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Original Article

Low KCNQ1 expression is associated with unfavorable outcome and metabolism of gastric cancer

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Abstract: Background: Potassium voltage-gated channel subfamily Q member 1 (KCNQ1), is implicated in the onset and progression of gastric carcinoma (GC), one of the most common types of stomach malignancies. This research aims to investigate the potential prognostic implications of KCNQ1 mRNA in GC using various databases such as The Cancer Genome Atlas (TCGA), The Human Protein Atlas (HPA), LinkedOmics, TISIDB, ESTIMATE, and TIMER. Methods: We searched the HPA database to obtain information on KCNQ1 levels in human normal tissues, organs, and cell lines as well as in pan-cancer tissues. Then, we used TIMER and UALCAN to comparatively analyze the KCNQ1 mRNA levels in different types of cancers relative to their adjacent normal counterparts. Based on TCGA and Gene Expression Omnibus, the correlation of clinical information with KCNQ1 expression was analyzed using logistic regression model. Univariable and Multivariate Cox analyses were then carried out to compare differences in survival among patients with different clinical characteristics. The multivariate methods, such as Kaplan-Meier plotter and GEPIA survival curves, were further employed to identify the correlation of KCNQ1 expression with overall survival (OS). Besides, LinkedOmics was used to identify differentially expressed genes for functional enrichment analysis. Results: KCNQ1 exhibited tissue-specific imprinting and expression in human normal tissues, organs and cell lines, while it was aberrantly expressed in pan-cancer tissues. Lower KCNQ1 mRNA expression was determined in GC tissue samples versus normal counterparts. In GC cases, elevated KCNQ1 levels were strongly linked to a longer OS and strongly correlated with invasion depth (χ^2=12.631, P=0.006), TNM stage (χ^2=8.750, P=0.033), differentiation grade (χ^2=7.426, P=0.024), and vital status (χ^2=5.676, P=0.017). Furthermore, KCNQ1 was identified by univariable and multivariate Cox analyses as an independent risk factor for GC. Based on Gene Ontology analysis, digestion as well as tricarboxylic acid metabolic, carbohydrate catabolic, and small molecule catabolic processes were differentially enriched in the up-regulated KCNQ1 phenotypic pathway. While carbon metabolism, fatty acid degradation, peroxisome, and citrate cycle (TCA cycle) were identified by the Kyoto Encyclopedia of Genes and Genomes-based analysis as pathways with differential enrichment. Conclusion: Being a prognostic biomarker, KCNQ1 may play an inhibitory role and involve in the metabolic process of GC.

Keywords: KCNQ1, gastric carcinoma (GC), TCGA, bioinformatics, prognosis, metabolic process

Introduction

Gastric carcinoma (GC), a high-incidence gastrointestinal malignancy, is the third leading cause of cancer-related deaths worldwide [1]. Despite continuous progresses in cancer-related therapies, the outcomes of GC patients remain unsatisfactory, partially due to the unclear pathogenesis, lack of effective prognostic markers, and late diagnosis in most patients. Meanwhile, given the heterogeneity of GC [2, 3], it is challenging to fully elucidate the different prognoses or therapeutic responses of GC based on microsatellite instability (MSI), HER2 mutation, and amplification [4, 5]. Traditional standard chemotherapy and targeted therapy based on precision medicine played an insufficient role in enhancing the overall survival (OS) of GC patients [6]. In recent years, immunotherapy (IT), as a novel therapeutic approach for most malignant tumors, has been widely applied as the first-line treatment for GC. However, nearly all patients eventually experience disease progression due to drug-induced

immune escape or resistance in the immune microenvironment [7, 8]. Tumor mutation burden and MSI are considered to be key factors in predicting the response of IT [9, 10]. In addition, key genes driving immune response can be considered as therapeutic targets and effective indicators [11]. Therefore, it is necessary to identify additional reliable indicators and biomarkers to predict the prognosis of GC patients undergoing IT.

Potassium voltage-gated channel subfamily Q member 1 (KCNQ1) is a protein coding gene that encodes voltage-gated potassium channels (VGPCs) required for the repolarization phase of cardiac action potential. It is widely expressed in various tissues, including the heart, inner ear, stomach, colon, and others [11-14]. Exhibiting tissue-specific imprinting, this gene is preferentially expressed from maternal allele in certain tissues and shows biallelic expression in others [15]. KCNQ1 mutations are associated with many diseases, including deafness, diabetes mellitus, Jervell and Lange-Nielsen Syndrome 1, long QT syndrome 1, and familial atrial fibrillation, as well as pathways involved in chemical synapse transmission and circadian entrainment. The annotations of KCNQ1 based on Gene Ontology (GO) are calmodulin and ion channel binding, with KCNQ5 being its important paralog. Aberrantly expressed K+ channels in human cancers have been previously reported [16, 17]. For instance, KCNQ1 has been shown to be an antioncogene in mouse and human colorectal carcinoma [18], and hypermethylated KCNQ1 plays a tumor-inhibiting role in hepatocellular carcinoma (HCC) [19]. However, there is limited research on the functional and molecular events linking KCNQ1 to GC.

Herein, we comprehensively analyzed KCNQ1 levels in pan-cancers and its correlations with the clinicopathological features of GC patients. In addition, the association between KCNQ1 differential expression and prognosis as well as the co-expression interaction (CEI) networks in GC were investigated using sequencing data sets. This study aimed to reveal the prognostic implications of KCNQ1 expression in pan-cancers and its potential role as a prognostic indicator, so that we can have a better understanding of GC progression and treatment resistance mechanisms.

Methods

Gene expression profiles and clinical features in databases

Data were retrieved from The Cancer Genome Atlas (TCGA) database (URL: https://genome-cancer.ucsc.edu/), which provides clinic and pathological information on 33 tumor types. The Human Protein Atlas (HPA) project can support proteome analysis based on 26941 antibodies against 17165 unique proteins, from which we collected information on KCNQ1 mRNA levels of normal human tissues, organs and cell lines. Differences in KCNQ1 mRNA between human pan-cancers and the adjacent counterparts were also identified by TIMER (URL: http://cistrome.org/TIMER/) and UALCAN (URL: http://ualcan.path.uab.edu/) as well as the xiantao.love platform (URL: https://www.xiantao.love/products). We collected clinicopathological details and general information of GC cases from the TCGA and GEO based on KCNQ1 expression profiling, and discussed its correlation with clinicopathological characteristics. Quantitative variables, statistically displayed as the mean ± SD, were analyzed using unpaired t-test. Fisher's exact or Pearson chi-square test was employed to determine the connection between KCNQ1 and clinical characteristics.

Survival and statistical analyses

Patients were categorized as either high or low KCNQ1 expression group based on the median. We used Kaplan-Meier (KM) plotter (URL: http://kmplot.com/analysis/) to evaluate the impact of individual genes on the survival of patients with GC or other cancers. GEPIA [20] (URL: http://gepia.cancer-pku.cn/), a web-based instrument with a standard processing pipeline, was utilized to analyze the data retrieved from TCGA and genotype-tissue expression. The correlation of KCNQ1 with OS of GC patients was evaluated using KM plotter for GEO data and GEPIA for TCGA data, respectively.

