Original Article

High expression of *SEZ6L2* as an independent prognostic Indicator in thyroid carcinoma

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Background: Seizure-related 6 homolog (mouse)-like 2 (*SEZ6L2*) is a type 1 transmembrane protein that is primarily expressed in the brain . In recent reports, *SEZ6L2* has been found to be overexpressed in some cancers and can drive the progression of tumors. However, its function and mechanism in thyroid cancer remain unclear.

Methods: In this article ,we searched for the *SEZ6L2* expressions in Pan-cancer on TCGA (The Cancer Genome Atlas) and evaluated these data using the TIMER2 method. Then, the immunohistochemical score (IHC score) of *SEZ6L2* in cancer tissue was collected in human protein mapping (HPA) data. And we used CIBERSORT to assess the association between the levels of *SEZ6L2* expression and the number of various immune cells in papillary thyroid carcinoma (PTC) tissue. Finally, Gene Expression Omnibus (GEO) analyses, real-time quantitative polymerase chain reaction (qRT-PCR) of tissues, and immunohistochemical staining were used to detect the result.

Results: The article illustrated that a large number of cancers had a higher expression of *SEZ6L2* compared to the control tissues. And the immunohistochemical score (IHC score) of *SEZ6L2* in cancer tissue was considerably elevated compared to that in normal tissues *SEZ6L2* was elevated in thyroid carcinoma (THCA) tissue, besides, GEO analyses, qRT-PCR of tissues, and immunohistochemical staining were conducted to test the results. Finally, the Kaplan-Meier survival analysis illustrated that the increased expression of *SEZ6L2* was correlated with a dismal prognosis-higher SEZ6L2 is associated with shorter survival. And the univariate analysis illustrated that T stage, SEZ6L2 and Pathologic stage were related to the overall survival (OS), multivariate analysis stated that elevated expression of *SEZ6L2* was an independent risk factor that affected progression-free interval (PFI) ($P<0.05$). Consequently, we found that the expression of *SEZ6L2* was correlated with tumor-infiltrating immune cells by TIMER.

Conclusions: *SEZ6L2* was upregulated in patients with THCA and that increased expression of *SEZ6L2* was related with clinical progression and was regarded as an independent risk factor for PFI. In THCA patients, the expression of *SEZ6L2* could be a significant prognostic factor, which is expected to be a prospective biomarker for THCA in the future.

Keywords: Seizure-related 6 homolog (mouse)-like 2 (*SEZ6L2*); thyroid carcinoma (THCA); the cancer genome atlas; diagnosis; prognosis

Submitted Dec 14, 2021. Accepted for publication Feb 18, 2022.
doi: 10.21037/gs-22-37
View this article at: https://dx.doi.org/10.21037/gs-22-37

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Introduction

Thyroid carcinoma (THCA) has been identified as the most commonly occurring type of endocrine malignancy. As indicated in the recent global cancer report, the number of diagnosed THCA patients in 2020 reached 586,000, representing 11 out of every 36 recently diagnosed malignancies worldwide (1). As the most common type of THCA, papillary thyroid carcinoma (PTC) accounts for almost 85–90% of all cases (2). PTC usually progresses slowly. Furthermore, with standard therapy, PTC frequently displayed an excellent prognosis, with a 93% 10-year survival rate (3). Nevertheless, more than 30% of cases of THCA displayed an increased propensity of recurrence or early lymph node metastasis (4). As a result, it is critical to find reliable and precise biomarkers for both the diagnosis and the prognosis of THCA.

Seizure-related 6 homolog (mouse)-like 2 (*SEZ6L2*) is a type 1 transmembrane protein that is primarily expressed in the brain and is affiliated with the seizure-related gene 6 (*SEZ6*) family, which comprises *SEZ6L2*, *SEZ6L*, and *SEZ6*. Members of the *SEZ6* family have primarily been reported in a variety of psychiatric and neurodevelopmental illnesses, including bipolar disorder, epilepsy, intellectual impairment, schizophrenia, and autism (5-12). *SEZ6L2* has been demonstrated to modulate differentiation and neurogenesis, as well as to regulate the modulatesα-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid-adducin (AMPA-ADD) signal transmission by controlling phosphorylation of adducin (13,14). Furthermore, a recent research report found the overexpression of AMPA-ADD in lung tumors, which suggests that AMPA-ADD can be used as a potential lung cancer prognostic biomarker (15). *SEZ6L2* has been found to be overexpressed in some cancers and can drive the progression of tumors. An *et al.* found that the up-modulation of *SEZ6L2* was strongly associated with the dismal prognosis in CRC patients (16). Wang *et al*. demonstrated a correlation between the elevated expression of *SEZ6L2* protein and tumor size, TNM stage, and tumor number, in hepatocellular carcinoma (HCC) patients. Overexpression of *SEZ6L2* was strongly related to the dismal OS and disease-free survival. Additionally, *SEZ6L2* was demonstrated to be an independent prognostic indicator for HCC patients' survival (17). And it is indicated that the expression of SEZ6L2 can drive the growth of tumor by mitochondria-related proteins. Besides, it's also prevents phosphorylation of adducin and neuritogenesis. However, its specific functions in THCA remain unknown. The aim of this research was to examine the expression of *SEZ6L2* and its function in patients with THCA. We present the following article in accordance with the REMARK reporting checklist (available at https://gs.amegroups.com/article/view/10.21037/gs-22-37/rc).

Methods

Patient data sets

Patient data sets mRNA expression data (510 samples, Workflow Type: HTSeq-FPKM) and related clinical information were downloaded from the TCGA database (https://portal.gdc.cancer.gov). A total of 510 patients with THCA with the corresponding clinical features were collected in this study. We used the online human protein mapping (HPA) portal website (https://www.proteinatlas.or) to. By inputting the word *SEZ6L2* into the Tissue Atlas and Pathology Atlas modules, protein expression data and histochemical staining images of different human normal tissues and tumor tissues were obtained. We standardized the data source according to the product of cell staining intensity, where scores of 0, 1, 2, and 3 meant not detected, low, medium, and high, respectively, and according to the number of positive cell expression scores, where 0, 1, 2, and 4 points were given when the number of positive cell expression was none, <25%, <75%, and >75%, respectively. The two integrals were added together to obtain the immunohistochemical score. If there were multiple data in the same tissue, the mean value was taken to compare the immunohistochemical score of normal tissue and tumor tissue according to tumor type, and the histogram was drawn.

