Original Article

Revealing the oncogenic role of elevated GNL3L expression in esophageal squamous cell carcinoma: insights into the STAT3 pathway

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Background: Esophageal squamous cell carcinoma (ESCC) patients carries a poor prognosis, with limited effective therapeutic targets. This study aimed to clarify the clinical significance of guanine nucleotide-binding protein like 3-like (GNL3L) protein expression in ESCC and its role in malignant progression.

Methods: GNL3L expression and associated cancer-promoting pathways in ESCC were interrogated via bioinformatics analysis through use of The Cancer Genome Atlas (TCGA) database. Subsequent verification of GNL3L protein expression in ESCC, coupled with clinical data, was conducted through immunohistochemistry and followed by a comprehensive prognostic analysis. We further investigated potential signaling pathways facilitating ESCC progression, employing a combination of bioinformatics analysis and immunohistochemical (IHC) experiments.

Results: Bioinformatics analysis unveiled a significant elevation in GNL3L expression, particularly in gastrointestinal tumors and ESCC. Immunohistochemistry confirmed elevated GNL3L expression in ESCC tissues. Regression analysis established a correlation between elevated GNL3L expression and advanced tumor node metastasis (TNM) stage, with high expression associated with poor prognosis in patients with ESCC. Our integrated approach of bioinformatics and IHC analysis indicated a potential role of the signal transducers and activators of transcription 3 (STAT3) signaling pathway in ESCC progression.

Conclusions: High GNL3L expression significantly contributes to the malignant progression of ESCC. This study further elucidates the mechanisms driving ESCC progression and offers possible insights for more effective diagnosis and treatment strategies.

Keywords: Guanine nucleotide-binding protein like 3-like (GNL3L); esophageal squamous cell carcinoma (ESCC); clinical significance; malignant progression; signal transducers and activators of transcription 3 (STAT3)

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Introduction

Esophageal cancer ranks as the eighth most prevalent cancer worldwide and the sixth leading cause of cancer-related mortality (1). The incidence of esophageal squamous cell carcinoma (ESCC) exhibits notable regional disparity, and within China, ESCC is the most predominant subtype of esophageal cancer (2). Despite advancements in treatment modalities such as surgery, radiotherapy, and systemic therapy (i.e., chemotherapy and immunotherapy), ESCC is associated with high rates in incidence and mortality, and overall poor prognosis. Locally advanced ESCC, in particular, has a 5-year survival rate below 40% (3). ESCC exhibits an aggressive biologic behavior with high rates of recurrence after curative intent treatment, which contributes to the poor prognosis (4). A comprehensive exploration of signaling pathways linked to the malignant progression of ESCC and a deeper understanding of their mechanisms can help propel breakthroughs in treatment leading to improvement in prognosis, thereby bearing a significant clinical and societal impact.

Guanine nucleotide-binding protein like 3-like (*GNL3L*), a member of the nucleolar guanosine triphosphate-binding protein (GTP) protease family, has garnered attention by virtue of its close association with tumor invasion and metastasis (5). However, the available literature pertaining to the role of GNL3L in esophageal cancer is relatively limited. The few existing papers on this subject either discuss the significance of GNL3L across various cancers or solely examine its relationship with patient prognosis (6,7). However, there is a paucity of literature examining the potential pathways that may drive the development of ESCC. Moreover, there is a lack of explicit focus on investigating GNL3L specifically in the context of ESCC. Therefore, this study aimed to elucidate the clinical significance of GNL3L in ESCC and identify the potential signaling pathways through which GNL3L may facilitate the malignant progression of ESCC. We present this article in accordance with the REMARK reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-473/rc).

Highlight box

Key findings

- This study found that elevated guanine nucleotide-binding protein like 3-like (GNL3L) expression in esophageal squamous cell carcinoma (ESCC) correlates with advanced stage and poor prognosis and plays a pivotal role in driving malignant progression via the signal transducers and activators of transcription 3 (STAT3) pathway.

What is known and what is new?

- Determining the prognosis of ESCC is challenging, and the clinical relevance of GNL3L in this specific context has been widely recognized. Our study identified a link between elevated GNL3L expression and certain pathways associated with ESCC progression.
- This paper provides novel insights by conclusively confirming increased GNL3L expression in ESCC. We found a correlation of elevated GNL3L expression with advanced stage and poor prognosis specifically in patients with ESCC. Additionally, our study clarified the previously unexplored role of GNL3L in driving ESCC progression through the STAT3 pathway, offering a unique perspective on potential therapeutic targets and diagnostic markers within the context of esophageal cancer.

