Original Paper

Stabilization of Slug by NF- κ B is Essential for TNF- α -Induced Migration and Epithelial-Mesenchymal Transition in Head and Neck Squamous Cell Carcinoma Cells

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Key Words

TNF- α • Slug • HNSCC • EMT • NF- κ B

Abstract

Background/Aims: Slug protein, a transcription factor for the induction of epithelial-mesenchymal transition (EMT) and cancer cell invasion and metastasis, is frequently upregulated in human epithelial cancers. However, mutation of this gene in cancer is rare, and the mechanism of its dysregulation remains unknown, especially in head and neck squamous cell carcinoma (HNSCC). **Methods:** We examined the role of TNF- α in the stabilization of Slug by immunoprecipitation-western blot analysis. Migration of HNSCC cells with or without knockdown of Slug gene expression was assayed by a wound healing assay. Immunohistochemical staining analysis was used to measurement Slug levels in both normal and HNSCC tumor tissues. **Results:** The inflammatory cytokine TNF- α stabilized Slug protein by inhibiting its ubiquitination through the NF- κ B pathway. Inhibition of NF- κ B or knockdown of p65 abrogated the TNF- α -induced stabilization of Slug. Knockdown of Slug expression inhibited cancer cell migration and EMT characteristics induced by TNF- α . Moreover, increased levels of Slug were found to correlate with lymph node metastasis and predict poor prognosis in patients with HNSCC. **Conclusions:** NF- κ B-mediated stabilization of Slug underlies the inflammation-induced EMT and metastasis in HNSCC, which may serve as a therapeutic target for metastatic HNSCC.

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Introduction

Approximately 90% of cancer deaths are caused by metastasis, yet it remains the most poorly understood component of cancer pathogenesis because of its complexity [1]. Emerging evidence suggests that epithelial-mesenchymal transition (EMT), which is an essential phenotypic conversion during embryonic development, tissue remodeling, and wound healing, plays an important role in cancer metastasis [2-4]. During EMT, epithelial cells acquire fibroblast-like properties, exhibit reduced intercellular adhesion, and show increased motility. Several transcription factors are key regulators of EMT, including the Snail/Slug family, Twist, δ EF1/ZEB1 and SIP1/ZEB2 [5-12].

Slug is known to promote tumor progression and metastasis through EMT, causing loss of cell adhesion and polarity while conferring migratory and invasive properties [13, 14]. Consistent with its vital role in cancer metastasis, an overabundance of Slug protein is observed in many types of cancer, including head and neck squamous cell carcinoma (HNSCC) [15, 16]. However, despite its frequent overabundance, Slug is rarely mutated or amplified in HNSCC. Slug is a labile protein that is degraded rapidly in normal tissue. Interestingly, increased Slug stability has been observed in cancer cells, which is linked to the repression of proteasomal degradation [16]. However, the mechanism by which Slug escapes proteasomal degradation in cancer cells remains unknown. Thus, elucidating the molecular mechanism underlying the phenomenon of increased Slug stability may provide a new target for cancer therapeutic intervention.

The processes involved in the invasive growth and metastasis of tumors are complex and vary depending not only on the intrinsic characteristics of the tumor cells themselves but also on the microenvironments where tumors originate. Tumor promotion by the microenvironment is largely orchestrated by inflammatory cells that facilitate extracellular matrix breakdown, angiogenesis, and tissue remodeling, thus promoting the motility of tumor cells [17, 18]. The complex interactions between a tumor and inflammatory cells mediated by inflammatory cytokines are an essential aspect of the tumor microenvironment. The association of inflammation and cancer has been well recognized in many types of cancer, and inflammation is regarded as the “seventh hallmark of cancer” [19, 20]. Accumulating evidence has shown that TNF- α is a key mediator of inflammation and cancer [21, 22]. Increased TNF- α expression and serum TNF- α levels are associated with clinical cancer stages, high-grade malignancies, and poor prognosis in HNSCC and several other cancers [23, 24]. However, the comprehensive mechanism by which TNF- α promotes HNSCC metastasis remains unclear.

In this study, we investigated the roles of TNF- α in the metastatic features of HNSCC. We found that stabilized Slug is essential for TNF- α -induced migration and EMT characteristics in HNSCC. Importantly, NF- κ B mediates the effect of TNF- α on the stabilization of Slug protein by inhibiting its ubiquitination-mediated degradation. Moreover, Slug overexpression was found to correlate with lymph node metastasis and poor prognosis in HNSCC. Thus, the TNF- α -NF- κ B-Slug axis represents a therapeutic target for metastatic HNSCC.

