REVIEW

Role of SLUG in Epithelial-Mesenchymal Transition of Oral Squamous Cell Carcinoma

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Abstract

Introduction: Oral squamous cell carcinoma (OSCC) is the most common malignancy which shows diverse heterogeneity. Epithelial-mesenchymal transition (EMT) is a biological process that involves the transformation of epithelial cells acquiring mesenchymal characteristics developing into malignancy. Several transcription factors are directly or indirectly involved in this cadherin switch of decreased expression of E-cadherin and increased expression of N-cadherin and vimentin which induces EMT and further invasion and metastasis in OSCC. SLUG is a zinc finger-type transcription factor that promotes tumour progression and metastasis through EMT.

Objectives: This comprehensive review describes the role of SLUG in the epithelial-mesenchymal transition of OSCC and also highlights the various molecular pathways involved.

Keywords: SLUG, Oral squamous cell carcinoma, EMT

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most prevalent cancer worldwide.[1] Oral squamous cell carcinoma (OSCC) is the most common histological subtype of head and neck cancer which accounts for 95% of all cases.[2,3] Even though the five-year survival rate has surged to 65%, diagnosis at an advanced stage leads to a five-year survival rate of only 27%, which necessitates the need for early diagnosis and prompt therapeutic implications in combating this disease.[4-6]

OSCC is a heterogeneous disease at the inter- and intratumor levels. The major cellular change responsible for heterogeneity is the epithelial-to-mesenchymal transition (EMT). EMT is a process that transforms polarized epithelial cells into mesenchymal cells morphologically by increasing their motility and invasive capacity.[7,8] Elisabeth Hay and her colleagues originated the first description of EMT.[9] The major hallmark of EMT is the loss of epithelial marker expression, typically expressed by E-cadherin, and a gain in the mesenchymal marker expressed by N-cadherin and vimentin, which is also accompanied by an invasive phenotype. Epithelial cells acquire an apicobasal polarity, with an attachment to the basal lamina and a tight cell-to-cell junction. Mesenchymal cells acquire a detached arrangement in the stroma with a front-back polarity and migration ability. Transcriptional factors that directly or indirectly down regulate E-cadherin expression and induce EMT are known as EMT-activating transcriptional factors (EMT-TFs) which are tightly regulated via oncogenic signaling pathways. They consist of non-coding RNAs, extracellular mediators and translational/post-translational regulations.[10]

Key regulators of EMT include several transcription factors such as the Snail/Slug family, Twist, δEF1/ZEB1 and SIP1/ZEB2. Many studies showed the role of Twist in oral mucosa carcinogenesis and development of OSCC. Nuclear localization of Twist is significantly correlated with lower E-cadherin expression and over expression of N-cadherin in OSCC. The ZEB family includes two proteins, ZEB1 and ZEB2, which function as either transcriptional activators or repressors depending on their target gene and tissue. Increased expression of ZEB1 or ZEB2 in epithelial cells induces EMT through the repression of E-cadherin and other epithelial markers. During EMT, expression of mesenchymal genes such as N-cadherin, vimentin and several matrix metalloproteinases (MMPs), are significantly upregulated

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with increased expression of ZEB proteins.[11]

Snail family genes are well-known transcription factors, which play critical roles in EMT during embryogenesis. Three snail family genes, such as SNAIL (Snail 1), SLUG (Snail 2), and SMUC (Snail 3), have been identified so far, and they are classified as zinc finger-type transcription factors. SLUG, also known as Snail[2], is located on chromosome 8q and contains five zinc finger domains at its C-terminal. Physiologically, SLUG has been reported in its presence in normal embryogenesis as a mediator in mesoderm and neural crest cell formation.[12,13]

SLUG in cancer:

SLUG has a variety of biological roles such as in cell growth, migration, and invasion. Oncologically, it is associated with tumour cell metastasis. Therefore, it is well known due to its increased expression in various solid tumours, including leukaemia, lung cancer, oesophagal cancer, gastric cancer, breast cancer, and glioma compared with normal tissue. Elevated slug expression reduces the survival rate in various cancers.[14]

Liu et al. in their study found that Slug expression was higher in lung cancer cells when compared to normal lung tissue and that increase was associated a with poorer survival rate and more aggressive clinicopathological parameters.[15] Chang et al. in their study showed the high correlation of Slug with tumor invasion and drug resistance in ovarian cancer cells.[16] Shioiri et al. found that increased slug protein expression was significantly elevated in colorectal cancers with a high T-stage, liver metastases, and lymph node metastases and it can be considered as a potential prognostic marker for colorectal cancer.[17]

