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Original Article

Expression and prognostic potential of TMEM204: a pan-cancer analysis

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Abstract: TMEM204 (Transmembrane Protein 204) is a member of the TMEM family that regulates cell function and angiogenesis. Previous studies showed that TMEM204 is related to pancreatic cancer, but its roles in other cancers remain unknown. To reveal this relationship, we conducted a pan-cancer analysis by several online databases. The expression of TMEM204 was analyzed by Oncomine and Tumor Immune Estimation Resource2.0 (TIMER2.0). The prognostic potential of TMEM204 was evaluated by the GEPIA2, UALCAN, and OncoInc. The methylation level of gene expression was analyzed by UALCAN, and the relationship between cancer and immune invasion was displayed by TIMER2.0. The Protein-Protein Interactions Network and functional analysis of TMEM204 and its related genes were conducted by STRING and Webgestalt. We found that TMEM204 expression was up-regulated and correlated with prognosis in multiple cancers. In liver hepatocellular carcinoma (LIHC), high TMEM204 expression was associated with a good prognosis, and with high infiltrating levels of $CD8^+$ T and $CD4^+$ T cells, macrophages, neutrophils, and myeloid dendritic cells. In addition, the methylation level in LIHC was higher than in normal tissues. p53 signaling pathway and Fanconi anemia pathway were implicated by KEGG pathway analysis. These results indicate that TMEM204 is associated with the prognosis, methylation, and immune invasion of cancers, especially LIHC. TMEM204 may act as a prognostic marker of LIHC and its role in other cancers should be studied.

Keywords: Expression, pan-cancer analysis, methylation, prognostic biomarker, TMEM204

Introduction

Cancer is the second leading cause of death in the United States [1]. It is worth noting that lung cancer is the most common cancer and the main cause of cancer death in men, while breast cancer is the most commonly diagnosed cancer in women and a major cause of cancer death [2]. Although surgery, chemotherapy, radiotherapy, immunotherapy, and traditional Chinese medicine treatment have been widely used in anti-tumor therapy, these methods are not enough to fight cancer [3]. An approximate 19.3 million new cases of cancer (18.1 million excluding nonmelanoma skin cancer) and about 10.0 million cancer deaths (9.9 million excluding nonmelanoma skin cancer) occurred in 2020 [4]. By 2030, it is estimated that the number of patients will increase by 50% to 21.6 million every year [5]. Therefore, it is urgent and significant to explore the pathogenesis of tumors and find new therapeutic targets.

TMEM (transmembrane protein) is a protein that straddles the lipid bilayer and is fixed permanently in it [6]. Plasma membrane proteins communicate with cells and the cell environment through the immobilization of ligands and receptors or the internalization of small biomolecules [7]. In recent years, the functions of TMEM family members have been discovered. Rare mutations in TMEM163 and TMEM175 may increase the risk of Parkinson's disease [8]. The expression of TMEM is also associated with tumors. For example, TMEM25 is a biomarker for colorectal cancer [9]. In renal cell carcinoma, TMEMs were identified as potential cancer grading classifiers [10].

TMEM204 protein contains four predicted transmembrane domains and a C-terminal protein-protein interaction domain. It is a novel hypoxia-regulated tetramer adhesion junction protein, which regulates cell adhesion, cell bypass permeability, and angiogenesis [11]. The expression of TMEM204 in endothelial-specific transcripts in developing endothelial cells was higher than that in normal endothelial cells in the control group, indicating that TMEM204 plays an important role in adult angiogenesis [12]. In addition, meta array and methylation-specific PCR analysis showed that 95% of TMEM204 in three typical pancreatic cancer cell lines AsPC-1, Mia PaCa-2, and PANC-1, was methylated [13]. The methylation level of TMEM204 was up-regulated in advanced non-alcoholic fatty liver disease [14]. Moreover, variations in the DNA methylation level of TMEM204 may indicate a change of metabolism or immune pathway during carcinogenesis [15]. These findings suggest that TMEM204 is related to cancers, especially regarding methylation level and immune infiltration. However, few studies have focused on TMEM204, and the specific mechanism is not clear. Therefore, it is necessary to further explore the role of TMEM204 in tumorigenesis and prognosis.

The pan-cancer analysis is a comprehensive method to investigate the roles of a specific gene or a group of genes in different kinds of cancer. One of the sources of data used for pan-cancer analysis is The Cancer Genome Atlas (TCGA) Research Network, which has profiled and analyzed large numbers of human tumors to discover molecular aberrations at the DNA, RNA, protein, and epigenetic levels [16, 17]. TCGA data combined with other published data can be analyzed by different tools for different purposes. Expression, methylation level, and survival are the most common indices in pan-cancer analysis.

In this study, we analyzed the expression, methylation levels, and survival curves of TMEM204 in different cancers by Oncomine, GEPIA2, UALCAN, and other databases. We discovered a correlation between TMEM204 and tumor-infiltrating immune cells in different tumor microenvironments by TIMER2.0. To analyze the functions of the TMEM204 network, we constructed a PPI network and performed pathway enrichment analysis. Our findings revealed a correlation between the expression of TMEM204 and tumorigenesis in liver hepatocellular carcinoma (LIHC), and provide new insight into research on tumor biomarkers.

Materials and methods

Oncomine database analysis

Oncomine database (https://www.oncomine.org/) collects, standardizes, analyzes cancer transcriptome data, and provides the data to the biomedical research community. It has identified genes, pathways, and networks in 18000 cancer gene expression microarrays and the database has a total of 86,733 samples of cancer tissue and normal tissue [18]. mRNA expression in different cancers and normal tissues was analyzed by the Oncomine database. Meta-analysis was used for statistical tests. The threshold was determined according to the following values: *P*-value of 0.001, fold change of 1.5, and gene ranking of all.

TIMER2.0 database analysis

Tumor Immune Estimation Resource (TIMER2.0) (https://cistrome.shinyapps.io/timer/) comprehensively studied the levels of 6 tumor-infiltrating immune subsets in 10897 tumors of 32 cancer types. It allows users to interactively explore the relationship between immune infiltration and broad-spectrum diseases [19]. Through this database, the correlation between expression and prognosis of TMEM204 and tumor immune infiltrates (including B cells, $CD4^+$ T cells, $CD8^+$ T cells, neutrophils, macrophages, and dendritic cells) was analyzed.

GEPIA2 analysis

Gene Expression Profile Interaction Analysis 2 (GEPIA2) (http://gepia2.cancer-pku.cn/) is a generic interactive website for drawing specific gene expression profiles. GEPIA2 contains 198,619 isoforms and 84 cancer subtypes from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) databases [20]. We used GEPIA2 to investigate the expression of TMEM204 in different cancers and the survival curves of cancer. The lower percentile and upper percentile were both set to 50. The HR and *P* or Cox *P* values from a log-rank test were used to determine the threshold.

UALCAN database analysis

Ualcan (http://ualcan.path.uab.edu) is an interactive tool for in-depth analysis of TCGA gene expression data, including the pathologic characteristics of different tumor subgroups and the impact on the survival rate of patients. This resource provides a platform for the verification of target genes and the identification of tumor subgroups [21]. We compared the expression of TMEM204 based on histologic subtype and gender. TPM (Transcripts Per Million) value was employed to estimate the *P*-value using Student's T-test, and $P < 0.0001$ was marked with ***. We also used this platform to explore the effect of TMEM204 expression on the survival of patients with different tumor subgroups. The top 25% was identified as high expression, and the rest as low expression. For methylation analysis, the Beta value is the ratio of the methylated probe intensity to the sum of methylated and unmethylated probe intensity, which indicates the level of DNA methylation ranging from 0 (unmethylated) to 1 (fully methylated). Different beta value cut-offs have been considered to indicate hypermethylation (Beta value: 0.7-0.5) or hypomethylation (Beta-value: 0.3-0.25). The boxplot represents the Beta values of CpG probes located up to 1500 bp upstream of the gene's start site.

Oncolnc database analysis

Oncolnc (http://www.oncolnc.org/) includes the survival data of 8647 patients with 21 kinds of cancer and can generate Kaplan-Meier graphs to further analyze the survival information [22]. In this study, we used the Oncolnc database to obtain an association between TMEM204 expression and survival prognosis. The lower percentile and upper percentile were both 50.

cBioPortal database analysis

The cBioPortal for Cancer Genomics (http://cbioportal.org) is a website for analyzing and visualizing cancer genomics data, transforming complex cancer tissue and molecular data into easily understanding graphs [23]. On this website, we estimated the gene mutations, deletions, mRNA upregulation, and mRNA downregulation, and survival curves were conducted to explore the genetic changes in genes. For gene alternation analysis, 729 LIHC samples from TCGA and other published data were included [24-28].

