procedure of Lipofectamine™2000 Transfection Reagent (Invitrogen, US). For the overexpression of NF-κB p65, full length of NF-κB p65 was obtained by PCR amplification and inserted into the pcDNA3.1 plasmid (Miaoling, China) to construct the NF-κB p65 overexpression plasmid. Then the NF-κB p65- pcDNA3.1 or the empty pcDNA3.1 plasmid were transfected to CAL27 and HN30 cells using Lipofectamine™2000 reagent. Follow-up experiments could be performed 48 h after the transfection.

2.7 CCK-8 assay

This assay was carried out to assess the cell viability according to the working manual of Cell Counting Kit-8 (Sangon Biotech, China). Briefly, 5×10^5 of CAL27 or HN30 cells were inoculated in 96-well plates. When all cells were completely adherent to the plate, 10 μL of CCK-8 reagent was injected to treat cells for another 2 h. Finally, the OD_{450} was measured by the microplate reader (Tecan, Swiss).

2.8 Transwell invasion assay

The basement membrane of Transwell chamber (Corning, US) was pre-coated with Matrigel (Corning, US) one day before the experiment, and the chambers were seated in 24-well plates. Then the transfected CAL27 and HN30 cells were placed in the upper chamber, and 500 μL/well of the complete DMEM was injected into the lower chamber. After 24 h of culture, the chamber was taken out and stained with 0.1% crystal violet (Aladdin, China). Then the remaining cells on the upside of the basement membrane were erased with Q-tips, and the cells invaded to the bottom side were observed with the microscope (Nikon, Japan).

2.9 Statistical analysis

All quantitative data were shown as Mean ± Standard deviation. The SPSS software (V 19.0) was used for the statistical analysis. The comparison between two groups was conducted using Student's *t* test, and one-way ANOVA was used for three groups. $P < 0.05$ was considered as statistically significant.

3 Results

3.1 NF-κB p65 is closely related to the carcinogenesis of oral mucosa

It is unclear that whether NF-κB p65 is associated with oral precancerous lesions. Therefore, we examined its expression in clinical tissues of patients with early, intermediate, and advanced OSF by IHC assay. PDE4, an enzyme that can specifically hydrolyze c-AMP, has been proved to be abundant in various tumor cells, and its level is positively correlated with the carcinogenesis process [15]. Therefore, we selected PDE4 as the positive control. The results revealed that NF-κB p65 and PDE4 in OSF tissues gradually increased with the progression of OSF (Fig. 1A and B), suggesting that NF-κB p65 is correlated with the carcinogenesis of oral mucosa.

3.2 hBD-3 and NF-κB p65 are highly expressed in OSCC cell lines

Next, we investigated the relative expression capacity of hBD-3 and NF-κB p65 in OSCC cells. On the one hand, the hBD-3 and NF-κB p65 mRNA expression in CAL27 and HN30 cells was found significantly higher than that in OMES (Fig. 2A). One the other hand, the protein level of hBD-3 and NF-κB p65 exhibited similar results, they were low expressed in OMES, but obviously higher in CAL27 and HN30 cells (Fig. 2B).

3.3 hBD-3 facilitates the proliferation and invasion of OSCC cells

To further investigate the role of hBD-3 in the occurrence and development of OSCC, we used siRNA technology to knockdown hBD-3 expression in CAL27 and HN30 cells. The knockdown efficiency of si-hBD3-#1 in CAL27 and HN30 cells was about 92.6% and 52.2%, respectively, and si-hBD3-#2 was about 59.5% and 58.7%, respectively (Fig. 3A). si-hBD3-#2 was chose for the further experiments. Next, we evaluated the function of hBD-3 on the viability of CAL27 and HN30 cells by CCK-8 assay. The results showed that 72 h after hBD-3 was knocked down, cell viability was dramatically inhibited (Fig. 3B). In addition, we found that the invasion ability of CAL27 and HN30 cells was obviously decreased after the

knockdown of hBD-3 (Fig. 3C). The above-mentioned results illustrated that hBD-3 could facilitate proliferation and invasion of OSCC cells.