Metastasis of tumour cells and resistance to antitumor therapy are the two main leading causes of poor prognosis in cancer patients. There are several molecular mechanisms by which slug promotes the metastasis of cancer underlying EMT. Liu et al. showed the inhibition effect of slug on the expression of miR-200b and miR-1, which evoked EMT and tumour cell invasion.[18]

SLUG in OSCC

In HNSCC, the biological functions of the slug are poorly defined. Liu et al observed the stabilization of SLUG via the activation of nuclear factor kappa B, which subsequently resulted in an inflammation-induced EMT and eventually metastasis in HNSCC.[11] Gao et al. in their study demonstrated a decreased expression of E-cadherin along with an increased expression of vimentin and SLUG at tumour margins was related to the activation of the EGFR/ ERK-pathway via the release of EGF by cancer-associated macrophages in the stroma.[19] Zhang et al reported an increased expression of SLUG in hypoxia-mediated cadherin switch and SLUG may be considered as an important predictor of poor clinical outcome in patients with HNSCC.[20]

SLUG and cancer stem cells

Cancer stem cells (CSCs) are referred to as a subpopulation of cancer cells that contain self-renewal and multipotent differentiation, as seen in embryonal stem cells. Cancer stem cells maintain tumour growth exclusively. Recent studies have shown the reversal induction of EMT with CSC-like phenotypes, indicating a strong association between EMT and CSC genesis. The well-established roles of SLUG in EMT during embryogenesis and tumour progression suggest a possible role of SLUG in CSC genesis.

Moon et al in their study revealed SLUG conferred with CSC features on cancer cells, such as increased self-renewal capacity, which was based on sphere-forming capacity in vitro, chemoresistance via increased ABC transporter gene expression, elevated tumour initiation and invasion in xenograft models. SLUG may also suppress diverse genes involved in differentiation. In addition to these, SLUG can also control stemness by interacting with other cofactors such as SOX9, which can prevent its ubiquitin-mediated proteasomal degradation, and YAP/TAZ which can enhance self-renewal and differentiation traits of mesenchymal stem cells. They also suggest the prognostic value of SLUG for patients with HNSCC post-treatment. SLUG expression can be considered a favourable prognostic indicator compared with the AJCC system. Thus, they suggest slug as an additional therapeutic target for HNSCC management.[13]

Molecular pathways of SLUG

EMT regulatory pathways such as canonical Wnt, Notch, Hedgehog (HH), Transforming growth factor beta (TGFb), Receptor tyrosine kinase (RTK), and others play critical roles in human malignancy. Among these, the canonical Wnt/β-catenin pathway is involved in multiple cancer development processes such as cell proliferation, migration, and invasions.[13] The canonical Wnt/β-catenin pathway induces Snail transcription and EMT through b catenin/T-cell factor (TCF)/LEF transcriptional complex. Normally, b-catenin is phosphorylated and subsequently degraded to maintain a constant intracellular level. The general assumption is that b-catenin phosphorylation results from a multiprotein 'destruction complex', involving kinase proteins such as glycogen synthase kinase-3b (GSK-3b), casein kinase 1 (CK1)] and scaffold proteins such as Axin and adenomatous polyposis coli (APC). Similarly, Snail also harbours GSK-3-dependent phosphorylation motif, ubiquitination and proteasomal degradation. Correspondingly, canonical Wnt signalling inhibits Snail degradation via Axin2-mediated nuclear export of GSK-3b. Also, the Wnt/b-catenin axis promotes Slug activity and BRCA1 downregulation in breast cancer cells. Axin2 and Snail are also highly expressed in oral precancerous lesions.[10]

Notch signalling directly activates EMT with nuclear translocation of the Notch intracellular domain (NICD). Interaction of Jagged-2 (JAG2) and Notch can extend to NCID cleavage by disintegrin and Metalloprotease (ADAM) and c-secretase. NICD can directly bond to the Snail promoter and can stimulate its expression. In addition to these, under hypoxic conditions, Notch can also indirectly stabilize Snail1 activation by binding hypoxia-inducible factor 1a (HIF-1a) to the lysyl oxidase (LOX) promoter.[10]

