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High expression of guanine nucleotide-binding protein-like-3-like is associated with poor prognosis in esophageal cancer

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Abstract

Guanine nucleotide-binding protein-like-3-like (GNL3L) is required for processing ribosomal pre-rRNA and cell proliferation and is upregulated in many types of cancer. This study is aimed to investigate the clinical significance of GNL3L in esophageal cancer. The mRNA and protein expression levels of GNL3L were determined by using quantitative real-time polymerase chain reaction and immunohistochemistry, respectively. GNL3L was localized in both cytoplasm and nucleus. The expression levels of GNL3L in esophageal cancer tissues were significantly higher than those in adjacent nonmalignant tissues. High GNL3L expression was associated with pathologic type and poor differentiation. Patients with high GNL3L expression had shorter overall survival (OS) than those with low GNL3L expression. Multivariate Cox regression analysis revealed that GNL3L expression was an independently predictive factor for the OS of patient with esophageal cancer. The Gene Expression Profiling Interactive Analysis (GEPIA) databases also showed that GNL3L was upregulated in esophageal cancer, which was closely associated with an unfavorable prognosis of patients with esophageal cancer. Taken together, our findings suggest that GNL3L is upregulated in esophageal cancer, which is linked to the progression of the disease. As a result, GNL3L could be used as a biomarker for esophageal cancer.

Abbreviations: 95% CI = 95% confidence interval, cDNA = complementary DNA, CSCs = cancer stem cells, FDR = false discovery rate, FFPE = formalin-fixed paraffin-embedded, GEPIA = Gene Expression Profiling Interactive Analysis, GNL3L = guanine nucleotide-binding protein-like-3-like, HR = hazard ratio, IHC = immunohistochemistry, LNM = lymph node metastasis, MDM2 = MDM2 proto-oncogene, NF-κB = nuclear factor-kappa B, OS = overall survival, PFS = progression-free survival, qPCR = quantitative real-time polymerase chain reaction, TNM = tumor node metastasis.

Keywords: cancer stem cell, esophageal cancer, guanine nucleotide-binding protein-like-3-like, prognosis

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1. Introduction

Esophageal cancer is one of the most common cancer worldwide, accounting for approximately 3% of cancer cases and 5% of cancer deaths.[1] China has a higher incidence rate of esophageal cancer. Although the incidence rate of esophageal cancer has declined in recent years, the mortality rate of esophageal cancer still ranks the fourth.[2] Therefore, esophageal cancer has always been a major cancer threatening the health of Chinese. To date, patients with esophageal cancer have very little choice other than cytotoxic chemotherapy due to very few targeted drugs. This emphasizes the critical importance of the identification of alternative targets for the development of novel anticancer treatments and biomarkers for the prognosis of esophageal cancer.

Guanine nucleotide-binding protein-like-3-like (GNL3L) is an evolutionarily conserved high molecular weight nucleolar GTPase which belongs to the YawG/YIqF/HSR1_MMR1 GTP-binding protein subfamily of GTPases. GNL3L is required for processing ribosomal pre-rRNA and cell proliferation.[3] Overexpression of GNL3L promotes the S phase progression by upregulating E2F1, cyclins A2 and E1, whereas inhibition of GNL3L leads to G2/M arrest.[4] Furthermore, GNL3L has been shown to exert an anti-apoptotic activity through modulating the expression and stability of subunit p65 of nuclear factor-kappa B (NF-κB).[5] GNL3L is upregulated in many types of cancer, including esophageal colorectal, esophageal, and gastric cancers.[6,7] Kannathasan et al[7] reported that GNL3L promoted tumorigenesis, cell cycle regulation, and anti-apoptosis through NF-κB activation colorec-

tal cancer (CRC) and was upregulated in chemoresistant CRC cells. In addition, GNL3L is also one of factors that are important for the maintenance of tumorigenic property of cancer stem cells (CSCs).[8] GNL3L plays an important role in cancer, including regulating cell proliferation, metastasis, and chemoresistance.[5–8] Whether the expression level of GNL3L affects the prognosis of cancer patients has not yet been reported.

To date, there is little knowledge about the role of GNL3L in esophageal cancer. Considering its important role in cancer, this study aimed to explore the clinical significance of GNL3L in esophageal cancer. The results demonstrated that GNL3L was upregulated in esophageal cancer, particularly in those poor differentiation cases. High GNL3L expression was associated with ulcerative type, poor differentiation, and worse prognosis. Therefore, GNL3L might be a potential prognostic biomarker and a potential therapeutic target for esophageal cancer.

