Signal peptidase complex catalytic subunit SEC11A upregulation is a biomarker of poor prognosis in patients with head and neck squamous cell carcinoma

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Abstract

In the current study, we aimed to investigate the expression of the five microsomal signal peptidase complex (SPC) subunit genes (SEC11A, SEC11C, SPCS1, SPCS2, and SPCS3) in head and neck squamous cell carcinoma (HNSC) and to explore their prognostic value. Data from the HNSC subset of The Cancer Genome Atlas (TCGA) and one previous single-cell RNA-seq dataset was used. Subgroup analysis was conducted in tumors from different anatomic sites. Gene set enrichment analysis (GSEA), and immune cell infiltration analysis were performed to check the influence of SEC11A on the tumor microenvironment. Among the genes significantly upregulated in the tumor group, only SEC11A expression (as a continuous variable) is independently associated with poorer progression-free survival (PFS) (HR: 2.075, 95%CI: 1.447–2.977, \( p < 0.001 \)) and disease-specific survival (DSS) (HR: 2.023, 95%CI: 1.284–3.187, \( p = 0.002 \)). Subgroup analysis confirmed the prognostic value in tumors from three anatomic origins, including laryngeal squamous cell carcinoma, oral cavity-related squamous cell carcinoma, and oropharynx-related squamous cell carcinoma. SEC11A is expressed in all subtypes of cells in the tumor microenvironment. Its expression showed a moderate positive correlation with its gene-level copy number (Pearson’s \( r = 0.53, p<0.001 \)). SEC11A expression was negatively correlated with CD8+ T cells and B cells, but was positively correlated with cancer-associated fibroblast and myeloid-derived suppressor cells (MDSCs) in the tumor microenvironment. In summary, SEC11A upregulation is a result of gene amplification in head and neck squamous cell carcinoma. Its upregulation might serve as an independent prognostic biomarker and a predictor of the infiltration of certain types of immune cells.

Introduction

The microsomal signal peptidase complex (SPC), which is an essential membrane component in the endoplasmic reticulum (ER), has five subunits, including SEC11A, SEC11C, SPCS1,
SPCS2, and SPCS3 [1]. Functionally, SPC removes signal peptides (SPs) from a series of secretory pre-proteins with high specificity [1]. Among the subunits, SEC11A has been characterized as a penitential oncogene and a valuable prognostic biomarker in multiple types of solid tumors. SEC11A upregulation is associated with worse survival of gastric cancer [2]. It facilitates gastric cancer cell growth \textit{in vitro} and \textit{in vivo} by promoting the expression of the secretion of TGF-\(\alpha\) secretion [2]. SEC11A can promote the phosphorylation of epidermal growth factor receptor (EGFR) and the downstream extracellular signal-regulated kinase (ERK) and protein kinase B (Akt) in colorectal cancer cells [3]. Its expression serves as an independent prognostic biomarker for patients with CRC [3], basal-Like bladder cancer [4] and esophageal squamous cell carcinoma [5]. One recent study found that inhibiting SEC11A can reduce proliferation, migration, and invasion and induce apoptosis of tongue squamous cell carcinoma [6], suggesting a potential oncogenic role of this gene in head and neck squamous cell carcinoma.

The dysregulation of the other SPC subunits was also observed in some tumors. SPCS1 expression can be affected by FOXM1-modulated gene promoter methylation in head and neck squamous cell carcinoma [7]. SPCS2 has a significant diagnostic value for hepatocellular carcinoma (HCC) [8]. SPCS3 expression can be used as a component of a risk score model to predict the survival of patients with pancreatic adenocarcinoma [9].

Head and neck squamous cell carcinoma is a group of heterogeneous carcinomas derived from different anatomic sites. Most patients are diagnosed in advanced stages (stage III and IV). More than half of the cases suffer locally advanced disease recurrence even after curative therapy. The median overall survival for the cases with recurrent or metastatic tumors is shorter than one year [10]. Therefore, it is necessary to explore reliable prognostic biomarkers to support better disease management.

In the current study, we aimed to investigate the expression profile of the SPC subunit genes in head and neck squamous cell carcinoma and explore their prognostic value. Subgroup analysis was conducted in tumors from different anatomic sites. Gene set enrichment analysis (GSEA), and immune cell infiltration analysis were performed to check the influence of SEC11A on the tumor microenvironment.

**Materials and methods**

This study is a secondary bioinformatic analysis based on online open datasets. No primary samples were collected in the current study. Therefore, ethical approval is not required. Data in the databases are fully anonymous. Informed consent was appropriately handled by the original studies relevant to the databases.