Sample collection

THCA and adjacent tissues were enrolled from 40 patients of Guizhou Medical University, immediately stored in liquid nitrogen, and keept at –80 ℃. All procedures performed in this study were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Ethics Committee of Human Trials, Guizhou Medical University [No. 245(2021)] and informed consent was taken from all the patients.

Quantitative real-time PCR of tissues

Total RNA was extracted from normal thyroid and THCA

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tissue specimens using TRIzol reagent (Invitrogen, Thermo Fisher Scientific, Shanghai, China) on the basis of the manufacturer's instructions. RNA was reversely

RNA was transcribed into cDNA using the Transcription First Strand cDNA synthesis kit (Takara, Dalian, Liaoning, China). Quantitative real-time PCR (qRT-PCR) analyses were quantified with SYBR® Green (Takara). As an internal reference, the hGAPDH was used to calculating the expression of *SEZ6L2* based on the $2^{-\Delta\Delta ct}$ method. The real-time quantitative polymerase chain reaction (qRT-PCR) primers used in the present study were as follows: *SEZ6L2* forward primer, 5'-ATGAAGCTGGGGATACGC-3'; *SEZ6L2* reverse primer, 5'-CCTCGTGGGATAGGGAGA-3'; hGAPDH forward primer, 5'-GGACCTGACCTGCCGTCTAG-3'; and hGAPDH reverse primer, 5'-GTAGCCCAGGATGC CCTT-3'.

Immunohistochemical staining

Immunohistochemical staining was used to determine the expression of *SEZ6L2* in the tissues. Paraffin slices of cancer tissues were taken from the Pathology Department of Guizhou Medical University Hospital, dewaxed with xylene, and hydrated with gradient alcohol (anhydrous ethanol, 95% ethanol, 80% ethanol, 70% ethanol, and 50% ethanol for 5 minutes each). The tissues were boiled in EDTA solution for 10 minutes and cooled to room temperature, then were washed with PBS. Goat serum working fluid was closed for 30 minutes at 37 ℃. Thereafter, the tissues were probed with 50 μL of *SEZ6L2* primary antibody (AF4916, 1:50, R&D Systems), and then reprobed with 50 μL of secondary antibody donkey anti-sheep IgG (a21060, 1:500, Abbkine) for 30 minutes at 37 ℃. PBS washing was followed by DAB development for 3 minutes. The tissues were then washed under tap water for 10 minutes, and then the tissues were counterstained with hematoxylin for 20 seconds, differentiated with hydrochloric acid alcohol, gradient alcohol dehydrated, xylene transparentized, and neutral gum sealed before the paraffin slices were observed under a microscope.

Statistical analysis

Box plots were used to evaluate the level of *SEZ6L2* expression in THCA patients. The cut-off value of *SEZ6L2* expression was chose as the median method of gene expression. Wilcoxon signed-rank test and logistic regression were used to analyze the association between clinical features and *SEZ6L2* expression in THCA. According to the Kaplan-Meier analysis, we compared the progression-free interval (PFI) between the low and high *SEZ6L2* expression groups by using the P value determined in the log-rank test. A received operating characteristic (ROC) curve was adopted to assess the diagnostic value of *SEZ6L2* expression, using the area under the ROC curve as the diagnostic value. We conducted statistical analysis and visualization of RNA SEQ data and clinical data of TCGH in TCGA through R language, then used univariate Cox analysis to filtrate potential prognostic factors, and multivariate Cox analysis to verify the influence of *SEZ6L2* expression on survival as well as other clinical variables. An alignment chart was constructed to predict 3-, 5- and 10-year THCA survival by combining the expression value of *SEZ6L2* with clinical variables. In order to test the results, we selected datasets containing both PTC and normal tissues in the Gene Expression Omnibus (GEO) database. The following three microarray datasets were downloaded from the National Center for Biotechnology Information GEO database (http://www.ncbi.nlm.nih.gov/geo): GSE129562 (P=3.1e-04), GSE65144 (P=6.4e-06), and GSE35570 (P=1.9e-07). The expression level of *SEZ6L2* in patients with THCA and normal individuals was further validated in the TIMER database (https://cistrome.shinyapps.io/timer/). All statistical analyses were performed using R statistical software (version 3.6.3), SPSS software (version 24.0) or GSVA (version1.34.0). A P value less than 0.05 was considered as statistically significant.

Results

Analysis of gene expression data

The TIMER2 technique was used to determine the expression of *SEZ6L2* in various kinds of cancer data retrieved from TCGA. As illustrated in *Figure 1A*, the expression of *SEZ6L2* was significantly elevated as opposed to the control tissues (P<0.05) of the following tumor types: lung squamous cell carcinoma (LUSC), uterine corpus endometrial carcinoma (UCEC), head and neck squamous cell carcinoma (HNSC), stomach adenocarcinoma (STAD), cervical squamous cell carcinoma, endocervical adenocarcinoma esophageal carcinoma (CESC), liver hepatocellular carcinoma (LIHC), rectum adenocarcinoma (READ), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), pheochromocytoma and paraganglioma (PCPG), breast invasive carcinoma (BRCA), esophageal carcinoma