2. Materials and methods

2.1. Patients

A total of 218 pairs of esophageal squamous cell carcinoma and adjacent nonmalignant esophageal tissues were obtained from patients who underwent surgery esophagectomy at Taizhou People's Hospital. No patient received chemotherapy, radiotherapy, or immunotherapy before surgery. All patients were treated with at least 2 cycles of platinum-combined adjuvant chemotherapy after surgery. The details of patient characteristics have been described previously.[9] In addition, 30 pairs of esophageal cancer and adjacent nonmalignant esophageal formalin-fixed paraffin-embedded (FFPE) tissues were used to detect the protein levels of GNL3L. The study was approved by the Ethical Committee of Taizhou People's Hospital. Informed consent was obtained from all patients.

2.2. Quantitative real-time polymerase chain reaction (qPCR)

The total RNA was extracted from tissues with Trizol reagent (Invitrogen, CA, USA) following the manufacturer's protocol. Complementary DNA (cDNA) was synthesized from 1 μg of total RNA using PrimeScript™ RT reagent Kit with gDNA Eraser (Takara, Dalian, China) according to the manufacture's instructions. QPCR was carried out using the SYBR Premix Ex Taq™ II kit (Tli RNaseH Plus) (Takara, Dalian, China) on ABI 7900 system (Applied Biosystems, CA, USA) according to the manufacturer's instructions. Assays were performed in triplicate for each sample. The relative expression levels of GNL3L were calculated and normalized using the $2^{-\Delta\Delta Ct}$ method relative to GAPDH.

2.3. Immunohistochemistry (IHC)

The protein levels of GNL3L were examined using IHC. The tissue slides were deparaffinized with xylene and dehydrated in a graded series of ethanol (100%, 95% and 80% ethanol) and PBS. After antigen retrieval, slides were incubated in 3% H_2O_2 to quench endogenous peroxidase activity. Nonspecific binding was blocked by incubation with 10% goat serum in PBS for 1 h at room temperature. The slides then were incubated with a primary monoclonal rabbit anti-GNL3L antibody (Abcam, Cambridge, MA, USA) and subsequently incubated with secondary antibody. The evaluation of IHC staining results was conducted blindly. Staining intensity was scored and the total histological score was calculated as described previously.[10]

2.4. Statistical analyses

Statistical analyses were performed using IBM SPSS software version 25.0 (SPSS Inc., IL, USA) and GraphPad Prism 7 (GraphPad Software, CA, USA). A P value < .05 was considered to be statistically significant for all statistical procedure. The differences in mRNA and protein expression levels of GNL3L between esophageal cancer and adjacent tissues were compared with the Mann–Whitney test. Spearman correlation test was used to determine the association between the expression level of GNL3L and tumor size. All patients were divided into low and high GNL3L expression groups according to its median value. Pearson χ^2 and Fisher's exact test were used to evaluated the association between clinicopathologic variables and GNL3L expression. Estimates of overall survival (OS) were from Kaplan–Meier curves and tests of differences by log-rank test. Clinicopathological variables with a value of P < .05 in the univariate Cox regression analysis were further analyzed using multivariate Cox regression.

3. Results

3.1. Upregulation of GNL3L expression in esophageal cancer tissues

The mRNA and protein expression levels of GNL3L in esophageal cancer were examined by qPCR and IHC, respectively. The mRNA expression levels of GNL3L in esophageal cancer tissues were significantly higher than those in adjacent nonmalignant tissues (P < .001, Figure 1A). In addition, we evaluated the difference in the mRNA expression level of GNL3L between different clinicopathological variable groups. As shown in Figure 1B, cases with poor differentiation (P = .002) had significantly higher level of GNL3L than those with well and moderate differentiation. Although there was no difference in the mRNA expression level of GNL3L between tumor size ≤ 4 cm and tumor size greater than 4 cm (P = .063), the mRNA expression level of GNL3L was positively correlated with tumor size (P = .037). With the development of biotechnologies and the continued reduction in costs, omics data is now massively produced.[11] Therefore, integrating online omics data will help to improve the reliability of the findings. The mRNA expression level of GNL3L in esophageal cancer was validated in the Gene Expression Profiling Interactive Analysis (GEPIA) database, in which 286 normal and 182 esophageal cancer tissues were included.[12] The results suggested that the mRNA expression level of GNL3L was significantly upregulated in esophageal cancer (Figure 1C). which was consistent with our result.