STRING database analysis

Search Tool for the Retrieval of Interacting Genes (STRING) (http://string-db.org/) is a database to provide protein-protein interactions including direct (physical) and indirect (functional) connections. It contains more than 2000 organisms, and can transfer interaction information between organisms [29]. We used STRING to construct a protein-protein interaction network (PPI) of TMEM204.

Gene Ontology and KEGG pathway enrichment analysis

Gene Ontology (GO) is a functional classification system that comprehensively describes the characteristics of genes and their products in organisms, and provides dynamically updated control vocabulary and strictly defined concepts [30]. GO annotation has been proven to be a reliable predictor of cancer genes [31]. Kyoto Encyclopedia of Genes and Genomes (KEGG) is a database that contains genes and their biochemical functions [32]. In this study, the online tool WebGestalt (http://webgestalt.org/) was used to analyze the functions of TMEM204. We discovered biological pathways that played key roles in tumorigenesis through functional enrichment analysis.

Statistical analysis

The results generated from Oncomine were displayed with *P*-values, fold changes, and ranks. Results from GEPIA2 and UALCAN were displayed with HR and *P* or Cox *P*-values from a log-rank test. Spearman correlation and statistical significance were used to evaluate the correlation of gene expression. *P*-value < 0.05 was indicative of a significant difference.

Results

TMEM204 expression levels in various types of cancer

To analyze the expression of TMEM204 in tumor and normal tissues, we used the Oncomine database to present the TMEM204 expression profile. The total unique expression of the database was 381, and the significant unique expression was 44, including 19 high

expressions and 24 low expressions. Compared to normal tissues, the expression was higher in breast cancer, melanoma, and pancreatic cancer, but lower in bladder cancer, kidney cancer, lung cancer, and myeloma (**Figure 1A**). The results showed that the TMEM204 expression was diverse in different cancers.

In addition, we further analyzed the TMEM204 expression in the TIMER2.0 database and found that TMEM204 was expressed differently in different tumors. TMEM204 was highly expressed in CHOL (Cholangiocarcinoma), GBM (Glioblastoma multiforme), HNSC (head and neck squamous cell carcinoma), KIRC (Kidney renal clear cell carcinoma), and LIHC (Liver hepatocellular carcinoma). In contrast, TMEM204 expression in BRCA (Breast invasive carcinoma), CESC (cervical squamous cell carcinoma and endocervical adenocarcinoma), KICH (Kidney chromophobe), KIRP (kidney renal papillary cell carcinoma), LUAD (lung adenocarcinoma), LUSC (lung squamous cell carcinoma) and UCEC (uterine corpus endometrial carcinoma) was significantly lower than that of normal tissues (**Figure 1B**).

Prognostic potential of TMEM204 in cancers

The prognostic value of TMEM204 in different cancers was analyzed based on the GEPIA2. Results are shown in Supplementary Figure 1. Results revealed that high TMEM204 expression levels were related to poor OS in BLCA (Log-rank P=0.044, HR=1.4) and LUSC (Log-rank P=0.008, HR=1.4). However, high expression of TMEM204 can lead to good prognosis of OS and DFS in KIRC (OS Log-rank P= 0.00013, HR=0.55; DFS Log-rank P=0.019, HR=0.65) and LIHC (OS Log-rank P=0.0061, HR=0.61; DFS Log-rank P=0.016, HR=0.69) (**Figure 2A-F**). After that, we searched for the relationship between TMEM204 and prognosis in the UALCAN database and the results are shown in Supplementary Figure 2. We found that there was a significant correlation between TMEM204 expression and prognosis in COAD (P=0.0032), KIRC (P=0.0029), KIRP (P=0.0029), and LIHC (P=0.028) (**Figure 2G-J**). Finally, we evaluated the relationship between TMEM204 and prognosis in the OncoInc database and the results are shown in Supplementary Figure 3. We obtained the graph related to the prognosis of KIRC (P=0.000212), LIHC (P=0.000298), LUSC (P=0.0248), and STAD (P=0.00588) (**Figure 2K-N**). In conclusion, TMEM204 expression in different tumors led to different prognoses. Clearly TMEM204 was significantly associated with a good prognosis in LIHC.

Further study on TMEM204 expression in LIHC

To further verify TMEM204 expression in cancer, we obtained box plots in LIHC through the UALCAN database. From the box plot, TMEM204 expression in LIHC tissues was significantly higher than in normal tissues (**Figure 3A**). After that, we investigated the expression of TMEM204 based on histologic subtype and gender. TMEM204 expression was higher in all histologic subtypes including hepatocellular carcinoma, fibrolamellar carcinoma, and hepatocholangiocarcinoma (**Figure 3B**). TMEM204 expression was also higher in LIHC patients of both genders (**Figure 3C**). Furthermore, in the Oncomine database, the same result was obtained (**Figure 3D**). Therefore, it can be concluded that TMEM204 was overexpressed in LIHC compared to normal tissues. These results indicate TMEM204 may be a diagnostic biomarker for LIHC.

Promoter methylation levels of TMEM204 in cancers

We further analyzed the relationship between TMEM204 expression and promoter methylation level of LIHC through the UALCAN database. We found that the promoter methylation level of TMEM204 in LIHC was higher than in normal tissues (**Figure 4**). In addition, TMEM204 promoter expression was linked to gender in LIHC. Thus, high promoter DNA methylation may lead to high expression of TMEM204 in LIHC.

Gene alterations in TMEM204 in cancer tissue

The oncoprint map of cBioportal showed that the mutation rates of LIHC in cancer were 0.5% (**Figure 5A**). The data in all mutations of TMEM204 in LIHC were summarized: there was a missense mutation (**Figure 5B**). In hepatocellular carcinoma. Deep deletion accounts for the majority and the rest are mutations (**Figure 5C**). We also made the survival curves for cancer gene mutations. However, there was no significant relationship between LIHC survival and TMEM204 mutation (**Figure 5D**).

TMEM204 expression is related to immune infiltration level

We used the TIMER2.0 database to explore the relationship between the expression of