Furthermore, we calculated the *P*-values as well as hazard ratios (HRs) with 95% confidence intervals (CIs) using log-rank tests. A logistic regression analysis was then conducted to identify the association of the clinical characteristics with KCNQ1 expression in different groups. Whether the KCNQ1 gene is an inde-

pendent prognostic factor for GC survival was determined using multivariate Cox analysis. A minimum significance level of *P*-value less than 0.05 was set. The aforementioned analytical methods and the R package were utilized with the R software *v*4.0.3 (The R Foundation for Statistical Computing Platform, 2020).

CEI and protein-protein interaction (PPI) network analysis

Thirty-two cancer-related multidimensional TCGA datasets were analyzed using LinkedOmics (URL: http://www.linkedomics.org/login.php) [21], in which the Link-Finder module was used to identify KCNQ1-related differentially expressed genes in GC in TCGA-STAD that were activated between high and low KCNQ1 level datasets. Correlations were tested by Pearson correlation coefficients, which are presented in volcano plots and heatmaps. We conducted enrichment analysis using the LinkInterpreter module to identify relevant pathways in both groups, such as Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and GO biological processes enriched by the top ranked genes. We used STRING database (URL: https://www.string-db.org/) to identify and build PPI networks and SWISS-MODEL (URL: https://swissmodel.expasy.org) [21] to model KCNQ1 protein homology based on its primary sequence.

Results

KCNQ1 expression in normal human tissues and organs

Exhibiting tissue-specific imprinting, KCNQ1 was preferentially expressed in heart, inner ear, pancreas, prostate, kidneys, stomach, small intestine, and peripheral blood white blood cells. It's also named as calmodulin binding protein, as well as voltage gated, ion, and potassium channel, with potassium transport as its main function. The HPA database provided information on KCNQ1 mRNA expression in various tissues, revealing its specific enhancement in the adrenal gland, ductus deferens, and seminal vesicle. However, the expression of KCNQ1 in human brain showed low regional specificity (**Figure 1A**). The genome-wide differential analysis of cell type specific protein-coding genes indicated that KCNQ1 mRNA expression was notably enhanced in undifferentiated cells and enterocytes (**Figure 1B**) and was detected in various blood and immune cells, but exhibted low cell type specificity in these cells (**Figure 1C**). Among KCNQ1-expressing cell lines, KCNQ1 mRNA was especially enhanced in HMC-1, OE19, RPMI-8226, U-937, and U-266/70 (**Figure 1D**). Protein expression analysis showed that KCNQ1 protein was mainly expressed in the cytoplasm and membrane of the adrenal and thyroid glands, as well as the stomach.

KCNQ1 mRNA differs between human pancarcinomatous tissues and adjacent counterparts

KCNQ1 mRNA expression was compared between diverse pancarcinomatous tissues and paracancerous counterparts in different databases, including HPA, TIMER and UALCAN. We assessed KCNQ1 levels in pancarcinomas using the HPA database and found its moderate cytoplasmic and membranous positivity in thyroid colorectal and carcinomas and in a small number of melanomas, ovarian, stomach, pancreatic and liver carcinomas, but it was weakly stained or negative in the remaining cancers (**Figure 2A**). KCNQ1-nagative was found in most tumor cells, with only varying levels of cytoplasmic and membranous immunoreactivity seen in colorectal and stomach carcinoma cells (**Figure 2B**). According to the analysis results of the TIMER database, KCNQ1 was found to be significantly downregulated in breast invasive carcinoma, kidney renal papillary cell carcinoma, lung squamous cell carcinoma, thyroid carcinoma, lung adenocarcinoma, HCC, prostate adenocarcinoma, skin cutaneous melanoma, and stomach adenocarcinoma (STAD) in comparison to their corresponding control tissues. By contrast, it showed significantly elevated levels in cholangiocarcinoma, colon adenocarcinoma, kidney clear cell carcinoma, head and neck cancer, kidney chromophobe, and rectum adenocarcinoma than those in adjacent counterparts (**Figure 2C**). Further, we assessed KCNQ1 expression in pancarcinomas using UALCAN data and xiantao.love platform and found similar results (**Figure 2D**, **2E**). Overall, KCNQ1 showed aberrant expression profiling in pancarcinomas.

Decreased expression of KCNQ1 in GC and its association with clinicopathological characteristics

Using TCGA and GEO databases, we found reduced KCNQ1 mRNA expression in GC tissue versus normal stomach tissue samples (**Figure**