**Data extraction**

Data extraction was performed using the UCSC Xena browser [11]. The five SPC subunit gene expression, gene-level methylation, gene-level copy number data, clinicopathological and survival data were extracted from the HNSC subset in The Cancer Genome Atlas (TCGA)-pancancer database, following the methods we described previously [12]. Single-cell RNA-seq data of SEC11A expression from more than 5000 cells from head and neck squamous cell carcinomas were extracted from Puram 2017 dataset [13].

**Cell culture and immunofluorescent staining**

SCC9 and TU212 cell culture and immunofluorescent staining were conducted as we previously described [14]. For immunofluorescent staining, cells grown on coverslips were fixed, permeabilized, and blocked. Then, coverslips were incubated with anti-SEC11A (1:100,
A10552, ABclonal, Wuhan, China) at 4 °C overnight. Then, the coverslips were washed and incubated with a secondary antibody (anti-rabbit IgG (H+L), F(ab’)2 Fragment, Alexa Fluor 647 Conjugate) (Cell Signaling, Danvers, MA, USA) for 1 h at 37 °C. Then, the coverslips were washed and mounted with a mounting medium containing DAPI. IF images were captured using a fluorescence microscopy (Nikon, 90i, Japan).

**Immunohistochemistry (IHC) staining**

IHC was performed using a commercial human paraffin-embedded head and neck squamous cell carcinoma tissue array and anti-SEC11A (1:200, A10552, ABclonal, Wuhan, China), on a BOND-III Automated IHC Stainer (Leica Microsystems GmbH, Wetzlar, Germany).

**Gene Set Enrichment Analysis (GSEA)**

GSEA was performed among patients with primary head and neck squamous cell carcinoma. Gene set enrichment was compared between the high (top 50%) and low (bottom 50%) SEC11A expression groups using the GSEA software (v 4.1.0). The h.all.v7.5.syntbols.gmt in Molecular Signatures Database (MSigDB) was selected as the reference gene set. Only the gene set with nominal (Nom) \( p < 0.05 \) and false discovery rate (FDR) \( q < 0.05 \) were included.

**Statistical analysis**

Survival curves (both progression-free survival, PFS and disease-specific survival, DSS) were estimated using the Kaplan-Meier method and median gene expression. Univariate and multivariate analysis by Cox proportional hazards model was performed to assess the independent prognostic value of SEC11A expression as a continuous variable. Only the variates with \( p \) value \( < 0.1 \) were included in the multivariate analysis. Log-rank test was used to compare the curves and calculate the \( p \) values. Correlation analysis was performed by calculating the Pearson’s correlation coefficients. \( p < 0.05 \) was considered statistically significant.

**Results**

**SEC11A expression independently predicts unfavorable survival of patients with head and neck squamous cell carcinoma**

We extracted RNA-seq data from primary head and neck squamous cell carcinoma (\( n = 518 \)) and tumor-adjacent normal tissues (\( n = 44 \)) and compared the expression profile of the five SPC subunit genes. Results showed that SEC11A, SPCS2 and SPCS3 were significantly upregulated in the tumor group than in the normal group (Fig 1A). K-M survival analysis revealed that patients with high (higher 50%) SEC11A expression had significantly shorter PFS and DSS compared to the low expression (lower 50%) group (Fig 1B and 1E). However, these trends were not observed between groups by median SPCS2 or SPCS3 separation (Fig 1C, 1D, 1F and 1G).

The clinicopathological characteristics of patients with high and low SEC11A expression groups were compared (Table 1). No significant difference was observed in histological grade, margin status, AJCC stage, gender and age between the two groups (\( p > 0.05 \), Table 1). However, the high SEC11A expression group had a significantly higher ratio of PFS events (117/259, 22.6% vs. 80/259, 15.4%, \( p = 0.001 \)) and DSS events (80/246, 16.3% vs. 50/246, 10.2%, \( p = 0.003 \)) compared to the low SEC11A expression group (Table 1).

Therefore, we further explored whether SEC11A expression serves as an independent prognostic biomarker. Univariate and multivariate analysis confirmed that SEC11A expression (as a continuous variable) is independently associated with poorer PFS (HR: 2.075, 95%CI: 1.447–
2.977, \( p < 0.001 \), after adjustment for histological grade, margin status, and AJCC stage (Table 2). Additionally, \( \text{SEC11A} \) expression also independently predicted poorer DSS (HR: 2.023, 95%CI: 1.284–3.187, \( p = 0.002 \), after adjusting histological grade, margin status, and AJCC stage (Table 3).