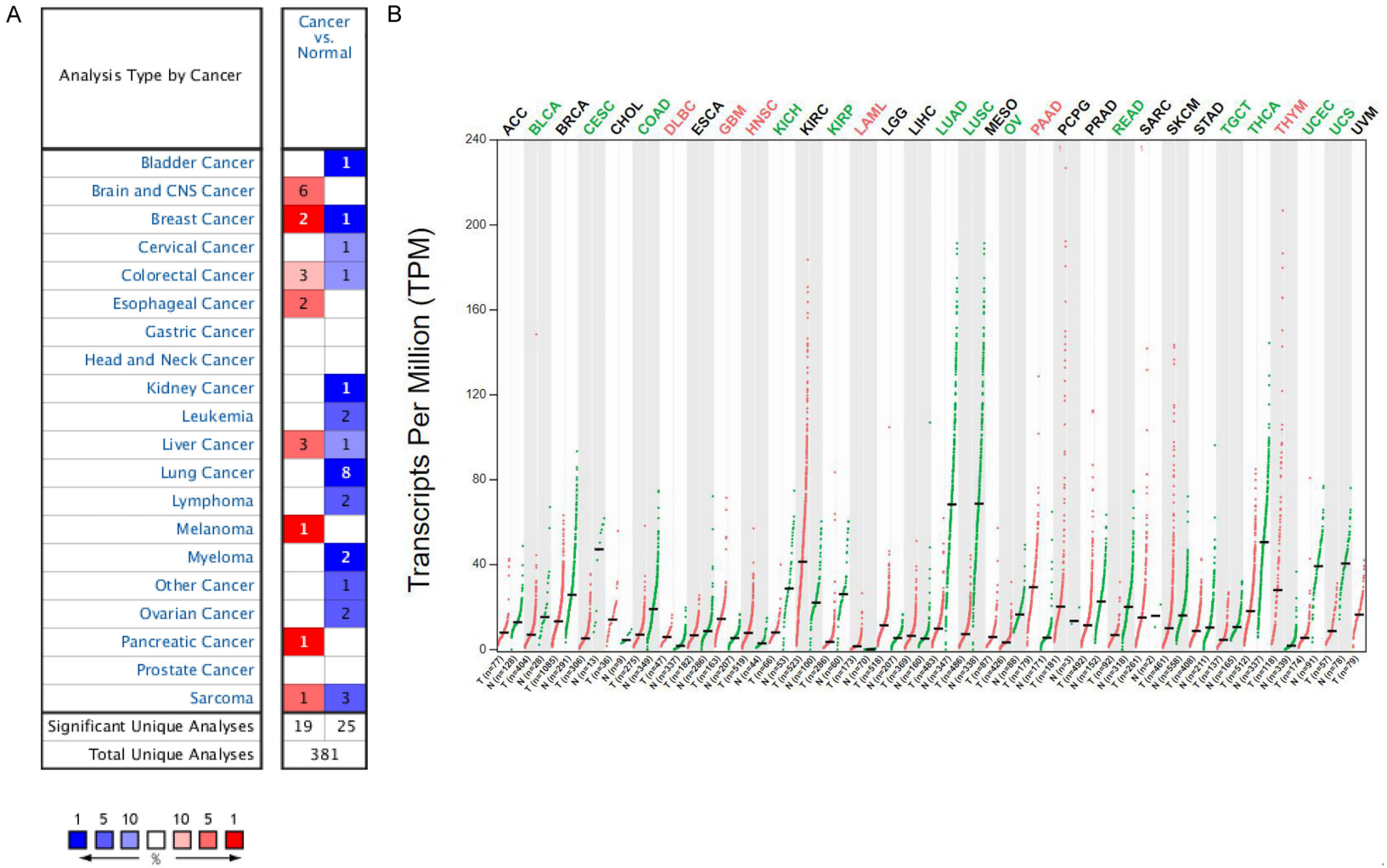

Figure 1. TMEM204 expression in cancers. A. TMEM204 data showing an increase or decrease in various cancers compared to normal tissues in the Oncomine database. B. TMEM204 expression profiles in all tumor samples and matched normal tissues were detected by TIMER2.0. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

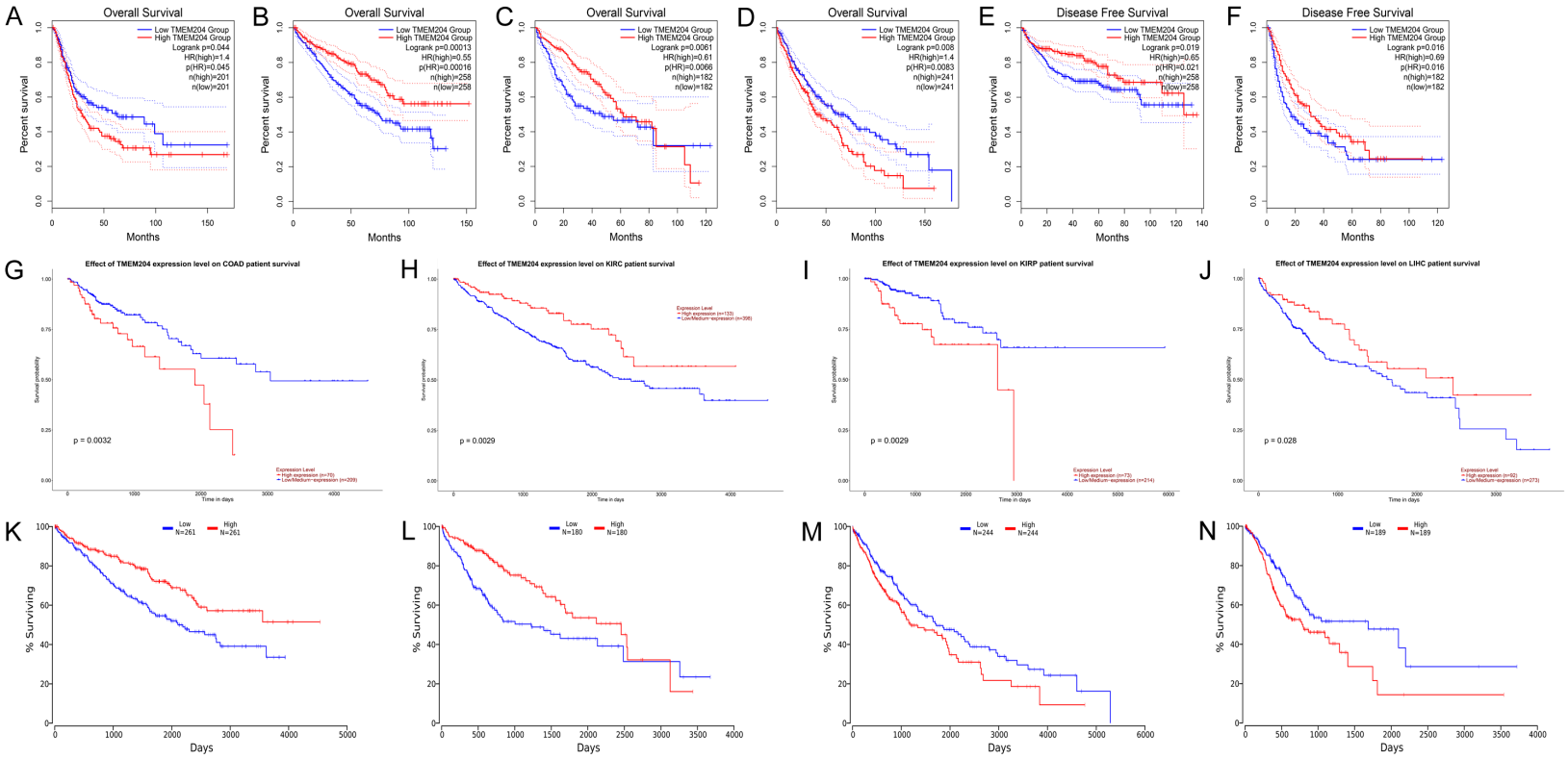

Figure 2. Survival curves of TMEM204 in different cancers obtained through GEPIA2, UALCAN, and Oncolnc databases. GEPIA2: (A-F), UALCAN: (G-J), Oncolnc: (K-N). (A) OS survival curve of BLCA (n=402). (B, E) OS and DFS survival curves of KIRC (n=516). (C, F) OS and DFS survival curves of LIHC (n=364). (D) OS survival curves of LUSC (n=482). (G-J) Survival curves of COAD, KIRC, KIRP, and LIHC in UALCAN. (K-N) Survival curve of KIRC, LIHC, LUSC, and STAD in Oncolnc.

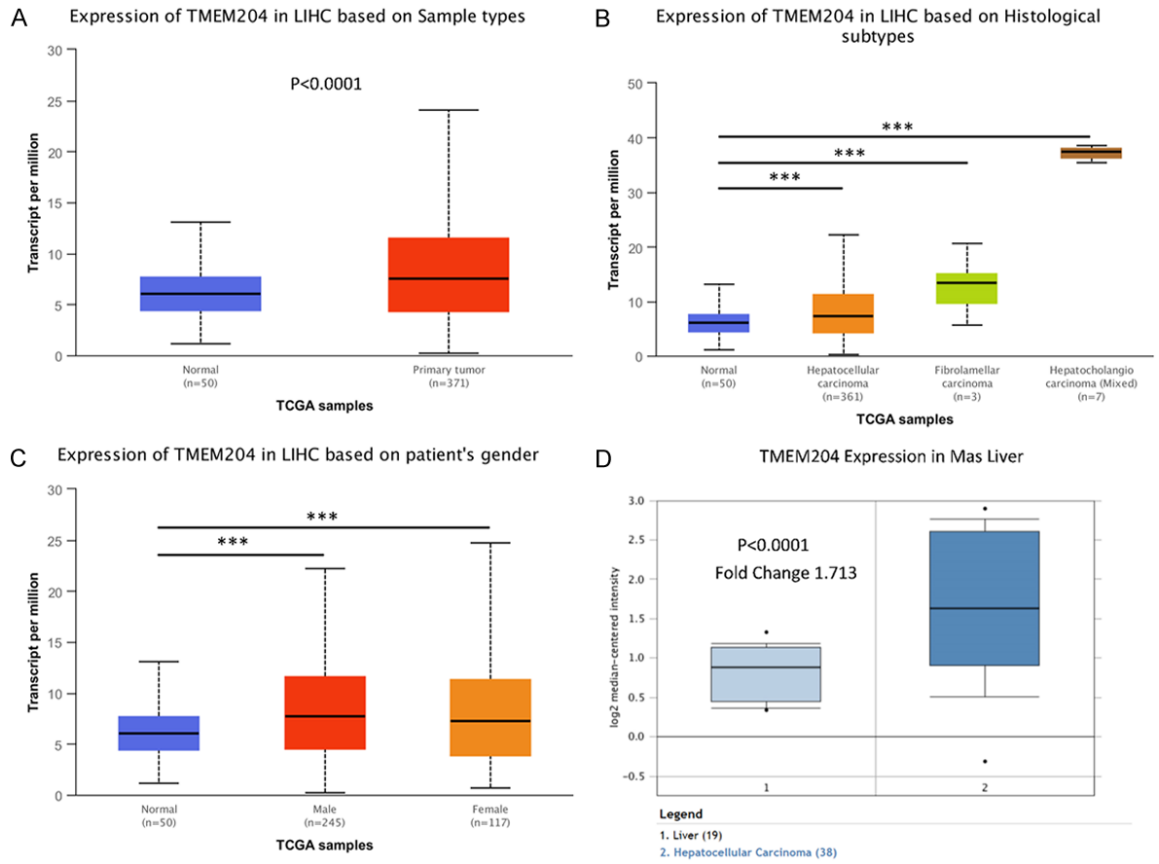

Figure 3. Box plot of TMEM204 expression levels in tumors based on UALCAN and Oncomine database. A. The box plot shows the relative expression of TMEM204 in normal and LIHC tissue. B. The box plot shows the relative expression of TMEM204 in LIHC based on histologic subtype. C. The box plot shows the relative expression of TMEM204 in LIHC based on gender. D. The box plot shows the relative expression of TMEM204 in the liver and hepatocellular carcinoma. ***P < 0.001.

