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ORIGINAL RESEARCH ARTICLE

Underexpression of SCN7A is associated with poor prognosis in lung adenocarcinoma

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Abstract

Lung cancer is one of the most common malignancies and the leading cause of cancer-related deaths worldwide. Elucidating the mechanism behind the development of lung cancer at a molecular level could reveal new biomarkers and clinical therapeutic targets. A growing body of evidence has shown an association between ion channel-related genes and the progression of various diseases, including cancer. However, the association is not well-understood. In this study, we identified ion channel-related genes differentially expressed between cancer and normal lung tissue cells. Data were extracted from GSE31552, GSE33532, and GSE103512 lung adenocarcinoma (LUAD) datasets, and the prognostic value of the differentially expressed genes between LUAD and normal lung tissue was evaluated using data in The Cancer Genome Atlas. Only sodium voltage-gated channel alpha subunit 7 (SCN7A) was found to be significantly correlated to prognosis. The expression of SCN7A in LUAD was assessed further by immunological analysis. We also constructed a competing endogenous RNA network of *SCN7A*. Further analyses revealed that the underexpression of SCN7A predicted a poor prognosis in LUAD, with a strong correlation between SCN7A expression and immune cell infiltration as well as immune checkpoint expression. We found that SCN7A is potentially regulated via the OTUD6B-AS1-miR-21-5p-SCN7A axis in LUAD cells and proved that SCN7A inhibits the proliferation and migration of lung cancer cells *in vitro*. Taken together, SCN7A, as an independent prognostic factor, is a promising diagnostic biomarker for LUAD, and the OTUD6B-AS1-miR-21-5p-SCN7A axis is a potential regulatory network of SCN7A expression.

Keywords: Lung adenocarcinoma; Gene Expression Omnibus; The Cancer Genome Atlas; Immune infiltration; Competing endogenous RNA; Prognosis

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1. Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide, accounting for nearly 2 million reported cases and 1.76 million deaths in 2020[1]. Non-small cell lung cancer accounts for 80% of all lung malignancies, and the overall 5-year survival rate of lung adenocarcinoma (LUAD) is 5%[2,3]. Despite the numerous treatments available, the prognosis of LUAD remains unsatisfactory. Due to the fact that early-stage lung cancer is usually asymptomatic, the majority of patients with lung cancer are diagnosed at a more

advanced stage[4]. Therefore, we need to understand the molecular mechanism of how lung cancer develops and progresses to identify accurate markers for early diagnosis and novel markers for treating the disease.

Ion channels are involved in many important biological processes (BPs). The association between ion channels and cancer development has been reported in many studies[5,6]. LUAD cells display specific changes in the expression of ion channel-related genes. Since novel diagnostic markers are urgently needed, these genes are promising clinical biomarkers for LUAD diagnosis and prognosis prediction. In addition, the therapeutic potential of ion channel-related genes for solid epithelial tumors and breast cancer have been reported[7-9]. However, we have not identified any ion channel-related gene that could be targeted for LUAD treatment.

In this study, differentially expressed genes (DEGs) related to ion channel between LUAD and normal tissues were identified from LUAD datasets (GSE31552, GSE33532, and GSE103512) in the Gene Expression Omnibus (GEO) database[10-12]. SCN7A was identified to be significantly correlated to prognosis. The expression profile, mutational landscape, immunological role, and prognostic value of SCN7A for LUAD were all analyzed. A competing endogenous RNA (ceRNA) network for SCN7A was also constructed. We found that SCN7A influences the survival of LUAD patients, suggesting that SCN7A may be a protective factor in LUAD and can be utilized to develop new therapeutic approaches.

2. Materials and methods

2.1. Data source and analysis

The GEO database (https://www.ncbi.nlm.nih.gov/geo/) was used to download GSE31552, GSE33532, and GSE103512 datasets for the expression of LUAD genes[13], which were then normalized using the quantiles function. DEGs were identified by "limma" R package based on $P < 0.05$ and $|\log FC| > 1$. The "ggplot2" R package was used to construct a volcano plot and heatmap. The genes related to ion channels were identified from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (hsa04040), and The Cancer Genome Atlas (TCGA) database (https://tcga-data.nci.nih.gov/tcga/) was used for downloading the TCGA pan-cancer RNA-seq data.

2.2. Gene ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analyses

The Metascape platform integrates gene annotation, enrichment analysis, and interactome analysis by leveraging 40 independent gene expression databases[14]. The BP, cellular component (CC), molecular function (MF), and DEGs-regulated pathways were identified from the Metascape database.

2.3. Expression and prognostic analysis

Wilcox test was performed to analyze the prognostic value of SCN7A for LUAD. Based on the plotted Kaplan-Meier (KM) survival curves, "survival" and "survminer" packages in the R software were used to conduct the survival analysis. The evaluation of the relationship between SCN7A expression and LUAD prognosis was by univariate Cox regression besides utilizing a forest plot to display it graphically.

2.4. Genes related to overall survival in lung adenocarcinoma

The genes significantly related to overall survival (OS) in LUAD were identified by univariate and multivariate analyses. Apart from SCN7A, the other parameters analyzed for inclusion in a predictive nomogram included calcium voltage-gated channel auxiliary subunit alpha2delta 2 (CACNA2D2) and glutamate ionotropic receptor AMPA type subunit 1 (GRIA1), age, gender, and pathological tumor-node-metastasis (pTNM) stage. A forest plot was used to display the effect estimates of the aforementioned parameters on LUAD prognosis.

2.5. Messenger RNA transcription and protein level expression analysis

A Sankey diagram was constructed to visually demonstrate the influence of age, gender, pTNM stage, and *SCN7A* mRNA expression on LUAD prognosis. A map of protein expression in 32 human tissues was taken from the Human Protein Atlas (HPA) database (http://www.proteinatlas.org/)[15]. SCN7A protein expression levels between lung cancer and normal tissues were analyzed in the HPA database. SCN7A expression in lung cancer tissues (167201_B_1_1, 167201_B_1_2, and 167201_B_1_3) and normal tissues (167203_A_1_4, 167203_A_2_4, and 167203_A_3_4) was analyzed.

2.6. Mutations in lung adenocarcinoma tissue cells

cBioPortal (https://www.cbioportal.org/) contains five published datasets and 15 provisional TCGA datasets and provides data for mutational analysis[16,17].

2.7. Immunological analysis

The correlation between immune score and LUAD prognosis was analyzed. TIMER2.0 (http://timer.cistrome.org/) contains data on immune cell infiltration to cancer tissues[18]. The impact of somatic copy number alteration (sCNA) of *SCN7A* on immune infiltration was analyzed using TIMER2.0 data.

2.8. Upstream non-coding RNA analysis

miRGator v3.0 (http://mirgator.kobic.re.kr/miRTargetNExpression.html) was used to predict microRNAs (miRNAs) targeting and binding *SCN7A*[19]. starBase (http://starBase.sysu.edu.cn/), a widely used database for ncRNA-related analysis[20], was used to predict long non-coding RNAs (lncRNAs) binding *SCN7A* and related LUAD prognosis.