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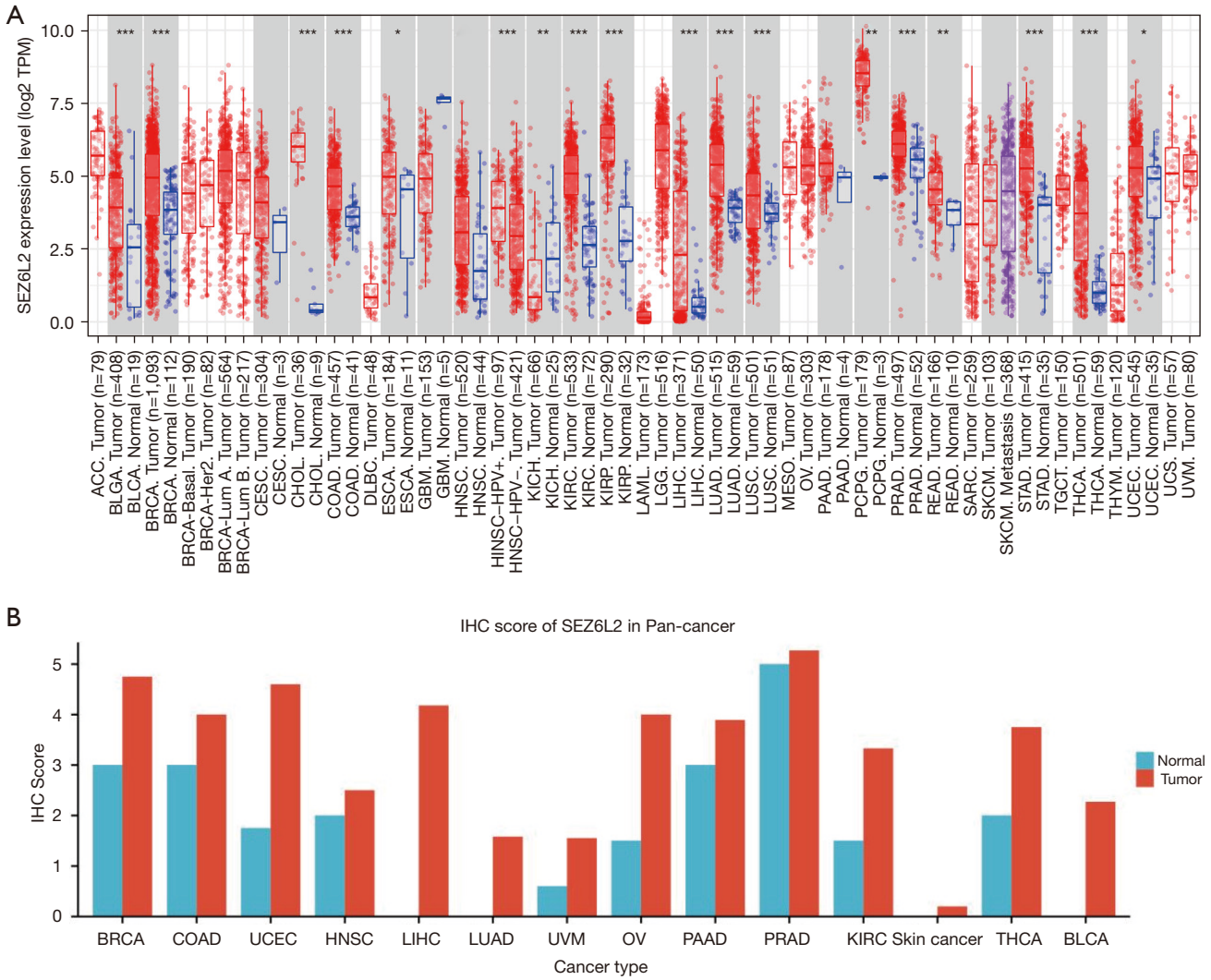

Figure 1 *SEZ6L2* expression in Pan-cancer. (A) Comparison of *SEZ6L2* expression between different cancerous and paraneoplastic tissues. (B) immunohistochemical score of *SEZ6L2* in Pan-cancer. *, P<0.05; **, P<0.01; ***, P<0.001. ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; 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; LAML, acute myeloid leukemia; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, Uterine carcinosarcoma; UVM, uveal melanoma.

(ESCA), THCA, lung adenocarcinoma (LUAD), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), prostate adenocarcinoma (PRAD), glioblastoma multiforme (GBM), kidney renal papillary cell carcinoma (KIRP), and bladder urothelial carcinoma (BLCA) (*Table 1*).

Then, we collected the data about the normal and tumor tissue comparisons in the HPA database. The immunohistochemical score (IHC score) of *SEZ6L2* in

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Table 1 31 types of human cancers employed in research

Abbreviation	Full name
ACC	Adrenocortical carcinoma
BLCA	Bladder urothelial carcinoma
BRCA	Breast invasive carcinoma
CESC	Cervical squamous cell carcinoma and endocervical adenocarcinoma
CHOL	Cholangiocarcinoma
COAD	Colon adenocarcinoma
DLBC	Lymphoid neoplasm diffuse large B-cell lymphoma
ESCA	Esophageal carcinoma
HNSC	Head and neck squamous cell carcinoma
KICH	Kidney chromophobe
KIRC	Kidney renal clear cell carcinoma
KIRP	Kidney renal papillary cell carcinoma
LAML	Acute myeloid leukemia
LGG	Brain lower grade glioma
LIHC	Liver hepatocellular carcinoma
LUAD	Lung adenocarcinoma
LUSC	Lung squamous cell carcinoma
MESO	Mesothelioma
OV	Ovarian serous cystadenocarcinoma
PAAD	Pancreatic adenocarcinoma
PCPG	Pheochromocytoma and paraganglioma
PRAD	Prostate adenocarcinoma
READ	Rectum adenocarcinoma
SARC	Sarcoma
SKCM	Skin cutaneous melanoma
STAD	Stomach adenocarcinoma
TGCT	Testicular germ cell tumors
THCA	Thyroid carcinoma
THYM	Thymoma
UCEC	Uterine corpus endometrial carcinoma
UCS	Uterine carcinosarcoma
UVM	Uveal melanoma

cancer tissue was considerably elevated compared to that in normal tissues among 14 tumors, including BRCA, COAD, UCEC, HNSC, UVM, OV, PAAD, PRAD, KIRC, THCA, and BLCA, as illustrated in *Figure 1B*.

Patients' baseline characteristics

Data from 510 THCA patients who met the criteria for the necessary clinical data was retrieved in June 2021 from TCGA. *Table 2* displays a detailed list of the clinical characteristics. The 510 patients were classified into two groups according to their median *SEZ6L2* expression value. The group with low expression of *SEZ6L2* had 304 cases, and the higher group had 206 cases. In terms of T stage, the low expression group included 99 patients (19.4%) at T1 stage, 100 patients (19.6%) at T2 stage, 88 patients (17.3%) at T3 stage, and 15 patients (2.9%) at T4 stage. In the high expression group, 44 patients (8.6%) were T1 stage, 67 patients (13.1%) were T2 stage, 87 patients (17.1%) were T3 stage, and 8 patients (1.6%) were T4 stage. As for the N stage, the low expression group included 141 patients (27.6%) at N0 stage and 125 patients (24.5%) at N1 stage. The high expression group contained 88 patients (17.3%) at N0 stage and 106 patients (20.8%) at N1 stage ($P<0.05$). In addition, in the low expression group 166 patients were M0 stage (32.5%) and 6 patients were M1 stage (1.2%), while the high expression group contained 120 patients at M0 stage (23.5%) and 3 patients at M1 stage (0.6%).

