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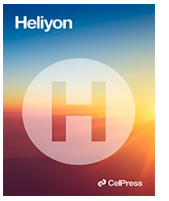

Research article

Ferritin light chain as a potential biomarker for the prognosis of liver hepatocellular carcinoma

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ABSTRACT

High expression of the ferritin light chain (FTL) in cancer promotes its onset and progression and is associated with tumour evolution. However, the significance of FTL in pan-cancer progression and prognosis in humans remains unclear. Therefore, we selected various bioinformatics databases to perform a pan-cancer analysis on a public dataset. Our results showed that FTL was differentially expressed in pan-cancer tissues compared to normal tissues. High FTL expression significantly correlated with the clinicopathological characteristics of patients with liver hepatocellular carcinoma (LIHC). The subsequent validation experiments confirmed these observations. Notably, our study found for the first time that FTLs are closely associated with LIHC and that FTLs have important clinical diagnostic and prognostic value for patients with LIHC. We confirmed that FTL expression was closely associated with altered DNA cycles and immune infiltration in LIHC. In conclusion, high levels of FTL expression are associated with poor prognosis in LIHC patients and are expected to be a potential prognostic and immune marker for LIHC.

1. Introduction

Liver hepatocellular carcinoma (LIHC) is the fourth leading cause of cancer-related deaths globally, and is expected to affect more than 1 million people annually by 2025. The pathophysiology of LIHC involves the interaction of multiple factors, such as susceptibility genes, fatty liver, and the tumour microenvironment (TME) [1]. Recent studies have shown that the prognosis of patients with LIHC is strongly influenced by the TME. The TME of LIHC is highly complex and can be attributed to crosstalk between the immune and vascular microenvironments as well as the tumour microenvironment, that is, the crosstalk between immune cells and LIHC cells [2,3]. Despite great progress in diagnosis and treatment, the problems of poor prognosis and high recurrence rate of LIHC remain very serious; therefore, the search for new diagnostic and therapeutic factors is essential to treat LIHC [4]. Therefore, it is necessary to explore the potential relationship between LIHC and the TME.

Ferritin is an iron storage protein involved in iron metabolism. Mammalian ferritin has two subunits: ferritin heavy chain (FTH) and ferritin light chain (FTL). The ferritin heavy chain (FTH) and ferritin light chain (FTL), as well as the mitochondrial subunit form (FtMt), exist only in the mitochondria. FTL consists of 174 amino acids and has a molecular weight of 19 kDa; therefore, it is

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structurally more stable than FTH. Different FTH/FTL ratios exhibit different characteristics. FTL is mainly found in iron storage organs, such as the liver or spleen, whereas FTH is mainly associated with antioxidant activity [5]. In recent years, many studies have shown that FTL is overexpressed in various malignant tumours and plays an important role in regulating the malignant progression of cancer [6–10]. FTL is closely associated with the degree of tumour malignancy. It can be used as a biomarker for predicting the prognosis of various tumours, such as gastric cancer [10] and colorectal cancer [11]. In gliomas, FTL affects epithelial-to-mesenchymal transition and chemotherapy resistance [6]. In addition, in colorectal cancer, FTL promotes the malignant progression of colorectal cancer through the Linc00467/miR-133b axis, leads to resistance of its cells to 5-FU, and promotes its metastasis [9]. FTL has been found to act as a biomarker to distinguish benign from malignant tumours and predict the prognosis of patients [10]. Although previous studies have confirmed the high expression of FTL in LIHC and its adverse effects on the prognosis of patients [7,8], the specific relationship and potential link between FTL and LIHC have not been elucidated.

This study aimed to analyse the expression and clinical value of FTL in pan-cancer using bioinformatics methods, which were validated by validation experiments. In this study, we systematically investigated the significance of FTL in pan-cancer for the first time and focused on the role of FTL in LIHC. Ultimately, the study revealed for the first time the potential biological function, clinical prognostic value, and immunotherapeutic value of FTL in LIHC (Fig. 1). We hope that this study will explore new avenues for the use of FTL in the treatment of LIHC and the improvement of patient prognosis.

2. Methods

2.1. Clinical samples

In accordance with the principles set out in the Declaration of Helsinki, patients undergoing this study provided informed consent, and patients agreed to have their tumor tissue samples used for the investigation. The LIHC tissue microarrays were purchased from Shanghai Outdo Biological Co. Ltd, and consisted of 37 LIHC tissues and 15 normal tissues adjacent to the tumor. Clinicopathological classification and staging were based on the American Joint Committee on Cancer (AJCC) criteria. The clinical information of the samples is summarised in Tables 1 and 2. This study was strictly evaluated and ethically reviewed by the Institutional Ethics Committee of Shanghai Outdo Biological Co., Ltd. (SHYJS-QT-2101).

2.2. Immunohistochemistry (IHC)

Tissue sections were placed at room temperature (RT), rewarmed, and conventionally dewaxed in an oven at 65 °C for 1 h, as well as deparaffinized with xylene and rehydrated in a graduated alcohol bath. The 1 % citric acid buffer was heated to boiling, and sections were placed in the buffer for antigenic thermal repair. After three flushes with PBS, 3%H_2O_2 was added to the tissue to block endogenous peroxidase. The FTL monoclonal antibody was then applied to the slides (1:100, Cat No.10727-1-AP, Proteintech, USA). Reaction enhancing reagent, secondary antibody reagent and DAB color solution were dropped to observe the staining degree under the microscope, and PBS buffer was placed to terminate color development. Stain with hematoxylin and reverse blue with PBS buffer. The sheet was sealed with neutral resin, the drawing was viewed through a microscope, and statistical staining results were obtained.

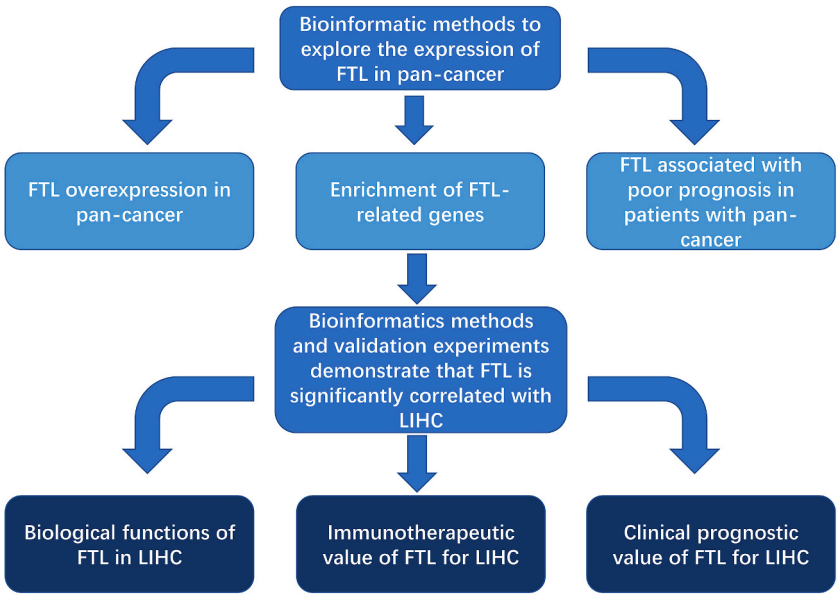

Fig. 1. Flowchart of this article.

Table 1
FTL protein expression in LIHC.