We further evaluated the protein expression of GNL3L in 30 pairs of esophageal cancer and adjacent nonmalignant tissues. IHC assays showed that GNL3L was localized in both cytoplasm and nucleus (Figure 2). The protein expression levels of GNL3L in esophageal cancer tissues was significantly higher than those in adjacent nonmalignant tissues (P < .001).

3.2. Association between GNL3L expression and clinicopathological variables of patients with esophageal cancer

As shown in Table 1, high GNL3L expression was significantly associated with clinicopathological variables including pathologic type (P = .020) and poor differentiation (P = .007). Furthermore, patients with high GNL3L expression had a tend towards larger tumor size than those with low GNL3L
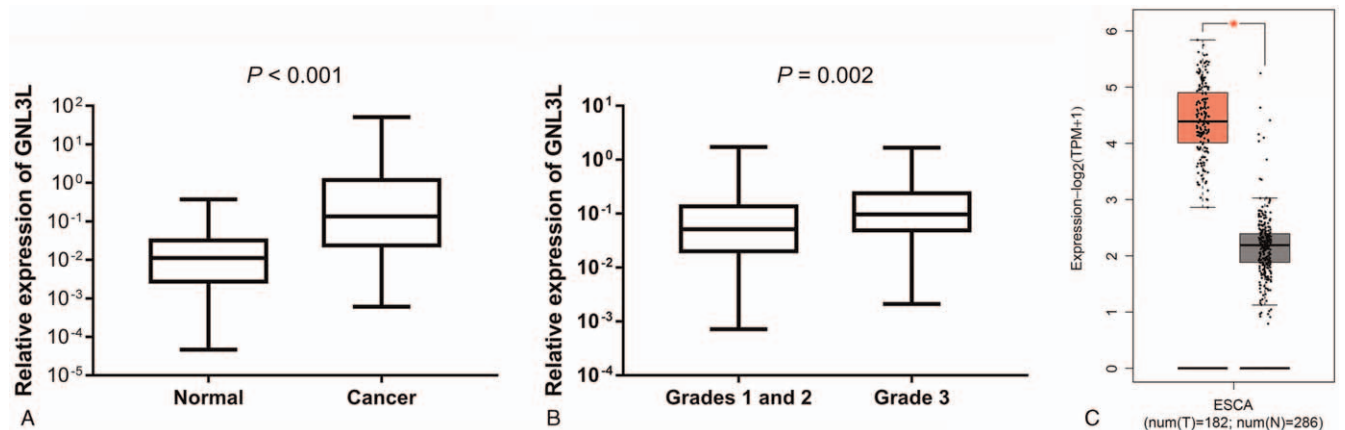
Figure 1. GNL3L was upregulated in esophageal cancer. (A) relative expression levels of GNL3L in esophageal cancer and adjacent nonmalignant tissues. (B) relative expression of GNL3L in esophageal cancer with different grades of differentiation. (C) the GEPIA database showed that GNL3L was significantly upregulated in esophageal cancer tissues (n=182) compared with normal esophageal tissues (n=286). *P < .05.

expression, but the difference did not reach statistical significance (P=.050). There was no correlation between GNL3L expression and other clinical variables including sex, age, drug response, tumor invasion, lymph node metastasis (LNM), and tumor node metastasis stage (P > .05).

3.3. Survival analyses

We also examined the effect of GNL3L on the prognosis of patients with esophageal cancer. There was a significant trend toward decreased survival time with increased expression level of GNL3L. As shown in Figure 3, high GNL3L expression demonstrated significant correlation with poor OS in patients with esophageal cancer (P=.008). Univariate and multivariate Cox regression analyses of clinicopathological variables regarding OS were listed in Table 2. The results revealed that pT category [hazard ratio (HR)=1.977, 95% confidence interval (CI): 1.244-3.142, P=.004], LNM (HR=2.349, 95%CI: 1.528–3.611, P < .001), tumor node metastasis stage (HR=2.050, 95% CI: 1.479–2.840, P < .001), and GNL3L expression (HR=1.504, 95%CI: 1.102–2.053, P=.010) had a remarkable impact on OS. Multivariate Cox regression analysis was subsequently performed based on the Clinicopathological variables with a value of P < .05 in the univariate Cox regression analysis. The results

revealed that LNM (adjusted HR=1.868, 95%CI: 1.118–3.122, P=.017) and GNL3L expression (adjusted HR=1.483, 95%CI: 1.066–2.063, P=.019) were independent risk factors affecting OS time. Subsequently, we validated the effect of GNL3L on the prognosis of patients with esophageal cancer using the GEPIA database. The results demonstrated that patients with high GNL3L expression had shorter progression-free survival (PFS) and OS than those with low GNL3L expression (P < .05).