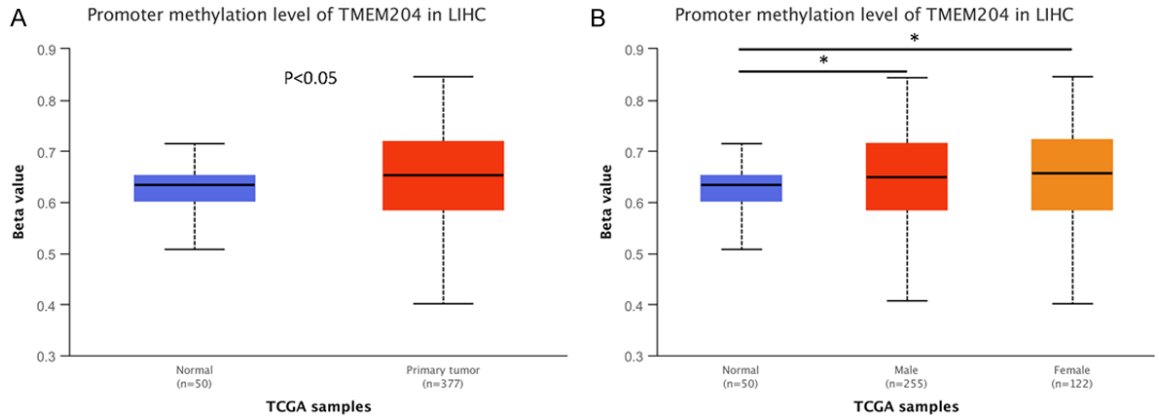

Figure 4. Promoter methylation level of TMEM204 in LIHC through UALCAN. *P < 0.05.

TMEM204 and immune cell infiltration in LIHC. TMEM204 expression was significantly correlated with $CD8^+$ T cells (Rho=0.202, P=1.58e-04), $CD4^+$ T cells (Rho=0.256, P=1.39e-06), macrophages (Rho=0.203, P=1.48e-04), neutrophils (Rho=0.157, P=3.56e-03) and myeloid dendritic (Rho=0.193, P=3.04e-04) cell infiltration (**Figure 6**).

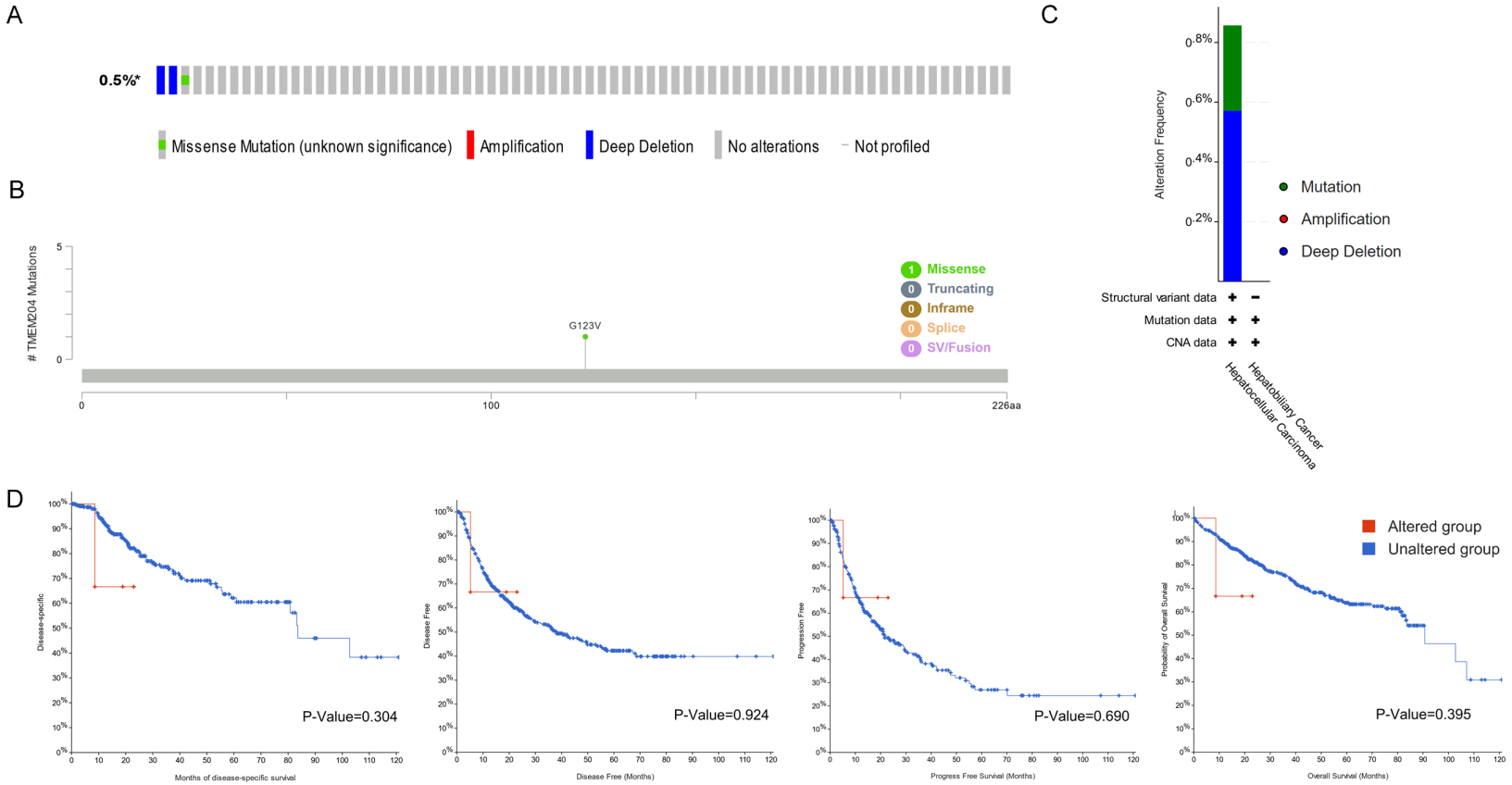

Figure 5. Gene alterations in TMEM204 in LIHC tissue. A. Showing mutations in TMEM204 in LIHC. B. Details of all mutations in LIHC. C. Frequency of genetic alterations in LIHC. D. Disease-specific survival curve, disease-free survival curve, progression-free survival curve, and overall survival curve of gene alterations in LIHC.

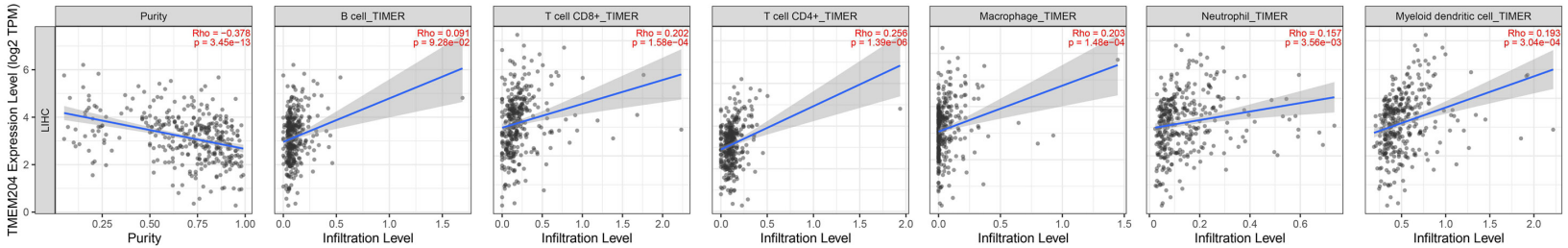

Figure 6. Correlation of TMEM204 expression with immune infiltration level in LIHC.