Materials and Methods

Cell cultures and reagents

HNSCC-derived cell lines HN4, HN12, HN13 was kindly provided by the University of Maryland, School of Dentistry. CAL27 cell line was purchased from the American Type Culture Collection (ATCC). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco) supplemented with 10% fetal bovine serum, 1% glutamine, and 1% penicillin-streptomycin. All cells were maintained in a humidified atmosphere of 5% CO₂ at 37°C. Antibodies for Slug (#9585), GAPDH (#9585), p65(#8242), E-cadherin (#3195), N-cadherin (#13116), Vimentin (#5741), Oct4 (#2750), Sox2 (#3579), GSK3 β (#12456) were from Cell

Signaling Technology Inc. HRP-conjugated secondary antibodies were from eBioScience (San Diego, CA). Human recombinant TNF- α was obtained from R&D system (Minneapolis, MN, USA).

Immunoprecipitation and Western Blotting

Experiment protocol was performed as described previously [25, 26]. Cells were lysed with RIPA lysis buffer. Immunoprecipitation was performed with 2 μ g of antibody against Slug, Ubiquitin or normal IgG (as a negative control) in 1.0-mg whole-cell lysate. Cell lysates and/or immunoprecipitation cellular proteins were separated by SDS-PAGE in a 10% acrylamide gel and transferred onto nitrocellulose membrane for immunoblot.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Experiment protocol was performed as described previously [27, 28]. Total RNA samples were extracted with TriPure Isolation Reagent (Roche, Switzerland) and cDNA prepared from 1 mg of total RNA using the SuperScript III System (Invitrogen Life Technologies). The mRNAs levels were determined by RT-PCR, using the following primers: Slug (F: 5'-GAGCATTTCGACAGGTCA-3' and R: 5'-CCTCATGTTTGTGCAGGAGA-3'); GAPDH (F: 5'-TCCACCACCTGTTGCTGTA-3' and R: 5'-ACCACAGTCCATGCCATCAC-3').

Wound healing assay

Experiment protocol was performed as described previously [29, 30]. Cells were plated to confluence in a 6-well plate, and the cell surface was scratched using a pipette tip. Accurate wounds measurements were taken at 0 and 24 hours to calculate the migration rate according to the equation. Three independent experiments were carried out.

Primary HNSCC samples

All of the methods were approved by the research medical ethics committee of Shanghai Jiao Tong University and were performed in accordance with the approved guidelines. We obtained archival, formalin-fixed and paraffin-embedded (FFPE) material from surgically resected HNSCC specimens from Ninth People's Hospital (Shanghai, CHINA), from 2007 to 2011. In total, 85 primary HNSCC patients without prior radiotherapy or chemotherapy were enrolled in this study. The age of these patients ranged from 18 to 83 years with an average 57.0 years. Histopathologic diagnosis of each neoplastic tissue was performed according to the World Health Organization criteria by the Department of Oral Pathology, Ninth People's Hospital of Shanghai. Clinicopathologic staging was determined by the TNM classification of the International Union against Cancer. This study was approved by the Ethics Committee of Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine and carried out according to the recommendations of the Declaration of Helsinki. All the patients involved in this study signed written informed consent in accordance with the institutional guidelines.

Immunohistochemistry

Immunohistochemistry was performed as previously described [31]. Immunohistochemical staining was performed on the sections (3- μ m thickness) of paraffin-embedded specimens. Briefly, the sections were dewaxed in xylene and hydrated with graded ethanol. Then antigen retrieval was carried out using 0.01 mM citrate buffer (pH 6.0) pressure-cooking, and endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 minutes at room temperature. The slides were incubated with anti-Slug in a moist chamber for overnight at 4°C. Upon incubation with the primary antibody, the specimens were washed three times in PBS and visualized using 3, 3'-diaminobenzidine detection kit (Dako Cytomation, Denmark). Samples were then counterstained with hematoxylin, a blue nuclear stain. Tumor cells were considered Slug-positive if the immunoreactivity was observed in the nucleus and/or cytoplasm. The mean percentage of positive tumor cells was determined at least five random fields at $\times 400$ magnification in each section. The percentage of positive tumor cells was assigned to one of the following categories: 0, <5%; 1, 5–25%; 2, 26–50%; 3, 51–75%; and 4, >75%. The intensity of Slug immunostaining was scored as follows: (a) weak, 1+; (b) moderate, 2+; and (c) intense, 3+. The percentage of positive tumor cells and the staining intensity were multiplied to produce a weighted score for each case. Cases with a weighted Slug score ≥ 2 were considered to be positive. These judgments were made by two independent pathologists, neither of whom had knowledge or information pertaining to the patients' clinical status.