What is the implication, and what should change now?

- The implication of elevated GNL3L expression in ESCC highlights the likely role of GNL3L as a prognostic marker in future clinical assessments for ESCC. Targeted therapeutic strategies altering the STAT3 pathway, may help to improve ESCC diagnosis, prognosis, and treatment.

Methods

Public database analysis

We conducted a comprehensive analysis of second-generation sequencing data from The Cancer Genome Atlas (TCGA) Program database using two widely employed platforms, the University of Alabama Cancer Portal (UALCAN) and Gene Expression Profiling Interactive Analysis (GEPIA). Our primary focus was on the sequencing data and the clinical information of patients diagnosed with esophageal cancer (8-10).

Data retrieval

We extracted expression data for multiple genes, including signal transducers and activators of transcription 3 (*STAT3*),

JAK1, *IFNAR1*, *IFNAR2*, and *EML4* from UALCAN.

Correlation analysis

To assess the relationship between *GNL3L* expression and the selected genes, we performed correlation analysis using the Spearman method. This statistical approach allowed us to determine the strength and direction of the association between *GNL3L* and the specified genes.

Tissue samples

We selected patients with ESCC undergoing radical surgery at the Fujian Medical University Union Hospital according to the following patient inclusion criteria: (I) pathology confirmed as ESCC via preoperative gastroscopy, (II) no neoadjuvant treatment such as radiotherapy or chemotherapy performed before surgery, and (III) postoperative pathological tumor node metastasis (TNM) stage I to IV. Meanwhile, the exclusion criteria were as follows: (I) previous neoadjuvant therapy received; (II) other types of esophageal cancer; and (III) distant metastasis of ESCC. Immunohistochemical (IHC) tissue samples (a total of 357 paraffin-embedded samples) were obtained from January 2015 to December 2017, and all pathological diagnoses were confirmed by two chief physicians in the Fujian Medical University Union Hospital's pathology department.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Ethics Committee of the Fujian Medical University Union Hospital (No. 2021KJCX068), and informed consent was obtained from all patients.

IHC

We used IHC SP method and diaminobenzidine (DAB) substrate staining, which is the same as the originally described method (4). Using tissue staining agents and UltraSensitive™ SP IHC Kit (MXB Biotechnologies, Fuzhou, China), we completed IHC staining on 5-mm-thick sections of paraffin-embedded specimens. Conventional paraffin sections were dewaxed and hydrated, and antigens were repaired with citric acid. Subsequently, 50 µL of monoclonal rabbit anti-human GNL3L antibody was added dropwise to paraffin sections (dilution 1:200; cat. no. bs-13472R; Beijing Biosynthesis Biotechnology Co., Ltd., China) or rabbit anti-human phosphorylated STAT3 (pSTAT3) (dilution 1:200; cat. no. AP0705; ABclonal Technology Co., Ltd., Wuhan, China) and left to stand at room temperature for 1 hour. The slide was gently washed three times with phosphate-buffered saline (PBS) and then incubated with a rabbit secondary antibody (dilution 1:200; cat. no. TA-005; Xiamen Tagene Biotechnology Co., Ltd., China) and allowed to stand at room temperature for 15 minutes. We rinsed the slide with PBS again and developed the color using DAB. The paraffin sections underwent DAB staining and counterstaining with hematoxylin, followed by routine mounting. It was noteworthy that PBS was used as a substitute for the primary antibody to serve as a negative control.

For results determination, 100 cells from 10 high-power fields of view were selected from each slice. GNL3L and STAT3 positivity were determined by evaluating the degree of staining in the cytoplasm and cell membrane. Slides were observed with a light microscope (Olympus, Tokyo, Japan). The findings were evaluated independently by two pathologists who were blinded to the clinicopathological information. A semiquantitative scoring method was used, with the staining intensity (0= no staining, 1= weak staining, 2= moderate staining, and 3= strong staining) and the proportion of cells stained (0, <1%; 1, 1–25%; 2, 26–50%; 3, 51–75%; and 4, >75%) being determined. These two scores were multiplied and classified as low expression (0–4 points) and high expression (5–12 points).

Clinical data collection and follow-up

The patient's demographic information and surgical details were obtained from case records, and all pathological data were reported by dedicated pathologists in the Fujian Medical University Union Hospital. The variables collected included the following: age, sex, smoking, drinking, tumor size, tumor location, histological grade, TNM stage, and venous thrombus. We collected relevant prognostic data for patients with minimally invasive resection of ESCC, with all variable information documented in the raw records. Postdischarge follow-up was carried out by specialized personnel who underwent rigorous training and assessment to ensure the authenticity and reliability of the data. The follow-up deadline for this study was December 2022.