2.9. Pathway correlation analysis

The "GSVA" R package was used for pathway correlation assessment, and ssgsea was selected as a parameter in gene set variation analysis (GSVA). Spearman's correlation was performed to evaluate the association between the selected gene expression and LUAD prognosis.

2.10. Quantitative polymerase chain reaction (qPCR)

Total RNA was extracted using Trizol. SynScript® III RT SuperMix for qPCR (Tsingke, TSK314S) was used for reverse transcription. 2×TSINGKE® Master SYBR Green I qPCR Mix-UDG (Tsingke, TSE204) was used for qPCR. The primers used were as follows: GAPDHF, CTGGGCTACACTGAGCACC; GAPDHR, AAGTGGTCGTTGAGGGCAATG; SCN7AF, GCTTCGTAGCAAGTCCTCCA; SCN7AR, GGGTCCACATCTTCCAAGGG.

2.11. Cell counting Kit 8 (CCK8) assay and wound healing assay

After the cell confluency reached 90% in a 6-well plate, it was changed to 2 mL serum-free medium for overnight culture to deplete the residual serum, and scratches were made the following morning. A549 cell was scrapped in a straight line at 0 h, and images at 0 h, and 48 h were acquired for analysis. CCK8 assay was performed using CCK8 (YZ-CK04) at day 1, day 2, day 3, and day 4, measuring the absorbance value at 450 nm.

2.12. Statistical analysis

Rstudio software (R 4.0.3) was used for data analysis. $P < 0.05$ or log-rank $P < 0.05$ was considered statistically significant.

3. Results

3.1. Differentially expressed genes between lung adenocarcinoma and normal tissues

GSE31552, GSE33532, and GSE103512 contained data for 35, 40, and 27 LUAD tissues and 39, 10, and 7 normal tissues, respectively. We identified 267 DEGs between LUAD and normal tissues across the three datasets, of which there were 67 overexpressed and 200 suppressed genes (Figure 1A and B). Relying on Metascape data, GO and KEGG enrichment analyses of the DEGs were performed. In GO enrichment analysis, the main processes were the extracellular matrix, circulatory system process, and response to glucocorticoid (Figure 1C), whereas the main pathways in KEGG enrichment analysis were complement and coagulation cascades and extracellular matrix (ECM)-receptor interaction (Figure 1D).

3.2. Prognostic value of key differentially expressed genes

Ion channel-related DEGs, including *GRIA1*, chloride intracellular channel 5 (*CLIC5*), potassium sodium-activated channel subfamily T member 2 (*KCNT2*), *SCN7A*, and *CACNA2D2*, were identified (Figure 2A). The expression of these five candidate genes in LUAD was validated using the data in TCGA. The findings revealed that *CLIC5* was overexpressed in tumors but underexpressed in adjacent normal tissues, whereas *GRIA1*, *KCNT2*, *SCN7A*, and *CACNA2D2* were underexpressed in tumor tissues but overexpressed in normal tissues (Figure 2B). Further analyses revealed that the overexpression of *GRIA1*, *SCN7A*, and *CACNA2D2* was associated with favorable prognosis (Figure 2C–E), whereas the expression of *CLIC5* and *KCNT2* had no significant effect on LUAD prognosis.

3.3. *SCN7A* as an independent factor for LUAD prognosis

Univariate Cox regression analysis confirmed that *CACNA2D2* (HR = 0.86896, $P = 0.00028$), *GRIA1* (HR = 0.6111, $P = 0.00063$), and *SCN7A* (HR = 0.83379, $P = 0.00409$) had significant associations with the OS of LUAD patients (Figure 3A). Further multivariate regression analysis revealed *SCN7A* (HR = 0.82429, $P = 0.03508$) to be the only independent predictor of LUAD prognosis (Figure 3B). At the same time, we extracted variables with significant differences to construct a nomogram prognostic model to predict the 1-year, 3-year, and 5-year overall survival of LUAD patients (Figure 3C,D). Sankey diagram also showed that the overexpression of *SCN7A* in LUAD was associated with better LUAD prognosis (Figure 4A). These results showed that *SCN7A* is a protective factor for LUAD prognosis.

3.4. Messenger RNA transcription and expression of SCN7A protein in lung adenocarcinoma tissues

TCGA data analysis indicated that *SCN7A* mRNA transcription and the expression of corresponding proteins differed between gender and among different age groups. Interestingly, there was a smaller proportion of patients with high SCN7A expression in stages III and IV than in Stages I and II. Furthermore, compared with the low-expression group, the high-expression group had a lower