3.4 NF-κB p65 reverses the effects of hBD-3-knockdown on OSCC cells

Furthermore, we intended to clarify the role of NF-κB p65 in the progression of OSCC. To this end, we overexpressed NF-κB p65 in CAL27 and HN30 cells in which hBD-3 was knocked down. We found that the cell viability of CAL27 and HN30 cells that knocked down hBD-3 and co-overexpressed NF-κB p65 was markedly enhanced compared with the group that only knocked down hBD-3 (Fig. 4A, $P < 0.05$). Besides, knockdown of hBD-3 and co-overexpression of NF-κB p65 also restored the invasive ability of CAL27 and HN30 cells (Fig. 4B and C). The above results indicate that NF-κB p65 can reverse the effects of knockdown of hBD-3 on OSCC cells, it is a potential promoter for the progression of OSCC.

3.5 c-myc and p21 may be downstream signaling molecules of hBD-3 and NF-κB p65

In order to elucidate how hBD-3 and NF-κB p65 regulate OSCC, we further investigated the expression of downstream signaling molecules of NF-κB p65 in CAL27 cells. After knockdown of hBD-3, the protein content of IκB and p21 was notably increased, and c-myc and NF-κB p65 was decreased. When NF-κB p65 was simultaneously overexpressed, the protein content of c-myc was dramatically increased, p21 was decreased, while the level of IκB was not significantly changed (Fig. 5A and B). These results demonstrate that c-myc and p21 may be downstream signaling molecules of hBD-3 and NF-κB p65.

4 Discussion

At present, the 5-year survival rate of OSCC patients is less than 50%, which can be improved by early detection and treatment [16]. OSCC usually develops from precancerous lesions, such as oral erythema, oral leukoplakia, oral lichen planus, and OSF [17]. OSF is a chronic mucosal inflammatory disease with unknown etiology, and it is considered as a precancerous lesion of oral mucosa with certain carcinogenic potential and recognized by the World Health Organization [18]. It has been reported that about 3% ~ 19% of OSF patients may develop oral cancer, and this probability is increasing year by year [19]. Recently, studies have found that NF-κB pathway is strongly linked to the occurrence of tissue fibrosis, and its abnormal activation can promote the formation of inflammatory fibrosis [20]. NF-κB has a certain relationship with oral precancerous lesions and the occurrence and metastasis of OSCC [21]. In this study, the expression of NF-κB p65 in the oral mucosa of OSF patients was found increased with the aggravation of OSF, which indicated that NF-κB p65 was closely related to the carcinogenesis of oral mucosa. In the process of normal oral mucosal carcinogenesis, activation of NF-κB p65 may accelerate the expression of genes that accelerate cell proliferation and inhibit apoptosis, thereby causing malignant transformation of oral mucosal cells to OSCC cells.

hBD-3 is also closely linked to the evolution of tumors [9]. Marco et al. found that hBD-3 is frequently overexpressed in OSCC by analyzing the expression profile of hBD-3 in cancerous and non-cancerous specimens of OSCC patients, and hBD-3 may be related to the pathogenesis of OSCC [22]. Mburu et al.

confirmed that hBD-3 can promote the metastasis of head and neck squamous cell carcinoma by up-regulating the expression of CCR7 through NF-κB pathway [23]. The correlation between hBD-3 and NF-κB p65 in the pathogenesis and progression of OSCC has not been reported. Here, we found that the knockdown of hBD-3 reduced the proliferative and invasive abilities of OSCC cells, which was in keeping with the findings of Shuyi et al [11]. To elucidate the correlation between hBD-3 and NF-κB p65 in OSCC, we overexpressed NF-κB p65 in CSCC cells while knocking down hBD-3. The inhibitory effect of hBD-3-knockdown on OSCC cells was reversed by overexpressed NF-κB p65 to a great extent. The decreased expression of NF-κB p65 was also observed in OSCC cells after the knockdown of hBD-3, suggesting that hBD-3 may be the activator of NF-κB p65.