Statistical analysis

Data analysis used SPSS (Statistic Package for Social Sciences) 13.0 for Windows (SPSS Inc., Chicago, IL, USA). Unpaired Student's t-tests or U-Mann Whitney tests determined statistical significance between groups with P values <0.05 considered significant.

Results

TNF- α induces Slug protein stabilization

To identify the factors that are important for the regulation of Slug, we established a HEK293F-Slug stable transfectant cell line and examined various pro-inflammatory cytokines for their ability to stabilize Slug. Strikingly, we found that TNF- α was the most potent cytokine for the stabilization of Slug (data not shown). To further validate this observation, we also examined the effects of TNF- α on two HNSCC cancer cell lines (CAL27 and HN13). TNF- α treatment increased the level of Slug protein in both cell lines (Fig. 1A). Interestingly, the mRNA level of Slug did not show a significant elevation with TNF- α treatment as measured by RT-PCR (Fig. 1B). These results suggest that the TNF- α -induced elevation of Slug is likely through protein stabilization. Next, we examined the kinetic features of Slug following TNF- α treatment. TNF- α treatment increased Slug level within 1 h and reached a maximum level at approximately 4 hr without significant mRNA elevation (Fig. 1C and 1D). These results strongly demonstrate that the pro-inflammatory cytokine TNF- α increases Slug in HNSCC cells in a transcription-independent manner.

TNF- α enhances Slug stability by inhibiting its ubiquitination

Slug is a labile protein. To determine the regulatory mechanism underlying the TNF- α -induced stabilization of Slug we examined the protein levels of Slug in cells treated with

Fig. 1. TNF- α induces the stabilization of Slug protein. (A) Western blot analyses of Slug in CAL27 and HN13 cells treated with 0–200 ng/mL TNF- α for 4 h (upper panel) and the relative expression of Slug protein for each sample after normalization to GAPDH (lower panel). (B) RT-PCR analyses of Slug mRNA levels in CAL27 and HN13 cells treated with 0–200 ng/mL TNF- α for 4 h. (C) Slug protein in CAL27 and HN13 cells treated with TNF- α (100 ng/mL) for different time intervals was analyzed by western blotting. (D) RT-PCR analyses of Slug mRNA levels in cells treated with TNF- α (100 ng/mL) for different time intervals.

or without TNF- α for different lengths of time (up to 4 h) in the absence or presence of the protein translation inhibitor cycloheximide (Fig. 2A and 2B). TNF- α treatment significantly slowed down the rate of Slug degradation (Fig. 2C). This observation was confirmed further via immunofluorescence microscopy, in which the staining intensity of Slug was dramatically increased in CAL27 and HN13 cells treated with TNF- α (Fig. 2D and 2E). Taken together, these results suggest that TNF- α treatment induces the stabilization of Slug.

As the stabilization of Slug by TNF- α occurs at the protein level, we next asked whether TNF- α -induced Slug stabilization is through regulation of the ubiquitination pathway. We examined Slug levels in several HNSCC cells lines treated with or without the proteasome inhibitor MG132, and found that Slug was stabilized by MG132 treatment (Fig. 3A). To determine whether Slug was regulated through ubiquitination, we performed immunoprecipitation-western blot analysis and found that ubiquitylation of FLAG-tagged Slug was significantly reduced in TNF- α treated cells compared with untreated cells (Fig. 3B). Similarly, TNF- α treatment also dramatically suppressed the ubiquitylation of endogenous Slug compared to with that on untreated cells (Fig. 3C). Taken together, these results demonstrated that the stabilization of Slug by TNF- α was mediated by the suppression of its ubiquitylation.