Diagnosis	No. of cases	Positive cases	Positive cases rates	Strongly positive rates
		- + ++ +++		
Normal	15	9 2 3 1	40.00 %	26.67 %
LIHC	37	2 10 12 13	94.60 %**	67.57 %**

Positive rate: percentage of positive cases with +~+++ staining score.
Strongly positive rate: percentage of positive cases with + + and +++ staining score.
*$P < 0.05$ and **$P < 0.01$: compared with normal tissues.
LIHC: liver hepatocellular carcinoma.

Table 2
Correlation between FTL expression and the clinicopathological features of LIHC.

Variables	No. of cases	FTL strongly positive cases (%)	χ^2	*P*-value
Sex	29	19 (65.5 %)	0.257	0.612
Male	8	6 (75.0 %)		
Female				
Age	17	13 (76.4 %)	1.138	0.289
<50	20	12 (60.0 %)		
≥50				
Histological grade	7	2 (28.5 %)	6.143	0.046[a]
Grade 1	27	21 (77.7 %)		
Grade 2	3	2 (66.7 %)		
Grade 3				
Clinical stage	18	10 (55.6 %)	4.937	0.026[a]
0-IIA	19	17 (89.4 %)		
IIB-IV				

[a] $P < 0.05$.

2.3. Evaluation of IHC staining

All tissue specimens were examined and scored by two pathologists (Yang Yang and Nan Li) using a double-blind control method. Immunohistochemical analysis was evaluated using semi-quantitative score combined with percentage of positive area and staining intensity. FTL positive staining intensity score (negative = 0, weak = 1, medium = 2, strong = 3) times the percentage of stained cells (≤25 % = 1, 26–50 % = 2, 51–75 % = 3, >75 % = 4). Calculate the staining index (value 0–12). We defined FTL immunostaining values 0–3 as normal expression and 4 or above as overexpression.

2.4. Cell culture

Human normal liver sinusoidal endothelial cell line (SK-HEP1) and human LIHC cell lines (HEP-G2, HEP-3B, and Huh7) were kindly provided by the laboratory of the Oncology Research Centre of Yanbian University. The cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % fetal bovine serum (FBS) and penicillin-streptomycin mixture (100 U/mL), while maintaining standard environmental conditions of 37 °C and 5 % CO_2 to ensure optimal growth conditions.

2.5. Western blot

Western blot analysis was performed using standard methods. Briefly, cells were lysed and proteins extracted. Equal amounts of proteins were separated by SDS/PAGE and then transferred to PVDF membranes (microtiter wells). After being closed with 5 % skimmed milk, the membrane was incubated with primary antibody at 4 °C for a period of time and then treated with horseradish peroxidase-conjugated secondary antibody (microtiter wells). The signal was determined by enhanced chemiluminescence (microtiter wells).

2.6. Gene expression analysis of FTL

The Human Protein Atlas HPA, (https://www.proteinatlas.org/), TIMER2.0 (http://timer.cistrome.org/), GEPIA (http://gepia.cancer-pku.cn/), Sangerbox (http://sangerbox.com/login.html), GSCA (http://bioinfo.life.hust.edu.cn/GSCA/#/) and UALCAN (https://ualcan.path.uab.edu/index.html) databases were used to explore FTL expression in pan-cancer.

2.7. Data analysis of FTL gene and clinicopathological characteristics of pan-cancer

The cBioportal website (https://www.cbioportal.org/) was used to investigate genetic alteration type and frequency of FTL in pan-cancer. The Sangerbox database was used to explore whether there is an intrinsic association between FTL and pan-cancer clinicopathological features. The relationship was explored by using Sangerbox for the expression of FTL in four typical pan-cancer clinicopathological features, including patient gender, age, tumor grade, and tumor stage.

2.8. Survival prognosis analysis of FTL gene

The survival analysis was performed by Kaplan-Meier survival curves and ROC curves ($P < 0.05$). We performed Cox analysis with the R packages "survival" and "forest plot" to identify the correlation between FTL expression and survival. FTL expression was examined for correlation with clinicopathological features by using the R packages "ggpubr" and "limma".

2.9. Analysis of FTL associated genes enrichment

The top 200 FTL-correlated genes were identified using the GEPIA2. The protein-protein interaction network of the top 50 FTL-correlated genes was constructed using STRING (https://string-db.org/). TIMER2.0' s "Gene_Corr" module is used to generate heatmap data for selected genes. Additionally, the two data sets were compared together for KEGG and GO pathway analysis.

2.10. FTL expression in LIHC and relationship to patients with LIHC

The CTR-DB (http://ctrdb.cloudna.cn/) database was used to probe drug sensitivity of LIHC patients with high FTL expression. ROC curves to explore the diagnostic value of FTL in LIHC. The GEPIA and UALCAN databases were used to explore FTL expression in LIHC and normal liver tissue. Three different databases: GEPIA, GSCA, and UALCAN were utilized to explore the effect of FTL on the clinical stages of LIHC, the UALCAN database alone was also used to explore the effect of FTL on age and the expression of the TP53

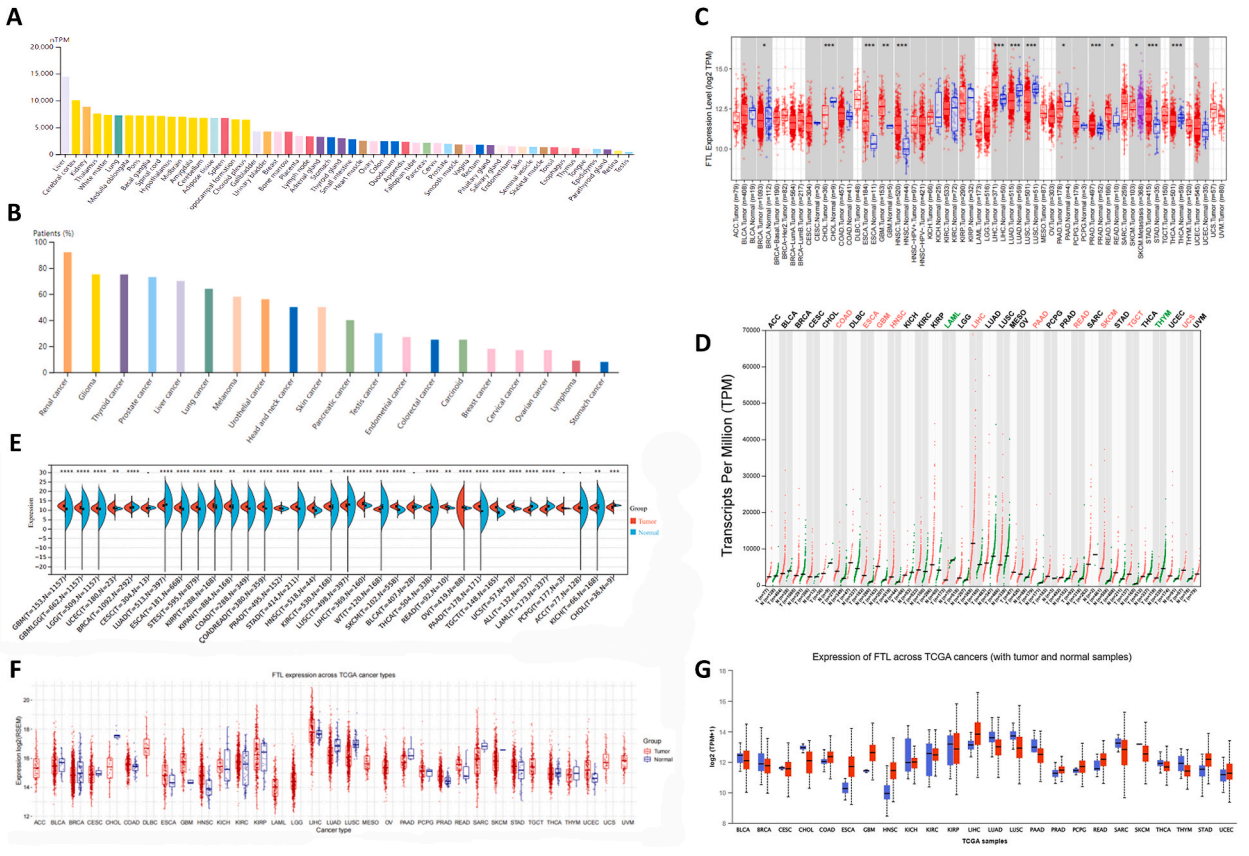

Fig. 2. Upregulation of FTL expression in human normal tissues and pan-cancer. (A) Expression of FTL in human normal tissues from the HPA database. **(B)** Expression of FTL in pan-cancer in the HPA database. **(C–G)** Expression of FTL in pan-cancers compared with normal tissues from TIMER2.0 (C), GEPIA (D), SangerBox (E), GSCA (F), and UALCAN (G) databases. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$.

