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Original Article

TMEM14C is a Novel Biomarker for Prognosis and Diagnosis of Liver Hepatocellular Carcinoma

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Abstract : Transmembrane proteins (TMEMs) are frequently used as prognostic biomarkers for various cancer types, making them a significant area of interest in cancer research. The prognostic significance of transmembrane protein 14C (*TMEM14C*) and its association with tumor-infiltrating immune cells (TIICs) has not been explored in liver hepatocellular carcinoma (LIHC). This study aimed to assess the potential prognostic value of *TMEM14C* by conducting a comprehensive analysis of online databases on LIHC. We evaluated the potential of *TMEM14C* as a prognostic biomarker using online databases such as TIMER, GEPIA2, UALCAN, OSlihc, and LinkedOmics. We observed that the mRNA expression of *TMEM14C* was upregulated in LIHC than that in normal tissues. Upregulated *TMEM14C* expression was associated with worse prognosis based on the clinicopathological characteristics of patients with LIHC. Furthermore, *TMEM14C* expression was positively associated with TIICs. In the co-expression and functional enrichment analyses of *TMEM14C*, 7,401 genes were positively associated with *TMEM14C*, whereas 12,520 genes showed negative associations. The results of gene set enrichment analysis (GSEA)-Gene Ontology showed that *TMEM14C*-related co-expression genes were involved in protein localization to translational elongation, protein localization to the endoplasmic reticulum, and mitochondrial gene expression. Furthermore, GSEA-Kyoto Encyclopedia of Genes and Genomes analysis showed that these genes regulated ribosomes, oxidative phosphorylation, and spliceosomes. A survival map revealed that 28 and 24 genes that were positively associated with *TMEM14C* showed significant hazard ratios for overall survival and disease-free survival, respectively. Our findings demonstrate that *TMEM14C* is a novel prognostic biomarker and a potential tumor immune therapeutic target in patients with LIHC.

Keywords : TMEM14C, Immune cells, Liver hepatocellular carcinoma, Prognosis, Biomarker

INTRODUCTION

Liver cancer is the third most common cause of cancer-related fatalities worldwide, and poses a significant global health challenge. Liver hepatocellular carcinoma (LIHC) is the most common type of liver cancer based on histological characteristics [1]. Notably, LIHC is among the most prevalent malignant tumors worldwide and the leading cause of

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mortality in individuals with cirrhosis [2]. Furthermore, LIHC is linked to clearly identifiable risk factors, including hepatitis B and C viruses, excessive alcohol consumption, metabolic syndrome, and diabetes [3]. Although the survival rate of patients with LIHC has improved due to advancements in diagnostic techniques, the effectiveness of treatment remains restricted. Moreover, early and conclusive diagnosis of LIHC is challenging. Most patients typically exhibit late-stage LIHC and have a poor prognosis. Additionally, LIHC has a high mortality rate owing to the failure of early detection. Therefore, novel approaches are required to enhance the early identification and prognosis of this condition [4]. Furthermore, innovative treatments for LIHC are urgently needed. Finding a new prognostic biomarker is crucial for improving early detection and increasing the survival rates of patients with LIHC. In recent years, tumor immunotherapy has become a promising therapeutic approach for LIHC [5].

The immune system is essential for regulating cancer advancement [6]. The tumor immune microenvironment (TIME) and tumor-infiltrating immune cells (TIICs) significantly affect the body. Notably, TIICs play a crucial role in host defense against cancer cell growth [7]. Therefore, TIICs have become significant research topics in the field of cancer [8] and have a strong correlation with the clinical outcomes of both tumors and immunotherapy [9,10]. Furthermore, the correlation between the characteristics of immune response and prognosis is emphasized [11]. The prognostic significance of TIICs and immune molecules, including tumor-associated dendritic cells, macrophages, and natural killer cells, has been specifically observed in LIHC [12]. Therefore, TIICs can substantially affect the management and prognosis of patients with LIHC.

Transmembrane proteins (TMEM) span the biological membranes. Several of them traverse the lipid bilayer of the plasma membrane, whereas others are situated on the membranes of organelles. The TMEM family comprises proteins with predominantly uncharacterized functions. In addition, TMEM expression can be either decreased or increased in tumor tissues compared to that in nearby healthy tissues. Several TMEMs have been suggested as potential prognostic biomarkers for lung cancer. Moreover, experimental evidence has shown that TMEM proteins can be classified as either tumor suppressors or oncogenes. Additionally, TMEMs have been associated with the advancement and infiltration of tumors [13]. Furthermore, TMEMs are used as prognostic biomarkers in many cancers. The critical role of TMEMs in cancer development and drug resistance suggests that the TMEM family is an important focus of cancer research. We examined *TMEM14C* expression and its prognostic implications in LIHC using publicly available databases including the Tumor Immune Estimation Resource (TIMER), University of ALabama at Birmingham CANcer (UALCAN), Gene Expression Profiling Interactive Analysis version 2 (GEPIA2), and Online consensus Survival for liver hepato-cellular carcinoma (OSlihc). Additionally, we evaluated the biological functions and co-expression patterns of *TMEM14C* in LIHC using the LinkedOmics database. Our results suggest that *TMEM14C* is a new prognostic biomarker and a possible target for tumor immunotherapy in patients with LIHC.

MATERIALS AND METHODS

1. TIMER database analysis

TIMER (https://cistrome.shinyapps.io/timer/) is a web-based tool that allows the analysis of immune cell infiltration in different cancer types. It uses information from 10,897 samples from The Cancer Genome Atlas (TCGA) to determine the immune cells present [1]. We investigated *TMEM14C* expression in various cancers and the correlation between *TMEM14C* expression and immune cells.

2. UALCAN database analysis

The UALCAN (http://ualcan.path.uab.edu) database utilizes RNA sequencing and clinical data from 31 different cancer types. This tool can be used to examine the comparative expression of particular genes in both tumor and normal samples, as well as within different tumor subcategories. Subgroups can be classified according to various clinicopathological characteristics such as tumor stage, grade, race, sex, histological subtype, age, nodal metastasis, and TP53 mutation status [1].