Subgroup analysis of PFS and DSS in laryngeal, oral cavity related, and oropharynx related squamous cell carcinoma

Since head and neck squamous cell carcinoma is a group of heterogeneous tumors with different anatomic origins, we compared \( \text{SEC11A} \) expression among different anatomic subgroups (Fig 2A). One-way ANOVA did not detect significant differences among the subgroups (\( p = 0.64 \), Fig 2A). Then, according to the anatomic origins, we divided the tumor cases into three categories, including laryngeal squamous cell carcinoma, oral cavity related squamous cell carcinoma (tumors from alveolar ridge, buccal mucosa, floor of mouth, hard palate, lip, oral cavity, and oral tongue), and oropharynx related squamous cell carcinoma (tumors from the base of tongue, hypopharynx, oropharynx and tonsil). K-M survival analysis showed that high (higher 50%) \( \text{SEC11A} \) expression was consistently associated with significantly shorter
PFS and DSS in these three subgroups (Fig 2B–2G). Since HPV positive and HPV negative head and neck squamous cell carcinoma tumors have distinct molecular profiles and biological behaviors [15], we further assessed whether SEC11A expression is consistently associated with PFS and DSS in these subgroups. In the cases with known HPV16 infection status in TCGA-HNSC. K-M survival curves suggested that the high SEC11A expression group might have worse survival (S1A–S1D Fig). However, the sample sizes of these subgroups are relatively small (38 HPV16 positive cases and 73 HPV16 negative cases). Statistical analysis only confirmed a significant difference in HPV negative subgroup in terms of DSS (S1D Fig).

Analysis of SEC11A expression in the tumor microenvironment

Using single-cell RNA-seq data (Puram 2017) from one previous publication [13], we checked SEC11A expression in the tumor microenvironment. Results confirmed its expression in all subtypes of cells, such as tumor cells, T cells, fibroblast, endothelial cells and B cells (Fig 3A). By performing immunofluorescent staining in a tongue squamous cell carcinoma cell line (SCC9) and a laryngeal squamous cell carcinoma cell line (TU212), we observed SEC11A expression in the nucleus membrane and cytoplasm (Fig 3B). Via IHC staining of SEC11A in a
Table 2. Univariate and multivariate analysis for PFS.

| Characteristics | Total (N) | Univariate analysis | | | Multivariate analysis | |
|-----------------|----------|---------------------|---|----------------------|---|
|                 |          | Hazard ratio (95% CI) | p-value | Hazard ratio (95% CI) | p-value |
| SEC11A          | 517      | 2.173 (1.584–2.982) | <0.001 | 2.075 (1.447–2.977) | <0.001 |
| Histological grade | 513 | | | | |
| G3              | 124      | Reference | | | |
| G1              | 61       | 0.768 (0.456–1.293) | 0.320 | | |
| G2              | 303      | 1.140 (0.815–1.593) | 0.445 | | |
| G4              | 7        | 0.658 (0.160–2.711) | 0.562 | | |
| GX              | 18       | 0.238 (0.058–0.978) | 0.046 | | |
| Margin status   | 456      | | | | |
| Negative        | 349      | Reference | | | |
| Positive        | 59       | 2.210 (1.532–3.188) | <0.001 | 1.891 (1.280–2.794) | 0.001 |
| Close           | 48       | 1.722 (1.079–2.747) | 0.023 | 1.868 (1.162–3.004) | 0.010 |
| AJCC stage      | 442      | | | | |
| Stage I         | 27       | Reference | | | |
| Stage IV        | 266      | 1.498 (0.760–2.953) | 0.243 | 1.488 (0.754–2.937) | 0.252 |
| Stage III       | 80       | 0.898 (0.417–1.933) | 0.783 | 0.959 (0.443–2.075) | 0.915 |
| Stage II        | 69       | 0.675 (0.303–1.504) | 0.336 | 0.698 (0.310–1.574) | 0.387 |
| Age             | 517      | 1.008 (0.995–1.021) | 0.226 | | |
| Gender          | 517      | | | | |
| Male            | 381      | Reference | | | |
| Female          | 136      | 0.986 (0.715–1.360) | 0.931 | | |

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Table 3. Univariate and multivariate analysis for DSS.

