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Comprehensive analysis of clinical and biological value of *ING* family genes in liver cancer

Shi-Cai Liu

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Abstract

BACKGROUND

Liver cancer (LIHC) is a malignant tumor that occurs in the liver and has a high mortality in cancer. The *ING* family genes were identified as tumor suppressor genes. Dysregulated expression of these genes can lead to cell cycle arrest, senescence and/or apoptosis. *ING* family genes are promising targets for anticancer therapy. However, their role in LIHC is still not well understood.

AIM

To have a better understanding of the important roles of *ING* family members in LIHC.

METHODS

A series of bioinformatics approaches (including gene expression analysis, genetic alteration analysis, survival analysis, immune infiltration analysis, prediction of upstream microRNAs (miRNAs) and long noncoding RNAs (lncRNAs) of *ING1*, and *ING1*-related gene functional enrichment analysis) was applied to study the expression profile, clinical relationship, prognostic significance and immune infiltration of *ING* in LIHC. The relationship between *ING* family genes expression and tumor associated immune checkpoints was investigated in LIHC. The molecular mechanism of *ING1* mediated hepatocarcinogenesis was preliminarily discussed.

RESULTS

mRNA/protein expression of different *ING* family genes in LIHC was analyzed in different databases, showing that *ING* family genes were highly expressed in LIHC. In 47 samples from 366 LIHC patients, the *ING* family genes were altered at a rate of 13%. By comprehensively analyzing the expression, clinical pathological parameters and prognostic value of *ING* family genes, *ING1/5* was identified. *ING1/5* was related to poor prognosis of LIHC, suggesting that they may play key roles in LIHC tumorigenesis and progression. One of the target miRNAs of *ING1* was identified as hsa-miR-214-3p. Two upstream lncRNAs of hsa-miR-214-3p,

U91328.1, and HCG17, were identified. At the same time, we found that the expression of *ING* family genes was correlated with immune cell infiltration and immune checkpoint genes.

CONCLUSION

This study lays a foundation for further research on the potential mechanism and clinical value of *ING* family genes in the treatment and prognosis of LIHC.

Key Words: Liver cancer; *ING* family genes; Noncoding RNAs; Immune cell infiltration; Prognosis

Core Tip: Liver cancer (LIHC) is a malignant tumor that occurs in the liver and has a high mortality in cancer. Comprehensive research of expression, mutation, prognosis, and biological mechanisms of *ING* family genes in LIHC is still lacking. We studied the expression profile, clinical relationship, prognostic potential and immune infiltration of *ING* family genes in LIHC. The relationship between *ING* family genes expression and tumor associated immune checkpoints was investigated in LIHC. The molecular mechanism of *ING1*-mediated hepatocarcinogenesis was preliminarily discussed. Our results highlight the potential mechanism and clinical value of *ING* family genes in treatment and prognosis of LIHC.

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INTRODUCTION

Liver cancer (LIHC) is a malignant tumor that occurs in the liver and has a high mortality in cancer[1,2]. At present, the main methods to treat LIHC are radiotherapy, chemotherapy, surgery and liver transplantation[3,4]. Recently, although immunotherapy has made significant progress, such as programmed cell death-1 (PD-1) targeted therapy, its prognosis is poor[5]. Therefore, the discovery of new markers is of importance for individualized therapy and effective improvement of prognosis.

The *ING* family genes (*ING1-5*) were identified as tumor suppressor genes[6,7]. Dysregulated expression of these genes can lead to cell cycle arrest, senescence and/or apoptosis[6]. *ING2* is highly expressed in colon cancer and induces colon cancer cells invasion through matrix-metalloproteinase-13-dependent pathway[8]. Studies have shown that knockdown of *ING2* with small interfering RNA (siRNA) leads to premature aging of normal fibroblasts[9], and to apoptosis or cell-cycle arrest of various adherent cancer cells[10]. These findings indicate that *ING2* plays a role in malignant transformation or progression of cancer. Knockdown of *ING5* and *ING4* with siRNA inhibits DNA replication and the transition from G2/M to G1 phase, respectively[11], indicating that *ING4* and *ING5* play a role in cell proliferation. However, the exact effects and the mechanisms of *ING* family genes in the occurrence and development of LIHC are still unclear. Comprehensive research on the expression, mutation and biological mechanisms of *ING* family genes in LIHC is still lacking, and the correlation between *ING* members and tumor immune infiltration in LIHC is unclear.