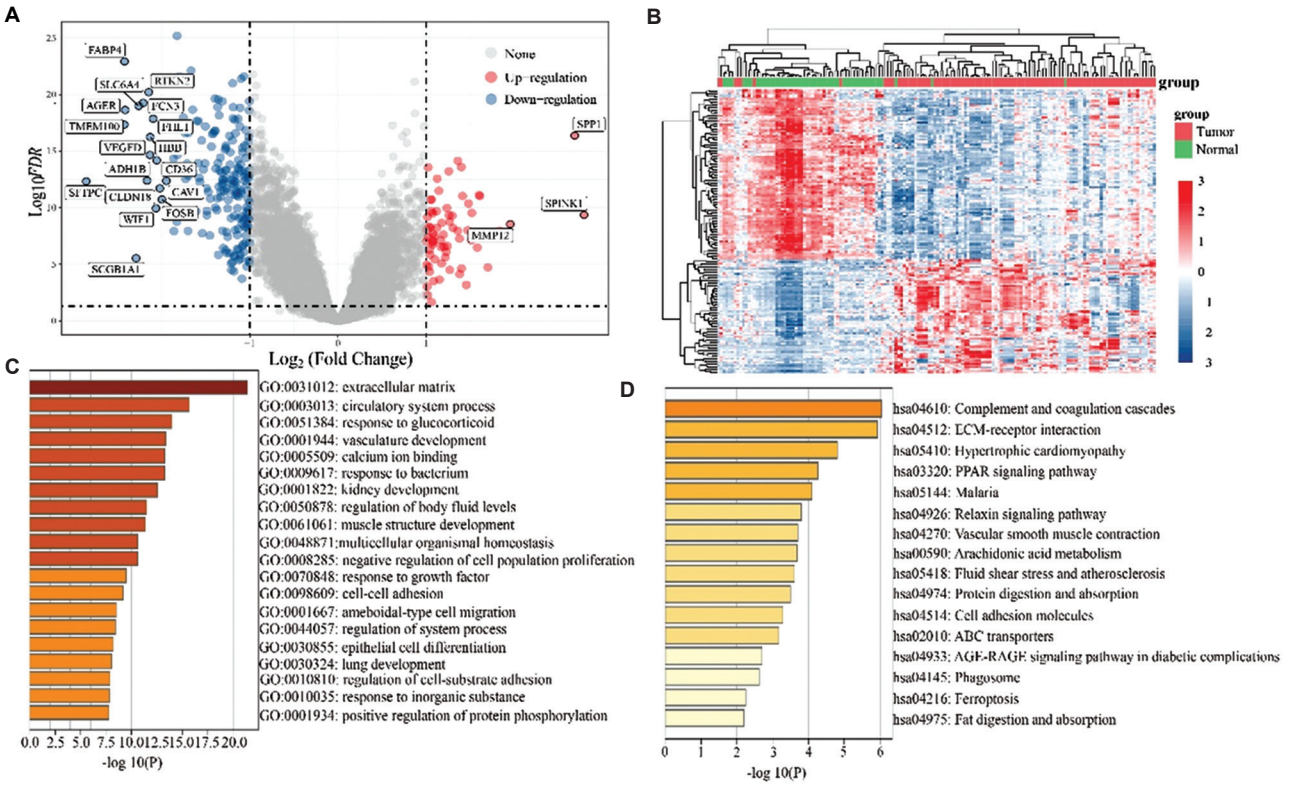

Figure 1. Identification of differentially expressed genes (DEGs) between lung adenocarcinoma and normal tissues from GEO datasets. (A–B) Volcano plot and heatmap of 267 DEGs. (C) Gene ontology enrichment analysis results inclusive of MF, BP, and CC. (D) Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis results.

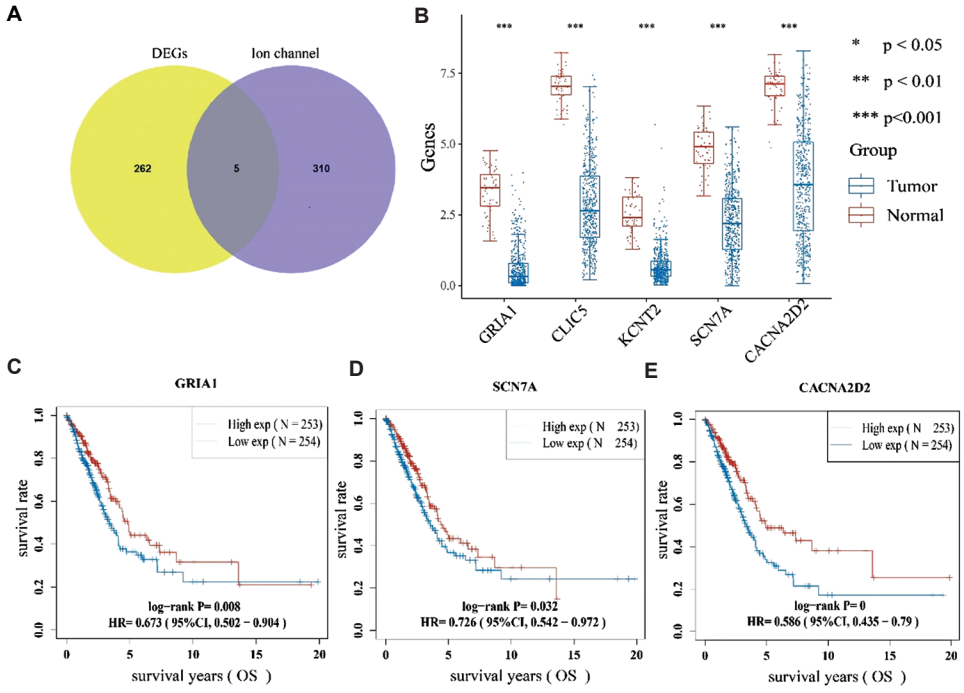

Figure 2. Expression and prognostic analysis of candidate genes. (A) Venn diagram showing five differentially expressed gene (DEGs) overlapping with ion channel-related genes (B) Expression analysis of five candidate genes using the lung adenocarcinoma (LUAD) data in The Cancer Genome Atlas (TCGA): *GRIA1* (*P* = 7.98e-34), *CLIC5* (*P* = 1.88e-35), *KCNT2* (*P* = 1.24e-32), *SCN7A* (*P* = 8.10e-31), and *CACNA2D2* (*P* = 2.03e-29). (C–E) Prognostic value of *GRIA1*, *SCN7A*, and *CACNA2D2* for LUAD using the LUAD data in TCGA.

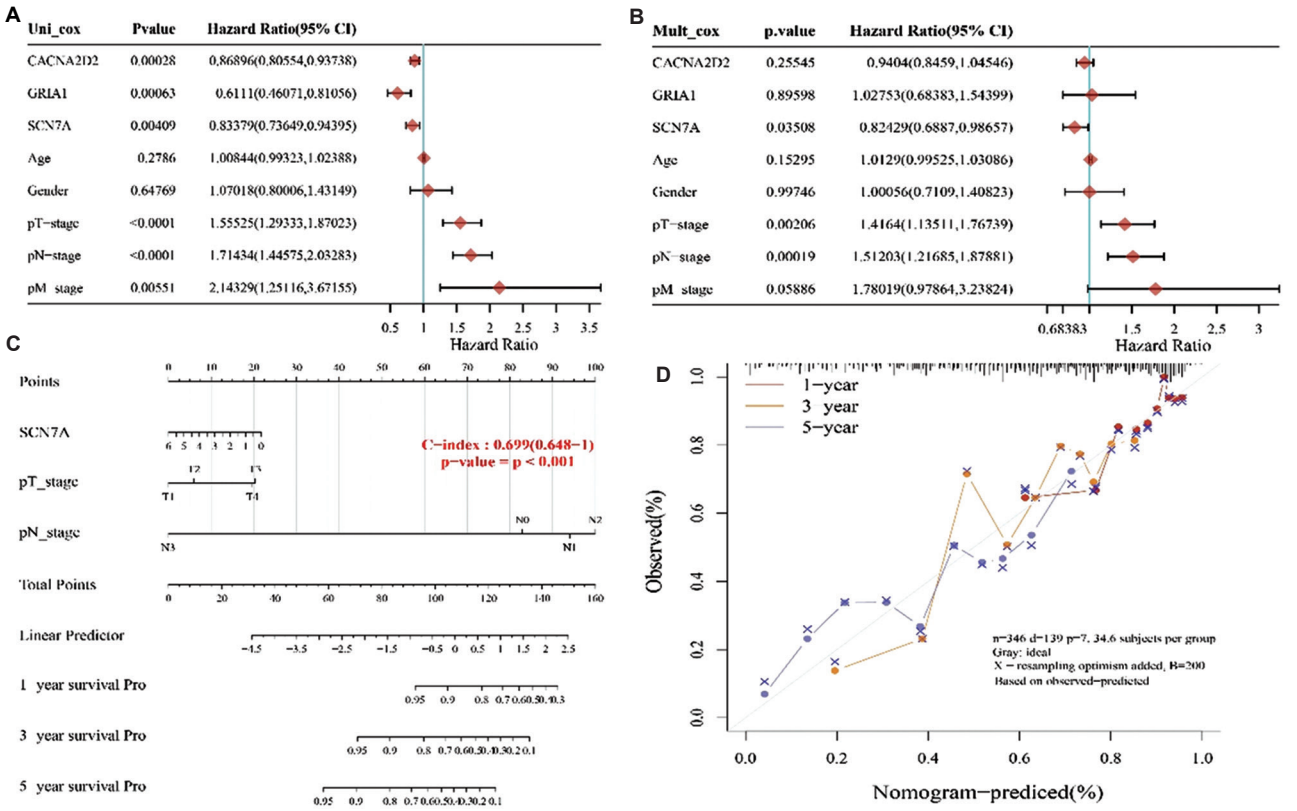

Figure 3. Predictive nomogram showing the prognosis prediction potential of *GRIA1*, *SCN7A*, and *CACNA2D2* for lung adenocarcinoma (LUAD). (A and B) Univariate and multivariate regression analyses of the relationship between *GRIA1*, *SCN7A*, and *CACNA2D2* expression and LUAD prognosis. (C and D) Nomogram of risk score and other clinical characteristics to predict the 1-year, 3-year, and 5-year overall survival (OS) of LUAD patients.