| Characteristics | Total (N) | Univariate analysis | | | Multivariate analysis | |
|-----------------|----------|---------------------|---|----------------------|---|
|                 |          | Hazard ratio (95% CI) | p-value | Hazard ratio (95% CI) | p-value |
| SEC11A          | 491      | 2.364 (1.599–3.494) | <0.001 | 2.023 (1.284–3.187) | 0.002 |
| Histological grade | 488 | | | | |
| G3              | 118      | Reference | | | |
| G1              | 58       | 0.656 (0.341–1.261) | 0.206 | | |
| G2              | 291      | 1.042 (0.700–1.553) | 0.838 | | |
| G4              | 7        | 0.000 (0.000–Inf) | 0.993 | | |
| GX              | 14       | 0.236 (0.032–1.721) | 0.154 | | |
| Margin status   | 433      | | | | |
| Negative        | 330      | Reference | | | |
| Positive        | 58       | 2.718 (1.784–4.141) | <0.001 | 2.318 (1.474–3.644) | <0.001 |
| Close           | 45       | 1.737 (0.960–3.144) | 0.068 | 1.884 (1.031–3.444) | 0.040 |
| AJCC stage      | 420      | | | | |
| Stage I         | 27       | Reference | | | |
| Stage IV        | 254      | 3.144 (0.993–9.950) | 0.051 | 3.141 (0.992–9.951) | 0.052 |
| Stage III       | 75       | 1.777 (0.514–6.141) | 0.363 | 1.895 (0.546–6.580) | 0.314 |
| Stage II        | 64       | 1.395 (0.389–5.003) | 0.610 | 1.446 (0.396–5.278) | 0.576 |
| Age             | 491      | 1.009 (0.993–1.025) | 0.272 | | |
| Gender          | 491      | | | | |
| Male            | 366      | Reference | | | |
| Female          | 125      | 1.063 (0.715–1.579) | 0.763 | | |

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In a commercial head and neck squamous cell carcinoma tissue array, we confirmed a similar subcellular location of SEC11A in immunofluorescent staining (Fig 3C). To explore the mechanisms leading to SEC11A dysregulation, we checked the methylation status of CpG sites within the SEC11A gene locus in Illumina HumanMethylation450 BeadChip and gene-level copy number in TCGA-HNSC (Fig 3D). No significant correlation was observed between SEC11A

Fig 2. Subgroup analysis of PFS and DSS in laryngeal, oral cavity related, and oropharynx related squamous cell carcinoma. A. The expression of SEC11A in tumors from different anatomic sites. B-G. K-M survival analysis was performed to compare the differences in PFS (B-D) and DSS (E-G) in patients grouped by anatomic sites, including laryngeal squamous cell carcinoma (B and E), oral cavity related squamous cell carcinoma (C and F) and oropharynx related squamous cell carcinoma (D and G). SCC, squamous cell carcinoma. Log-rank test was used to compare the curves and calculate the \( p \) values.

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commercial head and neck squamous cell carcinoma tissue array, we confirmed a similar subcellular location of SEC11A in immunofluorescent staining (Fig 3C). To explore the mechanisms leading to SEC11A dysregulation, we checked the methylation status of CpG sites within the SEC11A gene locus in Illumina HumanMethylation450 BeadChip and gene-level copy number in TCGA-HNSC (Fig 3D). No significant correlation was observed between SEC11A
expression and the methylation status of the CpG sites. However, \textit{SEC11A} expression showed a moderate positive correlation with its copy number (Pearson’s $r = 0.53$, $p < 0.001$, Fig 3E).

\textbf{SEC11A expression might predict the infiltration of certain types of immune cells in the tumor microenvironment}

Using immune cell estimation provided by Tumor Immune Estimation Resource 2.0 (TIMER 2.0) (http://timer.cistrome.org/) [16], we assessed the correlation between SEC11A expression and the abundance of immune cells in the tumor microenvironment. By setting $r > |0.2|$ as the cutoff, we found that \textit{SEC11A} expression was negatively correlated with CD8$^+$ T cells and B
cells, but was positively correlated with cancer-associated fibroblast and myeloid-derived suppressor cells (MDSCs) (Fig 4A). Then, we conducted GSEA to explore the dysregulated gene sets between high and low SEC11A expression groups. Results showed that the high SEC11A expression group had higher expression of the genes enriched in DNA repair and oxidative phosphorylation (Fig 4B).

**Discussion**

In this study, we examined the expression of the five SPC subunit genes and their prognostic value in head and neck squamous cell carcinoma. Among the five genes, SEC11A upregulation can serve as an independent prognostic biomarker regarding PFS and DSS. In addition, our subgroup analysis confirmed the prognostic value in tumors originating from different anatomic sites.