3.4. Prediction of interaction networks of GNL3L

A gene–gene interaction network for GNL3L was constructed using the STRING v11.0 (Figure 4),[13] which were further verified by the GEPIA database to enhance the accuracy and reliability of the network.[12] The node representing GNL3L was connected to the nodes of other genes in terms of co-expression and physical interactions. The protein–protein interaction (PPI) network of the top 10 genes was shown in Figure 4. The top 10 genes displaying the greatest correlations with GNL3L were listed in Table S1 Supplemental Digital Content (see Table, Supplemental Content, which lists top 10 genes co-expressed with GNL3L identified from the PPI network, http://links.lww.com/MD/G122). The results of STRING were consistent with that of GEPIA database. All 10 genes were positively correlated with
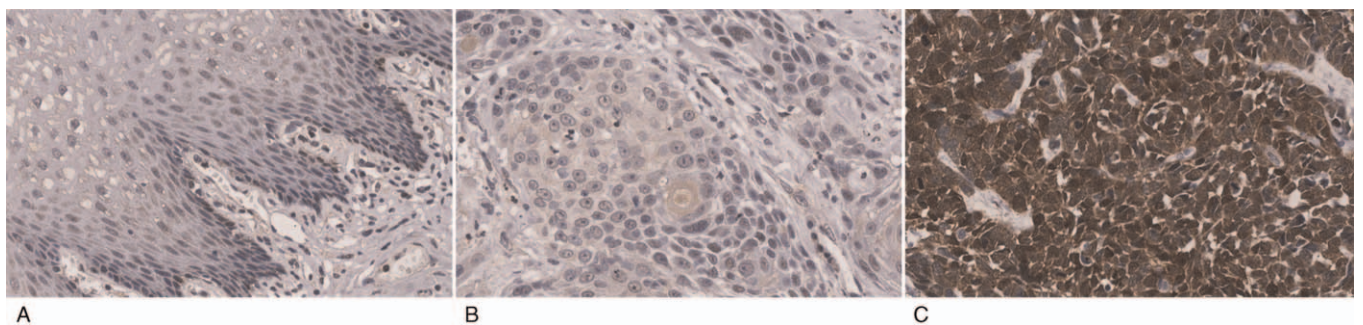

Figure 2. Representative images of immunohistochemical staining in esophageal cancer. (A) weak staining of GNL3L in normal esophageal tissue. (B) weak staining of GNL3L in esophageal cancer tissue. (C) strong staining of GNL3L in esophageal cancer tissue.

Association of GNL3L expression with clinico-pathologic parameters.

Variables	GNL3L expression		P value
	High	Low	
Age (years)			
< 65	54 (49.5)	54 (49.5)	1.000
≥ 65	55 (50.5)	55 (50.5)	
Sex			
male	85 (78.0)	86 (78.9)	1.000
female	24 (22.0)	23 (21.1)	
Pathologic type*			
ulcerative	65 (65.7)	46 (48.4)	.020
others	34 (34.3)	49 (51.6)	
Histologic grade			
1 + 2	68 (62.4)	87 (79.8)	.007
3	41 (37.6)	22 (20.2)	
Tumor size (cm)*			
≤ 4	36 (34.6)	51 (48.6)	.050
> 4	68 (65.4)	54 (51.4)	
Clinical response*			
sensitivity	40 (40.0)	36 (36.7)	.663
resistance	60 (60.0)	62 (63.3)	
pT categories*			
T1 + T2	21 (20.0)	18 (18.4)	.859
T3 + T4	84 (80.0)	80 (81.6)	
LNM*			
non-LNM	24 (22.0)	25 (23.1)	.872
LNM	85 (78.0)	83 (76.9)	
TNM stage*			
I + II	45 (41.3)	51 (50.5)	.213
III + IV	64 (58.7)	50 (49.5)	

* excluding missing data.
LNM = lymph node metastasis, TNM = tumor node metastasis.