Furthermore, we detected the expression levels of the downstream signaling molecules, including IκB, c-myc, and p21. Although the expressions of IκB, c-myc, and p21 were significantly up-regulated or down-regulated after knockdown of hBD-3, the expression of IκB did not change significantly after overexpression of NF-κB p65, which suggests that IκB is not the common downstream target of hBD-3 and NF-κB p65, and c-myc and p21 may be the key downstream signaling molecules that enrolled in the regulation of pathogenesis and progress of OSCC by hBD-3 and NF-κB p65. The study by Li et al. pointed out that high-expressed c-myc was found in OSCC tissues and cell lines, and it could accelerate the progress of OSCC [24]. As a negative regulator of cell cycle, abnormal expression of p21 can lead to cell cycle disorder and ultimately lead to tumorigenesis [25]. However, we did not investigate them in depth in this research. In the future, we will investigate how hBD-3 and NF-κB p65 regulate downstream targets to promote the progression of OSCC.

In conclusion, in the current study, we found that NF-κB p65 is strongly associated with the carcinogenesis of OSF, the proliferation and invasion of OSCC cells can be inhibited by the knockdown of hBD-3, and these processes can be reversed distinctly by overexpressed NF-κB p65, hBD-3 and NF-κB p65 may facilitate the occurrence and progression of OSCC by regulating the expression of downstream genes c-myc and p21.

Declarations

Author Contributions: YD and YY contributed equally to this study. PX conceived and designed this study. YD and YY performed the experiments, analyzed the data and drafted the manuscript. WZ contributed to data analysis. WZ, CY and PX discussed the results. YY and PX revised the manuscript.

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Data availability: The data used to support the findings of this study are available from the corresponding author upon request.

Ethics approval and consent to participate: The experimental protocols were approved by the Ethics Committee of the Affiliated Haikou Hospital of Xiangya Medical College of Central South University. This

paper has not been published elsewhere in whole or in part. All authors have read and approved the content, and agree to submit it for consideration for publication in your journal. There are no ethical/legal conflicts involved in the article. Informed consent was obtained from all individual participants included in the study.

Competing interests: The authors have declared no conflicts of interest in this work

References

1. Lu Y, Zheng Z, Yuan Y, Pathak JL, Yang X, Wang L, et al. The Emerging Role of Exosomes in Oral Squamous Cell Carcinoma. 2021:9;10.3389/fcell.2021.628103
2. Ling Z, Cheng BTao X. Epithelial-to-mesenchymal transition in oral squamous cell carcinoma: Challenges and opportunities. Int J Cancer. 2021:148;1548-61. 10.1002/ijc.33352
3. Eun YG, Lee JW, Kim SW, Hyun DW, Bae JWLee YC. Oral microbiome associated with lymph node metastasis in oral squamous cell carcinoma. Sci Rep. 2021:11;23176. 10.1038/s41598-021-02638-9
4. Gao X, Ding J, Liao C, Xu J, Liu XLu W. Defensins: The natural peptide antibiotic. Adv Drug Deliv Rev. 2021:179;114008. 10.1016/j.addr.2021.114008
5. Prasad SV, Fiedoruk K, Daniluk T, Piktel EBucki R. Expression and Function of Host Defense Peptides at Inflammation Sites. Int J Mol Sci. 2019:21;10.3390/ijms21010104
6. Harder J, Bartels J, Christophers ESchroder JM. Isolation and characterization of human beta -defensin-3, a novel human inducible peptide antibiotic. J Biol Chem. 2001:276;5707-13. 10.1074/jbc.M008557200
7. Gürsoy UK, Salli K, Söderling E, Gürsoy M, Hirvonen JOuwehand AC. Regulation of hBD-2, hBD-3, hCAP18/LL37, and Proinflammatory Cytokine Secretion by Human Milk Oligosaccharides in an Organotypic Oral Mucosal Model. Pathogens. 2021:10;10.3390/pathogens10060739
8. Xu D, Zhang B, Liao C, Zhang W, Wang W, Chang Y, et al. Human beta-defensin 3 contributes to the carcinogenesis of cervical cancer via activation of NF-κB signaling. Oncotarget. 2016:7;75902-13. 10.18632/oncotarget.12426
9. Uraki S, Sugimoto K, Shiraki K, Tameda M, Inagaki Y, Ogura S, et al. Corrigendum: Human β-defensin-3 inhibits migration of colon cancer cells via downregulation of metastasis-associated 1 family, member 2 expression. Int J Oncol. 2015:46;1858. 10.3892/ijo.2015.2868
10. Wang K, Wang JH, Baskaran H, Wang RJurevic R. Effect of human beta-defensin-3 on head and neck cancer cell migration using micro-fabricated cell islands. Head Neck Oncol. 2012:4;41. 10.1186/1758-3284-4-41
11. Shuyi Y, Feng W, Jing T, Hongzhang H, Haiyan W, Pingping M, et al. Human beta-defensin-3 (hBD-3) upregulated by LPS via epidermal growth factor receptor (EGFR) signaling pathways to enhance lymphatic invasion of oral squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2011:112;616-25. 10.1016/j.tripleo.2011.02.053