Statistical analysis

All data were analyzed using SPSS 26.0 (IBM Corp.). We analyzed the correlation between the expression of GNL3L and pSTAT3 and clinical data. The Fisher exact test was

used for continuous variables, the Chi-squared test was used for categorical variables, and P<0.05 was considered statistically significant. The Kaplan-Meier method was used for prognostic analysis.

Results

The GNL3L was closely associated with the progression and poor prognosis of ESCC

Pancancer analysis revealed a common occurrence of aberrant *GNL3L* expression, primarily a marked increase, in various tumors (*Figure 1A*). Specifically, in ESCC, elevated GNL3L expression was significantly higher than in corresponding normal tissue (*Figure 1B*). Further analysis indicated an increase in GNL3L expression level to be associated with tumor progression, although statistical differences were not consistently apparent due to limited sample size (*Figure 1C*). Additionally, poor histological differentiation was also correlated with higher GNL3L levels (*Figure 1D*), Notably, elevated GNL3L expression was correlated with a higher grade, and shorter overall survival period for patients with esophageal cancer (*Figure 1E*). Together, these findings indicate GNL3 expression as a poor prognostic factor in ESCC.

Clinical significance of the IHC analysis of GNL3L in ESCC

To further verify the changes and significance of GNL3L protein levels in ESCC, we collected 357 clinical tissues for IHC staining, including samples of ESCC tissue and adjacent tissue. We found that GNL3L was differentially overexpressed in ESCC tissue (*Figure 2*). Among the participants, there were 282 male cases and 75 female cases, with a mean age of 59.6±7.6 years. Further analysis of the clinical data from patients with ESCC revealed that the high expression rate of GNL3L in pathological staged T3/4 ESCC tissue as compared to T1/2 stage, and the high expression rate in N2/3 stage with more lymph node metastasis as compared to N0/1 tumors (*Table 1*). Overall survival (OS) analysis was used to further validate the correlation between GNL3L and the prognosis of patients with ESCC. Kaplan-Meier analysis showed that the OS of patients in the GNL3L high-expression group was significantly lower than that in the low-expression group (P=0.01; *Figure 3A*). The median OS for high and low expression of GNL3L was 24.5 and 29 months, respectively. Subsequent survival analysis was conducted using Cox regression analysis. Univariate analysis revealed that T stage, N stage, TNM stage, histological grade, venous thrombosis, high expression of GNL3L, and high expression of pSTAT3 were associated with poor prognosis. Multivariate analysis further identified T stage, N stage, and high expression of GNL3L as independent risk factors for poor prognosis (*Table 2*).

Bioinformatics analysis of the interaction between GNL3L and STAT3

The IHC results showed that pSTAT3 was also highly expressed in ESCC, and the high expression suggested an advanced TNM stage and poorer prognosis (*Table 1* and *Figure 3B*). A series of experiments confirmed that GNL3L could regulate STAT3 and its downstream pathways, and a positive correlation between GNL3L and STAT3 expression was demonstrated in clinical tissues (*Figure 4A*). In order to further validate the relationship between GNL3L and the molecular pathways associated with STAT3, we reviewed the literature of the selected genes including *JAK*, *IFN*, and *EML4* for analysis in TCGA and the Kyoto Encyclopedia of Genes and Genomes (KEGG) (11). We found that the genes including *JAK1*, *IFNAR1/2*, and *EML4* were correlated with the expression of *GNL3L* (*Figure 4B-4E*).

Discussion

In gastrointestinal tract tumors, particularly in ESCC, a pronounced upregulation of GNL3L at the transcriptional level has been observed in our research. Validation at the protein level confirmed that GNL3L expression in ESCC tissues is significantly higher than that in normal tissues. Furthermore, at both the transcriptional and protein levels, the expression of GNL3L correlated closely with the adverse prognosis of the tumor. By integrating bioinformatics analysis with IHC results, we hypothesize that GNL3L may promote the progression of ESCC through the activation of the STAT3 signaling pathway.