In this study, based on several large public databases, the expression profile, clinical relationship, prognostic significance and immune infiltration of *ING* family genes in LIHC were explored. The association of *ING* family genes expression with tumor associated immune checkpoints was investigated in LIHC. The molecular mechanism of *ING1* mediated hepatocarcinogenesis was preliminarily discussed. Our results highlight the potential mechanism and clinical value of *ING* family genes in the treatment and prognosis of LIHC.

MATERIALS AND METHODS

Gene expression analysis

Using the Gene_DE module of Tumor Immune Estimation Resource 2.0 (TIMER2)[12], the expression of *ING* family genes in different cancers in The Cancer Genome Atlas (TCGA) project was analyzed. TIMER2 is freely available at http://timer.cistrome.org/. The University of Alabama at Birmingham Cancer (UALCAN) web-portal[13] was applied to study the expression of *ING* family genes in normal, primary tumor based on clinical pathological parameters, including sample types, individual cancer stages, race, sex, body weight, age, tumor histology, nodal metastasis status, *TP53* mutation status and tumor grade. UALCAN is freely available at https://ualcan.path.uab.edu/. The protein expression data of the Clinical proteomic tumor analysis consortium (CPTAC) was applied to study the expression of ING proteins in normal and primary tumors tissues.

Survival analysis

GEPIA2[14] was applied to acquire significant maps of overall survival (OS) and disease-free survival (DFS) for *ING* family genes. Patients with LIHC were divided into low-risk and high-risk subgroups based on low (50%) and high (50%) threshold values. The Kaplan-Meier curve was obtained by GEPIA2 survival analysis module. GEPIA2 is freely available at http://gepia2.cancer-pku.cn/.

Genetic alteration analysis

The cBio cancer genomics portal (cBioPortal) is a publicly database that provides resources for analyzing, exploring and visualizing genomics data of cancer[15]. Based on cBioPortal, the LIHC (TCGA, Firehose Legacy) dataset containing 366 samples was selected to explore the *ING* family genes, and their genetic changes were evaluated. cBioPortal is available at https://www.cbioportal.org/.

Immune infiltration analysis

The Immune-Gene module of TIMER2[12] was applied to visualize the relationship between the expression of *ING* family genes and the level of immune infiltration in LIHC tissues. The relationship between *ING* family genes expression and immune checkpoints and immune cell chemotaxis was evaluated.

Prediction of upstream microRNAs and long noncoding RNAs of ING1

The ENCORI/starBase database[16] is a comprehensive database specifically designed for RNA interactions, which can be applied to predict potential microRNAs (miRNAs) and long noncoding RNAs (lncRNAs) that may bind to *ING1* and corresponding miRNAs. The upstream binding miRNAs identification of *ING1* was based on these principles: Present in at least five databases including microT, miRanda, miRmap, PicTar, TargetScan, PITA, and RNA22. Co-expression analysis of miRNA-target and RNA-RNA was performed using the Pan-Cancer module. ENCORI is available at https://rnasysu.com/encori/.

ING1-related gene functional analysis

The STRING database[17] was searched with *ING1* and *Homo sapiens* (main parameters: "experiments", "no more than 50 interactors" in first shell, "evidence"). The experimentally validated *ING1* binding proteins were obtained through the above search. Based on the dataset of LIHC tissues in TCGA, the top 100 *ING1*-related target genes were obtained using the similarity gene detection module of GEPIA2. Using the GEPIA2[14] correlation analysis module, pairwise gene correlation analysis between *ING1* and the selected genes was performed. Functional analysis was performed through DAVID[18] to determine the biological function of genes. The *P* value was adjusted using the Benjamini-Hochberg method, and $P < 0.05$ was the critical standard. STRING is available at https://string-db.org/.