High SEZ6L2 expression in THCA

We examined the level of *SEZ6L2* expression in normal thyroid tissues and in tumor specimens to ascertain the degree of *SEZ6L2* expression in THCA patients. The findings indicated that *SEZ6L2* gene expression was considerably elevated in THCA tissues (P=1e-23) compared to that in normal tissues (*Figure 2A*). These findings were confirmed in THCA tissues as well as in paired normal thyroid tissues (P=3.8e-10) (*Figure 2B*). Further research examined gene expression data from three GEO cohorts, and these findings corroborated the aforementioned results [GSE129562 (P=3.1e-04); GSE65144 (P=6.4e-06); and GSE35570 (P=1.9e-07)] (*Figure 2C-2E*). In order to test the results, we did a qRT-PCR of tissues and showed that the

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Table 2 Clinical features of the THCA

Characteristic	Low expression of *SEZ6L2* (N=304)	High expression of *SEZ6L2* (N=206)	P
T stage, n (%)			0.088
T1	99 (19.4)	44 (8.6)	
T2	100 (19.6)	67 (13.1)	
T3	88 (17.3)	87 (17.1)	
T4	15 (2.9)	8 (1.6)	
N stage, n (%)			0.032
N0	141 (27.6)	88 (17.3)	
N1	125 (24.5)	106 (20.8)	
M stage, n (%)			0.323
M0	166 (32.5)	120 (23.5)	
M1	6 (1.2)	3 (0.6)	

THCA, thyroid carcinoma.

level of *SEZ6L2* gene expression was considerably elevated in THCA tissues. These findings were validated in THCA tissues and paired normal thyroid tissues (P=1.3e-07) (*Figure 2F*). The immunohistochemistry findings also showed that *SEZ6L2* expression was significantly elevated in THCA tissues compared to that in normal tissues (*Figure 2G,2H*). The *SEZ6L2* expression in tumor tissues was considerably elevated compared to that in normal tissues in these 14 tumors from the HPA data, including THCA (*Figure 2I*).

Relationship between SEZ6L2 expression and clinical characteristics

Table 2 summarizes the association between *SEZ6L2* expression and clinical characteristics in THCA patients. Elevated expression of *SEZ6L2* was substantially associated with N classification (P=0.026), T classification (P=0.046) and clinical stage (P<0.001), as illustrated in *Figure 3*. With the aid of logistic regression, a univariate analysis illustrated that the *SEZ6L2* expression was a categorical dependent variable related to the dismal prognosis in terms of clinical outcomes (*Table 3*). Elevated expression of *SEZ6L2* was considerably correlated with T classification [T3 and T4 *vs.* T1 and T2 OR =1.441; 95% confidence interval (CI) =1.008–2.065; P=0.046), N classification (N1 *vs.* NO OR =1.521; 95% CI =1.053–2.200; P=0.026), and pathologic stage (stage III and IV *vs.* stage I and II OR =1.881; 95% CI =1.296–2.745; P<0.001]. According to the clinical features, multivariate and univariate analysis were finished. Univariate analysis illustrated that T stage, SEZ6L2 and pathologic stage were related to the overall survival (OS). And multivariate analysis stated that SEZ6L2 is the independent risk for it (P<0.05) (*Table 4*).

SEZ6L2 overexpression is an independent risk factor for PFI

Liu *et al.* summarised the recommendations for endpoint use for each cancer type. In the case of less severe tumor types such as THCA, the progression-free interval (PFI) and the disease-free interval (DFI) were suggested as measures of tumor response (18). As illustrated in *Figure 4*, a Kaplan-Meier survival analysis found that the elevated level of *SEZ6L2* expression was correlated with a dismal prognosis. Elevated *SEZ6L2* expression was demonstrated to have a strong link to a grim prognosis in clinical stage III/IV (P=0.023), N1 (P≤0.001), and T2/T3 (P=0.027) according to subgroup analysis based on distinct clinical characteristics. PFI was shown to be substantially correlated with increased *SEZ6L2* expression, according to a univariate Cox analysis [hazard ratio (HR) =1.34; 95% CI =1.16–1.54; P=4.86e-05]. The forest plot of the multivariate Cox regression analysis verified that *SEZ6L2* gene expression was an independent risk factor that affected the PFI in THCA patients (P<0.05), as demonstrated in *Figure 5*.

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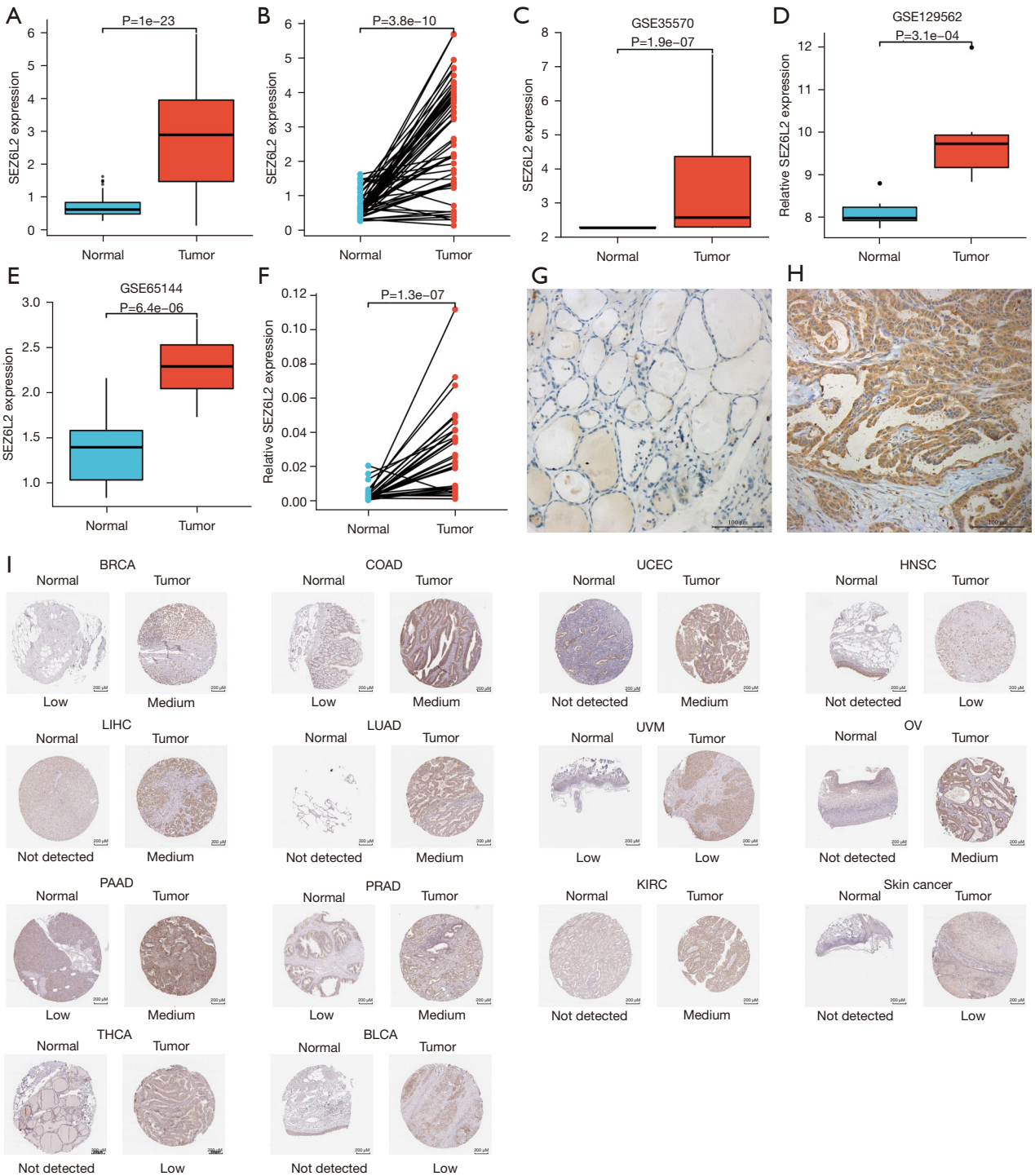