GNL3L. GNL3L showed the greatest correlation with snoRNA binding [false discovery rate (FDR) = 0.010], followed by GTPase activity (FDR = 0.019), RNA binding (FDR = 0.025), and GTP binding (FDR = 0.025).

4. Discussion

Although the combined application of surgery, chemotherapy, radiotherapy, and targeted therapy can improve the prognosis of patients with esophageal cancer, the therapeutic efficacy of esophageal cancer is still far from satisfaction. The mortality rate of esophageal cancer remains high for many years. In the present study, we found that GNL3L was upregulated in esophageal cancer. High GNL3L expression was an independent risk factor for an unfavorable OS in patients with esophageal cancer.

GNL3L contains a central GTP-binding domain and an N-terminal basic domain in which the activity of nucleoplasmic localization signal is dynamically controlled by the GTP-binding motifs.[14] In addition, the C-terminal domain is essential for the export of GNL3L from the nucleus that shuttles between cytoplasm and nucleus in CRM1-dependent manner.[4] GNL3L interacts with and stabilizes MDM2 proto-oncogene (MDM2) protein by preventing the ubiquitination.[6] Depletion of GNL3L not only inhibits proliferation and colony formation but also suppress invasion and migration of cancer cells.[7] In addition to its role in the regulation of cell proliferation, cell cycle, and anti-apoptosis, GNL3L may accelerate NF-κB-mediated inflammation by upregulating inflammatory molecules, such as IL-4 and IL-8,[5] whereas inflammation promotes carcinogenesis and tumor growth, and facilitates angiogenesis, tumor extravasation, and metastasis.[15] It has been revealed that GNL3L is overexpressed in many types of cancers, including esophageal colorectal, esophageal, and gastric cancers.[6] A recent study by Kannathasan et al[7] revealed that high GNL3L expression was associated with chemoresistance. GNL3L exhibits an oncogenic function that promotes cancer development and progression. However, little is known about the clinical significance of GNL3L in human cancer. In the present study, although GNL3L was upregulated in esophageal cancer, there was no association between GNL3L expression and drug response. Therefore, high GNL3L expression may not influence the chemoresistance of esophageal cancer, but confer a more aggressive phenotype to esophageal cancer than low GNL3L expression. These may explain at least partly why patients with high GNL3L expression had poor prognosis.
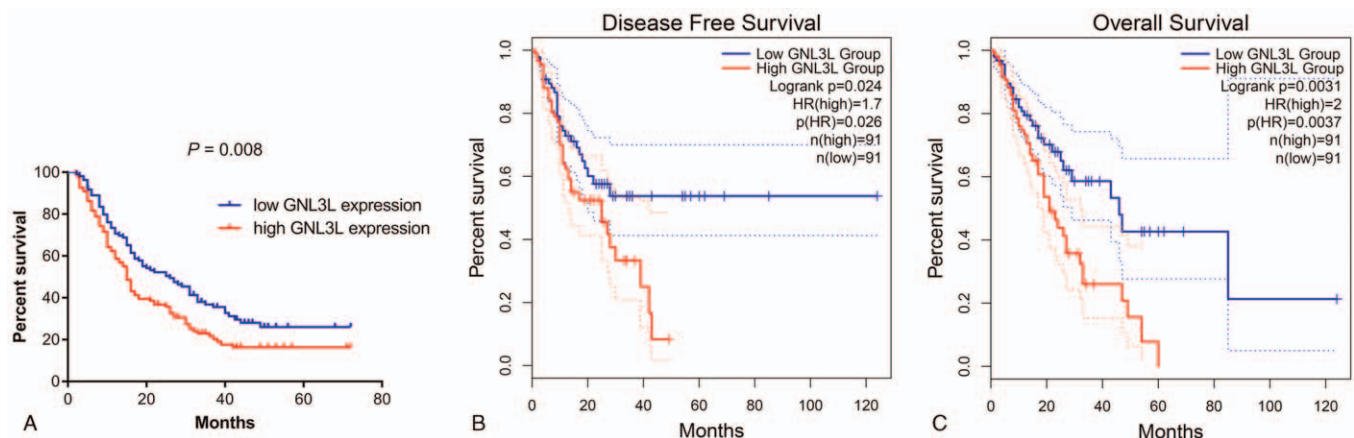

Figure 3. Kaplan–Meier survival curves. (A) OS curves stratified by GNL3L expression in 218 patients with esophageal cancer. (B) PFS curves stratified by GNL3L expression based on the GEPIA database. (C) OS curves stratified by GNL3L expression based on the GEPIA database.