PPI network and functional enrichment analysis of TMEM204

In the PPI network, colored nodes were query proteins and the first shell of interactors; white nodes were the second shell of interactors. Yellow links stood for text-mining, black links stood for co-expression, purple links stood for experimentally determined. We found 20 proteins closely related to TMEM204 proteins through the PPI network in the STRING database (**Figure 7A**). Among them, KLHDC8B, KBTBD12, ATR, TRAPPC6B, and CCDC36 showed the highest scores of interaction with TMEM204. Through GO and KEGG analysis of WebGestalt, the enrichment map of predicted gene functions was obtained. The cell organization component was the most correlated biologic progress of the 21 genes. The cell membrane accounted for the majority of the cellular component. Protein binding took the first place in the molecular function category (**Figure 7B**). GO terms from GO analysis were relevant to the proliferation and differentiation of cells. In addition, the p53 signaling pathway and Fanconi anemia pathway were in the top positions of KEGG pathway analysis of 21 genes (**Figure 7C**). Detailed data of KEGG and GO analysis are shown in **Table 1** and Supplementary Table 1, respectively.

Discussion

Cancer continues to be one of the most important causes of death in the world [33]. Liver hepatocellular carcinoma (LIHC) is the most common primary liver cancer, and it is also one of the main causes of death in patients with liver cirrhosis [34]. LIHC is an aggressive and complex cancer. The pathologic manifestations include changes in tumor cell behavior and the vascular system [35]. At present, the curative effect of surgical treatment is much better for early cancer. Because of the high risk of recurrence and metastasis, late-stage patients who cannot be operated on may have a poor therapeutic effect of chemoradiotherapy [36]. In clinical practice, new targeted drugs are seen to provide exciting therapeutic effects. In sum, the discovery of tumor markers may provide new insight into the therapy of cancer, and improve the survival in cancer.

Previous studies had shown that the TMEM204 genomic region was associated with the risk of pancreatic cancer. DNA methylation in this region was more closely associated with the risk of pancreatic cancer in lymphocyte subtypes, especially for $CD4^+$ and $CD8^+$ T cells [36]. Some studies had also shown that TMEM204 was related to angiogenesis, which suggested that TMEM204 may be related to the occurrence, development, survival, and prognosis of cancers. Therefore, we carried out research on TMEM204, explored its mechanisms in cancers, and analyzed the relationship between TMEM204 and cancer prognosis based on online databases.

In this study, we investigated the expression of TMEM204 in multiple tumors and normal tissues using the Oncomine and the TIMER2.0 databases. This revealed that expression of TMEM204 was significantly different in various types of cancer compared to normal tissues. We found that compared to the normal tissues, the expression was higher in breast cancer, melanoma, and pancreatic cancer, but significantly lower in bladder cancer, kidney cancer, lung cancer, and myeloma by Oncomine. TMEM204 expression was highly expressed in CHOL, GBM, HNSC, KIRC, LIHC and lower in BLCA, BRCA, CESC, KICH, KIRP, LUAD, LUSC, and UCEC compared with normal tissues by TIMER2.0. Afterward, we assessed the correlation between TMEM204 expression and prognosis of cancers through GEPIA2, UALCAN, and Oncolnc databases, and TMEM204 was significantly associated with good prognosis in LIHC. Therefore, further analyses of TMEM204 expression in LIHC were conducted through UALCAN and Oncomine. The expression of TMEM204 in LIHC was significantly higher in LIHC compared to normal tissue in both UALCAN and Oncomine databases. Using these results, we suggested that TMEM204 may act as a biomarker of LIHC for good prognosis.

For further investigation of the mechanisms of TMEM204 affecting the progress of LIHC, we analyzed the relationship between TMEM204 expression and promoter methylation level of LIHC through the UALCAN database. We found that the promoter methylation level of TMEM204 in LIHC was higher than in normal tissues. A previous study indicated that hypomethylation can result in oncogene activation or chromosomal instability in cancers [37], therefore, the results from investigating promoter methyl-

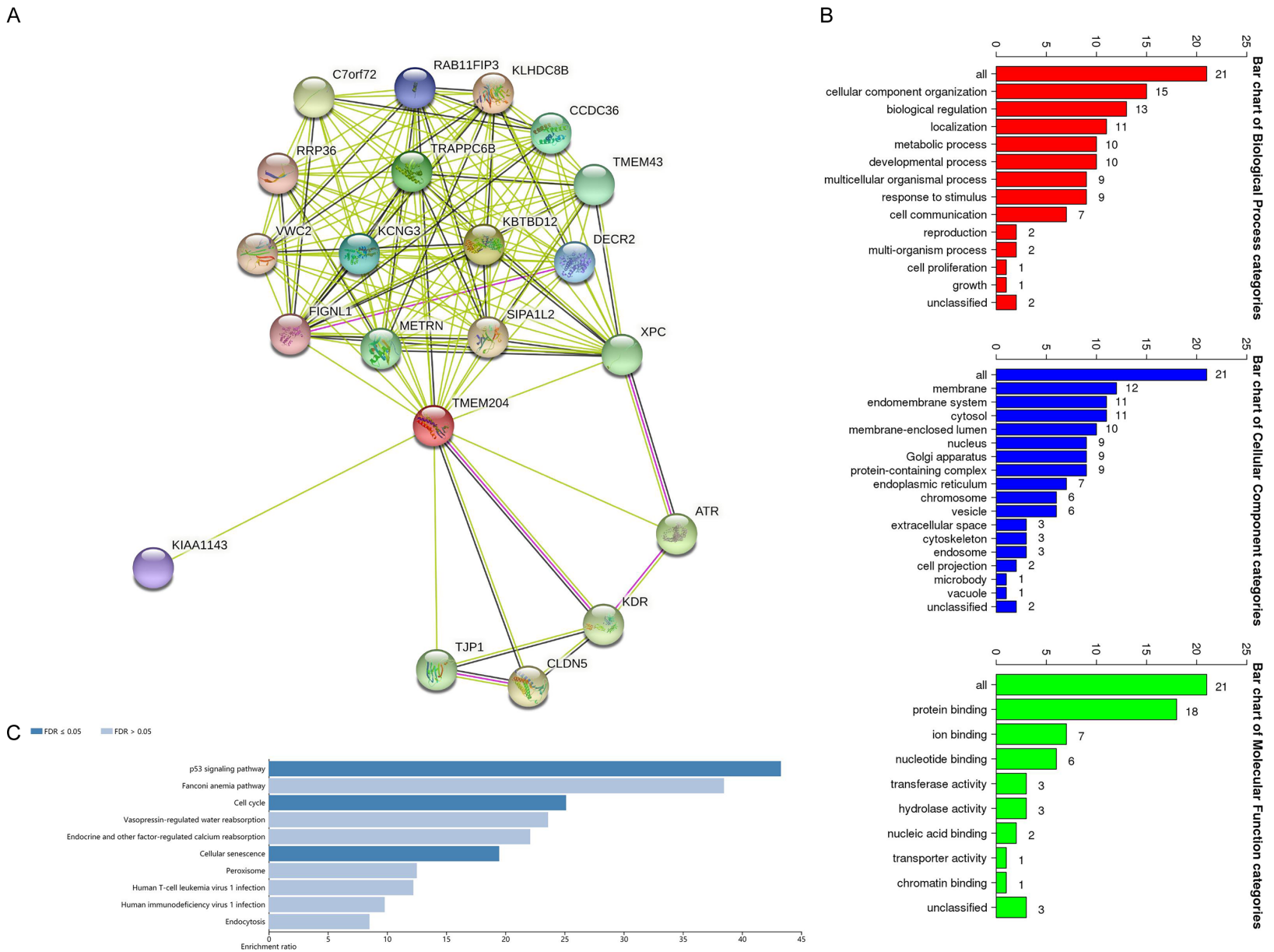
A
C7orf72
RAB11FIP3
KLHDC8B
CCDC36
RRP36
TRAPPC6B
TMEM43
VWC2
KCNG3
KBTBD12
DECR2
FIGNL1
METRN
SIPA1L2
XPC
TMEM204
ATR
KIAA1143
KDR
TJP1
CLDN5
B
all 21
cellular component organization 15
biological regulation 13
localization 11
metabolic process 10
developmental process 10
multicellular organismal process 9
response to stimulus 9
cell communication 7
reproduction 2
multi-organism process 2
cell proliferation 1
growth 1
unclassified 2
Bar chart of Biological Process categories
all 21
membrane 12
endomembrane system 11
cytosol 11
membrane-enclosed lumen 10
nucleus 9
Golgi apparatus 9
protein-containing complex 9
endoplasmic reticulum 7
chromosome 6
vesicle 6
extracellular space 3
cytoskeleton 3
endosome 3
cell projection 2
microbody 1
vacuole 1
unclassified 2
Bar chart of Cellular Component categories
all 21
protein binding 18
ion binding 7
nucleotide binding 6
transferase activity 3
hydrolase activity 3
nucleic acid binding 2
transporter activity 1
chromatin binding 1
unclassified 3
Bar chart of Molecular Function categories
C
FDR ≤ 0.05
FDR > 0.05
p53 signaling pathway
Fanconi anemia pathway
Cell cycle
Vasopressin-regulated water reabsorption
Endocrine and other factor-regulated calcium reabsorption
Cellular senescence
Peroxisome
Human T-cell leukemia virus 1 infection
Human immunodeficiency virus 1 infection
Endocytosis
Enrichment ratio

Figure 7. PPI network and functional enrichment of TMEM204. A. PPI network of TMEM204-related genes was constructed in STRING. B. Biological processes, cell composition, and molecular function map related to TMEM204. C. KEGG pathway figure of TMEM204.