Our bioinformatics analysis suggested that *GNL3L* is highly expressed in many cancers. Hence, GNL3L may be an important prognostic and therapeutic biomarker for malignancy. GNL3L is a newly discovered GTP binding nucleolar protein that can regulate the mitotic cycle of eukaryotic cells, affecting cell proliferation, migration, and apoptosis (12-14). In lung cancer, experiments have shown that regulation of LDOC1/GNL3L/NFκB pathway

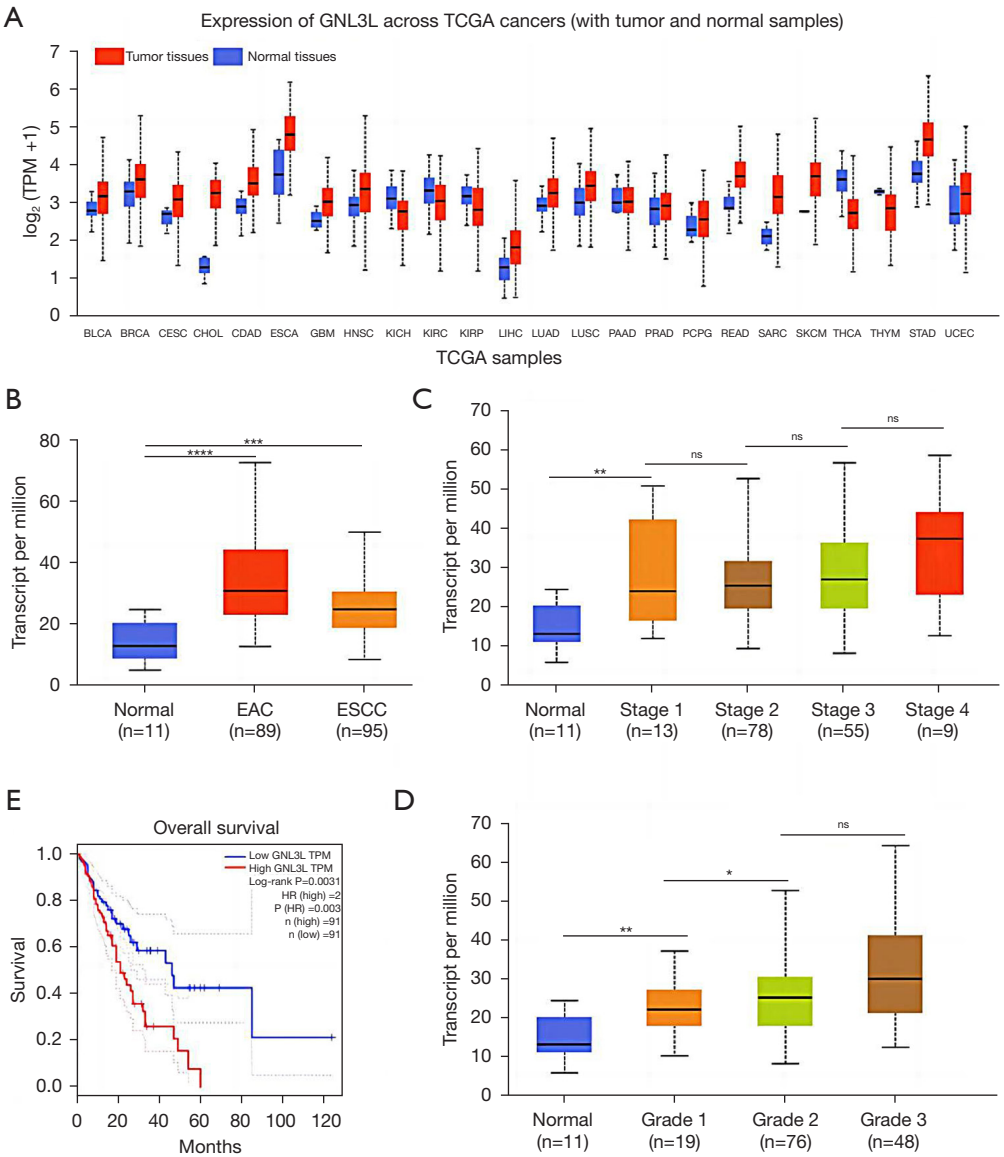

Figure 1 GNL3L expression was closely correlated with the progression and poor prognosis of ESCC. (A) Transcriptome data showed that GNL3L was dysregulated in pancancer. (B) The expression level of GNL3L is higher in EAC and ESCC compared to the control normal esophageal epithelial tissue. (C) The relationship between GNL3L and the progression of esophageal cancer tumors. (D) The relationship between GNL3L and the degree of differentiation of esophageal cancer. (E) High expression of GNL3L was associated with a poor prognosis for patients with esophageal cancer (P=0.003). (B-D) The sample numbers. *, significant difference with P<0.05; **, significant difference with P<0.01; ***, significant difference with P<0.001; ****, significant difference with P<0.0001; n, number; ns, no statistical difference. HR, hazard ratio; TCGA, The Cancer Genome Atlas; TPM, transcript per million; ESCC, esophageal squamous cell carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; PAAD, pancreatic adenocarcinoma; PRAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; THCA, thyroid carcinoma; THYM, thymoma; STAD, stomach adenocarcinoma; UCEC, uterine corpus endometrial carcinoma; EAC, esophageal adenocarcinoma.