Univariate and multivariate Cox regression analysis of overall survival in 218 esophageal cancer cases.

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Age (years), < 65 vs ≥ 65	1.279 (0.939–1.744)	.119		
Sex, male vs female	0.707 (0.479–1.043)	.081		
Pathologic type, ulcerative vs others	1.238 (0.888–1.726)	.208		
Histologic grade, 1+2 vs 3	1.058 (0.753–1.487)	.746		
Clinical response, resistance vs sensitivity	1.331 (0.956–1.851)	.090		
Tumor size (cm), > 4 vs ≤ 4	1.244 (0.902–1.716)	.092		
pT categories, T3+T4 vs T1+T2	1.977 (1.244–3.142)	.004	1.563 (0.939–2.602)	.086
LNM, positive vs negative	2.349 (1.528–3.611)	<.001	1.868 (1.118–3.122)	.017
TNM stage, III+IV vs I+II	2.050 (1.479–2.840)	<.001	1.243 (0.823–1.878)	.300
GNL3L expression, low vs high	1.504 (1.102–-2.053)	.010	1.483 (1.066–2.063)	.019

GNL3L = Guanine nucleotide-binding protein-like-3-like, LNM = lymph node metastasis, TNM = tumor node metastasis.

The traditional theory on cancer initiation and development is that cancer arises from the sequential accumulation of mutations. Since stem cells are the longest-living cells within an organism, the initial oncogenic mutations most likely occur in stem cells.[16] CSCs are small subpopulation of cancer cells within the entire tumor mass (0.001–0.1%) that have the properties of tumorigenesis, unlimited self-renewal, and multilineage differentiation potential and are able to form the bulk of the tumor even from a single cell, whereas the majority of cancer cells are differentiated and have little or no ability to generate new cancer cells.[17–19] It is widely believed that CSCs is responsible for cancer chemoresistance, metastasis, and relapse.[17,20,21] With the most recent study, Trevellin et al[22] have shown that stem cell markers CD34, CD133, and Nucleostemin are related to poor prognosis of patients with esophageal cancer. A study by Okamoto et al[8] revealed that the NS/GNL3L-TERT-BRG1 complex was important for the maintenance of CSCs. Therefore, cancer cells with high GNL3L expression might be more likely to have a stem cell phenotype. High GNL3L expression could confer higher proliferation capacity, chemoresistance, invasiveness, and metastasis to cancer cells. Although we did not observe an association between GNL3L expression and drug response, there was a significant difference in OS between patients with low and high GNL3L expression. GNL3L might be a potential therapeutic target for the elimination of esophageal CSCs.

In summary, this study revealed that GNL3L is evidently upregulated in esophageal cancer. Our findings provide the first evidence that high GNL3L expression is closely related to an unfavorable prognosis. GNL3L might serve as a biomarker and potential therapeutic target for esophageal cancer. Further studies are undoubtedly required to validate our findings and clarify the underlying molecular mechanism of GNL3L in esophageal cancer.

Author contributions

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Investigation: Hong Yu.
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Resources: Guihong Dai, Min Jiang, Huilin Zhou, Jingjing Bao.
Supervision: Junxing Huang.
Writing – original draft: Guihong Dai, Hong Yu, Junxing Huang.
Writing – review & editing: Hong Yu, Junxing Huang.

Figure 4. PPI network analysis of GNL3L. Top 10 candidate genes might have an interaction with GNL3L.

References

[1] Bray F, Ferlay J, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021.
[2] Zheng RS, Sun KX, Zhang SW, et al. [Report of cancer epidemiology in China, 2015]. Zhonghua Zhong Liu Za Zhi 2019;41:19–28.
[3] Du X, Rao MR, Chen XQ, Wu W, Mahalingam S, Balasundaram D. The homologous putative GTPases Grn1p from fission yeast and the human GNL3L are required for growth and play a role in processing of nucleolar pre-rRNA. Mol Biol Cell 2006;17:460–74.
[4] Thoompumkal IJ, Subba Rao MR, Kumaraswamy A, Krishnan R, Mahalingam S. GNL3L is a nucleo-cytoplasmic shuttling protein: role in cell cycle regulation. PLoS one 2015;10:e0135845.
[5] Thoompumkal IJ, Rehna K, Anbarasu K, Mahalingam S. Leucine Zipper Down-regulated in Cancer-1 (LDOC1) interacts with guanine nucleotide binding protein-like 3-like (GNL3L) to modulate nuclear factor-kappa B (NF-κB) signaling during cell proliferation. Cell cycle (Georgetown, Tex) 2016;15:3251–67.