Figure 2 *SEZ6L2* expression in PTC tissues. (A) The expression of *SEZ6L2* in tumor and normal tissues in TCGA. (B) The expression of *SEZ6L2* in paired tissues in TCGA. The expression of *SEZ6L2* in normal and tumor tissue in GEO (C-E). The expression of *SEZ6L2* in tumor and normal tissues by PCR (F). The expression of *SEZ6L2* in normal tissues (G) and THCA tissues (H) by immunohistochemical (scale bar =100 μm). The expression of *SEZ6L2* in normal and tumor tissues in the HPA database (method: SEZ6L2 was combined with antibody, diaminobenzidine showed the color; scale bar =200 μm) (I). PTC, papillary thyroid carcinoma; GEO, Gene Expression Omnibus; PCR, polymerase chain reaction; THCA, thyroid carcinoma; HPA, Human Protein Atls.

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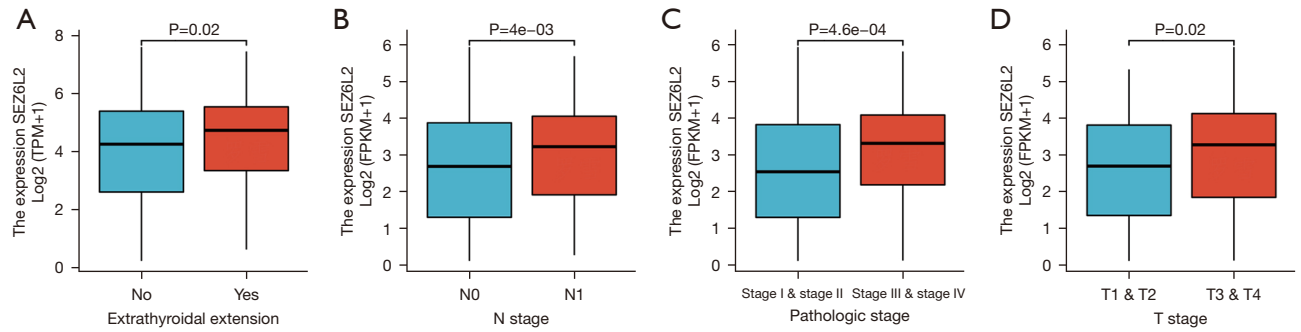

Figure 3 Box plot demonstrating the expression of *SEZ6L2* in thyroid cancer patients with various clinical features (A-D).

Table 3 The relationship between *SEZ6L2* expression and clinical features analyzed using a logistic regression model

Characteristics	Total (n)	Odds ratio (OR)	P
T stage (T3 & T4 *vs.* T1 & T2)	508	1.441 (1.008–2.065)	0.046
N stage (N1 *vs.* N0)	460	1.521 (1.053–2.200)	0.026
M stage (M1 *vs.* M0)	295	0.460 (0.096–1.778)	0.278
Pathologic stage (Stage III & Stage IV *vs.* Stage I & Stage II)	508	1.881 (1.296–2.745)	<0.001
Age (>45 *vs.* ≤45)	510	1.189 (0.840–1.685)	0.329
Gender (male *vs.* female)	510	1.294 (0.876–1.917)	0.197
Extrathyroidal extension (yes *vs.* no)	492	1.462 (0.997–2.150)	0.052

Table 4 Multivariate and univariate Cox regression analyses of clinical features related to OS

Characteristics	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
T stage (T3 & T4 *vs.* T1 & T2)	2.530 (1.410–4.540)	0.001*	1.717 (0.869–3.392)	0.119
N stage (N1 *vs.* N0)	1.578 (0.884–2.815)	0.122	1.152 (0.630–2.103)	0.644
SEZ6L2 (high *vs.* low)	1.034 (1.011–1.058)	0.003*	1.025 (1.002–1.049)	0.003
Pathologic stage (stage III & stage IV *vs.* stage I & stage II)	2.694 (1.522–4.771)	<0.001*	1.727 (0.857–3.479)	0.126
Gender (female *vs.* male)	1.361 (0.746–2.482)	0.313	1.143(0.622–2.101)	0.665
Age (>45 *vs.* ≤45)	1.784 (0.889–3.582)	0.103	1.145 (0.532–2.463)	0.727

*, P<0.05. OS, overall survival.

Diagnostic significance of SEZ6L2 expression in THCA

A ROC curve was used to examine the diagnostic significance of *SEZ6L2* expression in patients with THCA. As illustrated in *Figure 6A*, the AUC was 0.902, indicating that the *SEZ6L2* had an excellent diagnostic significance. Based on the subgroup analysis, the AUC values for the diagnostic significance of *SEZ6L2* gene expression in various clinical characteristics for PTC were as follows: 0.936 for M0, 0.891 for stage I/II, 0.924 for stage III/IV,