12. Ghosh SK, McCormick TSWeinberg A. Human Beta Defensins and Cancer: Contradictions and Common Ground. Front Oncol. 2019:9;341. 10.3389/fonc.2019.00341
13. Nishimura M, Abiko Y, Kusano K, Yamazaki M, Saitoh M, Mizoguchi I, et al. Localization of human beta-defensin 3 mRNA in normal oral epithelium, leukoplakia, and lichen planus: an in situ hybridization study. Med Electron Microsc. 2003:36;94-7. 10.1007/s00795-002-0206-8
14. Taniguchi KKarin M. NF-κB, inflammation, immunity and cancer: coming of age. Nat Rev Immunol. 2018:18;309-24. 10.1038/nri.2017.142
15. Hsien Lai S, Zervoudakis G, Chou J, Gurney MEQuesnelle KM. PDE4 subtypes in cancer. Oncogene. 2020:39;3791-802. 10.1038/s41388-020-1258-8
16. Jehn P, Dittmann J, Zimmerer R, Stier R, Jehn M, Gellrich NC, et al. Survival Rates According to Tumour Location in Patients With Surgically Treated Oral and Oropharyngeal Squamous Cell Carcinoma. Anticancer Res. 2019:39;2527-33. 10.21873/anticanres.13374
17. Scholtz B, Vo Minh D, Kiss C, Tar I, Kumar A, Tőzsér J, et al. Examination of Oral Squamous Cell Carcinoma and Precancerous Lesions Using Proximity Extension Assay and Salivary RNA Quantification. Biomedicines. 2020:8;10.3390/biomedicines8120610
18. Shih Y-H, Wang T-H, Shieh T-MTseng Y-H. Oral Submucous Fibrosis: A Review on Etiopathogenesis, Diagnosis, and Therapy. International Journal of Molecular Sciences. 2019:20;10.3390/ijms20122940
19. Kujan O, Mello FWWarnakulasuriya S. Malignant transformation of oral submucous fibrosis: A systematic review and meta-analysis. Oral Dis. 2021:27;1936-46. 10.1111/odi.13727
20. Markó L, Vigolo E, Hinze C, Park JK, Roël G, Balogh A, et al. Tubular Epithelial NF-κB Activity Regulates Ischemic AKI. J Am Soc Nephrol. 2016:27;2658-69. 10.1681/asn.2015070748
21. Kamperos G, Nikitakis N, Sfakianou A, Avgoustidis DSklavounou-Andrikopoulou A. Expression of NF-κB and IL-6 in oral precancerous and cancerous lesions: An immunohistochemical study. Med Oral Patol Oral Cir Bucal. 2016:21;e6-13. 10.4317/medoral.20570
22. Kesting MR, Loeffelbein DJ, Hasler RJ, Wolff KD, Rittig A, Schulte M, et al. Expression profile of human beta-defensin 3 in oral squamous cell carcinoma. Cancer Invest. 2009:27;575-81. 10.1080/07357900802620851
23. Mburu YK, Abe K, Ferris LK, Sarkar SNFerris RL. Human β-defensin 3 promotes NF-κB-mediated CCR7 expression and anti-apoptotic signals in squamous cell carcinoma of the head and neck. Carcinogenesis. 2011:32;168-74. 10.1093/carcin/bgq236
24. Li S, Zhang SChen J. c-Myc induced upregulation of long non-coding RNA SNHG16 enhances progression and carcinogenesis in oral squamous cell carcinoma. Cancer Gene Ther. 2019:26;400-10. 10.1038/s41417-018-0072-8
25. Xiao BD, Zhao YJ, Jia XY, Wu J, Wang YGHuang F. Multifaceted p21 in carcinogenesis, stemness of tumor and tumor therapy. World J Stem Cells. 2020:12;481-7. 10.4252/wjsc.v12.i6.481