proportion of stage II–IV cancers and a higher proportion of survival (Figure 4A). These results link high SCN7A expression with better survival outcomes.

Immunohistochemistry (IHC) analysis confirmed that SCN7A was moderately expressed in normal tissues but was entirely absent in lung cancer tissues (Figure 4B). These results showed that mRNA and protein levels of SCN7A are lower in LUAD tissues than in normal tissues.

3.5. Mutation studies of SCN7A in lung adenocarcinoma

Since ion channels are involved in many cellular processes, mutations in related genes could cause a variety of diseases[21]. We screened for *SCN7A* mutations in LUAD genes based on the data in cBioPortal database. *SCN7A* mutations were detected in 6% of LUAD tissues, most of which were missense mutations. A total of 28 mutation sites and seven truncation mutations were identified in the genome of LUAD cells (Figure 5).

3.6. Immunological analysis of SCN7A in lung adenocarcinoma

The prognostic value of SCN7A for LUAD was evaluated. We first analyzed how SCN7A expression correlates to immune cell infiltration to the tumor site based on the TIMER score. The results revealed a strong positive association between SCN7A expression and the infiltration of six immune cell subtypes: B cells (r = 0.33, P = 8.1e-15) (Figure 6A), $CD4^+$ T cells (r = 0.34, P = 1.98e-15) (Figure 6B), $CD8^+$ T cells (r = 0.33, P = 2.05e-14) (Figure 6C), neutrophils (r = 0.21, P = 2.04e-06) (Figure 6D), macrophages (r = 0.42, P = 2.36e-23) (Figure 6E), and myeloid dendritic cell (r = 0.35, P = 2.99e-16) (Figure 6F). In addition, the infiltration of the aforementioned immune cells was associated with favorable LUAD prognosis.

We then analyzed the sCNA of *SCN7A* in pan-cancer using data in TIMER2.0 database (Figure 7A). We found that the sCNA of *SCN7A* affects the infiltration of various immune cells, including $CD4^+$ T cells, macrophages, neutrophils, and dendritic cells, in LUAD (Figure 7B).

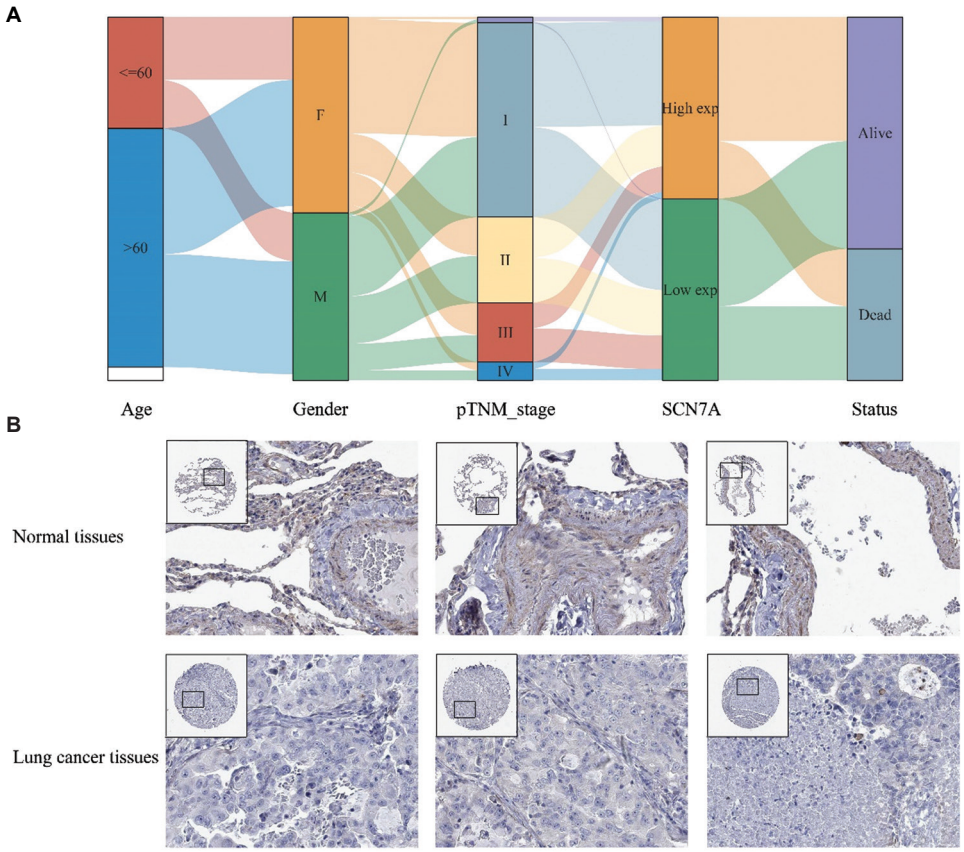

Figure 4. Messenger RNA (mRNA) transcription and expression of SCN7A protein in lung adenocarcinoma (LUAD). (A) Trend in the expression of *SNC7A* mRNA in LUAD among different stages, people of different age, and between gender; and the relationship between SNC7A expression and the overall survival (OS) of LUAD patients. (B) SCN7A protein level in lung cancer tissues and normal tissues from the Human Protein Atlas database.

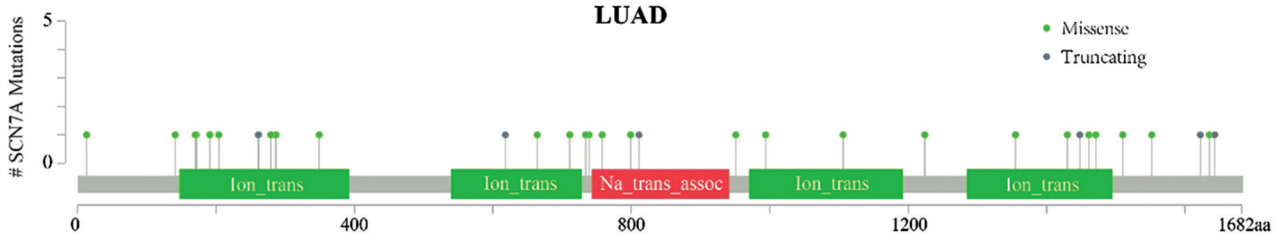

Figure 5. Somatic mutation landscape of *SCN7A* in lung adenocarcinoma (LUAD) cells.