3. OSlihc database analysis

The OSlihc (http://bioinfo.henu.edu.cn/DatabaseList.jsp) database functions as a platform for researchers to identify novel prognostic biomarkers and develop innovative targeted

therapies for different cancer types. The prognostic value of *TMEM14C* in overall survival (OS), disease-free interval (DFI), progression-free interval (PFI), and disease-specific survival (DSS) was assessed to determine survival outcomes using OSlihc [14].

4. LinkedOmics database analysis

LinkedOmics (http://www.linkedomics.org/) is an openly accessible platform that contains data for 32 different cancer types from TCGA. This resource provides a unique opportunity for biologists and clinicians to investigate cancer-related multiomics data. The Kyoto Encyclopedia of Genes and Genomes (KEGG) is a web-based tool that facilitates the systematic examination of gene function and genomic data. Gene Ontology (GO) analysis classifies gene functions into terms associated with cellular components (CC), biological processes (BP), and molecular functions (MF). The ranking criterion was set at a false discovery rate of <0.05, and 500 simulations were performed [15].

5. GEPIA2

GEPIA2 (http://gepia2.cancer-pku.cn/#index) was used to consolidate clinical data from 9,736 tumors and 8,587 normal tissues obtained from TCGA and GTEx pro-jects. The "Expression Analysis" module enabled the execution of clinical staging and survival analysis. Using the median value as the cut-off, we generated KM curves for OS and disease-free survival (DFS) associated with *TMEM14C* in LIHC. Survival results are shown as KM curves with HR and P-values from a log-rank test. $p<0.05$ using the Student's t test.

6. Statistical analysis

All results of this study were obtained from an accessible database, and all examinations were performed using online tools. The results are reported as P-values obtained from the log-rank test, with a significance level of $p<0.05$.

RESULTS

1. mRNA expression analysis of *TMEM14C* in LIHC

To assess the differential expression levels of *TMEM14C* between tumor and normal tissues, we analyzed *TMEM14C* expression levels in various cancer types, including LIHC, using the TIMER and GEPIA2 databases. We found that *TMEM14C* expression levels were upregulated in bladder cholangiocarcinoma, esophageal carcinoma, glioblastoma multiforme, head and neck squamous cell carcinoma, renal clear cell carcinoma, kidney renal papillary cell carcinoma, lung adenocarcinoma, stomach adenocarcinoma, and thyroid carcinoma tissues compared to normal tissues. Conversely, *TMEM14C* expression levels were downregulated in kidney chromophobe, rectal adenocarcinoma, and uterine corpus endometrial carcinoma tissues compared to those in normal tissues (Fig. 1A). In addition, *TMEM14C* expression levels were upregulated in LIHC (Fig. 1B). We also examined the relationship between *TMEM14C* expression and various clinicopathological features of LIHC, including tumor stage, tumor grade, histological subtype, and TP53 mutations. Our findings revealed a significant association between *TMEM14C* expression and primary tumor, tumor stage (I, II, III, and IV), tumor grade (I, II, III, and IV), histological subtype (hepatocellular carcinoma or fibrolamellar carcinoma), and TP53 mutations in LIHC (Fig. 1C). In summary, our data showed that *TMEM14C* expression is upregulated in LIHC.

2. Prognostic value analysis of *TMEM14C* in LIHC

We utilized the OSlihc database to investigate the potential prognostic value of *TMEM14C* expression. Survival rates, including OS, DFI, PFI, and DSS, were analyzed according to *TMEM14C* expression in LIHC. Our findings indicated that elevated *TMEM14C* expression was associated with a significantly poor prognosis in LIHC, as evidenced by OS (HR, 1.58; $p=0.0107$), DFI (HR, 1.68; $p=0.0008$), PFI (HR, 1.63; $p=0.0013$), and DSS (HR, 1.95; $p=0.004$) (Fig. 2A). Additionally, we utilized the GEPIA2 database. Elevated *TMEM14C* expression was associated with poor prognosis in LIHC, as evidenced by OS (HR, 1.9; $p=0.00058$) and DFI (HR, 1.6; $p=0.0027$) (Fig. 2B). Our results showed that upregulated *TMEM14C* expression predicts a poor prognosis in LIHC.

3. Analysis of correlation between *TMEM14C* and TIICs in LIHC

Next, we focused on the correlation between *TMEM14C* and TIICs in LIHC using the TIMER database. The results