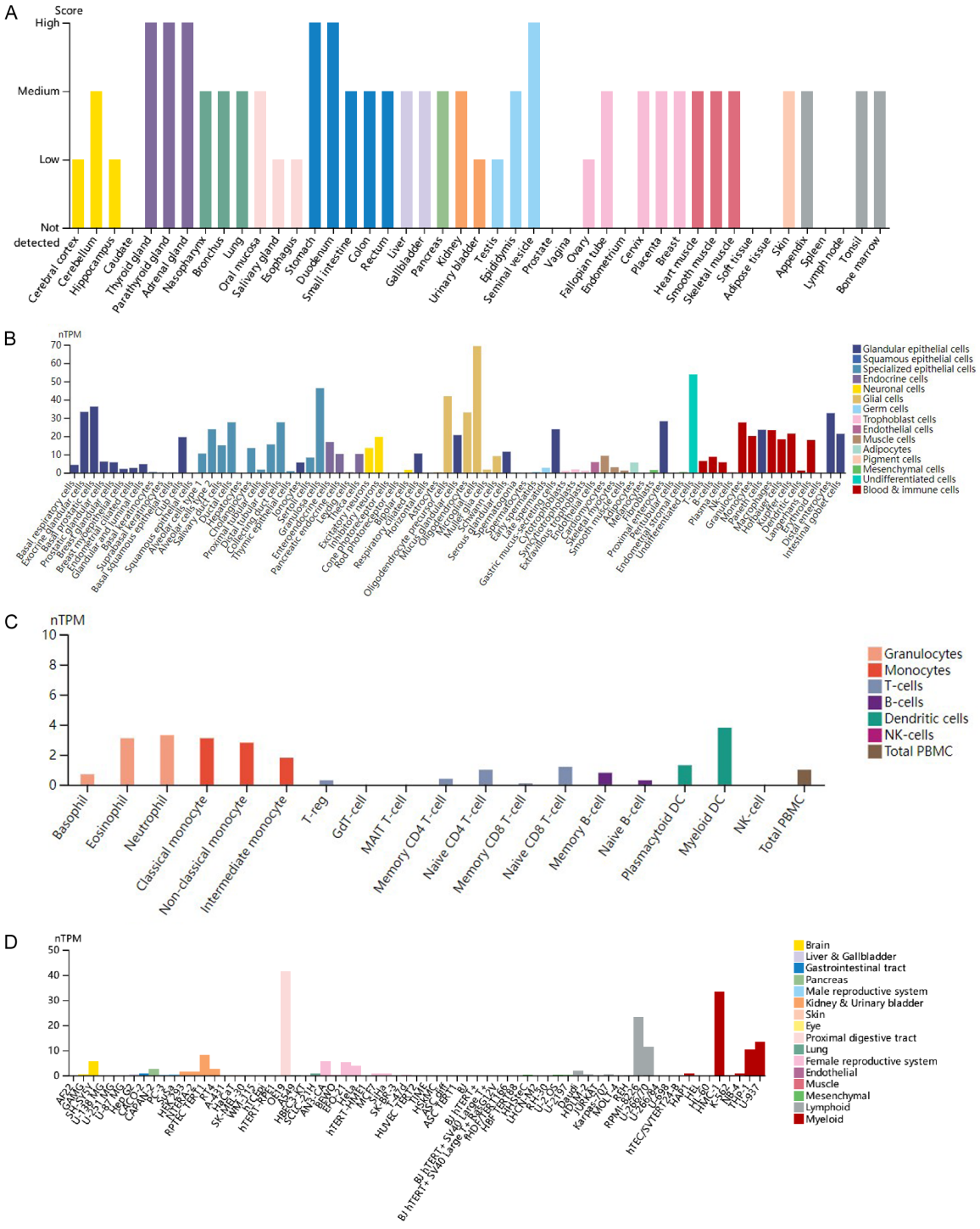
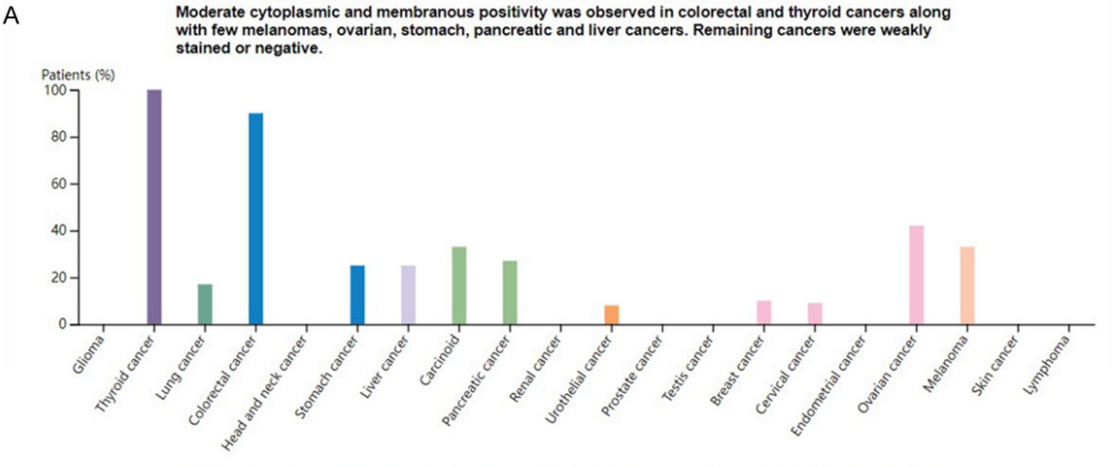


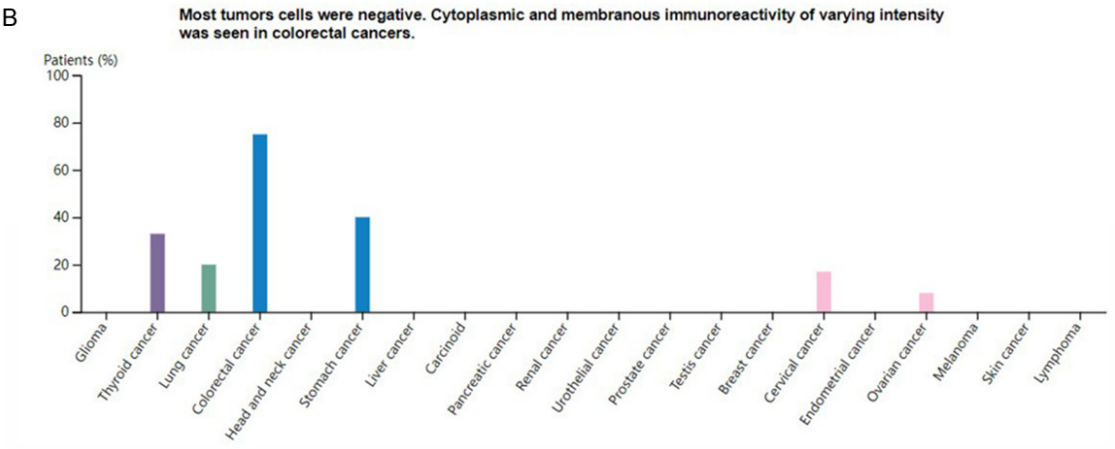
Figure 1. KCNQ1 expression levels in human normal tissues by The Human Protein Atlas. A. Expression in specific organs. B. Expression in specific cell types. C. Expression in blood and immune cells. D. Expression in HMC-1, OE19, RPMI-8226, U-266/70, and U-937.

3A), and the difference was even more significant in the matched tissue samples (**Figure 3B**), indicating under-expressed KCNQ1 in GC tissue samples versus normal counterparts. Meanwhile, HPA database showed that KCNQ1 protein was strongly expressed in normal gastric tissue (**Figure 3C**) while hardly expressed in cancer tissue (**Figure 3D**). The clinicopathologi-

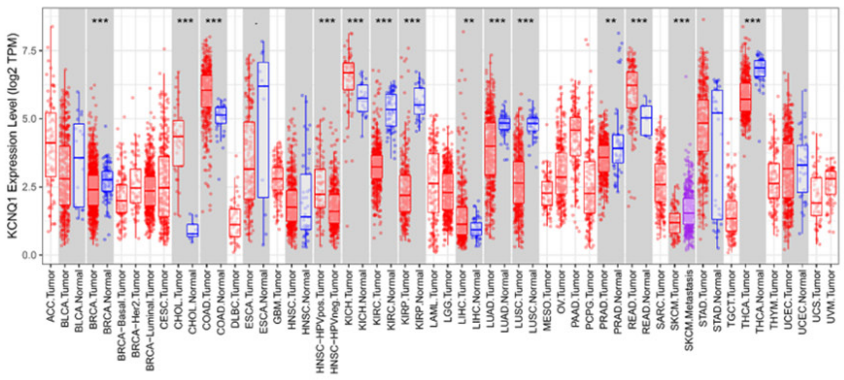
A


Moderate cytoplasmic and membranous positivity was observed in colorectal and thyroid cancers along with few melanomas, ovarian, stomach, pancreatic and liver cancers. Remaining cancers were weakly stained or negative.
Patients (%)
100
80
60
40
20
0
Glioma
Thyroid cancer
Lung cancer
Colorectal cancer
Head and neck cancer
Stomach cancer
Liver cancer
Carcinoid
Pancreatic cancer
Renal cancer
Urothelial cancer
Prostate cancer
Testis cancer
Breast cancer
Cervical cancer
Endometrial cancer
Ovarian cancer
Melanoma
Skin cancer
Lymphoma