To explore the correlation between SCN7A and immune checkpoint therapy in LUAD, we assessed the correlation between SCN7A expression and immune checkpoints. Cytotoxic T-lymphocyte associated protein 4 (CTLA4), hepatitis A virus cellular receptor 2 (HAVCR2), programmed cell death 1 ligand 2 (PD-L2), and T cell immunoreceptor with Ig and ITIM domains (TIGIT) expressions were positively correlated to SCN7A expression, whereas LAG3 expression showed a negative correlation (Figure 7C).

These results suggest a strong correlation between SCN7A expression and immune cell infiltration to LUAD tissues, consistent with the prognostic results. Thus, SCN7A is a potential biomarker for LUAD prognosis prediction.

3.7. Upstream non-coding RNAs of SCN7A

We searched through miRGator v3.0 database containing data for 25 miRNAs to identify the upstream regulatory mechanism of SCN7A in LUAD. Given how miRNAs regulate gene expression, miRNAs should be negatively correlated with SCN7A. Thus, we selected 15 miRNAs that were negatively correlated with SCN7A for further investigation. The expression of these miRNAs in LUAD

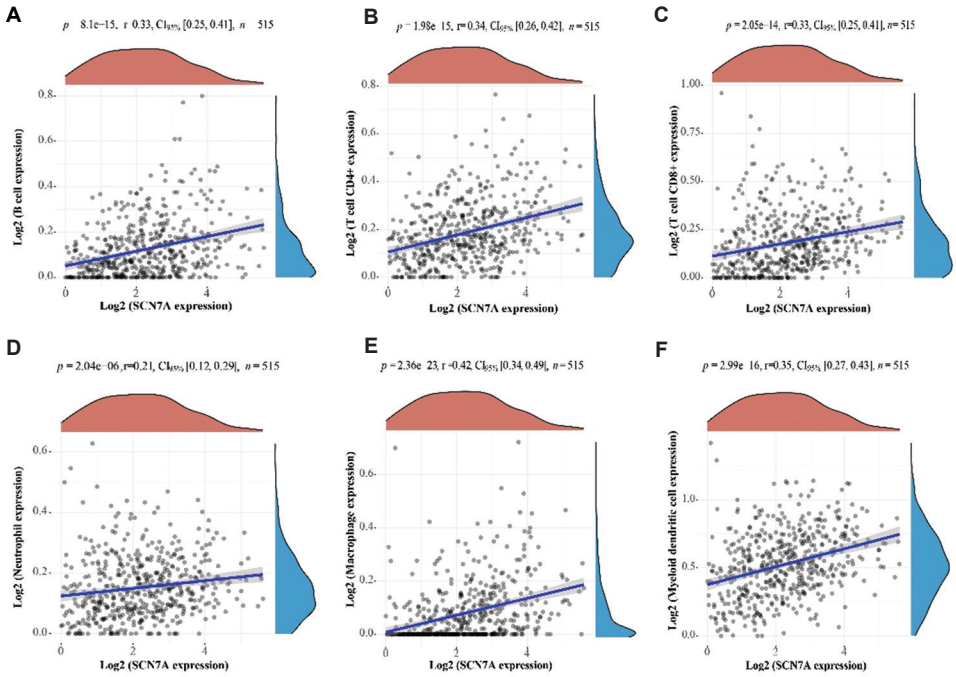

Figure 6. Correlation between SCN7A expression and infiltration of immune cells to the tumor site. (A) B cell. (B) $CD4^+$ T cell. (C) $CD8^+$ T cell. (D) Neutrophil. (E) Macrophage. (F) Myeloid dendritic cell.

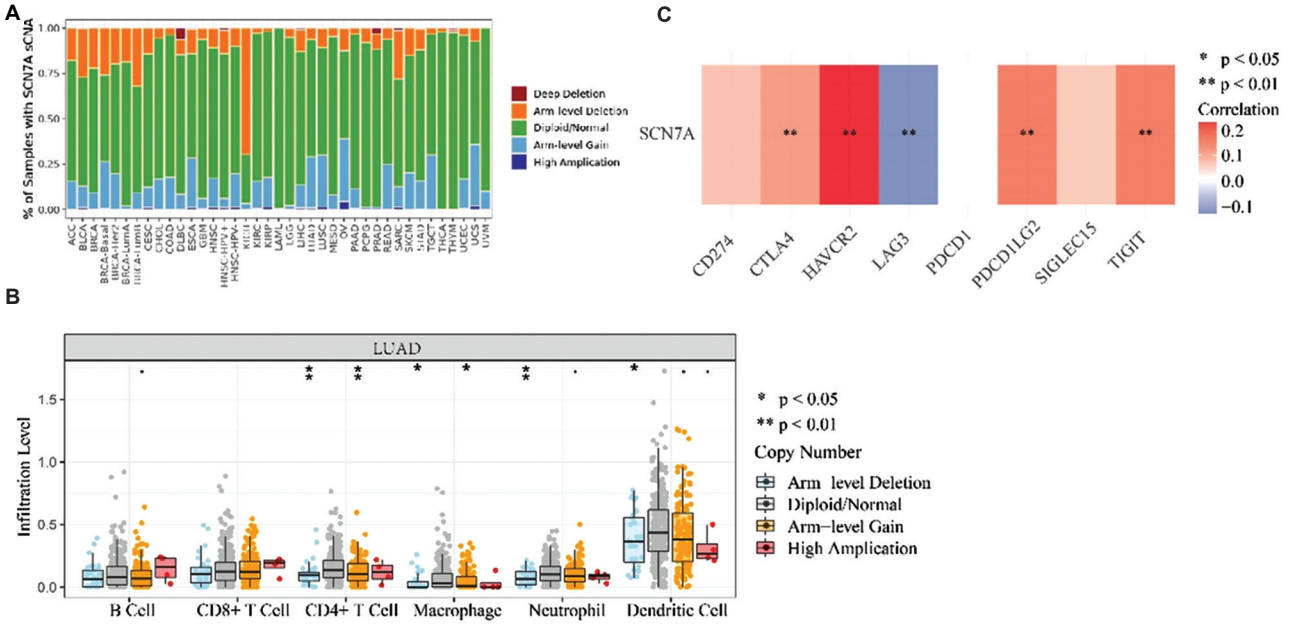

Figure 7. Immunological analysis of SCN7A in cancer. (A) Distribution of the somatic copy number alteration (sCNA) of SCN7A in pan-cancer. (B) Effect of SCN7A sCNA on the infiltration of immune cells to tumor site in lung adenocarcinoma (LUAD). (C) Correlation between the expression of SCN7A and immune checkpoints in LUAD.

was analyzed; 14 miRNAs were found to be overexpressed in LUAD (Figure 8A and Table 1). Further prognostic assessment indicated that high miR-21-5p levels in LUAD were associated with poorer prognosis (Figure 8B) but the other miRNAs had no significant impact on LUAD prognosis.