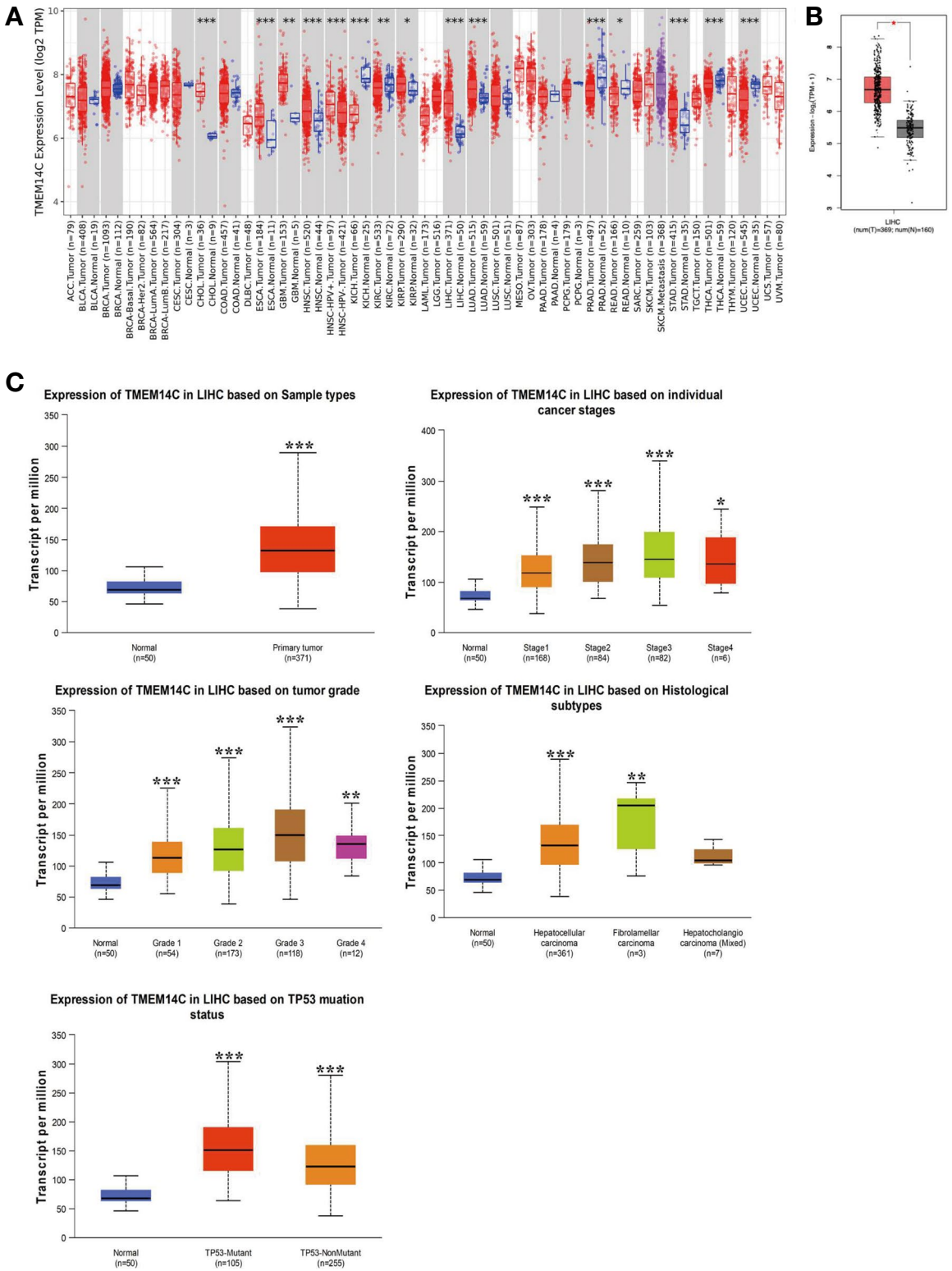

Fig. 1. mRNA expression levels of transmembrane protein 14C (*TMEM14C*) in several tissues including liver hepatocellular carcinoma (LIHC). (A) High or low expression of *TMEM14C* in tumor tissues compared with normal tissues analyzed using the TIMER database. (B) High or low expression of *TMEM14C* in tumor tissues compared with normal tissues analyzed using the GEPIA2 database. (C) *TMEM14C* expression according to the presence of clinicopathologic characteristics (primary tumor, tumor stage, tumor grade, histological subtype, and TP53 mutation) compared with normal tissues analyzed using the UALCAN database (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