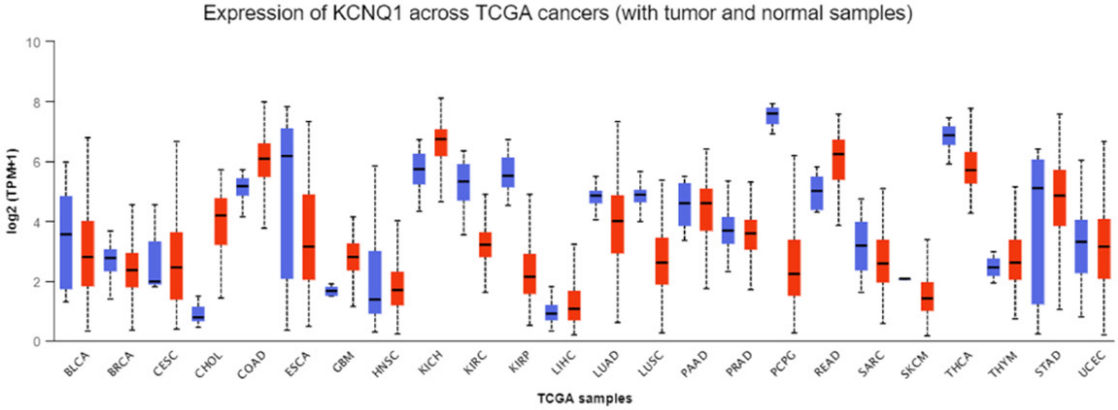
B


Most tumors cells were negative. Cytoplasmic and membranous immunoreactivity of varying intensity was seen in colorectal cancers.
Patients (%)
100
80
60
40
20
0
Glioma
Thyroid cancer
Lung cancer
Colorectal cancer
Head and neck cancer
Stomach cancer
Liver cancer
Carcinoid
Pancreatic cancer
Renal cancer
Urothelial cancer
Prostate cancer
Testis cancer
Breast cancer
Cervical cancer
Endometrial cancer
Ovarian cancer
Melanoma
Skin cancer
Lymphoma

C


KCNQ1 Expression Level (log2 TPM)
7.5
5.0
2.5
0.0
ACC.Tumor
BLCA.Tumor
BLCA.Normal
BRCA.Tumor
BRCA.Normal
BRCA−Basal.Tumor
BRCA−Her2.Tumor
BRCA−Luminal.Tumor
CESC.Tumor
CHOL.Tumor
CHOL.Normal
COAD.Tumor
COAD.Normal
DLBC.Tumor
ESCA.Tumor
ESCA.Normal
GBM.Tumor
HNSC.Tumor
HNSC.Normal
HNSC−HPVpos.Tumor
HNSC−HPVneg.Tumor
KICH.Tumor
KICH.Normal
KIRC.Tumor
KIRC.Normal
KIRP.Tumor
KIRP.Normal
LAML.Tumor
LGG.Tumor
LIHC.Tumor
LIHC.Normal
LUAD.Tumor
LUAD.Normal
LUSC.Tumor
LUSC.Normal
MESO.Tumor
OV.Tumor
PAAD.Tumor
PCPG.Tumor
PRAD.Tumor
PRAD.Normal
READ.Tumor
READ.Normal
SARC.Tumor
SKCM.Tumor
SKCM.Metastasis
STAD.Tumor
STAD.Normal
TGCT.Tumor
THCA.Tumor
THCA.Normal
THYM.Tumor
UCEC.Tumor
UCEC.Normal
UCS.Tumor
UVM.Tumor

D


Expression of KCNQ1 across TCGA cancers (with tumor and normal samples)
log2 (TPM+1)
10
8
6
4
2
0
BLCA
BRCA
CESC
CHOL
COAD
ESCA
GBM
HNSC
KICH
KIRC
KIRP
LIHC
LUAD
LUSC
PAAD
PRAD
PCPG
READ
SARC
SKCM
THCA
THYM
STAD
UCEC
TCGA samples

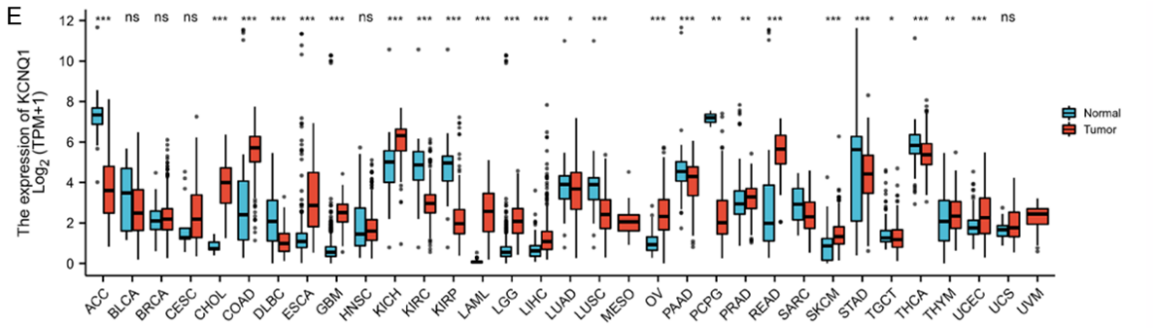


Figure 2. KCNQ1 expression in cancer tissues and tumor cells. A, B. KCNQ1 in different human cancer tissues and tumor cells in The Human Protein Atlas (TCGA). C. KCNQ1 in various cancers from TCGA data analyzed in TIMER. KCNQ1 presented low expression in breast invasive carcinoma (BRCA), kidney renal papillary cell carcinoma (KIRP), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), prostate adenocarcinoma (PRAD), skin cutaneous melanoma (SKCM), stomach adenocarcinoma (STAD), and thyroid cancer (THCA), and high expression in cholangiocarcinoma (CHOL), kidney clear cell carcinoma (KIRC), colon adenocarcinoma (COAD), head and neck cancer (HNSC), kidney chromophobe (KICH), and rectum adenocarcinoma (READ). D, E. KCNQ1 mRNA in cancerous and normal tissues across UALCAN data and xiantao.love platform (ns, P≥0.05; *, P<0.05; **, P<0.01; ***, P<0.001).