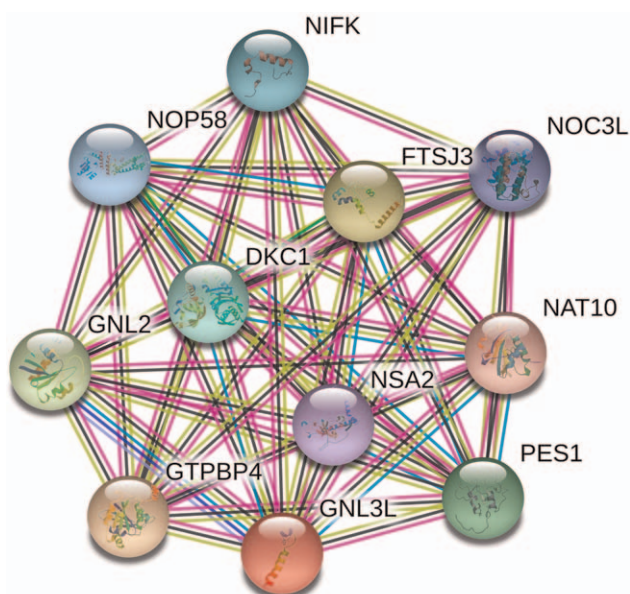[6] Meng L, Hsu JK, Tsai RY. GNL3L depletion destabilizes MDM2 and induces p53-dependent G2/M arrest. Oncogene 2011;30:1716–26.

[7] Kannathasan T, Kuo WW, Chen MC, et al. Chemoresistance-associated silencing of miR-4454 promotes colorectal cancer aggression through the GNL3L and NF-κB pathway. Cancers 2020;12:1231.

[8] Okamoto N, Yasukawa M, Nguyen C, et al. Maintenance of tumor initiating cells of defined genetic composition by nucleostemin. Proc Natl Acad Sci U S A 2011;108:20388–93.

[9] Jiang L, Wang W, Li G, et al. High TUG1 expression is associated with chemotherapy resistance and poor prognosis in esophageal squamous cell carcinoma. Cancer Chemother Pharmacol 2016;78:333–9.

[10] Zhang X, He C, He C, et al. Nuclear PKM2 expression predicts poor prognosis in patients with esophageal squamous cell carcinoma. Pathol Res Pract 2013;209:510–5.

[11] Kosvyra A, Maramis C, Chouvarda I. Developing an integrated genomic profile for cancer patients with the use of NGS data. Emerg Sci J 2019;3:157–67.

[12] Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res 2017;45:W98–102.

[13] Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res 2017;45:D362–8.

[14] Rao MR, Kumari G, Balasundaram D, Sankaranarayanan R, Mahalingam S. A novel lysine-rich domain and GTP binding motifs regulate the nucleolar retention of human guanine nucleotide binding protein, GNL3L. J Mol Biol 2006;364:637–54.

[15] Multhoff G, Molls M, Radons J. Chronic inflammation in cancer development. Front Immunol 2011;2:98.

[16] Sell S. On the stem cell origin of cancer. Am J Pathol 2010;176:2584–3494.

[17] Clarke MF. Clinical and therapeutic implications of cancer stem cells. N Engl J Med 2019;380:2237–45.

[18] Kreso A, Dick JE. Evolution of the cancer stem cell model. Cell Stem Cell 2014;14:275–91.

[19] Chae YC, Kim JH. Cancer stem cell metabolism: target for cancer therapy. BMB reports 2018;51:319–26.

[20] De Angelis ML, Francescangeli F, Zeuner A. Breast cancer stem cells as drivers of tumor chemoresistance, dormancy and relapse: new challenges and therapeutic opportunities. Cancers 2019;11:1569.

[21] Agliano A, Calvo A, Box C. The challenge of targeting cancer stem cells to halt metastasis. Semin Cancer Biol 2017;44:25–42.

[22] Trevellin E, Pirozzolo G, Fassan M, Vettor R. Prognostic value of stem cell markers in esophageal and esophagogastric junction cancer: a meta-analysis. J Cancer 2020;11:4240–9.