Through further searches in the starBase database, we identified 14 lncRNAs that bind to miR-21-5p. LncRNAs

Figure 8. Expression and prognostic value analysis of selected microRNAs (miRNAs) in lung adenocarcinoma (LUAD). (A) Expression analysis of 14 selected miRNAs in LUAD. (B) Prognostic value of miR-21-5p for LUAD.

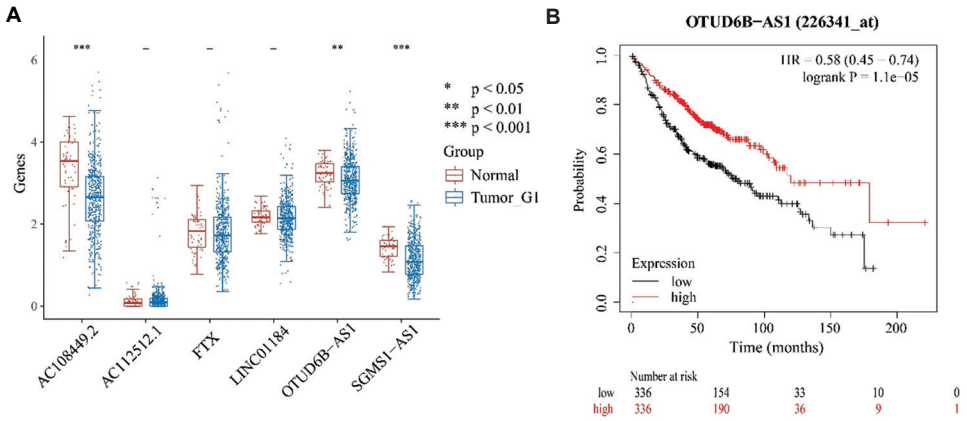

Figure 9. Expression analysis and prognostic potential of selected long non-coding RNAs (lncRNAs) for lung adenocarcinoma (LUAD). (A) Expression analysis of selected lncRNAs in LUAD. (B) Prognostic potential of OTUD6B-AS1 for LUAD.

also compete with miRNAs for binding mRNAs. We found that AC108449.2, AC112512.1, FTX, LINC01184, OTUD6B-AS1, and SGMS1-AS1 regulate the expression of SCN7A by binding to miRNA-21-5p. Further analyses showed that AC108449.2, OTUD6B-AS1, and SGMS1-AS1 were underexpressed in LUAD (Figure 9A). Prognostic analysis revealed an association between OTUD6B-AS1 overexpression and better LUAD prognosis (Figure 9B). Overall, SCN7A expression may be potentially regulated through the OTUD6B-AS1-miR-21-5p-SCN7A axis.

3.8. Pathways regulated by SCN7A in lung adenocarcinoma

We found that SCN7A closely participated in several cellular processes, including hypoxia (r = −0.4, P = 1.06e-21) (Figure 10B), proliferation of tumor cells (r = −0.66, P = 3.56e-66) (Figure 10C), expression of ECM (r = 0.61, P = 1.63e-54) (Figure 10E), angiogenesis (r = 0.29, P = 1.06e-11) (Figure 10F), apoptosis (r = 0.25, P = 1.24e-08) (Figure 10G), DNA repair (r = -0.65, P = 1.41e-62) (Figure 10H), and expression of G2M (r = -0.61, P = 4.25e-54) (Figure 10I). but not in tumor inflammation signature (r=-0.02, p=0.675) (Figure 10A) and EMT markers (r=-0.03, p=0.497) (Figure 10D).

3.9. SCN7A inhibits the proliferation and migration of lung cancer cells *in vitro*

To verify the role of SCN7A in lung cancer cells, SCN7A was overexpressed in A549 cells. We found that the expression of SCN7A increased by nearly 5 times compared with the control group (Figure 11A). We then evaluated the effect of SCN7A on the proliferation ability by CCK8 experiment and found that the overexpression of SCN7A significantly reduced the proliferation ability of A549 cells (Figure 11B). Through wound healing assay, we also found that the overexpression of SCN7A significantly reduced the migration ability of A549 cells (Figure 11C and D). Consistent with our analysis, these results strongly support the potential of SCN7A as a prognostic marker.

3.10. SCN7A expression in other cancer tissues

To investigate the role of SCN7A in other tumors, *SCN7A* gene expression in other cancer tissues was analyzed. Dysregulation of SCN7A was observed in 17 tumor tissues, including bladder cancer (BLCA), breast cancer (BRCA), cervical cancer (CESC), colon cancer (COAD), esophageal cancer (ESCA), head and neck cancer (HNSC), kidney chromophore (KICH), kidney clear cell carcinoma (KIRC), kidney papillary cell carcinoma (KIRP), liver cancer (LIHC), lung squamous cell carcinoma (LUSC), prostate cancer (PRAD), rectal cancer (READ), stomach cancer (STAD), thyroid cancer (THCA), and endometrioid cancer (UCEC) (Figure 12). Further analyses revealed that SCN7A could predict the prognosis of BLCA, LUSC, and ovarian cancer (OV), apart from LUAD (Figure 13). In addition, relying on the TIMER score, SCN7A expression was found to be positively correlated to immune cell infiltration in most cancers, including LUAD (Figure 14). These findings suggest the importance of SCN7A in cancer development and progression.

4. Discussion

At present, lung cancer is one of the most fatal malignancies in the world. Identifying prognostic markers and therapeutic targets for lung cancer are crucial to treating this cancer. Studies have shown that ion channels participate in the development and progression of tumors[22-25], underscoring the need to be aware of the molecular mechanisms behind these events.

The function of *SCN7A* differs from that of other related genes. Precisely, SCN7A does not act as a switch