gene in patients with LIHC. The impact of FTL on the survival of patients with LIHC was finally derived from the TIMER and UALCAN databases.

2.11. Data analysis relevance between FTL expression and tumor microenvironment

GO enrichment analysis of FTL at LIHC was performed using LinkedOmics (https://www.linkedomics.org/), which in turn validated the effect of FTL on LIHC immune cells by the TISCH database. Finally, the effect of FTL on LIHC immune infiltration was explored using the TIMER database.

2.12. Statistical analysis

In this study, the t-tests were used to assess the expression differences of FTL in tumor tissues and normal tissues. The univariate Cox regression analysis was used to derive HR values as well as p-values of the survival analysis. The analysis of the association between two variables was done using Spearman's test or Pearson's test. *P*-values less than 0.05 were regarded as being statistically significant. The following notes are used to illustrate statistical significance: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and $****P < 0.0001$.

3. Results

3.1. FTL was abnormally overexpressed in human pan-cancer

First, we used the HPA database to search for the expression of FTL in various normal human tissues and found that the expression level of FTL was high in a variety of tissues and organs, including the liver, cerebral cortex, kidney, thalamus, white matter, lung, medulla oblongata, pons, basal ganglia, spinal cord, hypothalamus, midbrain, amygdala, cerebellum, adipose tissue, spleen, and choroid plexus (Fig. 2A). The HPA cohort showed that FTL was highly expressed in most tumour tissues, and because FTL was predominantly expressed in the cytoplasm, the cytoplasm was moderately to highly positive for FTL in most cancer patients. FTL expression was weak or negative only in lymphoma, gastric cancer, breast cancer, ovarian cancer, and testicular cancer (Fig. 2B). Analyses using the TIMER2.0 database similarly showed high levels of FTL expression in various tumour subpopulations, including ESCA (Oesophageal carcinoma), GBM (Glioblastoma multiforme), HNSC (Head and Neck squamous cell carcinoma), COAD (colon adenocarcinoma), LIHC (Liver hepatocellular carcinoma), PRAD (Prostate adenocarcinoma), READ (rectum adenocarcinoma) and

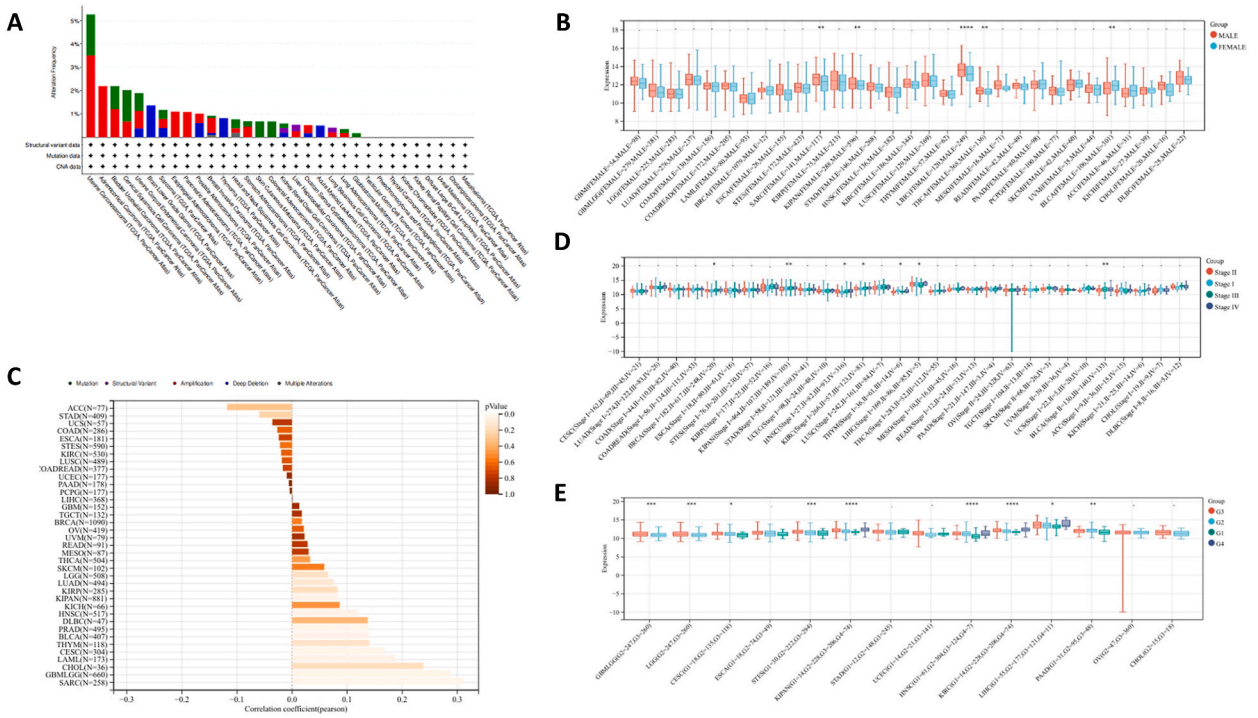

Fig. 3. Correlation between FTL expression and clinicopathologic features of pan-cancer. (A) Genetic alteration type and frequency of FTL in pan-cancer of TCGA by cBioportal website. **(B)** Gender-specific expression of FTL in the Sangerbox database. **(C)** FTL expression at different ages in the Sangerbox database. **(D)** FTL expression at different clinical stages in the Sangerbox database. **(E)** FTL expression in the Sangerbox database for different tumor grades. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and $****P < 0.0001$.

STAD (stomach adenocarcinoma) compared with the normal tissues (Fig. 2C, all $P < 0.05$). FTL expression levels in the significantly differentially expressed tumours and normal tissues were further analysed using various databases, including GEPIA, SangerBox, GSCA, and UALCAN, all of which revealed that FTL expression levels were high in various cancer types (Fig. 2D–G, all $P < 0.05$). Together, these data suggest that FTL is highly expressed in all cancers.

3.2. Association of FTL expression with clinicopathological characters of pan-cancer

We then explored the correlation between FTL and different clinicopathological features in patients with pan-cancer. Genetic alterations in FTL, leading to aberrant cancer expression, may affect the clinicopathological features of patients. According to search results from the cBioportal website, we found a high frequency of FTL alterations, mainly in the form of amplifications and mutations (Fig. 3A). According to Sangerbox data analysis, FTLs were correlated with sex, age, grading, and staging of tumour progression in patients with pancreatic cancer. We found that FTL expression was higher in men than in women in most tumours and was significantly higher in SARC, KIPAN, LIHC, and THCA (Fig. 3B, $P < 0.05$). Second, the study data showed that the relationship between FTL and age differed for different tumour types. FTL positively correlated with age in SARC, GBMLGG, and CHOL, whereas the opposite was true for ACC and STAD (Fig. 3C). In addition, FTL expression was strongly associated with the clinical stage and grade of most tumours in patients with pan-cancer, which was corroborated by data analysis (Fig. 3D–E, $P < 0.05$). In conclusion, FTLs have a profound impact on tumour progression in patients with pan-cancer.