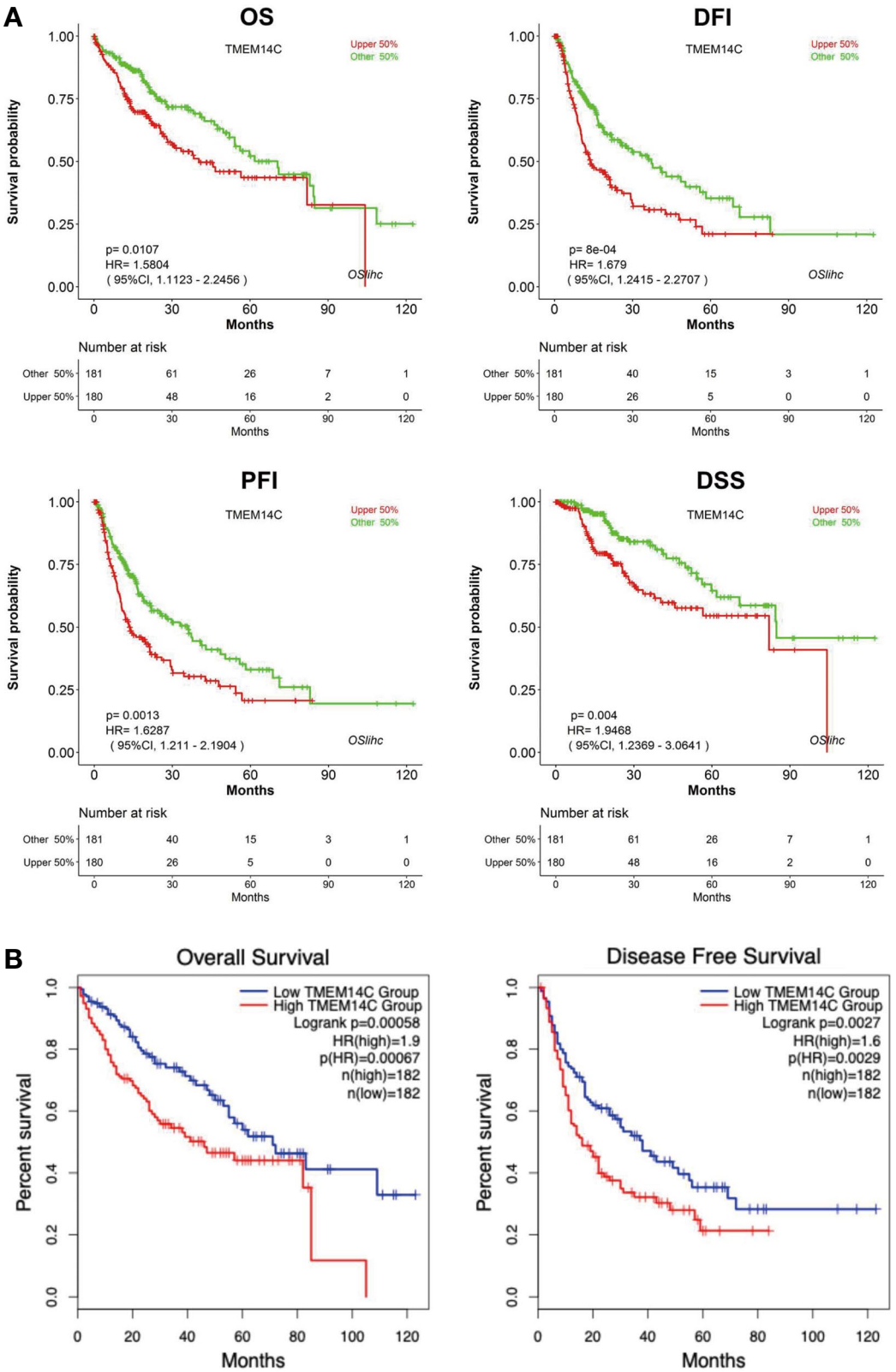

Fig. 2. Prognostic significance of *TMEM14C* expression in LIHC. The prognostic value of *TMEM14C* expression was analyzed using the OSlihc (A) and GEPIA2 (B) databases. OS, overall survival; DFI, disease-free interval; PFI, progression-free interval; DSS, disease-specific survival.

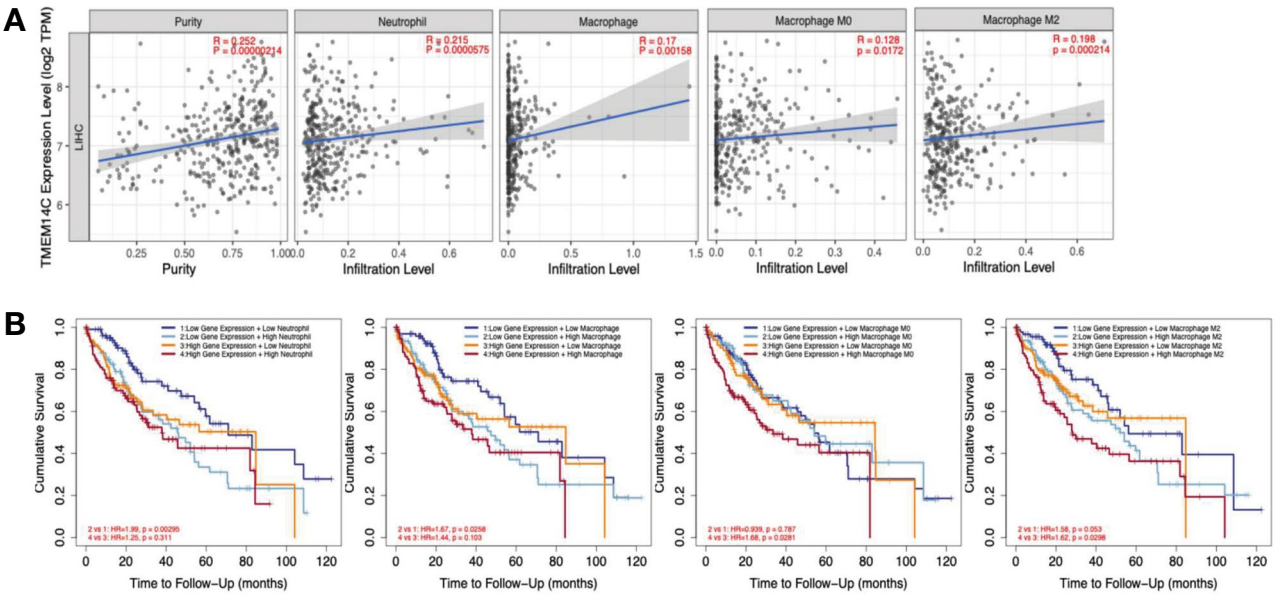

Fig. 3. Correlation between *TMEM14C* expression and tumor-infiltrating immune cells (TIICs) in LIHC. (A) Scatterplots showing the correlation between *TMEM14C* and TIICs, including neutrophils, macrophages, M0 macrophages, and M2 macrophages were analyzed using the TIMER database. (B) The prognostic value of *TMEM14C* and TIICs was analyzed using the TIMER database.

revealed that *TMEM14C* positively correlated with the infiltration levels of neutrophils (R = 0.215, p = 0.0000575), macrophages (R = 0.17, p = 0.00158), M0 macrophages (R = 0.128, p = 0.0172), and M2 macrophages (R = 0.198, p = 0.000214) in LIHC (Fig. 3A). Next, we explored the association among *TMEM14C* expression, prognosis, and TIICs in LIHC. Our findings showed that high *TMEM14C* expression combined with high neutrophil infiltration was associated with a poorer prognosis than low *TMEM14C* expression with low neutrophil infiltration. Low *TMEM14C* expression with low neutrophil infiltration was associated with poorer prognosis than low *TMEM14C* expression with high neutrophil infiltration. A worse prognosis was observed in patients with high *TMEM14C* expression and high macrophage infiltration than in those with low *TMEM14C* expression and low macrophage infiltration. Furthermore, low *TMEM14C* expression with low macrophage infiltration was associated with poorer prognosis than low *TMEM14C* expression with high macrophage infiltration. Similarly, high *TMEM14C* expression with high M0 macrophage infiltration was associated with a poorer prognosis than low *TMEM14C* expression with low M0 macrophage infiltration. Additionally, high *TMEM14C* expression with high M2 macrophage infiltration was associated with a poorer prognosis than low *TMEM14C* expression with low M2 macrophage infiltration (Fig. 3B). In summary, our results suggest that elevated *TMEM14C* expression is associated with TIICs and may influence tumor prognosis in patients with LIHC.