Table 1. KEGG pathways associated with TMEM204

Gene Set	description	size	overlap	expect	enrichment Ratio	*P* Value	FDR
hsa04115	p53 signaling pathway	72	3	0.069316	43.27976	3.17E-05	0.010327
hsa04110	Cell cycle	124	3	0.119378	25.13018	1.61E-04	0.026275
hsa04218	Cellular senescence	160	3	0.154037	19.47589	3.43E-04	0.037274
hsa03460	Fanconi anemia pathway	54	2	0.051987	38.4709	0.00111	0.08761
hsa05166	Human T-cell leukemia virus 1 infection	255	3	0.245496	12.22017	0.001344	0.08761
hsa05170	Human immunodeficiency virus 1 infection	212	2	0.204098	9.799191	0.016132	0.876489
hsa04144	Endocytosis	244	2	0.234906	8.514052	0.021068	0.981159
hsa04962	Vasopressin-regulated water reabsorption	44	1	0.04236	23.60714	0.041616	1
hsa04961	Endocrine and Other factor-regulated calcium reabsorption	47	1	0.045248	22.1003	0.044398	1
hsa04146	Peroxisome	83	1	0.079906	12.51463	0.077252	1

ation of TMEM204 suggest that TMEM204 may affect the prognosis of LIHC patients through hypermethylation.

Tumor cells usually acquire genetic and epigenetic alterations that always lead to oncogene dysregulation and widespread changes in gene expression [38]. The results from cBioPortal revealed that the mutation rates of LIHC in cancer were 0.5%. There was just a missense mutation of TMEM204 in LIHC and deep deletion accounted for the majority of mutation. Genetic mutations always cause phenotypic changes, which are usually related to carcinogenesis and aging [39]. Thus, analysis of TMEM204 alteration is crucial in understanding the roles of TMEM204 in LIHC and for therapy design.

In cancer patients, the degree of immune infiltration in the tumor can affect the survival rate and lymph node metastasis [40]. Hence, we used TIMER2.0 to explore the relationship between the expression of TMEM204 and immune cell infiltration in LIHC. These results indicated that the TMEM204 expression was significantly correlated with $CD8^+$ T cells (Rho=0.202, P=1.58e-04), $CD4^+$ T cells (Rho=0.256, P=1.39e-06), macrophages (Rho=0.203, P=1.48e-04), neutrophils (Rho=0.157, P=3.56e-03) and myeloid dendritic cell (Rho=0.193, P=3.04e-04) infiltration. Cancer immune surveillance, involving types of immune cells, is critical protection to inhibit tumorigenesis by means of identifying and destroying newly abnormal transformed cells. Our findings suggested that the TMEM204 plays an important role in the recruitment and regulation of immune infiltrating cells in LIHC, which may be related to the functions of identifying DNA damage and maintaining chromosomal stability mentioned below. However, the specific mechanisms of TMEM204 affecting immune infiltrating cells in LIHC are uncertain, and future studies are needed.

By PPI network analysis, twenty proteins with the highest degrees of communication with TMEM204 were identified. They were ATR, ATRIP, CCDC36, CHEK1, CHEK2, DECR2, FIGNL1, KBTBD12, KCNG3, KIAA1143, KLHDC8B, RAB11A, RAB11FIP3, RAD17, TRAPPC1, TRAPPC2, TRAPPC3, TRAPPC4, TRAPPC5, TRAPPC6B. Among them, KLHDC8B, KBTBD12, ATR, TRAPPC6B, CCDC36 showed the highest scores of interaction with TMEM204. KLHDC8B, expressed during mitosis, is essential for mitotic integrity and maintenance of chromosomal stability. There has been no relative study focusing on KBTBD12. TRAPPC6B is a component of TRAPP complexes, which are tethering complexes involved in vesicle transport [41]. CCDC36 had been identified as a direct interactor with HORMAD1, which is essential for DNA double-strand break (DBS) formation [42]. ATR could be activated to various types of DNA lesions through interacting with single-stranded DNA (ssDNA) [43]. Results from the PPI network imply that TMEM204 may be involved in the regulation of the progression of cancer. From KEGG pathway analysis, the p53 signaling pathway and Fanconi anemia pathway were

implicated among the 21 genes. The p53 signaling pathway plays a key role in tumor suppression [44] and the Fanconi anemia pathway is a fundamental DNA repair pathway that identifies DNA damage and orchestrates DNA damage response [45]. Combined with PPI network analysis, these findings strongly suggested TMEM204 inhibits tumorigenesis through identifying DNA damage, orchestrating DNA damage responses, and maintaining chromosomal stability.

There are some limitations in our study. First, the results in this study were based on the online database without verification of clinical samples. Secondly, there was more than one data source in the online databases, which may introduce error because of differences in pipelines, processing, or manual review. For example, GEPIA2 combines data from TCGA and GTEx, while cBioPortal integrates data from TCGA and published articles. Third, the different thresholds of the databases may conflict. In the survival analysis, the upper percentile of high expression was 50 in GEPIA2 and Oncolnc, while it was 25 in UALCAN. Future studies may include the verification of tumor samples from LIHC patients, and provide more reliable results.

Conclusions

We conducted a systemic analysis of the expression and the prognostic value of TMEM204 in multiple types of cancer. These results suggested that TMEM204 overexpression was significantly correlated with good prognostic values in LIHC patients. However, further experimental validation should be performed to identify the biological roles of TMEM204 in LIHC.

Data availability statement

All data in our study are available by online databases (Supplementary Table 2).

Acknowledgements

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Disclosure of conflict of interest

None.

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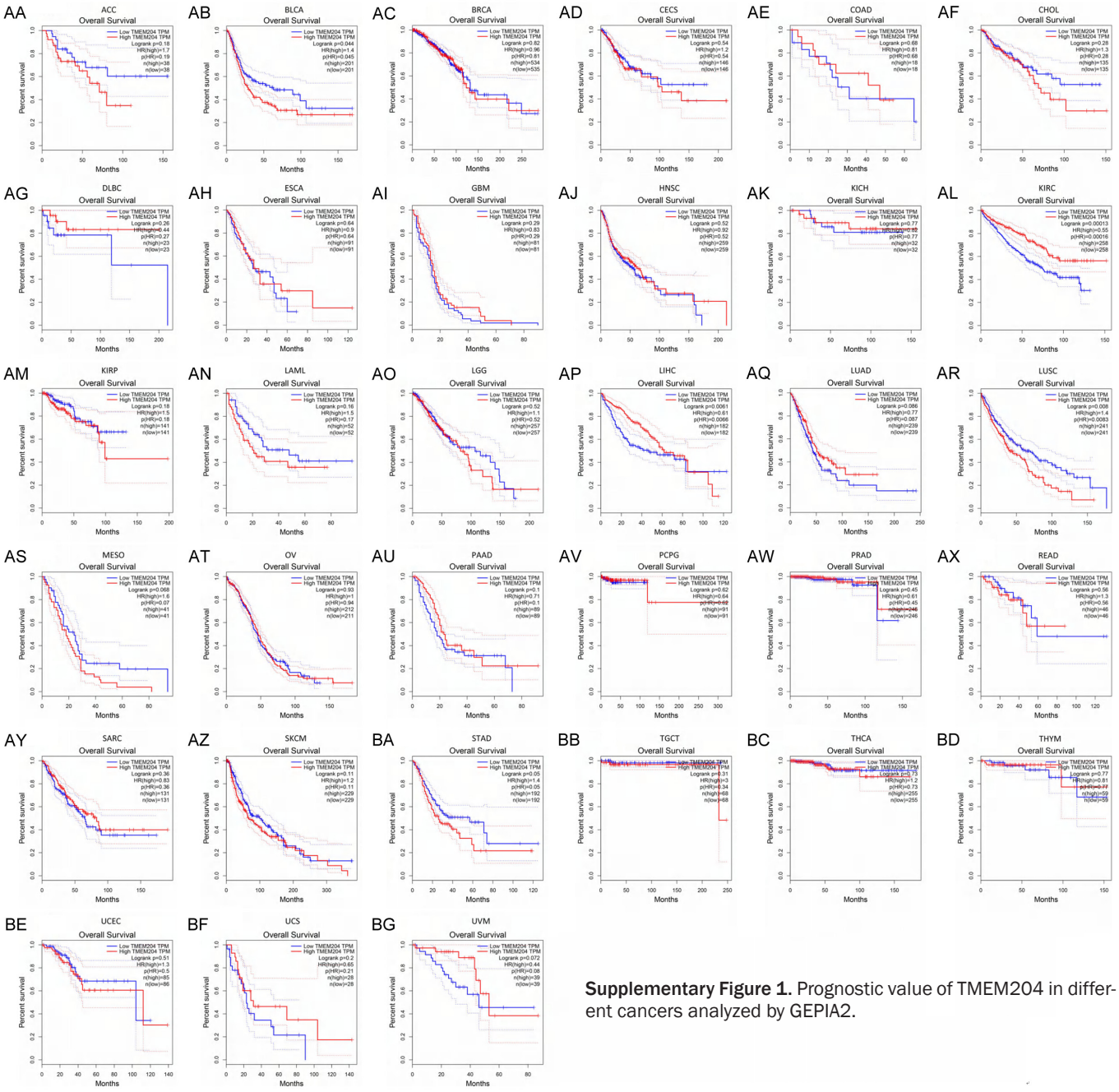
TMEM204 in pan-cancer analysis