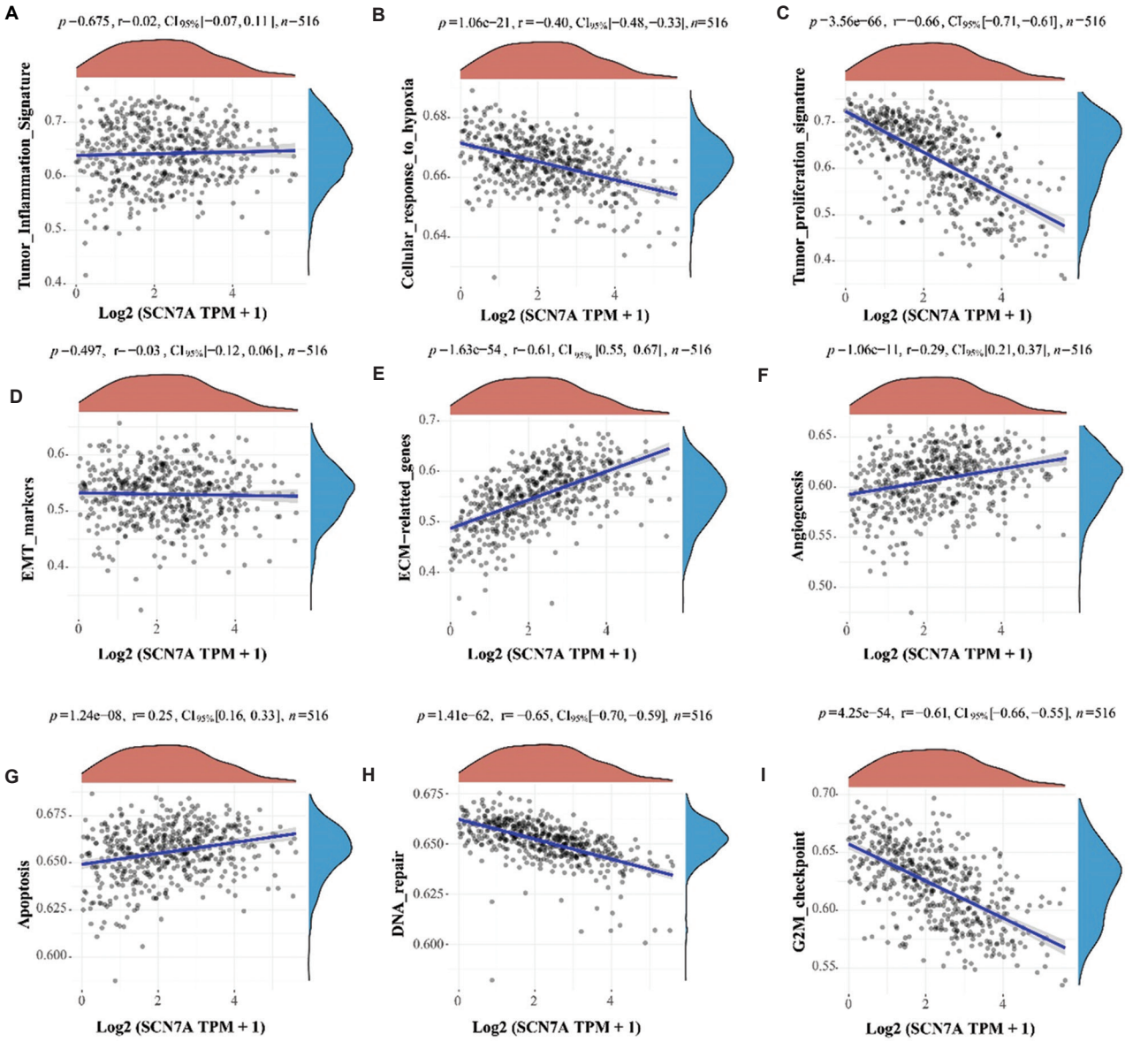

Figure 10. Pathways regulated by SCN7A in lung adenocarcinoma (LUAD) include (A) tumor inflammation, (B) cellular response to hypoxia, (C) tumor proliferation, (D) epithelial-mesenchymal transition (EMT) markers, (E) ECM-related genes, (F) angiogenesis, (G) apoptosis, (H) DNA repair, and (I) expression of G2M checkpoint.

for ion channels but regulates extracellular sodium ion concentration[26]. Current studies on SCN7A in tumors remain scanty. Consistent with the results of a previous study[24], we observed the downregulation of SCN7A protein in LUAD tissues, and this was associated with poor prognosis, implying that SCN7A could participate in LUAD development. Our results also showed that SCN7A has an inhibitory effect on the proliferation and migration of lung cancer cells.

Ion channels participate in inflammatory and immune responses in cancer cells[27,28]. Our results showed that SCN7A was strongly associated with immune cell infiltration. Rabbit models have revealed that SCN7A knockdown reduced the infiltration of macrophages and neutrophils[29,30], consistent with our results. These results indicate that SCN7A may affect the tumor immune microenvironment.

ceRNA mechanism has proven to be involved in regulating gene expression through ncRNAs[31-34]. We identified that SCN7A expression is regulated via the OTUD6B-AS1-miR-21-5p-SCN7A axis. Interestingly, OTUD6B-AS1 has been reported to suppress cancer

Table 1. MicroRNA (miRNA) expression between lung adenocarcinoma and normal tissues

miRNA	Fold change	*P*-value	False discovery rate
hsa-miR-21-5p	9.2	6.10e-101	1.60e-97
hsa-miR-93-5p	2.73	2.00e-11	3.60e-10
hsa-miR-570-3p	3.84	3.30e-06	3.10e-05
hsa-miR-590-3p	3.08	1.10e-23	6.10e-22
hsa-miR-182-3p	15.57	7.00e-05	0.00054
hsa-miR-17-3p	2	2.30e-09	3.30e-08
hsa-miR-576-5p	2.14	8.80e-07	9.00e-06
hsa-miR-577	124.3	1.30e-20	5.70e-19
hsa-miR-20a-5p	4.76	1.20e-26	7.20e-25
hsa-miR-155-5p	2.07	0.00048	0.0031
hsa-miR-556-5p	13.6	5.10e-06	4.60e-05
hsa-miR-331-5p	1.34	1.20e-06	1.20e-05
hsa-miR-106b-5p	1.38	1.20e-13	2.60e-12
hsa-miR-96-3p	15.09	0.00044	0.0029

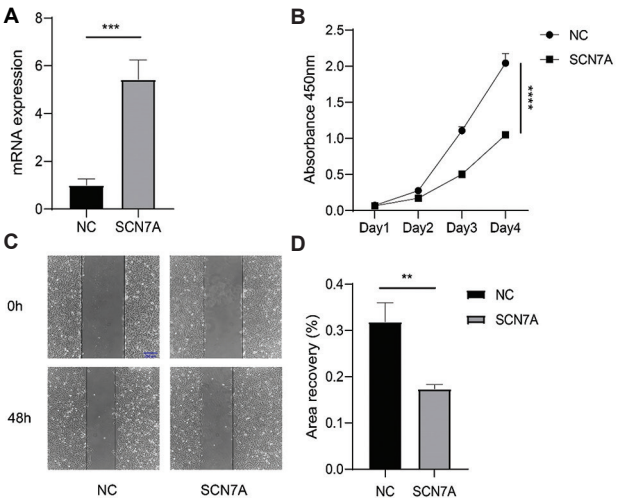

Figure 11. SCN7A as a protective factor in lung cancer cells. (A) *SCN7A* mRNA expression between the control group and SCN7A overexpression group. (B) Proliferation ability between the control group and SCN7A overexpression group. (C and D) Migration ability between the control group and SCN7A overexpression group (scale bar: 200μm). $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

progression in colorectal and thyroid cancers[35-37]. miR-21-5p contributes to lung cancer progression by inhibiting SMAD family member 7 (SMAD7) expression[38], while miR-21-5p inhibitors can effectively suppress lung cancer progression[39]. These studies suggest that the OTUD6B-AS1-hsa-mir-21-5p-SCN7A axis may have an important role in LUAD.