Fig. 2. TNF- α enhances the stability of Slug. (A) CAL27 cells were treated with or without TNF- α (100 ng/mL) for 4 h, followed by incubation with cycloheximide (CHX; 10 μ M) for an extended period of time. Slug levels were determined by western blot analysis. (B) HN13 cells were treated with or without TNF- α (100 ng/mL) for 4 h, followed by incubation with CHX (10 μ M) for an extended period of time. Slug levels were determined by western blot analysis. (C) Graphic representation of the densitometry results for Slug after CHX treatment (circle, with TNF- α ; square, without TNF- α). (D) Immunofluorescence staining for Slug in CAL27 and HN13 cells treated with or without 100 ng/mL TNF- α for 4 h. (E) Graphic representation of relative immunofluorescence staining intensity for Slug after treatment with or without 100 ng/mL TNF- α for 4 h.

Fig. 3. TNF- α inhibits the ubiquitination of Slug. (A) HEK293-Slug stable cells and HNSCC CAL27, HN12, and HN13 cells were treated with MG132 (10 mM) for 4 h, and Slug was examined by western blotting. (B) HA-ubiquitin was expressed in HEK293-Slug cells followed by treatment with either TNF- α (100 ng/mL) or MG132 (10 mM) for 4 h. Ubiquitin was immunoprecipitated from an equal amount of lysates, and ubiquitinated Slug was examined by western blotting. (C) CAL27 cells were treated with TNF- α (100 ng/mL) or MG132 (10 mM). Endogenous Slug was immunoprecipitated, and the bound endogenous ubiquitin was detected by western blotting.

TNF- α -mediated Slug stabilization is dependent on NF- κ B activation

To determine which signal transduction pathway is crucial for TNF- α -mediated Slug stabilization, we next examined the effects of inhibitors of various signaling pathways including NF- κ B, MAPK/ERK, mTOR, p38, and JNK kinases. Only NF- κ B inhibitors (BAY11-7082 and BMS-345541) completely blocked TNF- α -induced Slug stabilization (Fig. 4A), whereas the effects of the other inhibitors were minimal (data not shown). These results suggest that the NF- κ B pathway is crucial for TNF- α -mediated Slug stabilization. For further characterization, we examined the effects of overexpressing exogenous p65 in CAL27 and HN13 cells followed by TNF- α treatment. The expression of p65 dramatically increased Slug protein in these cells (Fig. 4B). Collectively, these results indicate that NF- κ B is crucial for the stabilization of Slug. To determine whether NF- κ B is required for Slug stabilization, we examined the levels of Slug in CAL27 and HN13 cells in which p65 was knocked down by small interfering RNA (siRNA). Knockdown of p65 expression dramatically suppressed the TNF- α -induced stabilization of Slug (Fig. 4C). Thus, NF- κ B is required for the TNF- α -induced stabilization of Slug. To analyze the molecular mechanism for Slug stabilization by p65, we coexpressed p65 with Slug in HEK293 cells. Interestingly, there was no physical association between Slug and

Fig. 4. Activation of the NF- κ B pathway is required for the stabilization of Slug. (A) CAL27 and HN13 cells were pretreated with NF- κ B inhibitors (10 μ M BMS-345541 and 50 μ M BAY11-7082) for 1 h followed by stimulation with TNF- α (100 ng/mL) for 4 h. Slug protein was examined by western blotting. (B) Myc-tagged p65 was expressed in CAL27 and HN13 cells that were treated with or without TNF- α (100 ng/mL) for 4 h. Expression of Slug and p65 was analyzed by western blotting. (C) Control or p65 siRNA was expressed in CAL27 and HN13 cells followed by TNF- α (100 ng/mL) or vehicle treatment for 4 h. Slug and p65 levels were examined by western blotting.

Fig. 5. Knockdown of Slug expression suppresses TNF- α -induced cell migration in vitro. (A) The motile behavior of CAL27 and HN13 cells and in their corresponding Slug knockdown cells that were treated with or without TNF- α (100 ng/mL) was analyzed by a wound healing assay. (B) The percentage of migrating cells is shown (mean $\pm$ standard error of the mean in 3 separate experiments). (C) Expression of Slug (treated with TNF- α), E-cadherin, N-cadherin, and GAPDH in CAL27 and HN13 cells and their corresponding transfectants with knockdown of Slug expression was analyzed by western blotting.

p65 (data not shown), suggesting that a mediator may be involved in the stabilization of Slug and that this potential mediator is induced by TNF- α or p65.