SEC11A can promote the phosphorylation of EGFR and the downstream kinases, including ERK and Akt (3). This association was confirmed in tongue squamous cell carcinoma, a major subtype of head and neck squamous cell carcinoma [6]. EGFR is overexpressed in approximately 50 to 90% of head and neck squamous cell carcinoma cases [17, 18]. Its activation results in a phosphorylation cascade involving a series of downstream signaling effectors, such as PI3K-AKT, MAPK, ERK, and Jak/STAT. Activation of these signaling pathways can enhance the growth, invasion, angiogenesis, and metastasis of the tumor [19–21]. EGFR can be overactivated via multiple machinimas, such as overexpression of EGFR and its ligands, genetic mutations/polymorphism and activation by other receptor tyrosine kinases (RTKs) [18–20]. In addition, activation of the EGFR signaling pathway can induce resistance to chemo-radiation in cancers by promoting DNA repair via multiple mechanisms, such as activating DNA dependent protein kinase, promoting the transcription of DNA repair genes (RAD51, ATM, and XRCC1) and phosphorylating proliferating cell nuclear antigen (PCNA) [22]. This mechanism helps explain why SEC11A upregulation is associated with increased genes enriched in the DNA repair set in our GSEA. Therefore, SEC11A upregulation might serve as an important mechanism for overactivated EGFR. Due to its critical regulative effects on cancer cell behaviors, EGFR has been considered a promising therapeutic target. Currently, the EGFR monoclonal antibody cetuximab is generally administrated in combination with radiation for patients with HPV-negative head and neck squamous cell carcinoma [23]. To understand the mechanisms underlying SEC11A upregulation, we checked its DNA methylation status and gene-level copy numbers. Our analysis showed that SEC11A upregulation might not be regulated by CpG methylation within its gene locus, but is associated with its gene-level amplification.

Using data from one previous single-cell RNA-seq database, we found that SEC11A is expressed not only in tumor cells, but also in other cells in the tumor microenvironment. Therefore, we hypothesized that SEC11A might also modulate the malignant behaviors of head and neck squamous cell carcinoma via other mechanisms. By checking immune cell abundance in the tumor microenvironment, we observed a significant positive correlation between SEC11A expression and the infiltration of MDSCs, a group of immature myeloid cells with immunosuppressive effects [24]. Previous studies showed that the abundance of MDSCs is associated with advanced stages of head and neck squamous cell carcinoma and poor prognosis [25, 26]. By conducting GSEA, we observed that the high SEC11A expression group had increased the expression of genes enriched in the oxidative phosphorylation set. Tumor-infiltrating MDSCs have increased fatty acid uptake, oxidation, and oxidative phosphorylation [27]. These alterations were associated with increased mitochondrial mass, upregulation of the key fatty acid oxidation enzymes, and an elevated oxygen consumption rate [28]. Therefore,
Fig 4. SEC11A expression might predict the infiltration of immune cells in the tumor microenvironment. **A.** Pearson’s rho values were calculated to assess the correlation between the expression of SEC11A and the infiltration of immune cells in the head and neck squamous cell carcinoma associated tumor microenvironment, including CD4+ T cells, CD8+ T cells, B cells, macrophages, cancer-associated fibroblasts and MDSCs. **B.** A summary (top) and representative images (bottom) of plot images of the gene sets significantly enriched in the high SEC11A expression group.

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we infer that SEC11A associated elevation of the oxidative phosphorylation genes might generate a favorable tumor microenvironment for the infiltration or conversion of MDSCs.

This study also has some limitations. Firstly, since we did not find other databases with large samples, detailed anatomic information and survival data together, no validation cohort was used to verify the prognostic significance of SEC11A expression. Secondly, the molecular mechanisms underlying the association among SEC11A expression, the elevation of the oxidative phosphorylation genes and the infiltration or conversion of MDSCs were not explored in the current study. Future studies are warranted for a better understanding of the regulatory mechanisms of SEC11A.

**Conclusion**

In this study, we revealed that SEC11A upregulation is a result of gene amplification in head and neck squamous cell carcinoma. Its upregulation serves as an independent prognostic biomarker regarding PFS and DSS. Besides, SEC11A expression might predict the infiltration of certain types of immune cells in the tumor microenvironment.

**Supporting information**

S1 Fig. Subgroup analysis of PFS and DSS in HPV16 positive or negative head and neck squamous cell carcinoma. A-D. K-M survival analysis was performed to compare the differences in PFS (A-B) and DSS (C-D) in patients grouped by known HPV16 infection status. Log-rank test was used to compare the curves and calculate the p values.

(TIF)

S1 Checklist. *PLOS ONE* clinical studies checklist.

(PDF)

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