4. Co-expression and functional enrichment analysis of *TMEM14C* in LIHC

To explore the potential biological roles and pathways of *TMEM14C* expression in LIHC, we assessed the co-expression of *TMEM14C* using the LinkedOmics database. A total of 7,401 genes demonstrated a positive association with *TMEM14C* expression, as indicated by dark red dots, whereas 12,520 genes exhibited a negative association, as represented by dark green dots, in Fig. 4A. Heat maps were used to highlight the top 50 genes that were positively or negatively associated with *TMEM14C* expression (Fig. 4B and C). The four most positively correlated genes were *TMEM14B* (R = 0.8373, p = 9.107e-99), *TOMM6* (R = 0.6843, p = 1.497e-52), *CUTA* (R = 0.6543, p = 3.039e-46), and *SNRPC* (R = 0.647, p = 2.249e-45) (Fig. 4D). Conversely, *CDC42EP2* negatively correlated with *SYNE1* (R = 0.4821, p = 5.506e-23), *HIVEP3* (R = 0.4429, p = 2.937e-19), *ASCC3* (R = 0.4378, p = 8.446e-19), and *SHROOM2* (R = 0.4279, p = 6.005e-18) (Fig. 4E). Biological process categories were identified using gene set enrich-

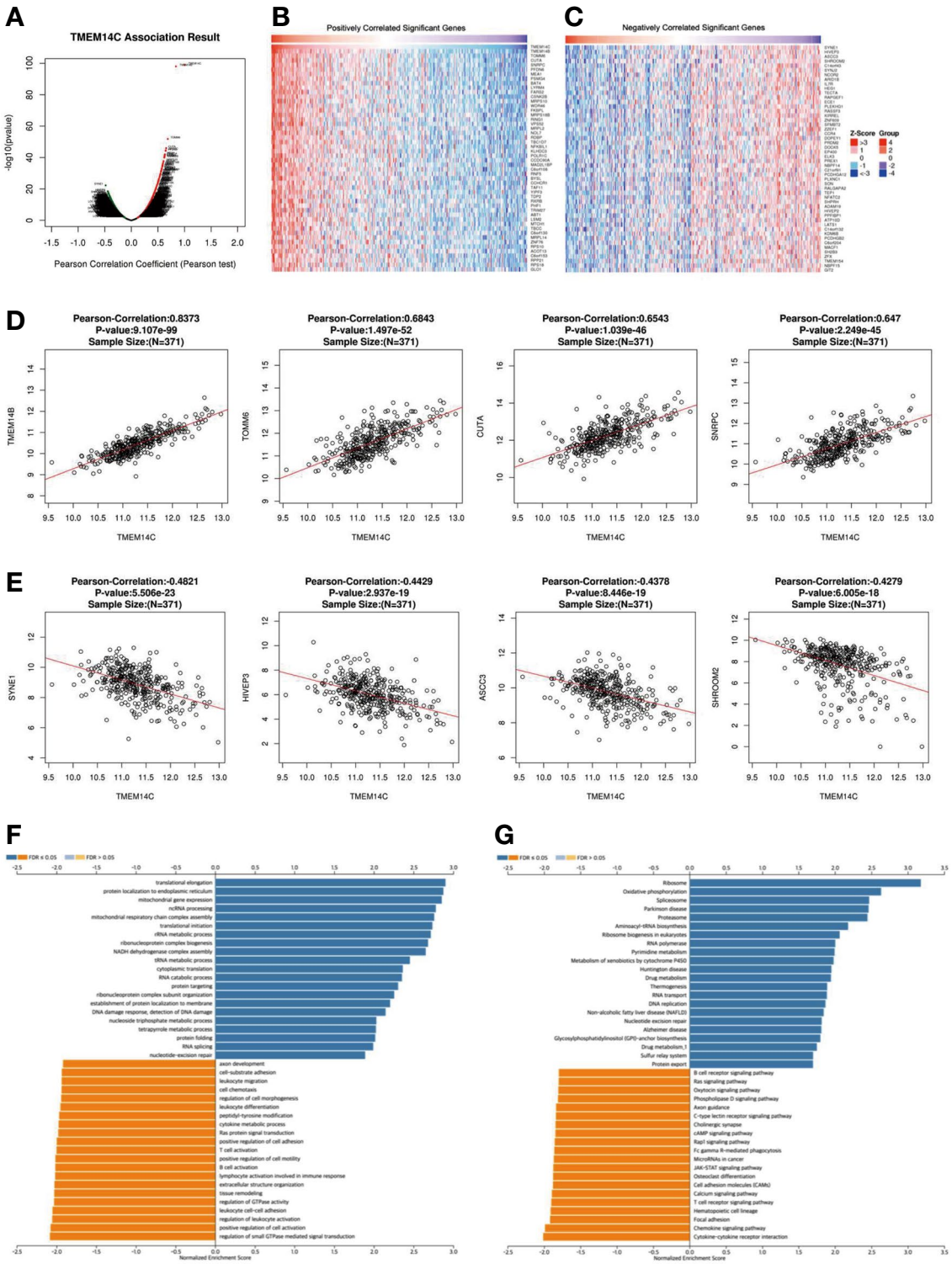

Fig. 4. Co-expression and functional enrichment for *TMEM14C* in LIHC. *TMEM14C* co-expression genes and Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) terms were analyzed using the LinkedOmics database. (A) Highly correlated genes for *TMEM14C* expression were assessed using the Pearson's test in the LIHC cohort. Heat maps showing the top 50 genes positively and negatively correlated with *TMEM14C* expression in LIHC. Red (B) and blue (C) indicate positively and negatively correlated genes, respectively. Correlation of the top 4 positively (D) and negatively related genes (E) with *TMEM14C* in LIHC. (F) Enriched biological processes associated with *TMEM14C* co-expressed genes by gene set enrichment analysis (GSEA). (G) KEGG pathway analysis for *TMEM14C* co-expressed genes by GSEA. Dark blue and orange indicate a false discovery rate (FDR) of ≤ 0.05 and light blue and orange indicate FDR > 0.05.