We also identified mutations and the role of SCN7A in pan-cancer prognosis. We found that SCN7A could

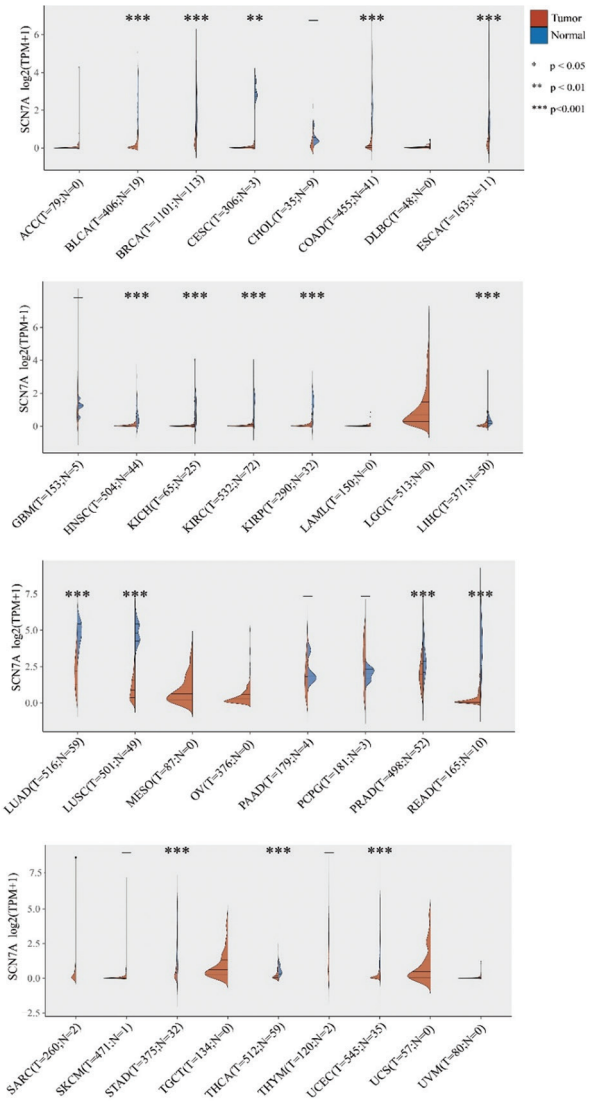

Figure 12. Expression of SCN7A in pan-cancer tissues.

predict the prognoses of LUSC and OV besides LUAD. Notably, SCN7A has been reported to be associated with poor prognosis in LUSC[40]. Our correlation analysis revealed a positive association between SCN7A expression and immune cell infiltration to most tumor tissues. These results suggest that SCN7A has significant research value in pan-cancer.

In conclusion, our work demonstrated that the underexpression of SCN7A predicts a poor prognosis in LUAD, thus indicating that SCN7A is a potential target in the treatment of LUAD. Notably, OTUD6B-AS1 and miR-21-5p are the ncRNAs that regulate SCN7A expression. The role of SCN7A in LUAD prognosis needs to be validated by *in vivo* studies.

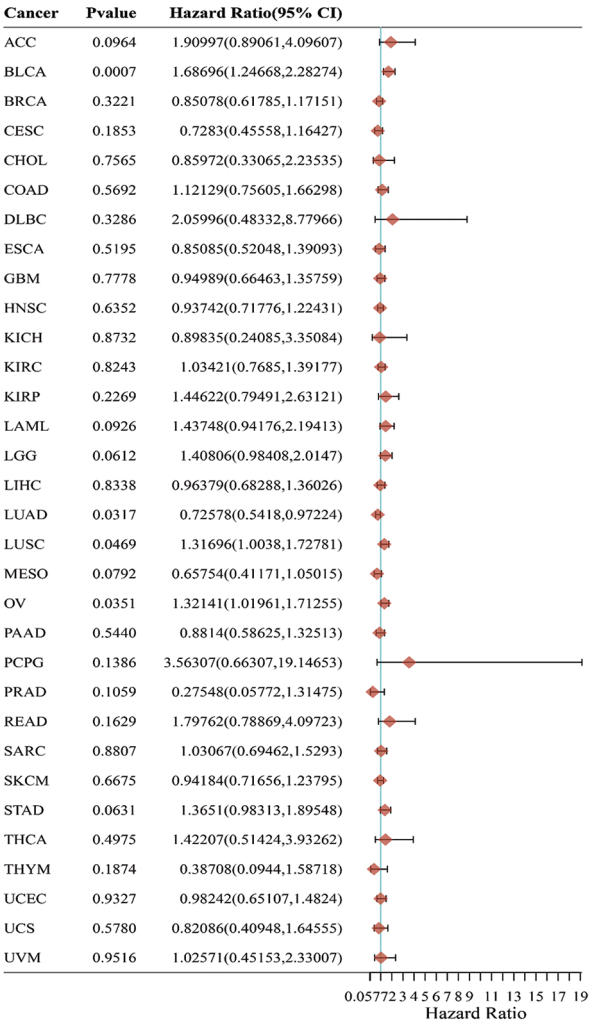

Figure 13. Prognostic value of SCN7A for pan-cancer.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: Hehui Lv, Hongyuan Song, Zhouping Qin, Yulian Chen
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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

The datasets in this study are available in online repositories, TCGA datasets (https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga), and GEO databases (https://www.ncbi.nlm.nih.gov/geo/).

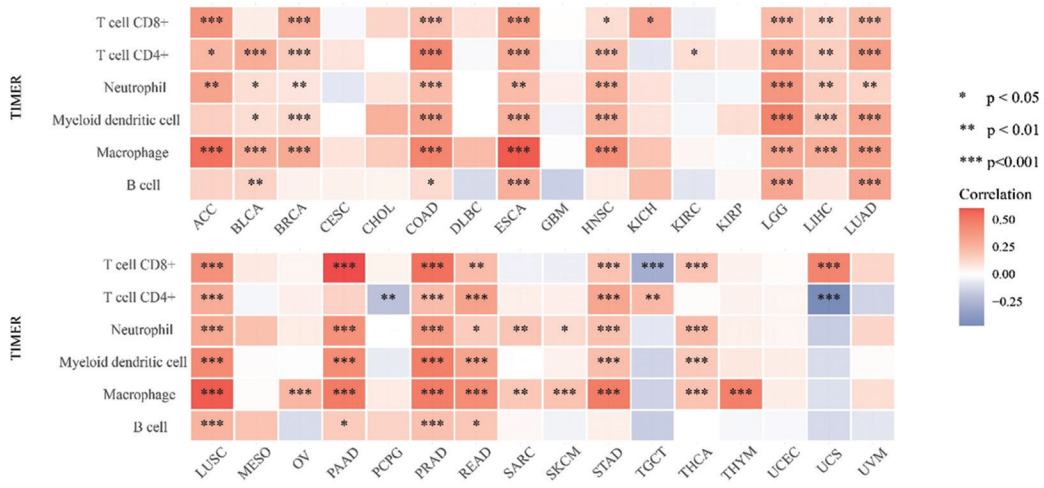

Figure 14. Relationship between SCN7A and immune cell infiltration to pan-cancer tissues.

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