Figures

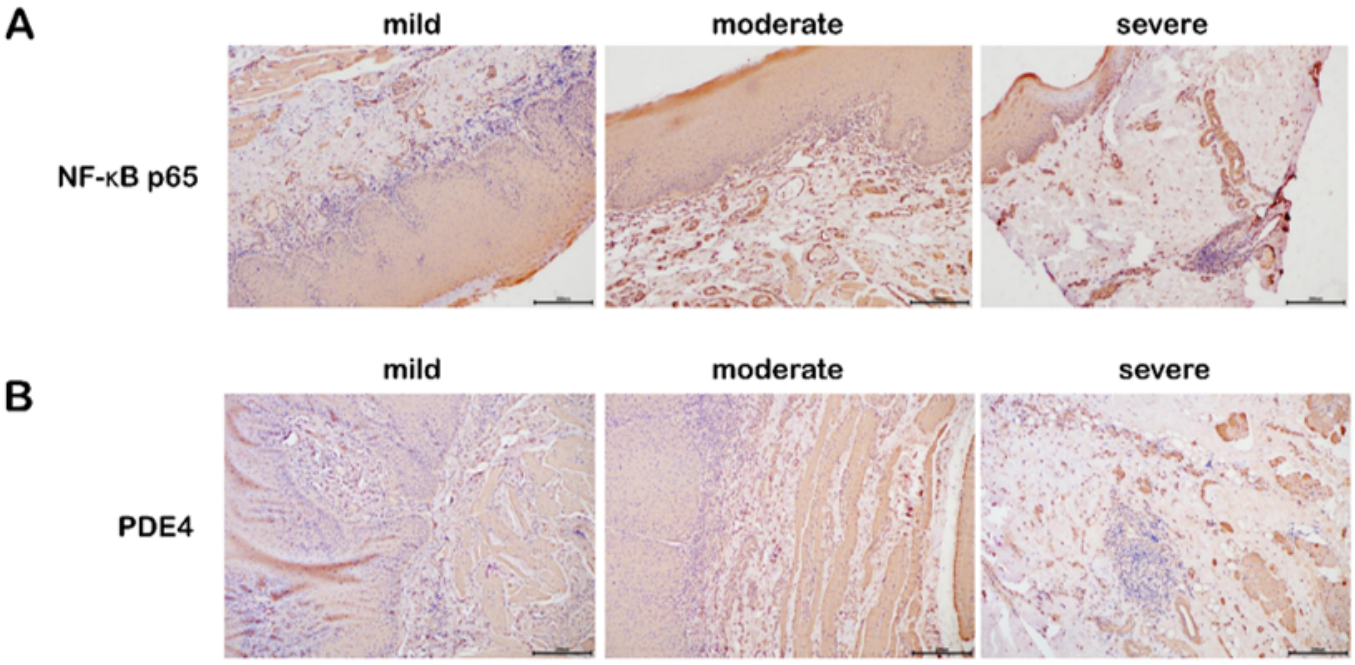

Figure 1

Immunohistochemical staining of **A** NF-κB p65 and **B** PDE4 in oral mucosa tissues of OSF patients with mild, moderate and severe degrees.