The Notch signalling pathway has diverse involvement in cell differentiation, regulating cell growth, proliferation, apoptosis, adhesion and EMT. Abnormality in the Notch signalling pathway is related to tumour formation. TGFb1 also

plays an important role in EMT by Smad3-induced activation of the Jagged1/Notch pathway. Tumour suppression or tumour-promoting effect differs depending upon the types of cells and tissue. Zhang et al confirmed that Slug is a direct target gene of Notch. TGFb1 and Smad3 also played an important role in the process of EMT. TGFb1 regulates Notch ligand expression by regulating the Notch signalling pathway. The notch signalling pathway, especially Jagged1, is closely related to tumour invasion, metastasis, chemotherapy resistance, and tumour immune escape, but the exact mechanism is not fully understood yet. The Notch signal emitted by the surrounding keratinocytes can initiate the differentiation of basal stem cells. Abnormal Notch signalling can cause epithelial cancer, such as squamous cell carcinoma, basal cell carcinoma, and malignant melanoma. Disordered stem cell distribution in the abnormal proliferation of oral epithelial cells indicated the significance of Notch signalling in the development of oral epithelial dysplasia and oral squamous cell carcinoma. Notch signaling which is activated by Jagged1 can promote EMT through the regulation of Slug to inhibit E-cadherin.[20]

SLUG and Cytokines

Roles of cytokines in EMT have been studied in various types of cancer cells. Among these, transforming growth factor (TGF)-β is a well-known inducer of EMT and is usually over expressed in some of the cancer tissues. Cancer cells express TGF-β extensively in the bone-invading area which is identified by the immunohistochemical techniques in human tissue specimens. TGF-β stimulation was directly related to the upregulation of Slug and Snail in human OSCC cells. A study in mouse normal mammary gland epithelial NMuMG cells has shown the upregulation of Snail by the TGF-β–Smad pathway by the treatment with cycloheximide. Also, HMGA2, which is induced by the TGF-β–Smad pathway, showed increased Snail expression in the same type of cells, which indicates that Snail is an indirect target for the TGF-β–Smad pathway. These studies demonstrated the induction of TGF- β by Snail in NMuMG cells is extensively upregulated by the Smad pathway and they are maintained by HMGA2, which leads to a biphasic manner of cellular response to TGF-β. In addition to these, the–Smad pathway also engaged in crosstalk with the STAT3 pathway in cancer tissues harbouring a KRAS mutation. They also observed Slug and Snail downregulate the expression of miR-34 and miR-203 in OSCC cells.[14]

IL-6 is a well-known multifunctional cytokine that regulates both immune and inflammatory responses. Increased expression of IL-6 has been observed in various human cancer tissues. IL-6 induces EMT through STAT3 activation in human breast cancer cells. IL-8 is a pro-inflammatory chemokine that is identified as a potent neutrophil activator and also a chemotactic factor, secreted from macrophages and monocytes. siRNAs against Slug and Snail upregulated IL-6 and IL-8 cytokine levels. However, the exact mechanism by which siRNAs act against Slug and Snail upregulation in IL6 and IL8 mRNA and promote STAT3 phosphorylation is not yet understood. Expression of SOCS3 and PIAS3, which are the negative regulators for STAT3, was not altered either by siRNA. STAT3 activation is dependent on autonomous IL-6 or IL-8 secretion only. STAT3 inhibitor, Stattic, alternatively repressed the induction of Snail by Slug siRNA and Slug by Snail siRNA. Stattic can also inhibit the expression of Slug even without Snail siRNA, which shows that STAT3 activation is essentially required for Slug expression in OSCC cells. Also, the phosphorylation levels of STAT3 were not completely consistent with Slug expressions in OSCC cells, suggesting a requirement for additional signalling pathways. Therefore, Slug and Snail could regulate reciprocally with each other's expression probably through or at least in part, by STAT3 activation which is induced by autonomously secreting IL-6 and IL-8. 14

SLUG and TNF- α

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". Studies have shown that TNF-α is an important key mediator of both inflammation and cancer. Increased expression of TNF-α and serum TNF-α levels are related to clinical cancer staging, high-grade malignancies, and poor prognosis in HNSCC and several other cancers. Persistent expression of TNF-α in the tumour microenvironment is an important feature of many malignant tumours, which is often associated with poor prognosis.[11]

TNF-α-induced Slug stabilization plays an important role in inflammation-induced EMT and cancer cell migration in HNSCC. The activity of NF-κB is essential for TNF-α-induced Slug stabilization. Also, TNF-α induces Slug stabilization by inhibiting its ubiquitination in HNSCC cells. Thus, the TNF-α-NF-κB-Slug axis represents a potential therapeutic target for inflammation-related metastasis in HNSCC. They also showed TNF-α induced EMT in HN13 cells and CAL27, along with the knockdown of Slug in these cells which reversed the EMT phenotype and aggressiveness induced by TNF-α. Slug undergoes acetylation-dependent protein degradation, and the key mediator of the posttranslational mechanism was detected as deacetylase SIRT2. These findings are in agreement with the notion that Slug is a labile protein that is subjected to delicate regulation by multiple signalling pathways to control its ubiquitination and degradation. The process of acetylation in TNF-α-mediated Slug protein stabilization requires Is not clearly understood yet, which requires further studies. 11