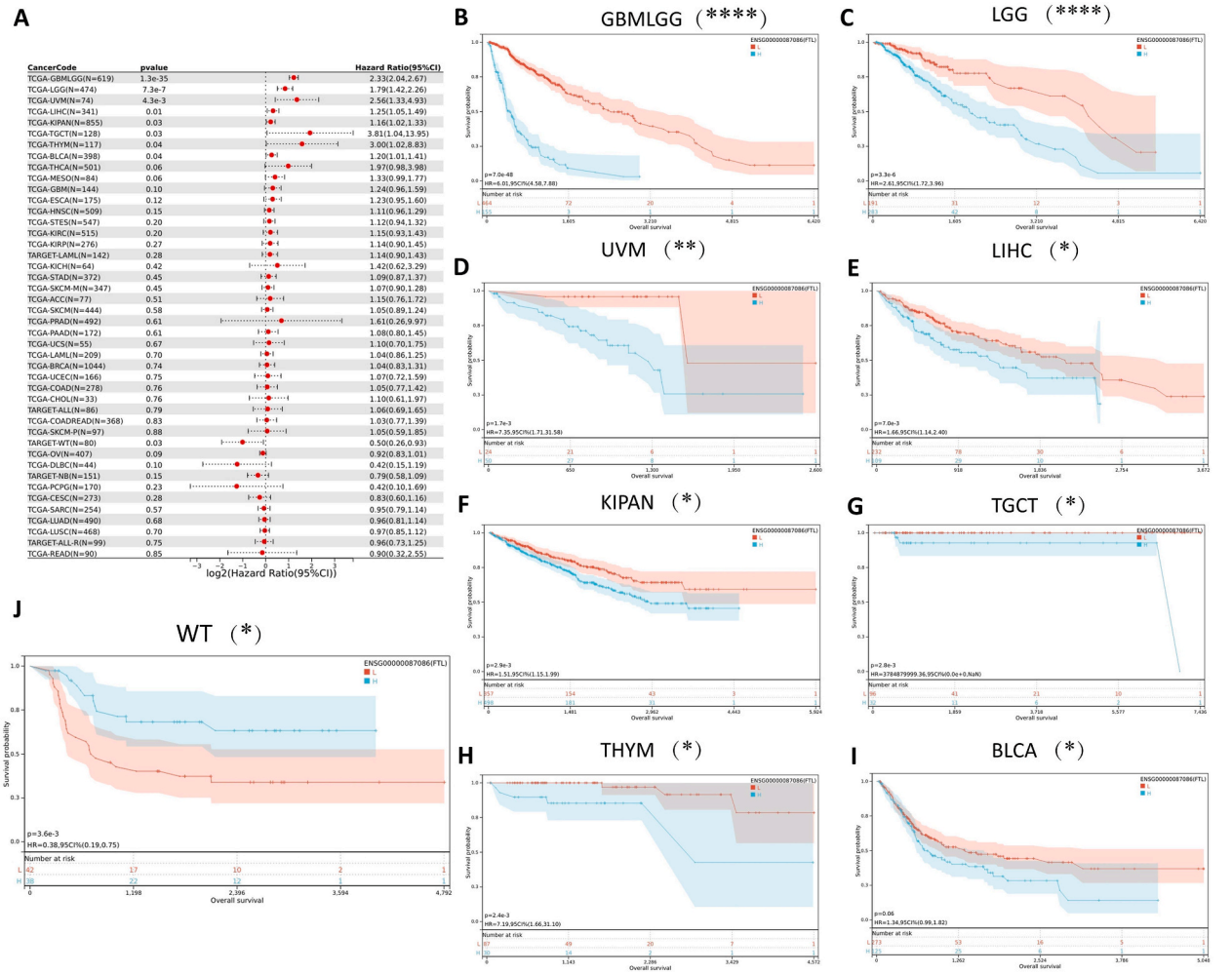

Fig. 4. Impact of FTL on survival as well as prognosis of patients with pan-cancer. (A) Relationship between FTL and pan-cancer survival by univariate COX analysis. **(B–J)** Impact of high FTL expression on survival and prognosis of patients 00with different tumours analysed by Kaplan-Meier overall survival (OS) including GBMLGG (B), LGG (C), UVM (D), LIHC (E), KIPAN (F), TGCT (G), THYM (H), BLCA (I), and WT (J) $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and $****P < 0.0001$.

3.3. Correlation of FTL with survival levels and prognosis in patients with pan-cancer

We investigated whether FTLs affect the survival and prognosis of patients with pan-cancer. According to univariate Cox analysis, FTL expression in these tumours, GBMLGG, LGG, UVM, LIHC, KIPAN, TGCT, THYM, BLCA, and WT, was correlated with overall patient survival (Fig. 4A, $P < 0.05$). The results of the Kaplan-Meier overall survival (OS) analysis showed that high FTL expression was significantly associated with shorter overall survival and poor prognosis in patients with GBMLGG, LGG, UVM, LIHC, KIPAN, TGCT, THYM, and BLCA (Fig. 4B–I, all $P < 0.05$). Interestingly, in the WT group, patients with high FTL expression had a better overall survival than those with low FTL expression (Fig. 4J, $P < 0.05$). Nevertheless, our study suggests that FTL expression in patients with pan-cancer has an important impact on both survival and prognosis.

3.4. Enrichment analysis of FTL-related genes

Enrichment analysis of target genes is an important piece of our study of the molecular mechanisms behind cancer-associated genes. We used STRING to analyse 50 experimentally verified proteins that may interact with FTL; the interaction network diagram of these proteins is illustrated in Fig. 5A. We then used the GEPIA2.0 database to screen 100 genes highly correlated with FTL and found six genes most closely linked to FTL (Fig. 5B). The accompanying heatmap further demonstrated that FTL were positively related to the above-mentioned five genes (FTH1, TKT, GPX2, PRDX2, and AKR1B10) in a wide range of tumours (Fig. 5C). Subsequently, cross-tabulation analysis of protein genes that interact with FTL and genes that are highly correlated with FTL revealed the following four genes: FTH1, PRDX1, TXNRD1, and RPS20 (Fig. 5D). Furthermore, Enrichment analysis of KEGG data suggests a mechanism by which FTL regulates tumour progression, including pathways such as Ferroptosis, and it is remarkable that LIHC was singled out as a pathway in the enrichment analysis results, suggesting that FTL and its related genes are closely related to LIHC (Fig. 5E).