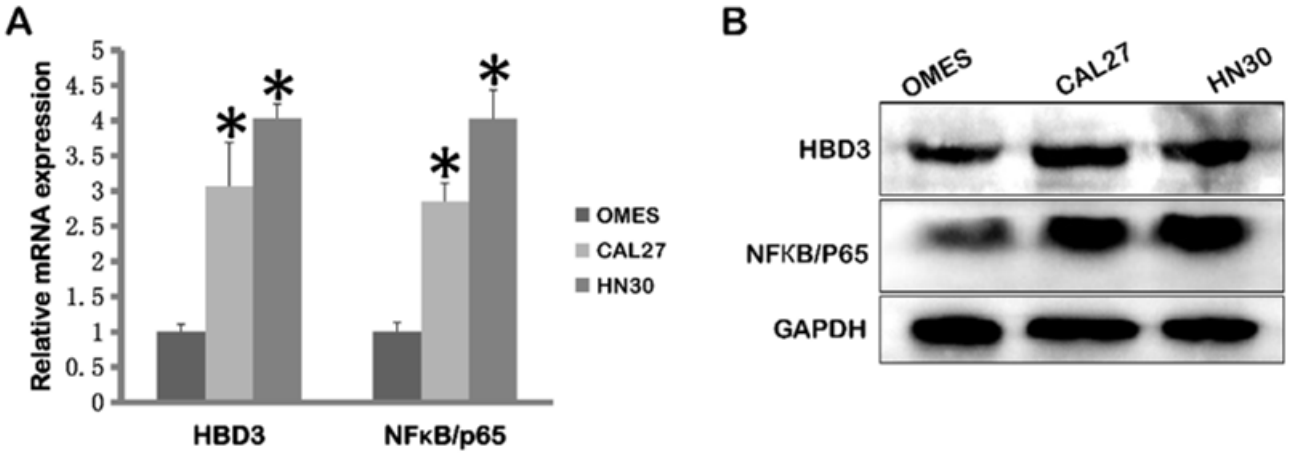

Figure 2

A qRT-PCR and **B** Western blot analysis of the expression of hBD-3 and NF-κB p65 in OMES, CAL27 and HN30 cells. * $P < 0.05$.