Slug and KLF4

In their study, Ingruber et al found a negative correlation between KLF4 and Slug gene expression, which is related to decreased KLF4 gene expression and at the same time, increased Slug expression when compared to normal mucosal tissue. In this form, KLF4 was replaced by Slug, which is recognized as KLF4/Slug switch. They also demonstrated the signalling properties of the TGF-β1-induced KLF4-Slug switch. TGF-β1-p38 MAPK signaling seems to be engaged with the replacement of KLF4 with Slug, whereas, the IL-6-STAT3 pathway might be at a lower level to upregulate Slug, but also increase or stabilize KLF4. In their study Slug gene expression was seen in normal mucosa and also in HNSCC, but increased expression of Slug in HNSCC. This upregulation was correlated with the induction of EMT by TGF-β1 or IL-6 with sequence mutation of the p53 coding region or with loss of the p53 gene product.[21]

SLUG and EGFR:

A high degree of heterogeneity at the transcriptional level is demonstrated by next-generation sequencing techniques which revealed the existence of classical epithelial-like, basal-like, mesenchymal enriched, and atypical molecular subtypes. Based on single-cell RNA sequencing of oral cancers, molecular subtypes were refined as malignant basal, classical, and atypical subtypes. The malignant basal subtype comprises tumours with a high expression of EGFR, which are frequently seen in its activated phosphorylated form, that have undergone a partial EMT. Activation of EGFR cancer cells induces EMT through the extracellular-regulated kinases ERK1/2, resulting in an enhanced expression of EMT TFs including Slug, Snail, and Zeb1. EGFR expression and activating phosphorylation were also seen in a sub-cluster of cancer cells that was defined by a low cytotoxic immune phenotype and reduced expression of programmed death ligand 1 (PD-L1) and interferon-gamma (IFNc). So, EGFR-mediated EMT is involved in the regulation of metastasis and therapy resistance and might potentially be involved in the interactions with immune cells.[22]

SLUG and pEMT-Singscores

Schinke et al in their study suggested a term, partial EMT (pEMT) due to the incomplete, transitional, and reversible nature of EMT in cancer cells. They developed a single-cell RNA sequencing signature-based pEMT quantification through cell type-dependent deconvolution of bulk RNA sequencing and microarray data combined with single-sample scoring of molecular phenotypes (Singscoring). In their study, they showed that SLUG mRNA expression was correlated best with pEMT-Singscores and common pEMT genes in clinical cohorts and cell lines. All other EMT-TFs were either poorly expressed or not correlated to pEMT-SingScores. They also suggested that SLUG is not only a surrogate for pEMT but also actively contributes to inducing a pEMT phenotype in HNSCC. This was also supported by the results which show ectopic SLUG expression in cell lines of the upper aerodigestive tract induces functions such as invasion and decreased sensitivity to irradiation, which is commonly attributed to pEMT.[23]

SLUG and CD271

CD271 is a low-affinity nerve growth factor receptor of p75NTR and is a functional marker of tumour-initiating cell subpopulation in HNSCC. In their study, Chung et al showed that activation of CD271 in OSCC results in increased expression of Snai2/Slug subsequently resulting in more invasion and increased capacity for metastasis to regional lymph nodes. These findings explain the function of CD271 in the tumour environment and suggest CD271 as a promising target for therapeutics. The authors also showed that when Snai2 was inhibited by shRNA transduction, the enhanced invasiveness induced by CD271 (by rhNGF-β) activation was significantly decreased. These findings suggest that EMT induction promotes not only tumor cell invasion and metastasis but also, the cells with the phenotype and properties of tumor-initiating cells.[24]

Conclusion

Our review discussed the diverse mechanisms of SLUG in various malignancies, particularly in OSCC. There are various molecular mechanisms by which SLUG directly or indirectly promotes the metastasis of cancer underlying EMT. Decreased expression of E-cadherin along with increased expression of N-cadherin, vimentin and SLUG are seen in EMT. SLUG inhibited the expression of miR-200b and miR-1 which promotes EMT and metastasis. Increased expression of SLUG is seen in hypoxia-mediated cadherin switch in HNSCC. Inflammation-mediated EMT which progresses into metastasis was also noted in HNSCC. Some studies have shown the association between EMT and cancer stem cells. SLUG along with so many factors such as cytokines TGF-β, IL-6, IL-8, TNF-α, KLF4, EGFR, and CD271 plays important roles in EMT and metastasis. The presence of TGF-β and Slug regulates invasiveness and chemo-resistance in OSCC cells.

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