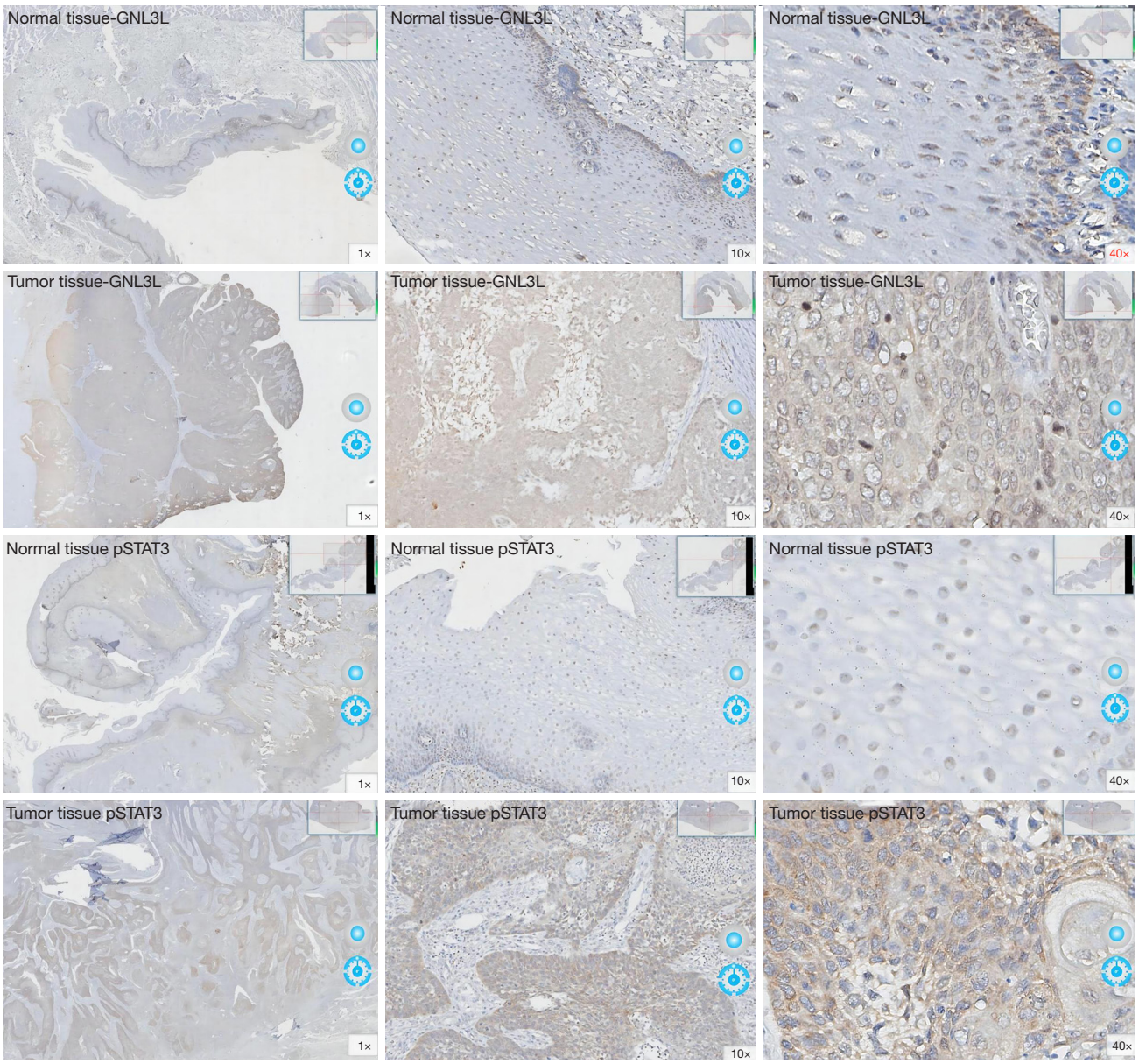

Figure 2 Low expression of GNL3L and pSTAT3 proteins in normal tissues; high expression of GNL3L and pSTAT3 proteins in esophageal squamous cell carcinoma; we used the diaminobenzidine substrate staining method. GNL3L, guanine nucleotide-binding protein like 3-like; pSTAT3, phosphorylated signal transducers and activators of transcription 3.

can inhibit the proliferation of gemcitabine-resistant cell lines (15), and the abnormal activation of GNL3L has been associated with chemotherapy resistance in colon cancer (16).