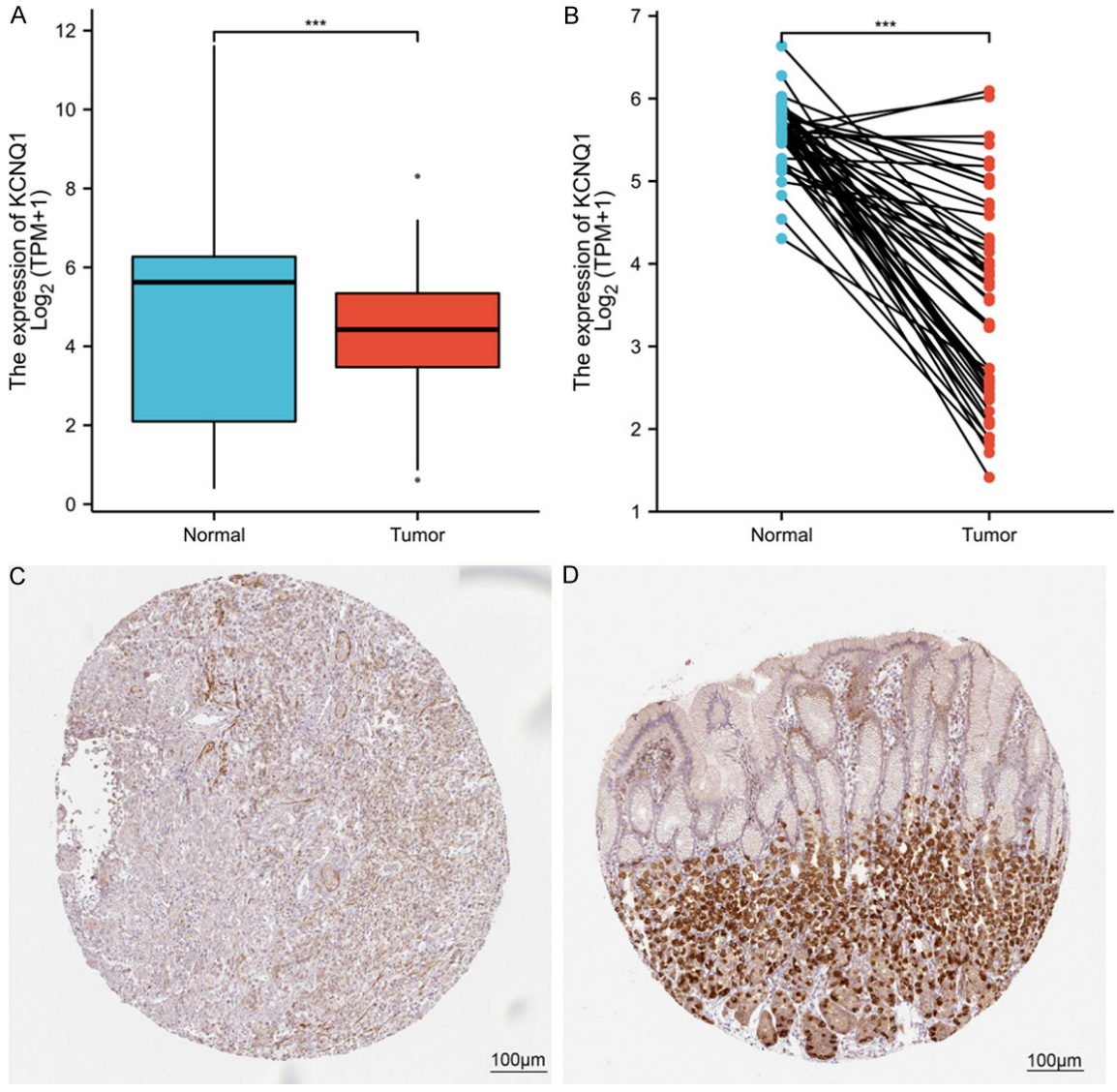


Figure 3. KCNQ1 is decreased in GC in the TCGA datasets. A. KCNQ1 mRNA in GC and normal gastric tissue specimens; B. KCNQ1 mRNA in GC and matched normal tissue specimens; C. KCNQ1 protein expression in gastric cancer tissue in HPA database; D. KCNQ1 protein expression in gastric normal tissue in HPA database. (*, P<0.05; **, P<0.01; ***, P<0.001).

Table 1. Association of KCNQ1 with the clinicopathological characteristics of gastric cancer patients

Characteristic	Total	KCNQ1 expression		χ^2	p
		Low (n=187)	High (n=188)		
Age				2.313	0.128
≥65	227	106	121		
<65	148	81	67		
Sex				0.811	0.368
Male	241	116	125		
Female	134	71	63		
Invasion depth				12.631	0.006
T1	23	7	16		
T2	113	45	68		
T3	148	86	62		
T4	91	49	42		
Lymph node metastasis				5.947	0.114
N0	111	46	65		
N1	97	53	44		
N2	85	41	44		
N3	82	47	35		
Distant metastasis				2.1	0.147
M0	330	160	170		
M1	45	27	18		
TNM stage				8.750	0.033
I	54	20	34		
II	111	49	62		
III	160	88	72		
IV	50	30	20		
Differentiation grade				7.426	0.024
G1	18	13	5		
G2	137	58	79		
G3	220	116	104		
Vital status				5.676	0.017
Alive	233	105	128		
Dead	142	82	60		

P<0.05, statistically significant.

cal characteristics of GC patients (age, sex, invasion depth, lymph node/distant metastasis, differentiation grade, tumor-node-metastasis (TNM) stage, and vital status) were collected from TCGA data through HOME for Researchers platform. A total of 375 GC patients (241 males and 134 females) were assigned to either a low or high KCNQ1 expression group based on the median KCNQ1 mRNA expression. There were 227 patients aged ≥65 years and 148 patients <65 years. As can be seen from **Table 1**, there was a significant connection between KCNQ1 expression and invasion depth (χ^2=12.631, P=0.006), TNM stage (χ^2=8.750, P=0.033), differentiation grade (χ^2=7.426, P=0.024), and vital status (χ^2=5.676, P=0.017) in GC patients. It is indicated that KCNQ1 may play a tumor-suppressing role in GC.

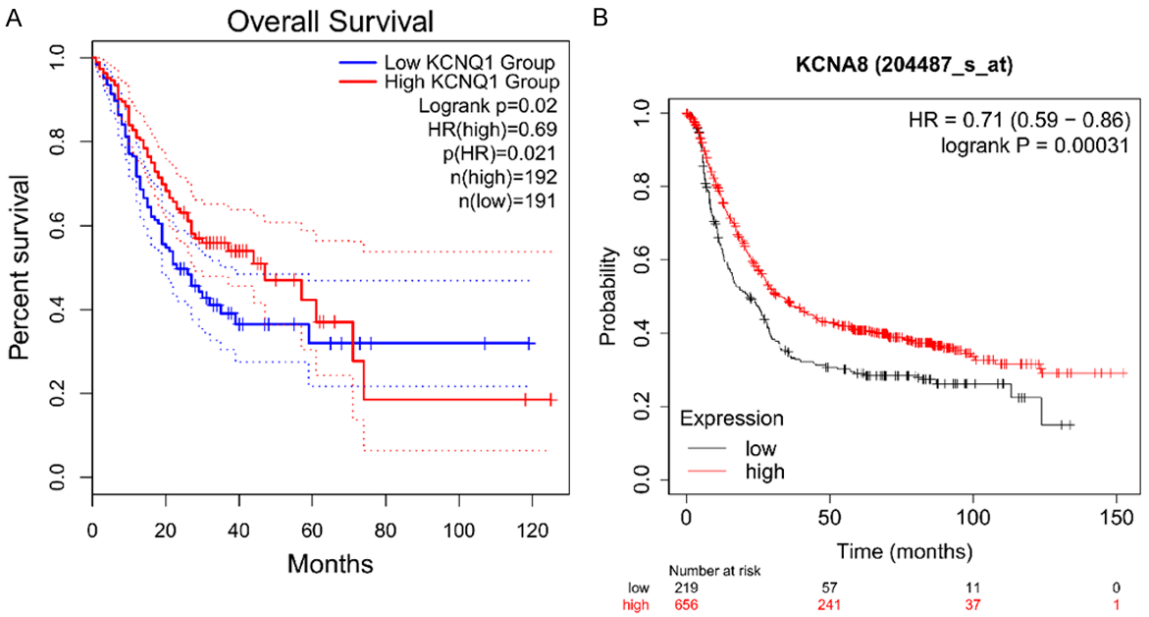


Figure 4. Survival curves of GC patients with high or low KCNQ1 expression. A. By GEPIA2. B. By Kaplan-Meier Plotter.