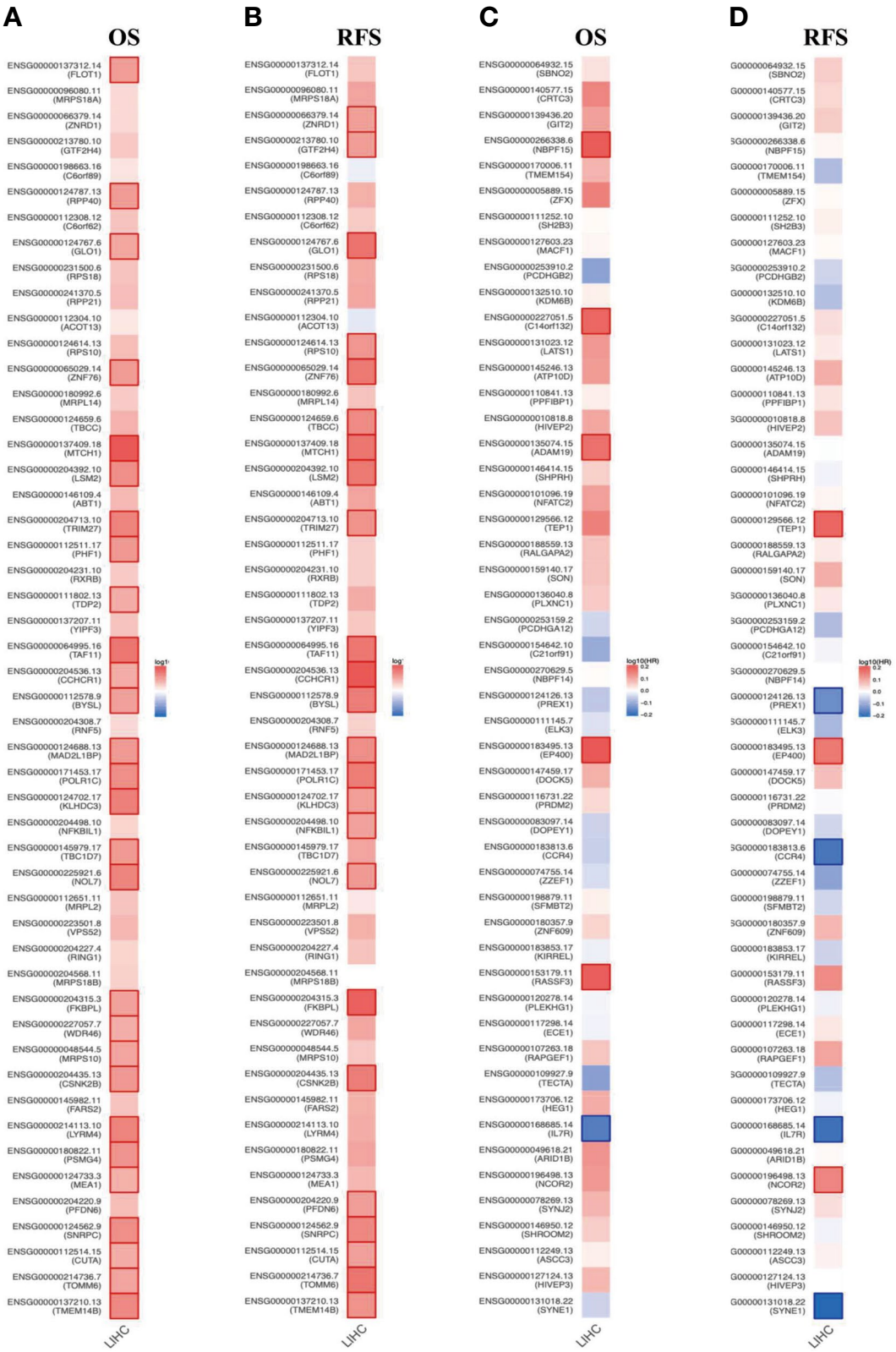

Fig. 5. Prognostic significance of *TMEM14C*-related genes was analyzed using the GEPIA2 database. Survival map of the positively-related genes of *TMEM14C* according to OS (A) and RFS (B). Survival map of the negatively-related genes of *TMEM14C* according to OS (C) and RFS (D). Heat map presenting the log10 (HR) of genes in LIHC. A square with bold border represents $p < 0.05$ in the survival analysis.

ment analysis (GSEA) based on GO. The analysis revealed that genes co-expressed with *TMEM14C* were involved in processes such as translational elongation, protein localization to endoplasmic reticulum, and mitochondrial gene expression (Fig. 4F). Additionally, GSEA of the KEGG pathways indicated that the co-expressed genes were predominantly enriched in the ribosomes, oxidative phosphorylation, and spliceosomes (Fig. 4G). In summary, our findings indicated that *TMEM14C* may affect LIHC prognosis by altering the overall transcriptome.

5. Prognostic value of *TMEM14C*-related gene in LIHC

We investigated the prognostic value of *TMEM14C*-related genes in LIHC by analyzing data from the GEPIA2 database. The *TMEM14C*-related genes appear to be high risk factors for LIHC. Among the genes positively associated with *TMEM14C*, 28 showed a significant hazard ratio (HR) for OS, whereas 24 showed a high HR for DFS (Fig. 5A and B). Conversely, Among the genes negatively associated with *TMEM14C*, seven showed a significant HR for OS, whereas three showed a significant HR for DFS (Fig. 5C and D). Therefore, *TMEM14C* and its related genes are of prognostic importance in LIHC.