The elevated expression of GNL3L holds significant value in assessing the clinical severity and poor prognosis of patients with ESCC. Dai *et al.* also observed the upregulation of GNL3L in esophageal cancer, which was correlated with adverse prognosis (6). He *et al.* reported that GNL3L positively regulates cell proliferation and autophagy in esophageal cancer cells via regulating the AMPK signaling (17). However, the current literature lacks a detailed discussion on the specific promotional mechanisms of GNL3L in the progression of ESCC. In gastric cancer, patients with GNL3L-positive expression exhibited significantly shorter overall survival than do GNL3L-negative patients, with studies suggesting a pivotal

Table 1 The expression and clinical significance of GNL3L and pSTAT3 in esophageal squamous cell carcinoma

Feature	No. of patients	GNL3L expression			pSTAT3 expression		
		Low	High	P value	Low	High	P value
All patients	357	117	240		128	229	
Age (years)				0.06			0.34
≤60	168	47	121		56	112	
>60	189	70	119		72	117	
Sex				0.002*			0.004*
Male	282	81	201		90	192	
Female	75	36	39		38	37	
Smoking				0.003*			0.03*
Yes	213	57	156		67	146	
No	144	60	84		61	83	
Drinking				0.003*			0.25
Yes	79	15	64		24	55	
No	278	102	176		104	174	
Tumor size (cm)				0.001*			0.01*
≤4	206	82	124		85	121	
>4	151	35	116		43	108	
Tumor location				0.30			0.56
Upper	32	14	18		14	18	
Middle	226	69	157		81	145	
Lower	99	34	65		33	66	
T stage				<0.001*			0.001*
T1/2	178	80	98		79	99	
T3/4	179	37	142		49	130	
N stage				<0.001*			<0.001*
N0/1	293	108	185		123	170	
N2/3	64	9	55		5	59	
Histological grade				0.054			0.50
G0/1	159	61	98		60	99	
G2/3	198	56	142		68	130	
TNM stage				0.001*			0.001*
I/II	241	93	148		100	141	
III/IV	116	24	92		28	88	

*, a significance different at P<0.05. GNL3L, guanine nucleotide-binding protein like 3-like; pSTAT3, phosphorylated signal transducers and activators of transcription 3; TNM, tumor node metastasis; T, tumor; N, node; G, grade.

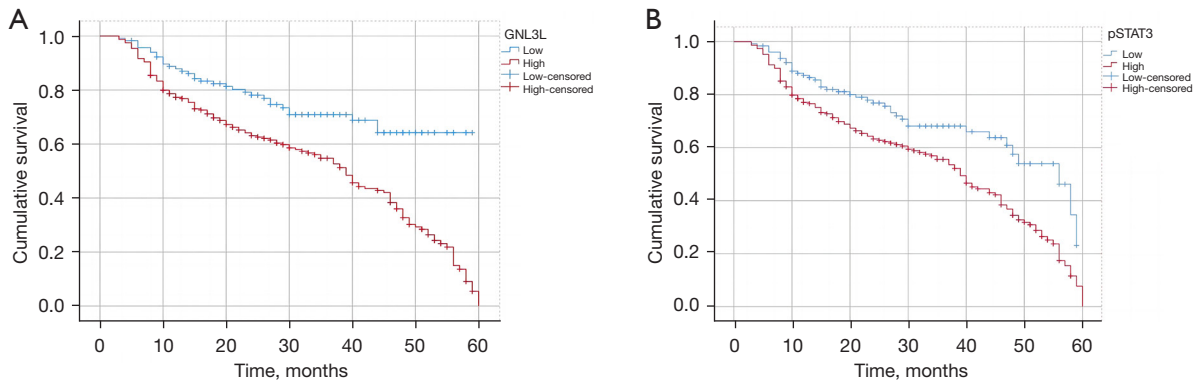

Figure 3 Patients with a high expression of GNL3L and pSTAT3 had poor prognosis. (A) High expression of GNL3L was associated with poor prognosis (P=0.01); (B) high expression of pSTAT3 was associated with poor prognosis (P<0.001). Low, low expression; High, high expression; GNL3L, guanine nucleotide-binding protein like 3-like; pSTAT3, phosphorylated signal transducers and activators of transcription 3.