Slug is required for TNF- α -mediated cell migration in HNSCC

Next, we asked whether Slug is required for the TNF- α -induced migration of HNSCC cells. We examined the effects of TNF- α on the migration of HNSCC cells that were treated with or without siRNA-Slug. TNF- α treatment significantly increased cell migration in a wound healing assay. However, this effect was significantly suppressed when Slug was knocked down in both CAL27 and HN13 cell lines (Fig. 5A and 5B). Consistent with the biological changes, the level of Slug altered the expression pattern of EMT markers, in which E-cadherin expression was increased whereas N-cadherin expression was decreased following knockdown of Slug (Fig. 5C). Taken together, these results demonstrated that Slug is required for cell migration mediated by inflammation in HNSCC.

High Slug expression correlates with lymph node metastasis and predicts poor prognosis in patients with HNSCC

Lymph node metastasis is associated with a poor clinical outcome in HNSCC patients and it contains many features that are characteristic of EMT. To determine if Slug plays an important role in lymph node metastasis in HNSCC, we examined Slug protein levels in 85 paired normal and primary HNSCC samples by immunohistochemistry (IHC) staining analysis. A summary of patient demographics and clinicopathological data is shown in Table 1. IHC staining analysis demonstrated that normal tissues had low Slug protein expression. In contrast, Slug protein was expressed at a high level in HNSCC tissues (Fig. 6A and 6B). Of note, Slug protein expression (IHC score) was significantly higher in patients with lymph node metastases (M-tumor) than in patients without lymph node metastasis (NM-tumor) (Fig. 6A and 6B). Taken together, the results of IHC staining analysis showed that Slug upregulation was strongly associated with lymph node

Table 1. The baseline characteristics of HNSCC patients include in the study

Characteristics	Patients	
	No.	%
Age, years		
≤60	53	62.3
>60	32	37.7
Sex		
Male	45	52.9
Female	40	47.1
T-primary tumor size		
T1	24	28.2
T2	35	41.2
T3	14	16.5
T4	12	14.1
N-regional lymph node		
Negative	55	64.7
Positive	30	35.3
TNM stage		
I	25	29.4
II	23	27.1
III	20	23.5
IV	17	20.0
Histopathological type		
Grade 1	36	42.3
Grade 2	40	47.1
Grade 3	9	10.6
Smoking history		
Yes	29	34.1
No	56	65.9
Alcohol history		
Yes	22	25.9
No	63	74.1
Total	85	100

Fig. 6. High Slug expression is associated with lymph node metastasis and predicts poor prognosis in patients with HNSCC. (A) Normal and tumor samples from HNSCC patients without lymph node metastasis showed low Slug expression, whereas tumor samples from HNSCC patients with lymph node metastasis showed high Slug expression. Bar = 500 μ m. (B) IHC scores of Slug expression in normal and HNSCC samples with or without metastasis. Statistically significant differences were detected using measures analysis of the Wilcoxon signed-rank test ($P < 0.001$). (C) High Slug expression was significantly correlated with a poor survival rate of HNSCC patients. The survival rates of patients with Slug-positive and Slug-negative tumors ($P < 0.05$) were calculated using the Kaplan–Meier survival test.

metastasis in HNSCC patients. As regional lymph node metastasis is considered a major risk factor for poor survival in patients with HNSCC, we investigated the relationship between Slug expression and survival rate of HNSCC patients. By performing survival analysis for the prognostic relationship of Slug expression in HNSCC via the Kaplan–Meier test, we found that patients with high Slug expression had a significantly shorter overall survival rate than patients with low Slug expression ($P < 0.05$) (Fig. 6C). Taken together, these results demonstrate that Slug overexpression is associated with metastasis to lymph nodes and poor survival rate in patients with HNSCC.

Discussion

In this study, we found that TNF- α -induced Slug stabilization plays a critical role in inflammation-induced EMT and cancer cell migration in HNSCC. NF- κ B activity is essential for TNF- α -induced Slug stabilization. Furthermore, TNF- α induces Slug stabilization by inhibiting its ubiquitination in HNSCC cells. Thus, the TNF- α -NF- κ B-Slug axis represents a potential therapeutic target for inflammation-related metastasis in HNSCC (Fig. 7).