DISCUSSION

LIHC is a globally prevalent malignancy, and is characterized by a poor prognosis and significant mortality rate [16]. Furthermore, LIHC is the predominant form of primary liver cancer, accounting for approximately 90% of all cases of liver cancer. Following initial therapies, including surgical resection and transplantation, LIHC shows a significant tendency to recur, with a 5-year survival rate ranging from 50% to 70% [17]. The prognosis of LIHC is highly unfavorable mainly because it is often diagnosed at an advanced stage and very few treatment options are available. Therefore, promptly identifying significant biomarkers for the diagnosis and prognosis of LIHC as well as targets for treatment is crucial [18,19]. To date, limited research has focused on *TMEM14C*; however, our findings are the first to demonstrate its relationship with LIHC. This study aimed to predict the prognosis of patients with LIHC and to enhance future outcomes.

The immune system plays a crucial role in controlling cancer growth, and the activity of immune cells has the potential to promote cancer progression [20,21]. The TIME, which is regulated by TIICs, is pivotal for cancer progression and promotion [22]. Furthermore, TIICs play a crucial role in the development and progression of cancer cells and tumors. The number and specific characteristics of TIICs directly affect clinical outcomes [23]. Several studies have examined the attributes of various types of immune cells and their correlation with prognosis [24] as well as the prognostic importance of TIICs and immune molecules in LIHC [25]. Therefore, TIICs significantly influence the treatment and prognosis of patients with LIHC.

A TMEM is a protein that extends across biological membranes. Some of these proteins span the lipid bilayer of the plasma membrane, whereas others are localized on organelle membranes. The TMEM family comprises proteins with predominantly uncharacterized functions. Furthermore, TMEMs are found in various cell types and play crucial physiological roles, including epidermal keratinization (*TMEM45A*) [26], autophagy, smooth muscle contraction (*TMEM16*) [27], protein glycosylation (*TMEM165*) [28], and liver development and differentiation (*TMEM97*) [29]. Differential regulation of TMEM expression has been observed in various cancer types, including lymphoma (*TMEM176*) [30], colorectal cancer (*TMEM25*) [31], hepatic cancer (*TMEM7*) [32], and lung cancer (*TMEM48*) [33]. Numerous TMEMs are highly expressed in cancer cells. Certain genes are involved in tumor progression, invasion, and development of metastasis, whereas others are associated with poor prognosis and may act as prognostic biomarkers. Many TMEMs have been linked to the development of cancer and drug resistance, indicating that the TMEM family is a prominent target for cancer investigation. In addition, certain proteins function as tumor suppressors [34]. Therefore, a more accurate description of these proteins would enhance our understanding of their involvement in cancer and facilitate the development of effective therapeutic approaches.

In this study, we investigated the mRNA expression levels of *TMEM14C* in LIHC. We found that *TMEM14C* expression was upregulated in LIHC. Various clinicopathological factors were associated with this upregulation. Elevated *TMEM14C* levels were associated with poor prognosis in patients with LIHC. Our data strongly indicated that elevated *TMEM14C* levels could serve as a novel prognostic biomarker for LIHC. We examined the relationship

between *TMEM14C* and TIICs in LIHC. Our findings revealed a positive correlation between *TMEM14C* expression and the presence of neutrophils and macrophages. Furthermore, elevated *TMEM14C* levels were associated with a worse prognosis. In our investigation of the co-expression and potential biological functions of *TMEM14C* in LIHC, we found that 7,401 genes were positively correlated with *TMEM14C*, whereas 12,520 genes were negatively correlated. This indicates that *TMEM14C* has an extensive impact on gene expression patterns in LIHC. In this study, we investigated the prognostic significance of *TMEM14C*-related genes in LIHC. Both *TMEM14C* and its related genes have prognostic value in LIHC. These findings highlight the limitations of big data analyses and emphasize the importance of conducting further research to gain a better understanding of the functional implications of these genes.

In conclusion, our findings indicate that high *TMEM14C* expression is associated with worse prognosis and the presence of TIICs in LIHC. Therefore, *TMEM14C* is a novel prognostic biomarker that provides information about possible targets for immune therapy in patients with LIHC. Further investigations are required to examine the complex mechanisms of *TMEM14C* as a potential prognostic biomarker through in vitro and in vivo studies involving living organisms.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