Supplementary Figure 1. Prognostic value of TMEM204 in different cancers analyzed by GEPIA2.

TMEM204 in pan-cancer analysis

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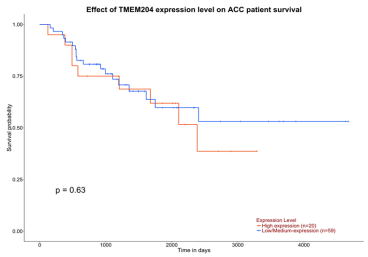

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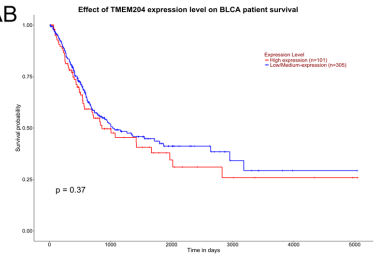

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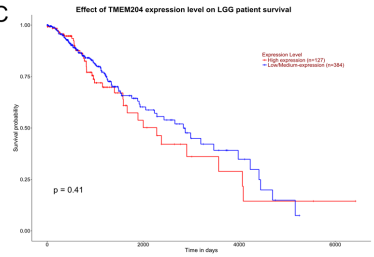

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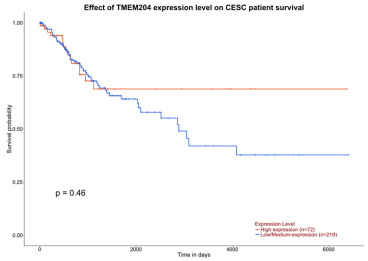

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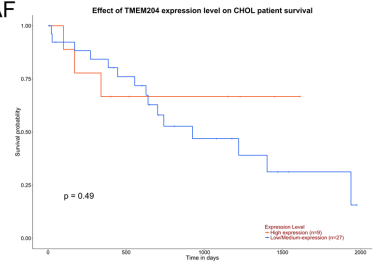

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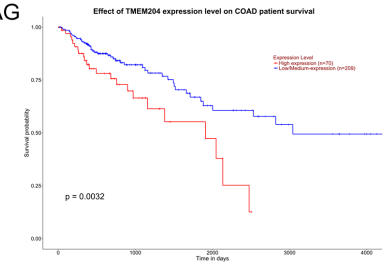

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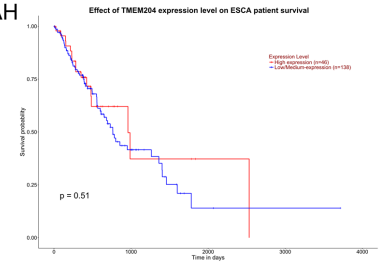

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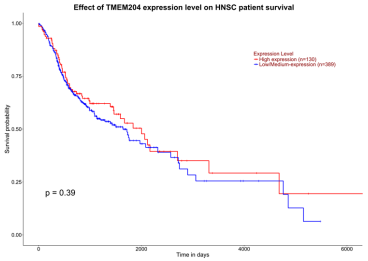

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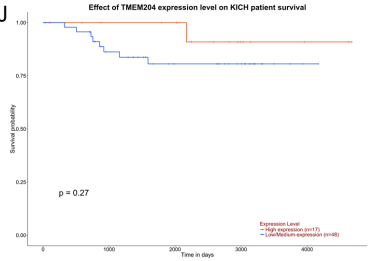

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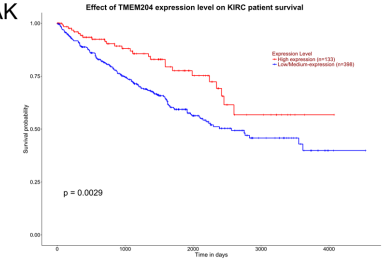

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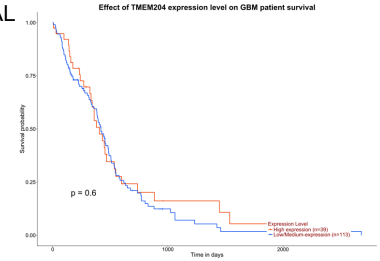

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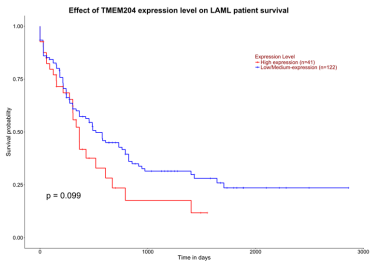

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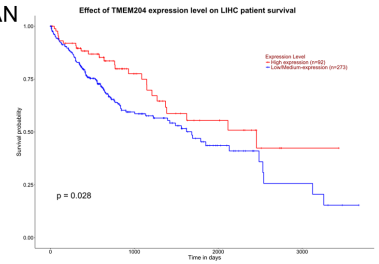

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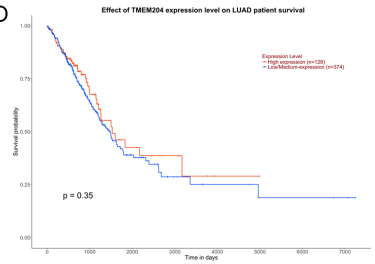