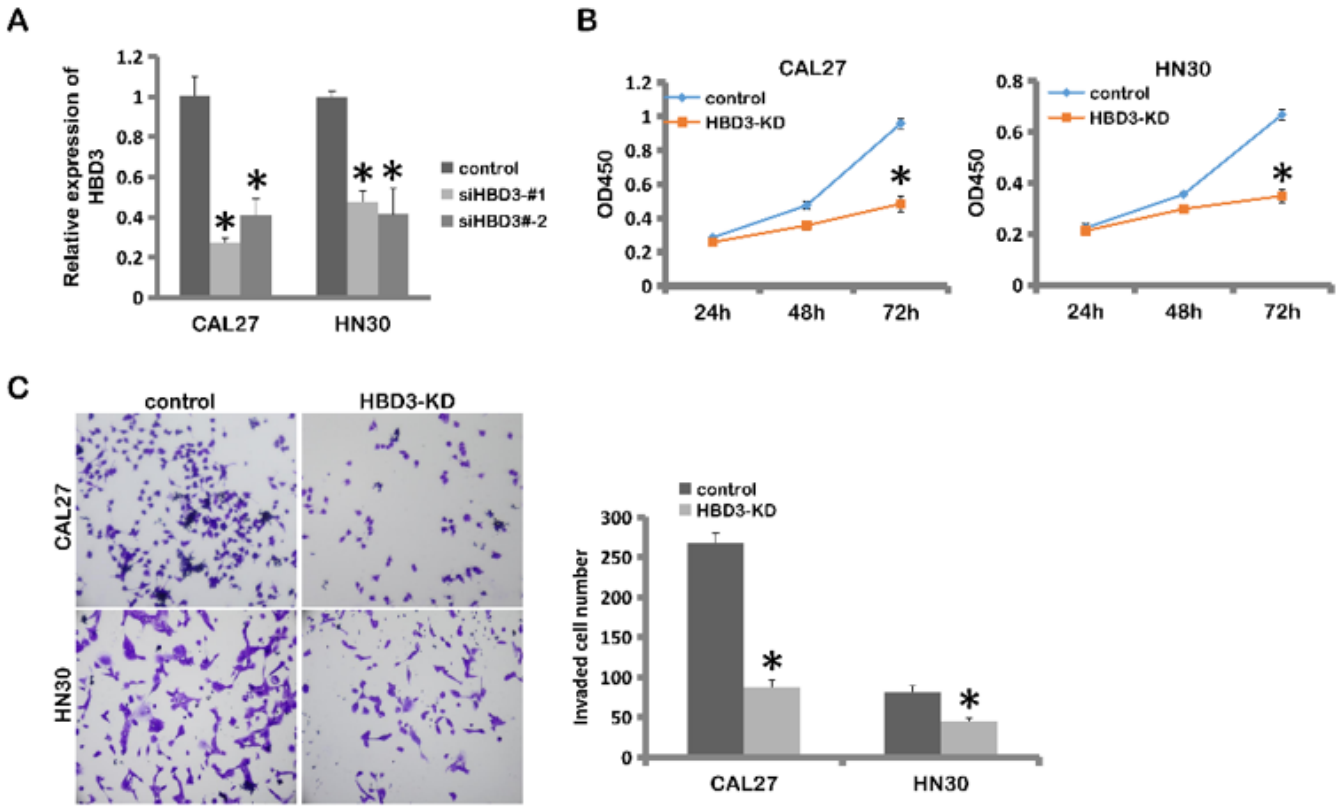

Figure 3

Effects of the knockdown of hBD-3 on proliferation and invasion of OSCC cells. **A** The efficiency of si-hBD-3 was verified by qRT-PCR. **B** Cell viability was tested by CCK-8 assay 48 and 72 h after the transfection. **C** The invasion ability of the transfected OSCC cells was evaluated by Transwell assay. * $P <$ 0.05.