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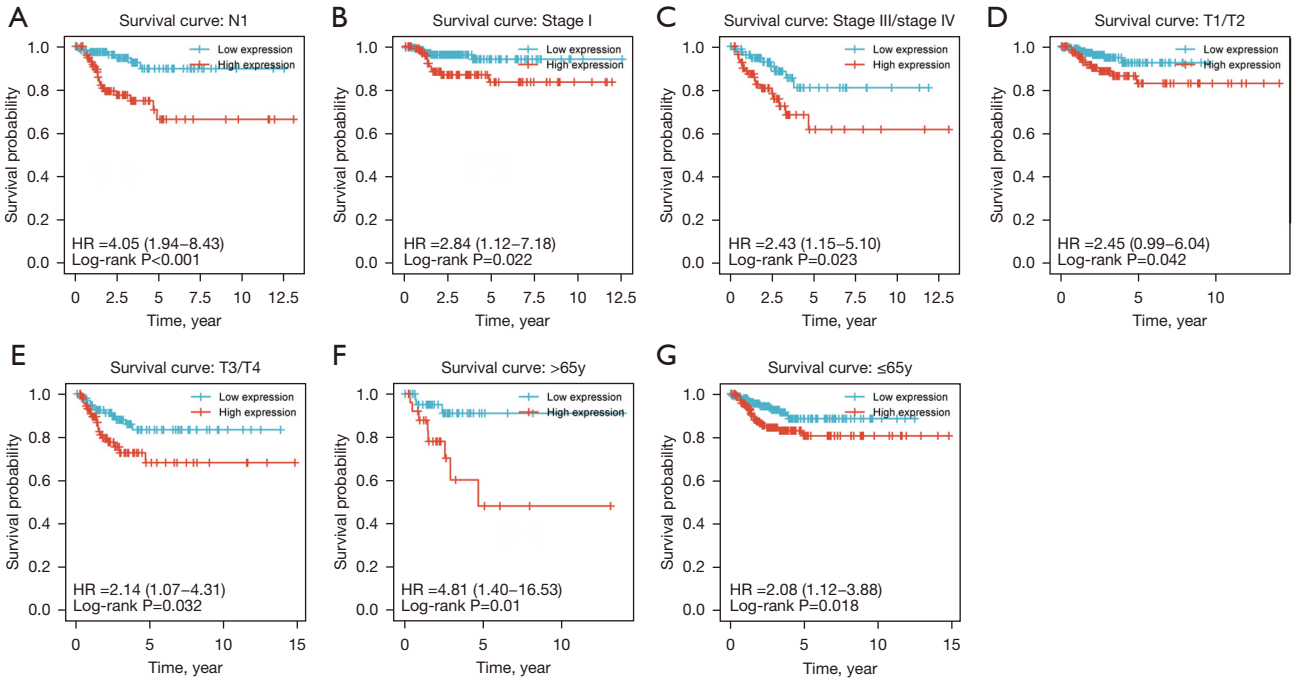

Figure 4 Kaplan-Meier curve for OS in THCA. (A-G) Subgroup analysis for N1, stage I, stage III/stage IV, T1/T2, T3/T4, >65 years, and ≤65 years. OS, overall survival; THCA, thyroid carcinoma.

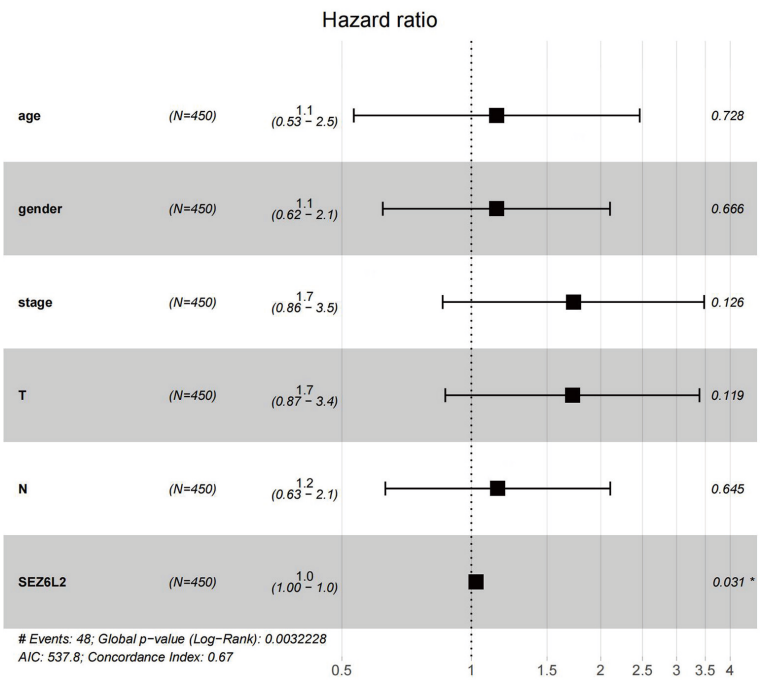

Figure 5 The forest plot illustrating the results of the multivariate Cox regression analysis in THCA. *, P<0.05. THCA, thyroid carcinoma.

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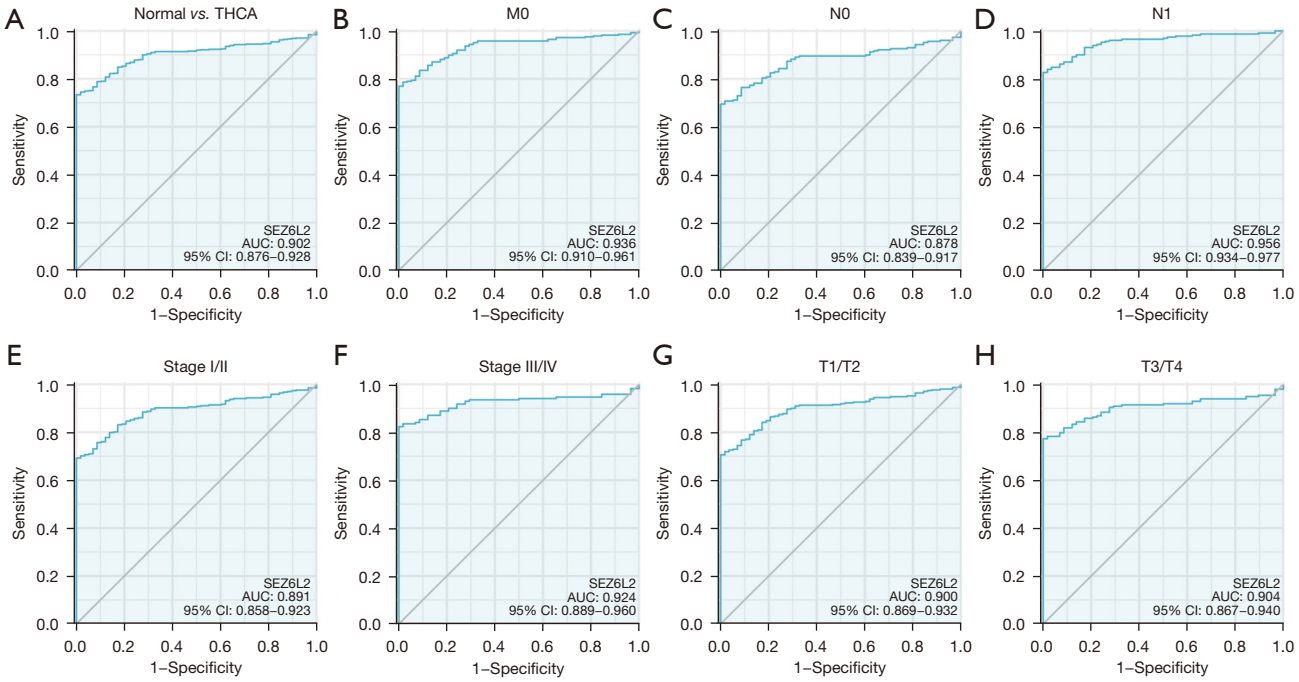