Table 2 Univariate and multivariate Cox regression analysis of overall survival in 357 cases of esophageal squamous cell carcinoma

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Age (>60 *vs*. ≤60 years)	0.816 (0.610–1.090)	0.16		
Sex (male *vs*. female)	1.389 (0.952–2.027)	0.08		
Smoking (yes *vs*. no)	1.266 (0.938–1.708)	0.12		
Drinking (yes *vs*. no)	1.382 (0.983–1.943)	0.06		
Tumor size (>4 *vs*. ≤4 cm)	1.302 (0.975–1.740)	0.07		
Venous thrombus (yes *vs*. no)	1.729 (1.231–2.43)	0.002*		0.058
T stage (T3/4 *vs*. T1/2)	1.776 (1.321–2.387)	<0.001*	1.446 (0.988–2.118)	0.002*
N stage (N2/3 *vs*. N0/1)	2.163 (1.522–3.072)	<0.001*	1.656 (1.209–2.267)	0.03*
Histological grade (G2/3 *vs*. G0/1)	1.393 (1.033–1.88)	0.03*	1.774 (1.040–3.027)	0.22
TNM stage (III/IV *vs*. I/II)	1.461 (1.079–1.978)	0.01*	1.211 (0.887–1.653)	0.26
GNL3L expression (low *vs*. high)	2.308 (1.582–3.367)	<0.001*	0.777 (0.499–1.209)	0.001*
pSTAT3 expression (low *vs*. high)	1.819 (1.288–2.570)	0.001*	1.939 (1.305–2.883)	0.08

*, a significance different at P<0.05. HR, hazard ratio; CI, confidence Interval; TNM, tumor node metastasis; T, tumor; N, node; G, grade. GNL3L, guanine nucleotide-binding protein like 3-like; pSTAT3, phosphorylated signal transducers and activators of transcription 3.

role for GNL3L in gastric cancer progression (18). In humans, the expression level of estrogen-related receptor alpha (ERRα) is associated with adverse prognosis in ovarian and breast cancer (19). Research by Yasumoto *et al.* further demonstrated that GNL3L-mediated mechanisms can regulate ERR protein transcriptional activity (20).

Okamoto *et al.* found that BJ-hTERT, MCF7, and HeLa cells expressing GNL3L exhibited increased expression of the tyrosine phosphorylated form of STAT3 and higher levels of twist, snail, and vimentin (21). A study has indicated that increased STAT3 signaling orchestrates the expression of the master regulator TWIST, leading to epithelial-mesenchymal transition (EMT) and metastasis (22). These observations raise the question as to whether a comparable signaling axis involving GNL3L, pSTAT3, EMT exists in ESCC. Our research findings revealed a