Slug plays an essential role in development [32] and EMT-associated cancer metastasis [33–35]. Mechanistically, Slug binds to *cis*-elements in the promoter of a number of target genes (e.g., E-cadherin) via its C-terminal “zinc finger” domain and represses gene expression by recruiting chromatin-modifying proteins via its N-terminal “SNAG” domain. Previous studies showed that many factors, such as TGF- β 1, CD271, CCL18, and the autocrine factor epiregulin, could contribute to the increased expression of Slug in HNSCC cells [36–39]. Although increased Slug expression has been linked to altered transcriptional programs that confer tumor-initiating and invasive capabilities, resistance to apoptosis, and therapeutic failure [40–44], the molecular basis for Slug stabilization in cancer has remained unclear. Here, we explored the mechanism by which the pro-inflammation factor TNF- α acts as a critical factor for Slug protein stabilization. Chronic inflammation is closely correlated to carcinogenesis and metastasis, and TNF- α acts as a major pro-inflammatory cytokine [21, 45, 46]. TNF- α was originally identified for its ability to induce rapid necrosis in experimental cancers [21]. The persistent expression of TNF- α in the tumor microenvironment is an important feature of many malignant tumors, which is often associated with poor prognosis [23, 24]. The tumor-promoting effects of TNF- α are facilitated through the enhancement

of cancer cell proliferation and survival, angiogenesis, and the formation of an invasive phenotype through various oncogenic pathways. In addition, TNF- α has also been reported to enhance cancer migration and invasion through EMT by stabilizing Snail protein in breast cancer [47]. In this study, we found that Slug was required for TNF- α -mediated EMT and metastatic features in HNSCC. Our study provides a mechanistic link between NF- κ B activation and Slug overexpression in metastatic HNSCC cells.

Snail and Slug are both members of the Snail family, have a critical role in EMT, and encode transcriptional repressors for the E-cadherin promoter [8, 48, 49]. Upregulated Snail and Slug are associated with aggressiveness, chemotherapy resistance, and poor survival in many cancers [50-52]. A previous study showed that the inflammation-triggered migration, invasion, and metastasis of tumor cells are mediated through NF- κ B-induced Snail stabilization in breast cancer. Furthermore, TNF- α -mediated Snail stabilization occurs through the induction of CSN2, which blocks the phosphorylation and ubiquitylation of Snail by disrupting its binding to GSK-3 β and β -Trcp, resulting in Snail stabilization in a non-phosphorylated and non-ubiquitylated state [47]. In the present study, we showed that TNF- α induced EMT in CAL27 and HN13 cells, while the knockdown of Slug in these cells reversed the EMT phenotype and aggressiveness induced by TNF- α . This suggests that Slug plays a major role in mediating TNF- α -induced EMT in HNSCC cells. Thus, Slug and Snail, members of the same protein family, may play the same role in different tissues. However, the detailed regulatory mechanisms for these two molecules in different tissues remain to be investigated. Slug reportedly undergoes acetylation-dependent protein degradation, and the deacetylase SIRT2 was identified as a key mediator of this posttranslational mechanism [53]. These findings are in agreement with the notion that Slug is a labile protein that is subjected to delicate regulation by multiple signaling pathways to control its ubiquitylation and degradation. However, whether acetylation is involved in TNF- α -mediated Slug protein stabilization still requires further study.

Clinically, lymph node metastasis is considered one of the most important adverse prognostic factors for HNSCC [54, 55]. The 5-year survival rate for patients with HNSCC at an early stage with a localized site is greater than 80%, but this decreases to 40% when the disease has spread to the neck nodes [56]. A recent study showed that chronic inflammation is closely correlated with cancer and metastasis through inflammatory cytokines such as TNF- α [57]. However, the mechanism underlying the correlation between inflammation and lymph node metastasis in HNSCC is still unclear. Here, we showed that the overexpression of Slug correlated with lymph node metastasis and predicted poor prognosis in patients with HNSCC. Moreover, Slug was required for TNF- α -mediated cancer cell migration and induction of the EMT program. Thus, targeting the TNF- α -NF- κ B-Slug axis represents a novel therapeutic strategy for metastatic HNSCC with chronic inflammation.

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Fig. 7. Proposed model to illustrate the stabilization of Slug by TNF- α leading to EMT and metastasis.

Our study was approved by the ethical committee of the Ninth People's Hospital of Shanghai, China.

Disclosure Statement

The authors declare that they have no competing interests.

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