Figure 6 Diagnostic value of *SEZ6L2* expression in THCA. (A) ROC curve for *SEZ6L2* in normal thyroid tissue and THCA. (B-H) Subgroup analysis for M0, N0, N1, stage I/II, stage III/IV, T1/T2, T3/T4. THCA, thyroid carcinoma; ROC, receiver operating characteristic.

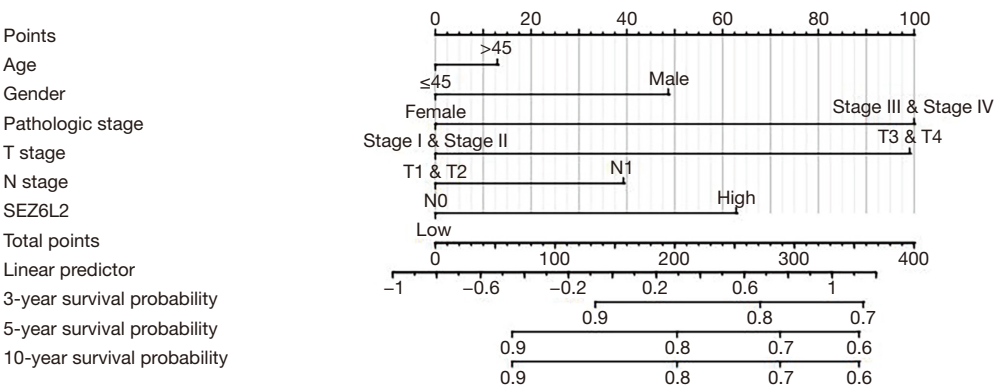

Figure 7 Nomogram anticipating the 3-, 5- and 10-year OS of patients. To estimate the risk, the status of each SEZ6L2 expression value and the clinical feature was determined by sketching a straight line up towards the point axis and looking at the points generated by individual features. This was repeated until the final scores for all variables have been determined. We added up all of the points and plotted the resulting point on the Total Points axis. Subsequently, by drawing a straight line downwards down the risk axis, the 3-, 5-, and 10-year associated survival rates were yielded.

0.900 for T1/T2, and 0.904 for T3/T4 (*Figure 6B-6H*). As a result, a nomogram was developed to anticipate the 3-, 5-, and 10-year survival rates of patients by integrating the level of *SEZ6L2* expression with clinical factors (*Figure 7*).

Relationship between SEZ6L2 expression and different immune cells

We used CIBERSORT to assess the association between the levels of *SEZ6L2* expression and the number of various

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Table 5 Spearman and Pearson correlation analyses between *SEZ6L2* and immune cells

Molecule	Cell	Pearson	P(Pearson)	Spearman	P(Spearman)
SEZ6L2	aDC	0.295	<0.001	0.303	<0.001
SEZ6L2	B cells	0.097	0.028	0.124	0.005
SEZ6L2	CD8 T cells	0.023	0.604	0.037	0.402
SEZ6L2	Cytotoxic cells	0.069	0.121	0.086	0.053
SEZ6L2	DC	0.299	<0.001	0.312	<0.001
SEZ6L2	Eosinophils	0.193	<0.001	0.182	<0.001
SEZ6L2	iDC	0.309	<0.001	0.301	<0.001
SEZ6L2	Macrophages	0.347	<0.001	0.344	<0.001
SEZ6L2	Mast cells	0.287	<0.001	0.274	<0.001
SEZ6L2	Neutrophils	0.305	<0.001	0.308	<0.001
SEZ6L2	NK CD56bright cells	0.090	0.042	0.083	0.060
SEZ6L2	NK CD56dim cells	0.053	0.231	0.059	0.184
SEZ6L2	NK cells	–0.041	0.352	–0.062	0.162
SEZ6L2	pDC	–0.017	0.704	–0.027	0.546
SEZ6L2	T cells	0.133	0.003	0.161	<0.001
SEZ6L2	T helper cells	0.049	0.269	0.093	0.035
SEZ6L2	Tcm	0.046	0.301	0.050	0.257
SEZ6L2	Tem	0.195	<0.001	0.173	<0.001
SEZ6L2	TFH	0.151	<0.001	0.190	<0.001
SEZ6L2	Tgd	–0.089	0.044	–0.072	0.106
SEZ6L2	Th1 cells	0.272	<0.001	0.265	<0.001
SEZ6L2	Th17 cells	–0.010	0.823	–0.064	0.146
SEZ6L2	Th2 cells	0.254	<0.001	0.244	<0.001
SEZ6L2	TReg	0.353	<0.001	0.341	<0.001

immune cells in PTC tissue in order to further investigate the correlation between the levels of *SEZ6L2* expression and the immune response. The findings showed that *SEZ6L2* overexpression was associated with a greater proportion of macrophages, treg, DC, neutrophils, aDC, iDC, Mast cells, Th1 cells, Th2 cells, TFH, eosinophils and Tem (P<0.05), while *SEZ6L2* overexpression was associated with a decrease in the proportion of NK cells, Th17 cells, and Tgd. To examine if the expression of *SEZ6L2* was associated with the immune cell, Spearman and Pearson analyses of association were performed. As illustrated in *Table 5*, the expression of *SEZ6L2* was associated with aDC, neutrophils, DC, Mast cells, iDC, macrophages, T cells, B cells, Tem, Tfh, Th1 cells, Th2 cells, eosinophils, and TReg [P (Spearman, Pearson) <0.05]. According to the findings, *SEZ6L2* expression was strongly associated with innate immune cells (*Figure 8*).