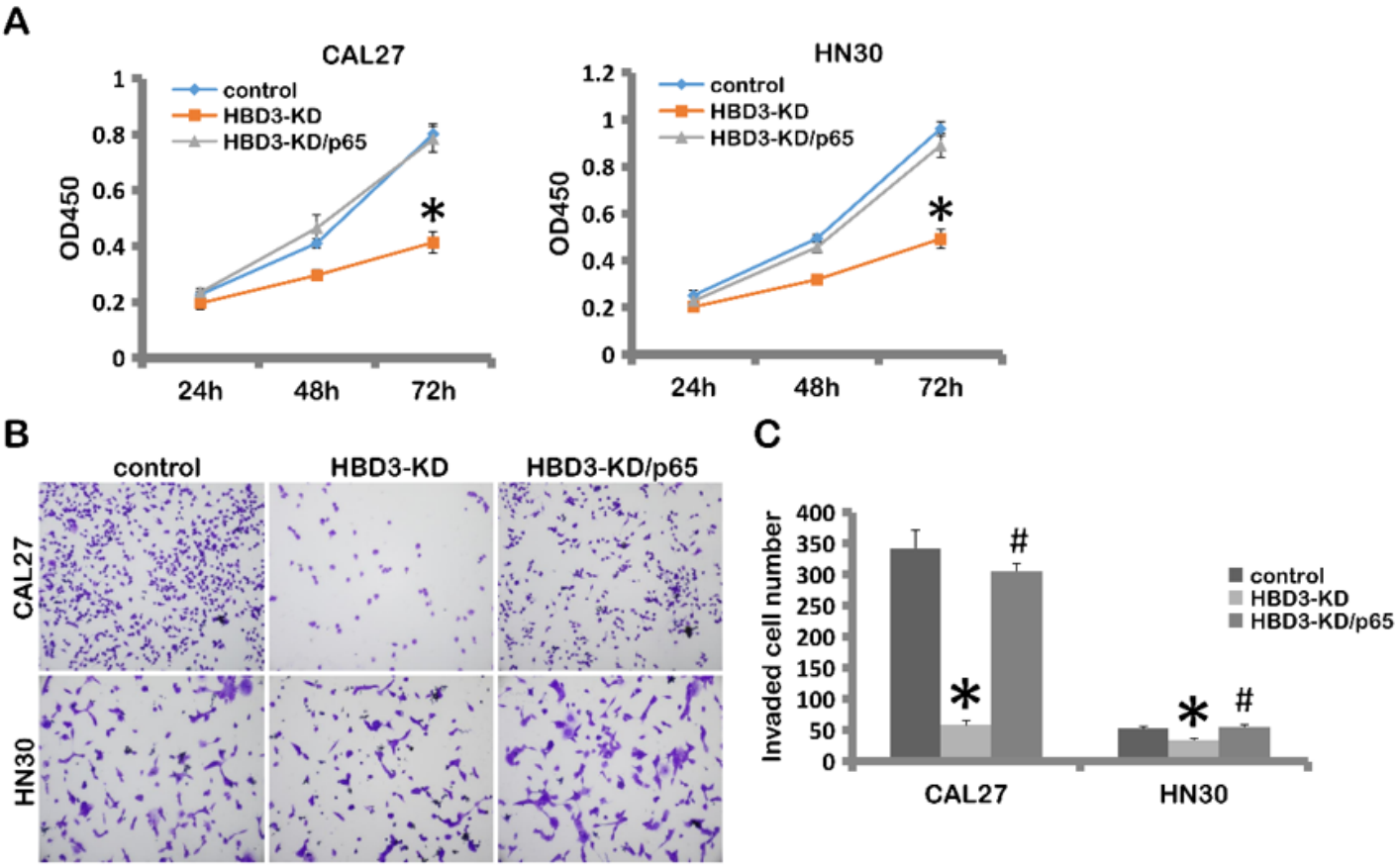

Figure 4

Overexpression of NF-κB p65 reversed the effect of si-hBD-3. **A** The proliferation ability and **B, C** the invasion ability of OSCC cells in control, hBD-3 knockdown and simultaneous overexpression of NF-κB p65 group was evaluated by CCK-8 and Transwell assay, respectively. * $P < 0.05$ *vs* control group, # $P < 0.05$ vs HBD3-KD group.

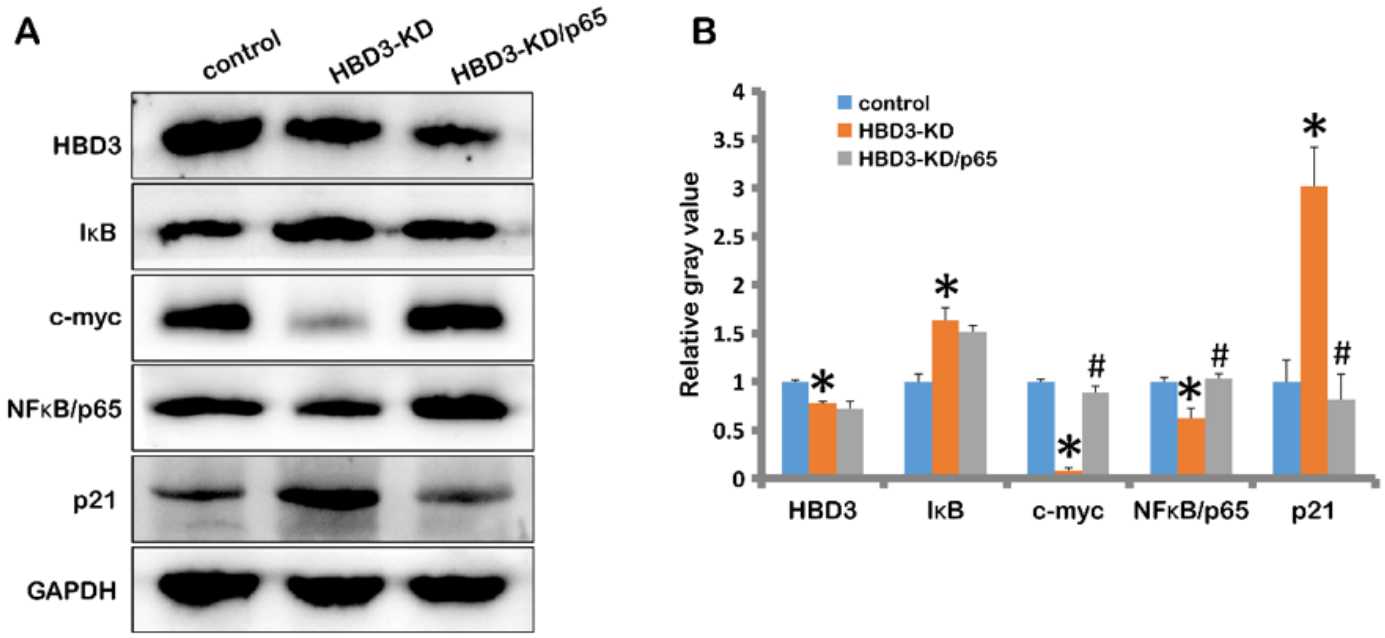

Figure 5

A Western blot analysis and **B** relative gray analysis of the changes in protein expression of hBD-3 and signaling molecules of NF-κB p65 pathway in OSCC cells. * $P < 0.05$ *vs* control group, # $P < 0.05$ vs HBD3-KD group.