3.5. FTLs are overexpressed in LIHC and play an important role in the evolution of LIHC

The expression levels of FTL were higher in LIHC tissues than in normal tissues according to the GEPIA and UALCAN databases (Fig. 6A, all $P < 0.05$). We analysed UALCAN and found that the expression level of FTL was different in LIHC patients of different ages, and the older the LIHC patients were, the more significant was the expression of FTL (Fig. 6B, all $P < 0.05$). FTL mRNA expression positively correlated with LIHC tumour grade (Fig. 6C, all $P < 0.05$). In addition, using multiple databases, including GEPIA, GSCA, and UALCAN, we further revealed that the expression trend of FTL was from the early to late stages in patients with LIHC (Fig. 6D, all $P < 0.05$). Moreover, using the UALCAN database, we found that high expression of FTL promoted mutation of the TP53 gene in liver tissue and expression of TP53 in LIHC (Fig. 6E, all $P < 0.05$). To examine FTL expression in LIHC, FTL immunohistochemical staining was performed on 37 LIHC tissues and 15 adjacent normal tissues. FTL-positive staining was mainly observed in the cytoplasm of the cancer cells (Fig. 6F). The positive rate of FTL protein expression was dramatically higher in LIHC (94.6 %, 35/37) than in adjacent normal tissues (40 %, 6/15) ($P < 0.01$). In addition, the strongly positive rate of FTL was 67.57 % (25/37) in LIHC, which was also markedly higher than that in adjacent normal tissues (26.67 %, 4/15) ($P < 0.01$) (Table 1). Consistent with this result, FTL protein expression was increased in LIHC cell lines and was higher than that in the normal liver sinusoidal endothelial cell line (SK-HEP1). Moreover, the expression level of FTL in the primary LIHC cell line (HEP-G2) was higher than that in the other two LIHC cell lines (HEP-3B and Huh7) (Fig. 6G, all $P < 0.05$). FTL expression positively correlated with histological grade ($P = 0.046$) and clinical stage ($P = 0.026$) (Table 2). These results support the overexpression of the FTL protein in LIHC and its possible essential role in the occurrence and progression of LIHC.

3.6. High FTL expression predicts poor prognosis in patients with LIHC

The diagnostic value of the FTL in patients with LIHC was evaluated using receiver operating characteristic (ROC) curve analysis. The diagnostic value revealed that the AUC at 1 year was 0.61, that at 3 years was 0.69, and at 5 years was 0.77 by the time-dependent ROC curve analysis of LIHC (Fig. 7A, $P < 0.05$). The CTR-DB database was used to explore the sensitivity of LIHC patients with high expression of FTL to sorafenib, a commonly used chemotherapeutic agent in the clinic; LIHC patients with high expression of FTL showed a better response to sorafenib. (Fig. 7B and C; all $P < 0.05$). The survival probability of 365 LIHC cases was analysed using the UALCAN method, which confirmed that patients with high FTL expression had a lower survival probability than those with low FTL expression. Correspondingly, both the Kaplan-Meier and TIMER databases showed that the survival of patients with LIHC with higher FTL expression was lower than that of patients with lower FTL expression (Fig. 7D, all $P < 0.05$). Subsequently, the expression level of FTL and tumour grade of LIHC in the UALCAN database, as well as the sex differences in patients with FTL and LIHC, had a significant impact on patient survival, especially in male patients with LIHC (Fig. 7E, all $P < 0.05$). Therefore, we can conclude that the FTL has an important diagnostic value in patients with LIHC and is significantly associated with poor prognosis in patients with LIHC. FTL overexpression may also serve as a marker for the targeted use of chemotherapeutic agents. FTL may be an important diagnostic, therapeutic, and prognostic indicator of LIHC.

3.7. FTL is correlated with immune infiltration in LIHC

To further investigate the mechanism of FTL in LIHC, the researchers first examined the genes co-expressed with FTL in LIHC patients in the TCGA dataset using LinkedOmics. The study results showed that a significant number of genes were significantly

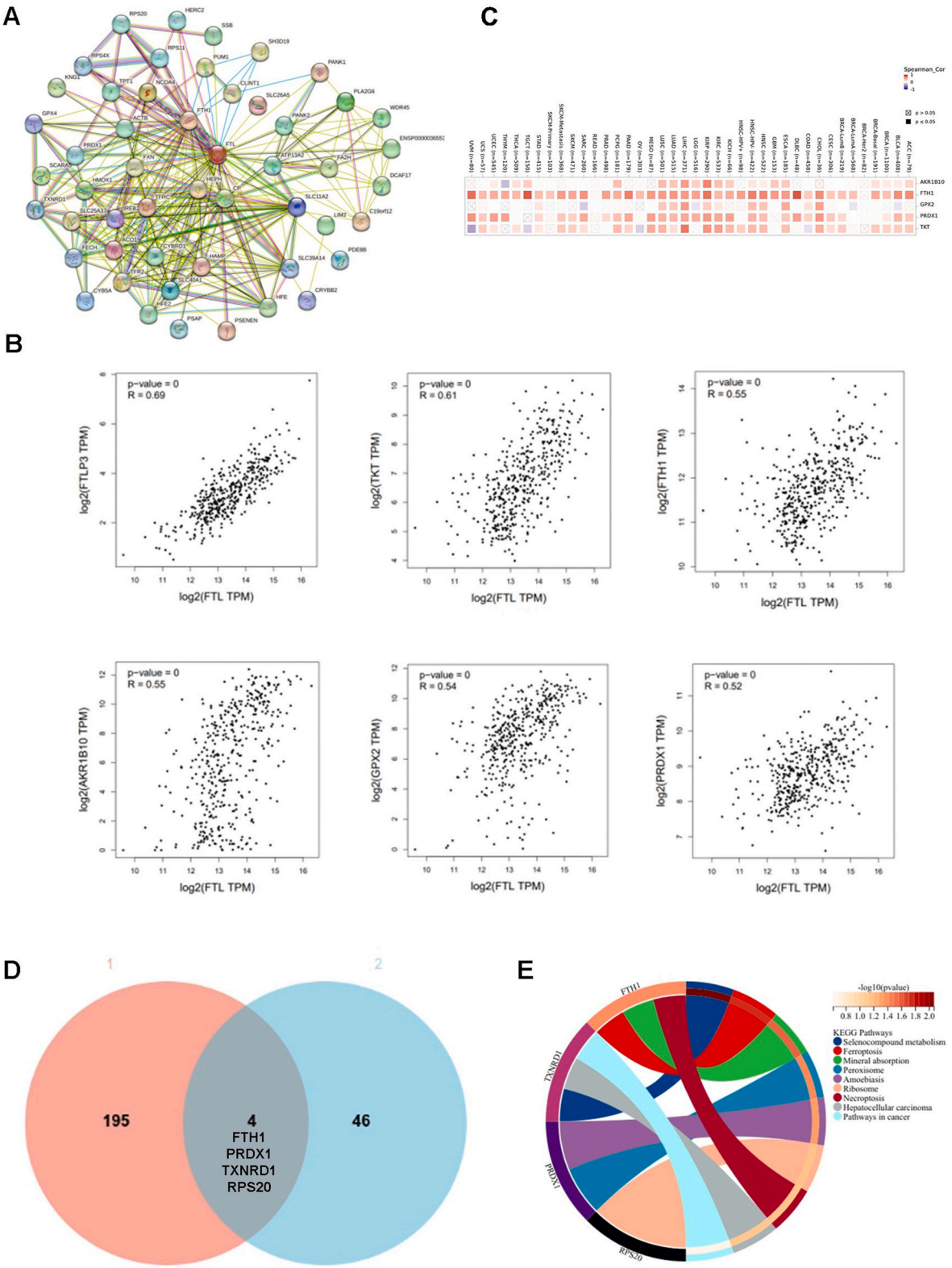

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Fig. 5. Enrichment analysis of FTL and its related genes. (A) Analysis of 50 proteins that potentially interact with FTL by STRING. **(B)** Six genes with the highest correlation were screened from 200 genes strongly associated with FTL by the GEPIA2 database. **(C)** Further evidence of the close association of these genes with FTL is provided by the heat map of GEPIA2. **(D)** The 50 proteins that interact with FTL and the 200 genes with the highest relevance were taken to the intersection by Sangerbox. **(E)** KEGG data enrichment analysis resulted in pathways with a high correlation with FTL. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$.