Discussion

The Sez6 family comprises three members, Sez6, Sez6L, and *SEZ6L2*, all of which have been found to have an effect on synapse numbers and dendritic morphology. These genes have also been correlated with a variety of neurological and mental diseases (19). Members of the Sez6 family have been regarded as indicators of dismal prognosis

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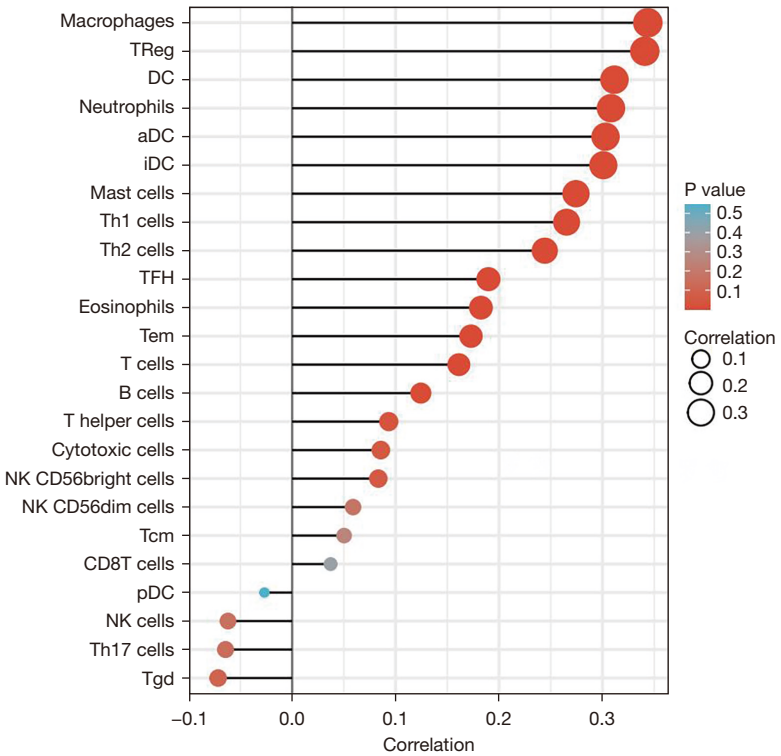

Figure 8 The association between *SEZ6L2* and immune cells in THCA. THCA, thyroid carcinoma.

in cancers that are not located in the nervous system (20-23). Wang *et al.* demonstrated that HCC samples had a greater level of the *SEZ6L2* protein expression, which was associated with tumor size, tumor-node-metastasis (TNM) stages, and tumor number. Moreover, overexpression of *SEZ6L2* was strongly correlated with the dismal OS and DFS in patients with HCC (17). An *et al.* illustrated that *SEZ6L2* expression was substantially elevated in the tumor tissues of CRC patients as opposed to adjoining normal tissues. They also found that SEZEL2 performs an integral function in the onset, progression, and malignant behavior of tumors (16). Nevertheless, the *SEZ6L2* gene and the relationship between the expression levels of the *SEZ6L2* gene and therapeutic value in THCA patients remain poorly understood.

As far as we know, ours is the first research report that assesses the association between the expression of *SEZ6L2* and clinical parameters in THCA patients. In our research, we searched for the *SEZ6L2* expressions among various forms of cancer data present on TCGA and evaluated these data using the TIMER2 method. The results illustrated that a large number of cancers had a higher expression of *SEZ6L2* compared to the control tissues. Moreover, the immunohistochemical score (IHC score) of *SEZ6L2* in cancer tissue was considerably elevated compared to that in normal tissues of HPA data, including THCA. *SEZ6L2* had a high predictive significance in the context of pan-cancer.

Subsequently, we searched for RNA sequencing data in the TCGA, and the findings indicated that *SEZ6L2* was elevated in THCA tissue. In addition, we investigated gene expression data from three GEO cohorts, and all of the findings corroborated the conclusions stated above [GSE129562 (P=3.1e-04), GSE65144 (P=6.4e-06), and GSE35570 (P=1.9e-07)] (*Figure 2C-2E*). In order to test the results, we conducted 3 GEO analyses, qRT-PCR of tissues, and immunohistochemical staining, which indicated that *SEZ6L2* expression was substantially elevated in THCA tissues compared to the normal tissues. Subsequently, we summarized the association between *SEZ6L2* expression and the clinical features of THCA patients by logistic analysis. The elevated expression of *SEZ6L2* was correlated with clinical, T, and N stages. The Kaplan-Meier survival analysis illustrated that the increased expression of *SEZ6L2* was correlated with a dismal prognosis. Moreover, the elevated expression of *SEZ6L2* was an independent risk factor that affected PFI. Consequently, we used the TIMER

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algorithms to examine the immune cell infiltrations. We found that the expression of *SEZ6L2* was correlated with tumor-infiltrating immune cells and was positively correlated with the immune infiltration of macrophages, Treg, DC, neutrophils, aDC, iDC, Mast cells, Th1 cells, Th2 cells, TFH, eosinophils, and Tem.

Conclusions

We demonstrated that *SEZ6L2* was upregulated in patients with THCA and that increased expression of *SEZ6L2* was correlated with clinical progression and was regarded as an independent risk factor for PFI. In patients with THCA, the expression of *SEZ6L2* could be a significant prognostic factor. However, our research has limitations, since we did not investigate the molecular processes and the relative function of *SEZ6L2* in THCA. In view of this, we plan to perform further in-depth mechanistic and functional research on cell lines and mice in the future. Taken together, our data showed that *SEZ6L2* is overexpressed in THCA tissue and that *SEZ6L2* may be regarded as a major independent prognostic indicator as well as a promising treatment target for THCA.

Acknowledgments

Funding: The study was supported by the National Natural Science Foundation of China (No. 81860478).

Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://gs.amegroups.com/article/view/10.21037/gs-22-37/rc

Data Sharing Statement: Available at https://gs.amegroups.com/article/view/10.21037/gs-22-37/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://gs.amegroups.com/article/view/10.21037/gs-22-37/coif). The 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. All procedures performed in this study were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Ethics Committee of Human Trials, Guizhou Medical University [No. 245(2021)]_and informed consent was taken from all the patients.

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(English Language Editor: C. Mullens)

Cite this article as: Luo X, Chen X, Chen S, Gao Q, Yang H, Zhao D. High expression of *SEZ6L2* as an independent prognostic Indicator in thyroid carcinoma. Gland Surg 2022;11(2):412-425. doi: 10.21037/gs-22-37

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