Table 2. Cox univariate and multivariate analyses of overall survival of gastric cancer patients

Characteristics	Univariate		Multivariate	
	HR (95% CI)	P	HR (95% CI)	P
Age (≥65 vs. <65)	1.620 (1.154-2.276)	0.005	1.681 (1.129-2.504)	0.011
Sex (female vs. male)	1.267 (0.891-1.804)	0.188		
Invasion depth (T3/T4 vs. T1/T2)	1.719 (1.131-2.612)	0.011	1.938 (1.028-3.653)	0.041
LNM (N2/N3 vs. N0/N1)	1.650 (1.182-2.302)	0.003	1.346 (0.782-2.317)	0.284
Distant metastasis (M1 vs. M0)	2.254 (1.295-3.924)	0.004	1.691 (0.810-3.533)	0.162
KCNQ1	0.871 (0.781-0.971)	0.013	0.853 (0.743-0.979)	0.024
TNM stage (III-IV vs. I-II)	1.947 (1.358-2.793)	<0.001	0.980 (0.523-1.834)	0.949
H pylori infection (Yes vs. No)	0.650 (0.279-1.513)	0.317		
PTO (SD/PR/CR vs. PD)	0.241 (0.165-0.352)	<0.001	0.251 (0.168-0.376)	<0.001

Abbreviations: CI, confidence interval; HR, hazard ratio; LNM, lymph node metastasis; PTO, primary therapy outcome. P<0.05, statistically significant.

KCNQ1 is associated with better prognosis in GC

The correlation of KCNQ1 with OS of GC patients was evaluated using KM plotter for GEO data and GEPIA for TCGA data, respectively. Upregulated KCNQ1 was strongly correlated with longer OS in both GEO data (HR: 0.71, P=0.00031) and TCGA data (HR: 0.69, P=0.021), as shown in **Figure 4A** and **4B**. Additionally, using the Cox model, univariable and multivariate analyses assessed the correlation of clinicopathological characteristics with OS. According to the univariate Cox model, age (P=0.005), invasion depth (P=0.011), LNM (P=0.003), distant metastasis (P=0.004), TNM stage (P<0.001), primary therapy outcome (P<0.001), and KCNQ1 expression (P=0.013) were factors significantly influencing the survival. Furthermore, T stage (P=0.041), primary therapy outcome (P<0.001), and KCNQ1 (P=0.024) were identified by the multivariate analysis as independent prognostic factors (**Table 2**). Overall, low expression KCNQ1 was an independent risk factor for poor prognosis in GC.

KCNQ1 CEI networks in GC

To more comprehensively clarify the biological role of KCNQ1, we used Link-Finder to identify the CEI pattern of KCNQ1-related genes in TCGA-STAD that were differentially expressed in GC between high and low KCNQ1 expression datasets. The results revealed 13017 genes (in dark red) and 7208 genes (in dark green) that were positively and negatively associated with KCNQ1, respectively (**Figure 5A**). The top 50 genes in STAD are presented in heat maps (**Figure 5B**, **5C**). Based on GO term annotations, the co-expressed genes of KCNQ1 were concentrated in various biological processes, such as digestion, carbohydrate derivative transport, peroxisome organization, execution phase of apoptosis, tricarboxylic acid metabolism, carbohydrate catabolism, small molecule catabolism, cofactor biosynthesis, and cellular aldehyde metabolism (**Figure 5D**). According to KEGG pathway analysis, KCNQ1 enrichment was primarily found in peroxisome organization, carbon metabolism, citrate cycle (TCA cycle), fatty acid degradation, fructose and mannose metabolism, glycolysis/gluconeogenesis, sphingolipid metabolism, aminoacyl-tRNA biosynthesis, and sulfur metabolism (**Figure 5E-G**). The above results suggest the extensive influence of KCNQ1 expression networks on the outcome and metabolic process of GC.

KCNQ1 PPI analysis and spatial structure composition

We utilized STRING to identify the PPI networks of KCNQ1 protein, and formed a core network comprised of 11 nodes and 23 edges, with an average node degree of 4.18 and a local clustering coefficient of 0.859 (P=0.0005; **Figure 6A**). The proteins interacted with KCNQ1 were AKAP9, KCNE1, CALM3, KCNE2, KCNE3, CALM2, KCNH2, KCNJ2, KCNE4 and ADCY9. Of these, AKAP9, KCNE4, and CALM2 were at the core of the KCNQ1 protein network. These genes were primarily implicated in potassium ion export and cyclic-nucleotide signaling, and function as activators of adenylate cyclase. All of the above pathological processes play critical roles in the metabolic process of GC cells.

Using Swiss Model's online web-based client, the 3D structure of the KCNQ1 protein was modeled and validated. The original (wild-type) model of the KCNQ1 protein is shown in **Figure 6B**, where blue and red colors represent the coiled peptide chain from the protein's N-to-C-terminus. This chain by itself has no function but can form a heterotetramer with CaM and has VGPC activity [22] (**Figure 6C**). KCNQ1 can form heteromultimers with CaM and KCNE3, inducing voltage-dependent currents through the rapid activation and slow inactivation of potassium-selective outward currents (**Figure 6D**).

Discussion

Expressed in multiple tissues throughout the body, KCNQ1 potassium channels can modulate many key physiological functions. Due to its tissue-specific imprinting, KCNQ1 is essential in various tissues, including the heart, inner ear, stomach and colon [11, 12]. The differential KCNQ1 mRNA levels also play a vital part in pan-cancer progression. To better clarify the role of KCNQ1 in humans, we comprehensively examined KCNQ1 in normal human tissues, organs and cell lines based on the HPA data. We confirmed the differences in KCNQ1 mRNA levels between 33 different human tumors and their corresponding adjacent counterparts based on HPA, TIMER and UALCAN databases.

Previously, the expression pattern of KCNQ1 was measured in many tissues and found to be enhanced in adrenal gland, ductus deferens and seminal vesicle but had low brain region specificity in human. Meanwhile, KCNQ1 mRNA expression was found to be specifically enhanced in undifferentiated cells and enterocytes. Moreover, among cell lines expressing KCNQ1, KCNQ1 mRNA was observed to be particularly enhanced in HMC-1, RPMI-8226, OE19, U-937, and U-266/70. We also used three different databases to perform analysis of differential expression in varying tumors and all the results revealed the aberrant expression of KCNQ1 in pan-cancers. Therefore, KCNQ1 expression in GC was further assessed using TCGA and GEO databases and was determined to be lower in GC tissue samples versus normal counterparts.