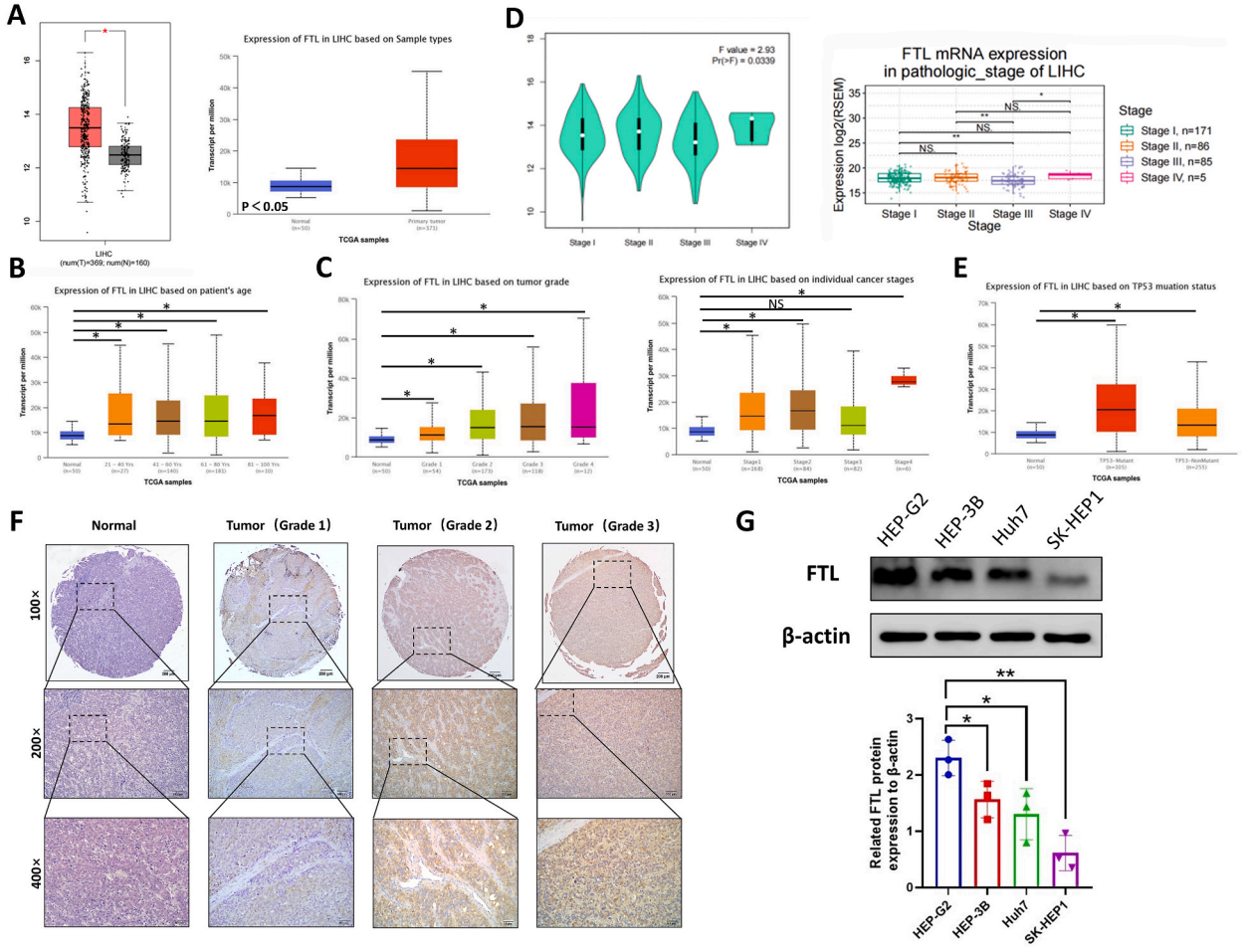

Fig. 6. Overexpression of FTL in LIHC and role in LIHC evolution. (A) Expression of FTL in LIHC and normal liver tissues in GEPIA and UALCAN databases. **(B)** Expression of FTL in LIHC patients of different ages by UALCAN database. **(C)** Analysis of FTL expression in different tumor grades of LIHC by UALCAN database. **(D)** FTL expression in different LIHC clinical stages was analysed by GEPIA, GSCA, and UALCAN databases. **(E)** Expression of FTL by the UALCAN database promotes mutation and expression of the TP53 gene in LIHC. **(F)** FTL expression in adjacent normal tissues and LIHC tissues as examined by IHC (× 100, × 200, × 400). Representative examples of FTL staining were shown. **(G)** Expression of FTL protein in normal liver sinusoidal endothelial cell line and LIHC cell lines were indicated by Western blot. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$.

correlated with FTL in LIHC (*FDR* < 0.05, $P < 0.05$). Among these 9234 genes, 4216 genes were positively correlated with FTL expression, while 5018 genes were negatively correlated with these factors (Fig. 8A, $P < 0.05$). The heatmap visualized the 50 genes that were positively and negatively correlated with FTL (Fig. 8B, $P < 0.05$). In the enrichment analysis, FTL was significantly correlated with response to interleukin-12, humoral immune response, response to transforming growth factor β, and axonal development in LIHC (Fig. 8C, $P < 0.05$). Therefore, it can be inferred that FTL is significantly associated with immune responses in LIHC. The effect of FTL on the infiltration level of various immune cells in LIHC was further explored. FTL was found to affect the infiltration levels of various immune cells in LIHC, including CD4T cells, CD8T cells, B cells, NK cells, Treg cells, DC cells, ILC cells, Mast cells, Mono/Macro cells, and Plasma cells (Fig. 8D). This shows the profound impact of FTL on LIHC immunity. To investigate further the connection between FTL expression and LIHC immune infiltration, we investigated the relationship between FTL and pan-cancer immunomodulators and found a positive correlation between FTL and CTLA4, an immune inhibitor of LIHC among pan-cancer immunoinhibitors (Fig. 8E, $P < 0.05$). However, among the pan-cancer immunostimulators, FTL was negatively correlated with TNFSF15, an