REFERENCES

1. Kim HR, Seo CW, Kim J. The value of CDC42 effector protein 2 as a novel prognostic biomarker in liver hepatocellular carcinoma: a comprehensive data analysis. Osong Public Health Res Perspect. 2023;14:451-67.
2. Paul C, Khera L, Kaul R. Hepatitis C virus core protein interacts with cellular metastasis suppressor Nm23-H1 and promotes cell migration and invasion. Arch Virol. 2019;164:1271-85.
3. Sangiovanni A, Del Ninno E, Fasani P, De Fazio C, Ronchi G, Romeo R, et al. Increased survival of cirrhotic patients with a hepatocellular carcinoma detected during surveillance. Gastroenterology. 2004;126:1005-14.
4. Zheng X, Jin W, Wang S, Ding H. Progression on the Roles and Mechanisms of Tumor-Infiltrating T Lymphocytes in Patients With Hepatocellular Carcinoma. Front Immunol. 2021;12:729705.
5. Lee YT, Fujiwara N, Yang JD, Hoshida Y. Risk stratification and early detection biomarkers for precision HCC screening. Hepatology. 2023;78:319-62.
6. Gentles AJ, Newman AM, Liu CL, Bratman SV, Feng W, Kim D, et al. The prognostic landscape of genes and infiltrating immune cells across human cancers. Nat Med. 2015;21:938-45.
7. Bremnes RM, Al-Shibli K, Donnem T, Sirera R, Al-Saad S, Andersen S, et al. The role of tumor-infiltrating immune cells and chronic inflammation at the tumor site on cancer development, progression, and prognosis: emphasis on non-small cell lung cancer. J Thorac Oncol. 2011;6:824-33.
8. Cabrita R, Lauss M, Sanna A, Donia M, Skaarup Larsen M, Mitra S, et al. Tertiary lymphoid structures improve immunotherapy and survival in melanoma. Nature. 2020;577:561-5.
9. Angell HK, Lee J, Kim KM, Kim K, Kim ST, Park SH, et al. PD-L1 and immune infiltrates are differentially expressed in distinct subgroups of gastric cancer. Oncoimmunology. 2018;8:e1544442.
10. Hao X, Luo H, Krawczyk M, Wei W, Wang W, Wang J, et al. DNA methylation markers for diagnosis and prognosis of common cancers. Proc Natl Acad Sci U S A. 2017;114:7414-9.
11. Sia D, Jiao Y, Martinez-Quetglas I, Kuchuk O, Villacorta-Martin C, Castro de Moura M, et al. Identification of an Immune-specific Class of Hepatocellular Carcinoma, Based on Molecular Features. Gastroenterology. 2017;153:812-26.
12. Tian MX, Liu WR, Wang H, Zhou YF, Jin L, Jiang XF, et al. Tissue-infiltrating lymphocytes signature predicts survival in patients with early/intermediate stage hepatocellular carcinoma. BMC Med. 2019;17:106.
13. Schmit K, Michiels C. TMEM Proteins in Cancer: A Review. Front Pharmacol. 2018;9:1345.
14. An Y, Wang Q, Zhang G, Sun F, Zhang L, Li H, et al. OSlihc: An Online Prognostic Biomarker Analysis Tool for Hepatocellular Carcinoma. Front Pharmacol. 2020;11:875.
15. Vasaikar SV, Straub P, Wang J, Zhang B. LinkedOmics: analyzing multi-omics data within and across 32 cancer types. Nucleic Acids Res. 2018;46:D956-63.
16. Ouyang X, Fan Q, Ling G, Shi Y, Hu F. Identification of Diagnostic Biomarkers and Subtypes of Liver Hepatocellular Carcinoma by Multi-Omics Data Analysis. Genes (Basel). 2020;11:1051.
17. Lee JG, Kang CM, Park JS, Kim KS, Yoon DS, Choi JS, et al. The actual five-year survival rate of hepatocellular carcinoma patients after curative resection. Yonsei Med J. 2006;47:105-12.
18. Dapito DH, Mencin A, Gwak GY, Pradere JP, Jang MK,

Mederacke I, et al. Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. Cancer Cell. 2012; 21:504-16.
19. Uehara T, Ainslie GR, Kutanzi K, Pogribny IP, Muskhelishvili L, Izawa T, et al. Molecular mechanisms of fibrosis-associated promotion of liver carcinogenesis. Toxicol Sci. 2013;132:53-63.
20. Ostrand-Rosenberg S. Immune surveillance: a balance between protumor and antitumor immunity. Curr Opin Genet Dev. 2008;18:11-8.
21. Munhoz RR, Postow MA. Recent advances in understanding antitumor immunity. F1000Res. 2016;5:2545.
22. Lazăr DC, Avram MF, Romoşan I, Cornianu M, Tăban S, Goldiş A. Prognostic significance of tumor immune microenvironment and immunotherapy: Novel insights and future perspectives in gastric cancer. World J Gastroenterol. 2018; 24:3583-616.
23. Choi Y, Kim JW, Nam KH, Han SH, Kim JW, Ahn SH, et al. Systemic inflammation is associated with the density of immune cells in the tumor microenvironment of gastric cancer. Gastric Cancer. 2017;20:602-11.
24. Kurebayashi Y, Ojima H, Tsujikawa H, Kubota N, Maehara J, Abe Y, et al. Landscape of immune microenvironment in hepatocellular carcinoma and its additional impact on histological and molecular classification. Hepatology. 2018;68:1025-41.
25. Sun H, Huang Q, Huang M, Wen H, Lin R, Zheng M, et al. Human CD96 Correlates to Natural Killer Cell Exhaustion and Predicts the Prognosis of Human Hepatocellular Carcinoma. Hepatology. 2019;70:168-83.
26. Hayez A, Malaisse J, Roegiers E, Reynier M, Renard C, Haftek M, et al. High TMEM45A expression is correlated to epidermal keratinization. Exp Dermatol. 2014;23:339-44.
27. Thomas-Gatewood C, Neeb ZP, Bulley S, Adebiyi A, Bannister JP, Leo MD, et al. TMEM16A channels generate Ca^{2+}-activated Cl^- currents in cerebral artery smooth muscle cells. Am J Physiol Heart Circ Physiol. 2011;301:H1819-27.
28. Foulquier F, Amyere M, Jaeken J, Zeevaert R, Schollen E, Race V, et al. TMEM165 deficiency causes a congenital disorder of glycosylation. Am J Hum Genet. 2012;91:15-26.
29. Malhotra K, Luehrsen KR, Costello LL, Raich TJ, Sim K, Foltz L, et al. Identification of differentially expressed mRNAs in human fetal liver across gestation. Nucleic Acids Res. 1999;27:839-47.
30. Cuajungco MP, Podevin W, Valluri VK, Bui Q, Nguyen VH, Taylor K. Abnormal accumulation of human transmembrane (TMEM)-176A and 176B proteins is associated with cancer pathology. Acta Histochem. 2012;114:705-12.
31. Hrašovec S, Hauptman N, Glavač D, Jelenc F, Ravnik-Glavač M. TMEM25 is a candidate biomarker methylated and down regulated in colorectal cancer. Dis Markers. 2013;34:93-104.
32. Zhou X, Popescu NC, Klein G, Imreh S. The interferon-alpha responsive gene TMEM7 suppresses cell proliferation and is downregulated in human hepatocellular carcinoma. Cancer Genet Cytogenet. 2007;177:6-15.
33. Qiao W, Han Y, Jin W, Tian M, Chen P, Min J, et al. Overexpression and biological function of TMEM48 in non-small cell lung carcinoma. Tumour Biol. 2016;37:2575-86.
34. Doolan P, Clynes M, Kennedy S, Mehta JP, Germano S, Ehrhardt C, et al. TMEM25, REPS2 and Meis 1: favourable prognostic and predictive biomarkers for breast cancer. Tumour Biol. 2009;30:200-9.