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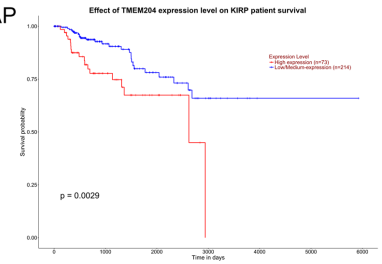
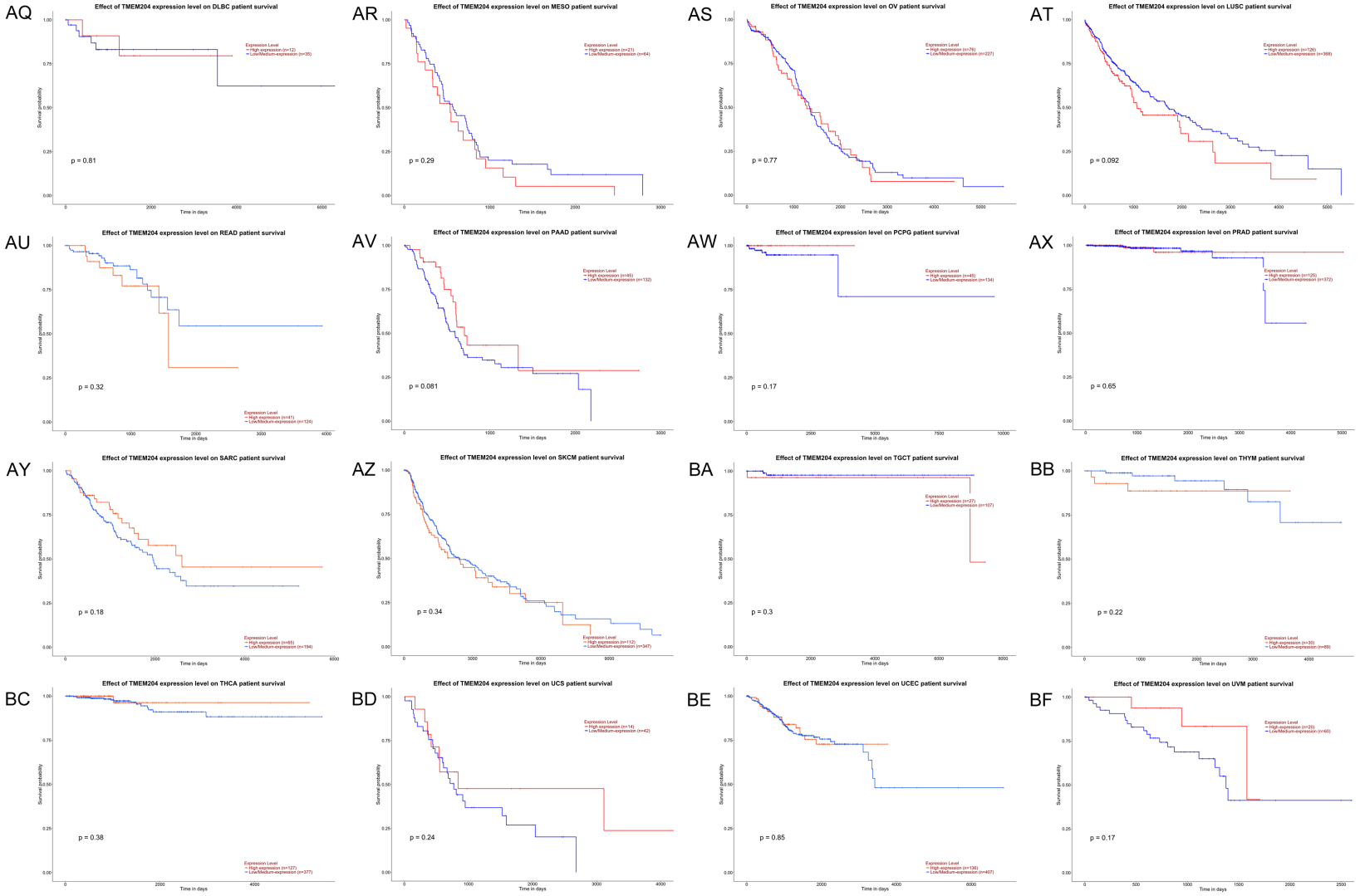
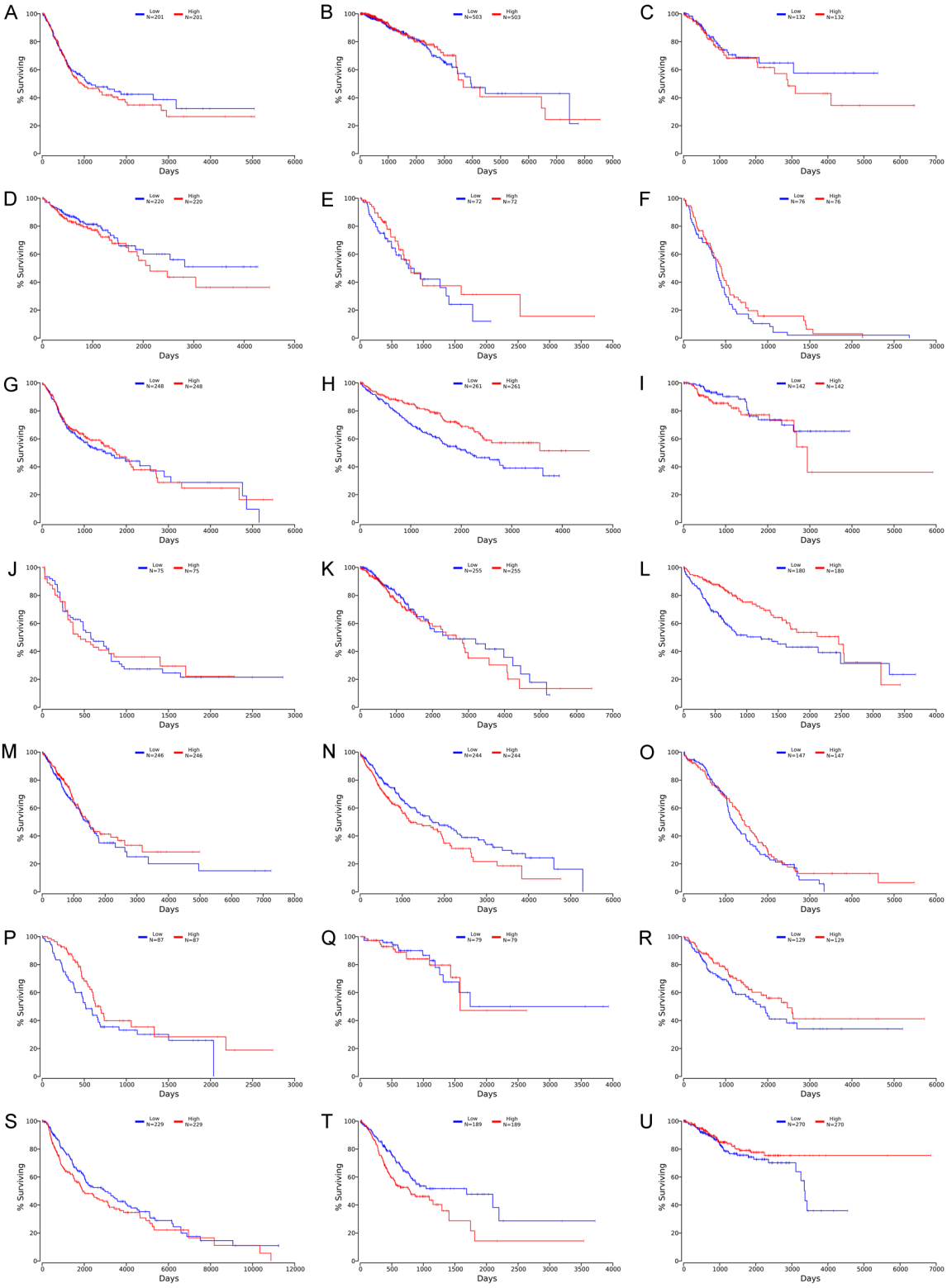




Supplementary Figure 2. Prognostic value of TMEM204 in different cancers analyzed by UALCAN. AA-BF. Survival curves of ACC, BLCA, LGG, BRCA, CESC, CHOL, COAD, ESCA, HNSC, KICH, KIRC, GBM, LAML, LIHC, LUAD, KIRP, DLBC, MESO, OV, LUSC, READ, PAAD, PCPG, PRAD, SARC, SKCM, TGCT, THYM, THCA, UCS, UCEC, and UVM.



Supplementary Figure 3. Prognostic value of TMEM204 in different cancers analyzed by OncoInc. A-U. Survival curves of BLCA, BRCA, CESC, COAD, ESCA, GBM, HNSC, KIRC, KIRP, LAML, LGG, LIHC, LUAD, LUSC, OV, PAAD, READ, SARC, SKCM, STAD, and UCEC.

Supplementary Table 1. Enrichment Results of TMEM204 by GO analysis

Biologic progress		
GOW16043	Cellular component organization	15
G0:0065007	biological regulation	13
G0:0051179	localization	11
G0:0008152	metabolic process	10
GOW32502	developmental process	10
G0:0032501	Multicellular organismal process	9
G0:0050896	response to stimulus	9
G0:0007154	cell communication	7
G0:0000003	reproduction	2
G0:0051704	multi-organism process	2
G0:0008283	cell proliferation	1
G0:0040007	growth	1
Cellular component		
G0:0016020	membrane	12
G0:0012505	endomembrane system	11
G0:0005829	cytosol	11
G0:0031974	membrane-enclosed lumen	10
G0:0005634	nucleus	9
G0:0005794	Golgi apparatus	9
G0:0032991	protein-containing complex	9
G0:0005783	endoplasmic reticulum	7
G0:0005694	chromosome	6
G0:0031982	vesicle	6
G0:0005615	extracellular space	3
G0:0005856	cytoskeleton	3
G0:0005768	endosome	3
G0:0042995	cell projection	2
G0:0042579	microbody	1
G0:0005773	vacuole	1
Molecular function		
G0:0005515	protein binding	18
G0:0043167	ion binding	7
G0:0000166	nucleotide binding	6
G0:0016740	transferase activity	3
G0:0016787	hydrolase activity	3
G0:0003676	nucleic acid binding	2
G0:0005215	transporter activity	1
G0:0003682	chromatin binding	1

Supplementary Table 2. Data availability

Database	Website
Oncomine	https://www.oncomine.org/
TIMER2.0	https://cistrome.shinyapps.io/timer/
GEPIA2	http://gepia2.cancer-pku.cn/
Ualcan	http://ualcan.path.uab.edu
Oncolnc	http://www.oncolnc.org/
cBioPortal	http://cbioportal.org
STRING	http://string-db.org/
WebGestalt	http://webgestalt.org/