Since this study was primarily intended to investigate the role of KCNQ1 in GC, we further analyzed the association of KCNQ1 with the clinicopathological characteristics of patients using TCGA. KCNQ1 was determined to be significantly associated with invasion depth, TNM

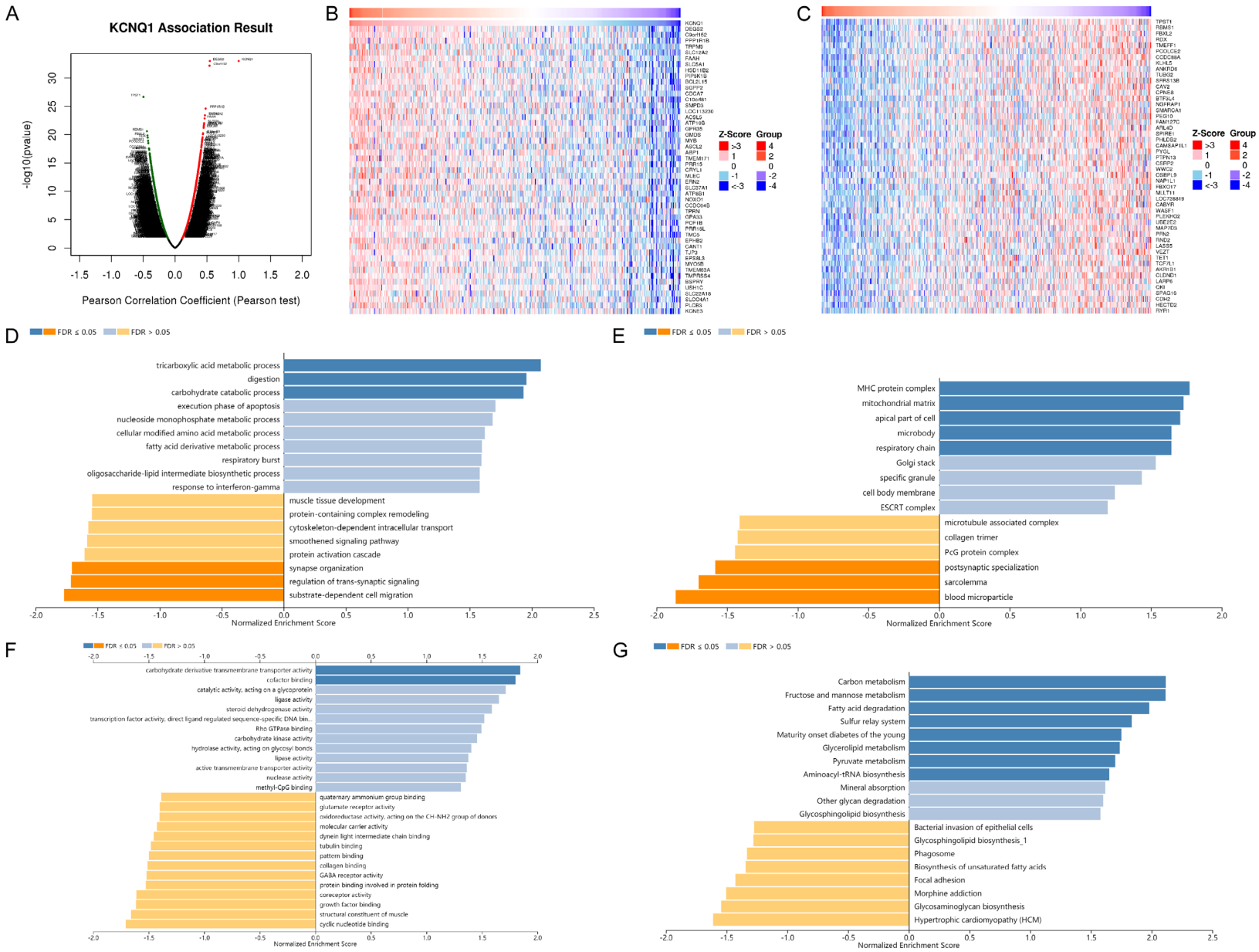

A
KCNQ1 Association Result
-log10(pvalue)
Pearson Correlation Coefficient (Pearson test)
B
Z-Score
Group
C
Z-Score
Group
D
FDR ≤ 0.05
FDR > 0.05
tricarboxylic acid metabolic process
digestion
carbohydrate catabolic process
execution phase of apoptosis
nucleoside monophosphate metabolic process
cellular modified amino acid metabolic process
fatty acid derivative metabolic process
respiratory burst
oligosaccharide-lipid intermediate biosynthetic process
response to interferon-gamma
muscle tissue development
protein-containing complex remodeling
cytoskeleton-dependent intracellular transport
smoothened signaling pathway
protein activation cascade
synapse organization
regulation of trans-synaptic signaling
substrate-dependent cell migration
Normalized Enrichment Score
E
FDR ≤ 0.05
FDR > 0.05
MHC protein complex
mitochondrial matrix
apical part of cell
microbody
respiratory chain
Golgi stack
specific granule
cell body membrane
ESCRT complex
microtubule associated complex
collagen trimer
PcG protein complex
postsynaptic specialization
sarcolemma
blood microparticle
Normalized Enrichment Score
F
FDR ≤ 0.05
FDR > 0.05
carbohydrate derivative transmembrane transporter activity
cofactor binding
catalytic activity, acting on a glycoprotein
ligase activity
steroid dehydrogenase activity
transcription factor activity, direct ligand regulated sequence-specific DNA bin...
Rho GTPase binding
carbohydrate kinase activity
hydrolase activity, acting on glycosyl bonds
lipase activity
active transmembrane transporter activity
nuclease activity
methyl-CpG binding
quaternary ammonium group binding
glutamate receptor activity
oxidoreductase activity, acting on the CH-NH2 group of donors
molecular carrier activity
dynein light intermediate chain binding
tubulin binding
pattern binding
collagen binding
GABA receptor activity
protein binding involved in protein folding
coreceptor activity
growth factor binding
structural constituent of muscle
cyclic nucleotide binding
Normalized Enrichment Score
G
FDR ≤ 0.05
FDR > 0.05
Carbon metabolism
Fructose and mannose metabolism
Fatty acid degradation
Sulfur relay system
Maturity onset diabetes of the young
Glycerolipid metabolism
Pyruvate metabolism
Aminoacyl-tRNA biosynthesis
Mineral absorption
Other glycan degradation
Glycosphingolipid biosynthesis
Bacterial invasion of epithelial cells
Glycosphingolipid biosynthesis_1
Phagosome
Biosynthesis of unsaturated fatty acids
Focal adhesion
Morphine addiction
Glycosaminoglycan biosynthesis
Hypertrophic cardiomyopathy (HCM)
Normalized Enrichment Score

Figure 5. KCNQ1 co-expressed genes in GC from LinkedOmics. A. Significantly KCNQ1-associated genes in STAD cohort distinguished by the Pearson test. B, C. Heatmap of the top 50 genes most positively (red) and negatively (blue) related to KCNQ1. D-G. GO annotations and KEGG pathway analysis of KCNQ1 in STAD cohort.