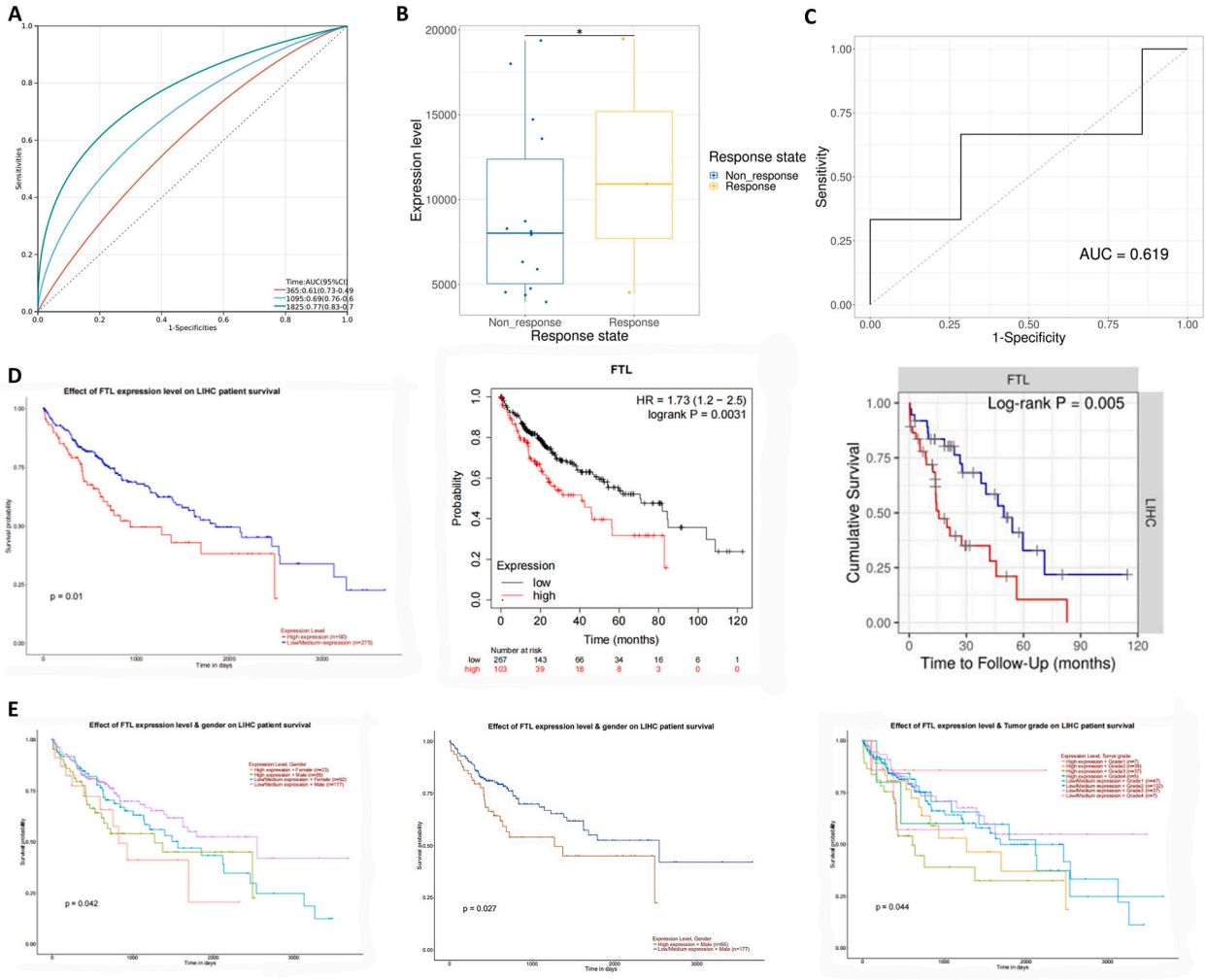

Fig. 7. High FTL expression leads to poor survival in patients with LIHC. (A) Diagnostic value of FTL in LIHC patients by ROC curve analysis. **(B–C)** Application of the CTR-DB database to explore the sensitivity of FTL overexpressed patients with LIHC to sorafenib. **(D)** Association between FTL expression and survival of LIHC patients in the UALCAN database. Association of FTL expression with survival in LIHC patients in the Kaplan-Meier and TIMER databases. **(E)** Survival of patients with different tumor grades and different sexes and exclusively male LIHC according to FTL expression in the UALCAN database. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and $****P < 0.0001$.

immunostimulator of LIHC (Fig. 8F, $P < 0.05$). The above results confirm that FTL affects the immunotherapeutic response in LIHC by influencing the level of immune cell infiltration in LIHC. FTL may be a potential immune marker for LIHC.

4. Discussion

Many previous studies have shown that high FTL expression is associated with poor prognosis in a variety of cancers, including glioma [6], liver hepatocellular carcinoma [7,8], gastric cancer [10] and malignant mesothelioma [12]. In addition, by targeting p21, p27, CDK2, and pRb, FTL may promote the proliferative capacity of malignant mesothelioma cells by arresting them in G1 phase [13]. Cujic et al. reported that FTL can bind to ferritin and improve the diagnostic efficiency of LIHC when serum alpha-fetoprotein levels are low in vivo [14]. Ren et al. found that FTL expression was increased in both liver cirrhosis and LIHC; however, FTL is not suitable as a marker for the early diagnosis of LIHC due to its insufficient specificity [15]. Our study provides a more intuitive and powerful demonstration of the specific relationship and potential link between FTL and LIHC. In this study, we noted a significant increase in FTL protein levels in LIHC tissues compared to adjacent non-tumour tissues ($P < 0.05$). We systematically confirmed the diagnostic value of FTL in LIHC using ROC analysis. However, previous studies have revealed that preoperative serum ferritin (SF) levels are not reliable predictors of survival in LIHC patients treated with transarterial chemoembolisation (TACE) [16]. One limitation of this study is that it was a retrospective single-group analysis with a relatively small sample size, which may have been subject to bias. Moreover, for the first time, we utilized various scientifically reliable bioinformatics datasets in combination with in vitro cell function experiments to confirm a potential link between FTL and LIHC. First, we used bioinformatics databases to confirm that mRNA formed by the