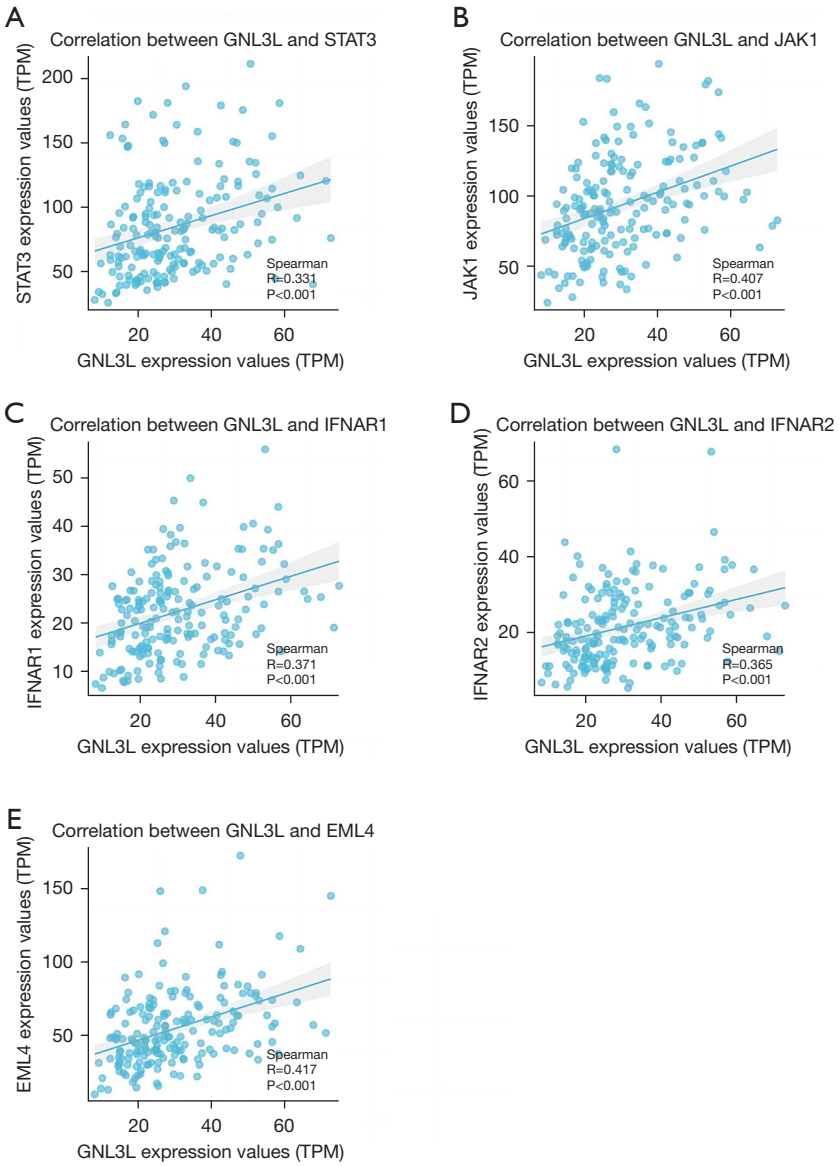

Figure 4 There was a positive correlation at the transcriptome level between the expression of GNL3L and that of STAT3, JAK1, IFNAR1, IFNAR2, and EML4. GNL3L, guanine nucleotide-binding protein like 3-like; STAT3, signal transducers and activators of transcription 3; TPM, transcript per million.

significantly positive correlation between GNL3L and pSTAT3 expression in ESCC, with elevated pSTAT3 levels associated with an unfavorable prognosis. Interestingly, our protein-protein interaction (PPI) analysis did not confirm a direct interaction between GNL3L and STAT3. Subsequent literature review uncovered evidence indicating that *JAK1*, *IFNAR1*, *IFNAR2*, and *EML4* can activate the STAT3 signaling pathway, contributing to tumor progression (23-25). Correspondingly, our bioinformatics analysis substantiated a positive correlation between GNL3L expression and that of *JAK1*, *IFNAR1*, *IFNAR2*, and *EML4*. Therefore, collectively, these studies underscore the complexity of the interaction between GNL3L and STAT3, implicating multiple regulatory mechanisms.

In comparison with previous studies (6,7,17), the research done so far has primarily focused on esophageal cancer without highlighting the role of ESCC, and there was no literature suggesting that GNL3L might have

promoted the malignant progression of ESCC through the STAT3 signaling pathway. Our research, through comprehensive bioinformatics analysis and IHC validation, indicates notable overexpression of GNL3L in ESCC. Furthermore, we identified a potential association of this phenomena with the STAT3 signaling pathway. A synthesis with other research findings suggests a distinct role of GNL3L in the malignant progression of ESCC. However, it is crucial to acknowledge certain limitations in our study, such as a lack of analysis clarifying the precise mechanisms through which GNL3L induces STAT3 phosphorylation to promote the malignant progression of ESCC. These aspects necessitate further exploration in future research through cellular functional experiments, animal models, or organoid systems.

Conclusions

Increased expression of GNL3L was significantly associated with ESCC progression. Moreover, GNL3L holds promise as a potential tumor marker, and as a therapeutic target and may be valuable in obtaining crucial insights into the diagnosis, treatment, and prognostic assessment of ESCC.

Acknowledgments

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-473/rc

Data Sharing Statement: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-473/dss

Peer Review File: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-473/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-473/coif). A.M. received $2,000 honoraria from ASCO Advantage Program for being a speaker and panelist for Upper GI Tumor Coarse in October 2023 in Alexandria, Virginia; and received $200 Visa gift card for being a speaker at DC-CCP-Pharmacy Lecture in Washington DC. F.D. received consulting fees from AstraZeneca, Eisai; and honoraria from Astellas, Deciphera, Exelixis, Ipsen, Servier, Sirtex, Takeda. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Ethics Committee of the Fujian Medical University Union Hospital (No. 2021KJCX068), and informed consent was obtained from all patients.

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