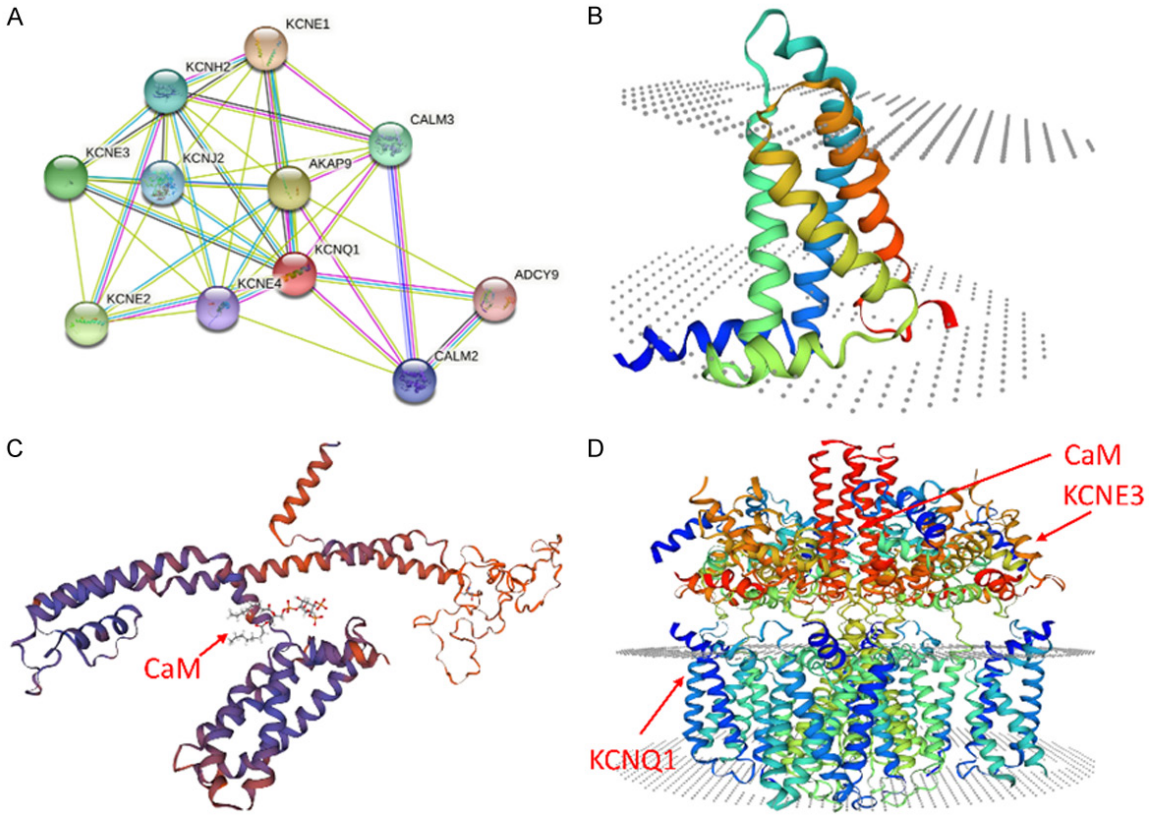


Figure 6. KCNQ1 protein-protein interaction (PPI) analysis and spatial structure composition. A. The core KCNQ1-associated PPI networks in gastric cancer, consisting 11 nodes (represent proteins) and 23 edges (represent interactions between proteins in the network) with a mean node degree of 4, were built by STRING database. KCNQ1, Potassium voltage-gated channel subfamily KQT member 1; AKAP9, A-kinase anchor protein 9; KCNE1, Potassium voltage-gated channel subfamily E member 1; CALM3, Calmodulin-1; Calmodulin 3 (phosphorylase kinase, delta); KCNE2, Potassium voltage-gated channel subfamily E member 2; KCNE3, Potassium voltage-gated channel subfamily E member 3; CALM2, Calmodulin 2 (phosphorylase kinase, delta); KCNH2 Potassium voltage-gated channel subfamily H member 2; KCNJ2, Inward rectifier potassium channel 2; KCNE4, Potassium voltage-gated channel subfamily E member 4; ADCY9, Adenylate cyclase type 9; B. The original model of KCNQ1 protein; C. Human KCNQ1 protein in complex with CaM; D. Structure of human KCNQ1-KCNE3-CaM complex.

stage, differentiation grade, and vital status in GC patients, suggesting a possible inhibitory role of KCNQ1 in GC procession. Additionally, we also found that higher level of KCNQ1 was significantly related to longer OS. Multivariate analysis further revealed that T stage, primary therapy outcome and KCNQ1 expression were independent prognostic factors for OS. Overall, the under-expressed KCNQ1 gene in GC is associated with unfavorable outcomes and malignant phenotypes in GC patients [23]. KCNQ1 acts as an anti-oncogene in inhibiting tumor progression through modulating metabolic process in GC. Studies have shown that people carrying the KCNQ1 germline mutation can develop a range of disorders, including hearing loss, gastrin elevation, gastric hyperplasia and, in some cases, tumors [19, 24, 25]. Recently, KCNQ1 has been suggested to play a critical part in colorectal carcinoma cell growth, EMT, invasion, and tumorigenesis, with an association with adverse disease-free survival via regulating the Wnt/β-catenin axis [26, 27]. In addition, KCNQ1 functions as an anti-oncogene in mouse and human carcinomas of the digestive tract [18]. Such phenotypes have also

been modeled in KCNQ1 knockout mice. As a result, the animals developed inner ear defects, chronic gastritis, imbalances, as well as gastric hyperplasia and metaplasia [28], demonstrating the relationship between KCNQ1 and the carcinogenesis and progression of GC.

Subsequently, we explored the CEI network of KCNQ1 in GC using the LinkedOmics portal to test KCNQ1's biological role in GC. KCNQ1 is a protein coding gene that can generate heteromultimers with KCNE1 and KCNE3, two potassium channel proteins [29]. Its role in human cancers has also been established [30]. This study demonstrated the extensive impact of KCNQ1 expression networks on the metabolic processes and outcomes of GC, including tricarboxylic acid metabolism, digestion, carbohydrate catabolism, peroxisome, carbon metabolism, and the citrate cycle (TCA cycle), as validated by GO term annotation and KEGG pathway analyses. These findings provide insight into the potential anti-oncogene role of KCNQ1 in GC cells. To explore the function of KCNQ1, we constructed its PPI networks and homologous function model, and found that KCNE3 and CaM were the functional subunits of KCNQ1. However, the pathway mechanisms and homology modeling involved still need further in vitro and in vivo validation.

Several limitations of this study remain to be noted. While KCNQ1 has been suggested to be crucial in GC and a candidate therapeutic target, the conclusions are obtained by analyzing data from public databases without validation using experiments. In the present study, KCNQ1 mRNA expression was found to be associated with the survival in GC patients, and KCNQ1 protein levels were correlated with OS. But based on the conflicting results of ID1, KCNQ1 has been suggested to be essential in GC onset and development. Hence, it is necessary to further investigate its specific role in GC tumorigenesis and progression to gain a better understanding of the underlying molecular mechanisms of KCNQ1.

Conclusion

This study is the first to reveal the prognostic implications of the expression patterns and biological role of KCNQ1 in GC and its promising prognostic significance, contributing to a better understanding of the mechanisms underlying GC recurrence and metastasis. KCNQ1 was downregulated in GC tissue, and low KCNQ1 expression is associated with adverse prognosis. KCNQ1 channels are expected to be a feasible prognostic index and a candidate target for GC therapy.

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Disclosure of conflict of interest

None.

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