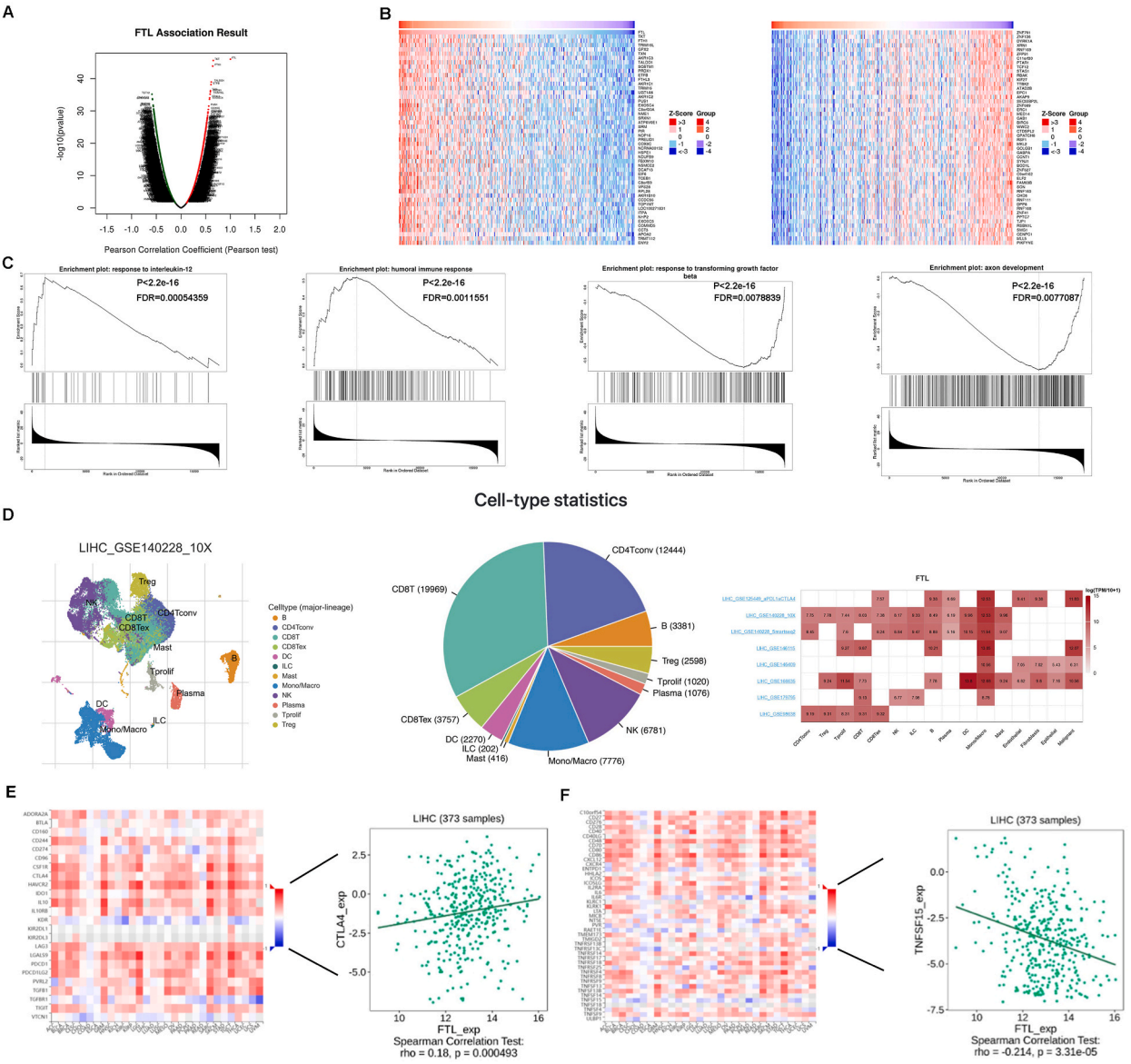

Fig. 8. The strong association between FTL and immune infiltration in LIHC. (A) Pearson's test revealed highly associated FTL genes in a LIHC cohort. **(B)** The most significant 50 genes positively and negatively correlated to FTL. **(C)** The findings of the GO enrichment analysis. **(D)** The impact of FTL on the levels of infiltration of various immune cells in LIHC was revealed by the TISCH database. **(E)** Relationship of FTL to LIHC immunoinhibitor in pan-cancer immunomodulators. **(F)** Relationship of FTL to LIHC immunostimulator in pan-cancer immunomodulators. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$.

transcription of the FTL gene was highly expressed in 10 tumour tissues and weakly expressed in two tumour tissues compared to that in neighbouring normal tissues. Second, in pan-cancer survival analysis, we found that FTL expression was associated with poor prognosis in a variety of cancers. Subsequently, we found that the upregulation of FTL expression was significantly associated with poor prognosis in patients with LIHC using Kaplan-Meier and univariate Cox regression analyses. Therefore, we specifically explored the relationship between FTL and LIHC and found that high expression of FTL in LIHC not only led to poor prognosis of patients but also correlated with the tumour grade of LIHC patients and even with the sex of LIHC patients. These findings clearly indicate that the FTL is a potential biomarker for predicting the prognosis of patients with LIHC.

To explore the potential mechanisms by which FTL plays a role in LIHC and therapeutic strategies associated with FTL, we investigated related genes that interact with and are highly similar to FTL and identified four genes with the highest relevance to FTL: (FTH1, PRDX1, TXNRD1, and RPS20). The ferritin heavy chain (FTH1), FTH1 has iron oxidase activity that specifically oxidises ferrous iron (Fe^{2+}) to trivalent iron (Fe^{3+}), whereas FTL is mainly associated with the stabilisation of ferric nucleophiles and assembly of ferritin. The ratio of these two chains varies from tissue to tissue, controlling iron storage and supply, and FTH1 has been investigated

as a possible link to iron death in several cancers [5,17]. Peroxiredoxin 1 (PRDX1) is a member of the peroxidase-dismutase family. PRDX1 has been reported to be aberrantly expressed in a variety of tumours. Peroxiredoxin 1 (PRDX1) is a classic 2-cysteine (2-Cys) peroxide dismutase and the most abundant and widely distributed peroxide dismutase isoform. PRDX1 is abnormally expressed at elevated levels in various tumours, including LIHC. Previous studies have shown that PRDX1 is a pro-oncogenic protein in LIHC [18,19]. Both these points suggest exploring potential therapeutic strategies for LIHC in future studies using FTL and the role of both in LIHC as an entry point. Thioredoxin reductase 1 (TXNRD1) is a selenocysteine-containing protein that is highly expressed in various malignancies. Multifactorial analysis has shown that TXNRD1 is an independent factor affecting the prognosis of patients with LIHC [20]. Therefore, TXNRD1 is a potential biomarker for the prognosis of LIHC [4,20]. Ribosomal protein S20 (RPS20) is also considered an oncogene that plays a role in LIHC [21]. Consistent with previous studies, FTL may act synergistically with the above four genes to promote tumour evolution [22–24]. Sorafenib, a commonly used clinical chemotherapeutic agent for the treatment of LIHC, is often rendered ineffective due to drug resistance [25]. In this study, we found that the FTL may be a potential marker for sorafenib in the treatment of LIHC and may help ameliorate drug resistance, providing new ideas for existing clinical treatment strategies. Understanding the functions of FTL-related genes and their interactions may provide new and effective treatment strategies for LIHC.

Increasing evidence suggests that macrophages play crucial roles in the development and progression [26–28]. Wang et al. have explored ways to improve the prognosis of patients with LIHC through immune cells by constructing models relating different immune cells to the prognosis of LIHC using bioinformatics [29,30]. Therefore, we investigated the relationship between FTL and immune cell infiltration in patients with LIHC. Our results showed that FTL positively correlated with immune B cells, $CD8^+$ T cells, $CD4^+$ T cells, and macrophages in patients with LIHC. Previous studies have found that M1-type tumour-associated macrophages (TAMs) can induce the TME to secrete classical inflammatory cytokines, which kill the tumour through necrosis of tumour cells and infiltration of immune cells. In contrast, M2-type TAMs display potent pro-tumour functions, including degradation of the tumour extracellular matrix, promotion of neovascularisation, and recruitment of immunosuppressive cells [31–34]. Hu et al. found that FTL levels were significantly and positively correlated with tumour invasion by TAMS and T regulatory cells in many cancers [35]. Similarly, our study found a close relationship between FTL and the aforementioned immune cells in LIHC. A growing number of studies have shown that the immune status of a tumour is closely related to the cellular composition and infiltration level of its environment [36–39]. Our results showed that FTL expression in LIHC was negatively correlated with the stromal score and tended to be negatively correlated with the immune and ESTIMATE scores, suggesting that LIHC tumours with high FTL expression were purer and more malignant. This suggests that high FTL expression is closely associated with immune infiltration in LIHC, and that FTL is likely to be a potential immune marker for future LIHC immunotherapy.

In conclusion, our study demonstrated for the first time systematically the high expression of FTL in pan-cancer and the diagnostic and prognostic value of FTL for LIHC. Abnormal expression of FTL predicts poor prognosis in patients with liver hepatocellular carcinoma and is associated with immune cell infiltration in TME of liver hepatocellular carcinoma. Furthermore, this study identified for the first time the significant value of highly expressed FTL for early diagnosis of LIHC and clinically targeted drugs. Various bioinformatics analyses and in vitro cell function experiments demonstrated that FTL was closely associated with LIHC. Therefore, it is reasonable to believe that FTL is very likely to become an effective biomarker for the diagnosis and treatment of LIHC in the future, thus benefiting liver hepatocellular carcinoma patients.

Ethical approval

This study was conducted in accordance with the principles of the Declaration of Helsinki. This study was approved by the Institutional Ethics Committee of Shanghai Otto Biotechnology Co. (approval number: SHYSJS-QT-2101). All patients were obtained informed consent before the experiment.

Data and code availability

Data included in article/supp. Material/referenced in article.

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CRediT authorship contribution statement

Aoqun Li: Writing – review & editing, Writing – original draft. **Yue Li:** Formal analysis. **Xiaoqing Li:** Data curation. **Chunxiao Tang:** Methodology. **Yang Yang:** Visualization. **Nan Li:** Resources. **Yun Jin:** Investigation, Funding acquisition.

Declaration of competing interest

All authors declare that there is no conflict of interest in the publication of this article.

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