RESULTS

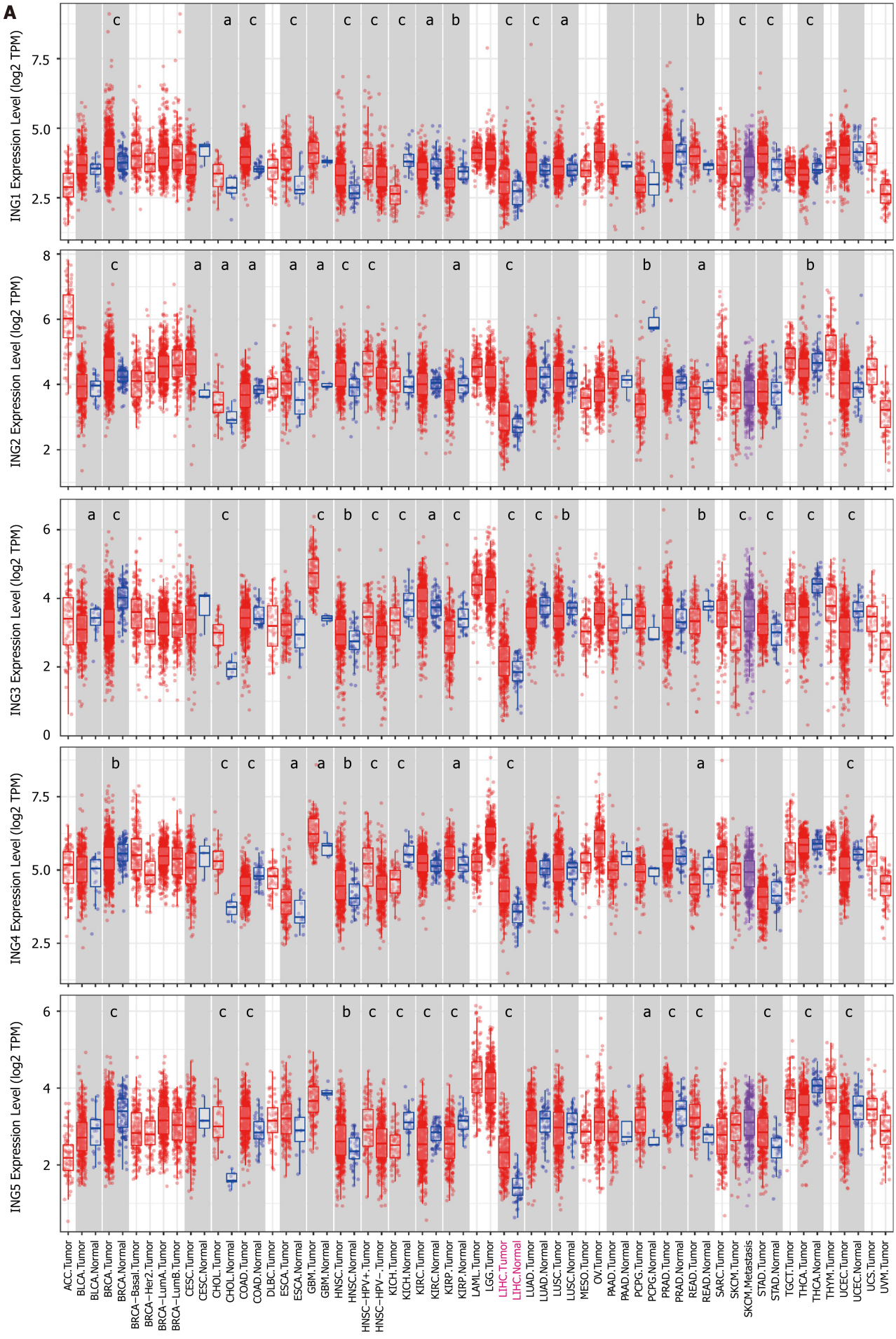
Expression of ING family genes in LIHC

To study the expression of *ING* family genes in LIHC, based on multiple databases, mRNA and protein expression was studied. Analysis by TIMER2[12] showed that mRNA expression of the *ING* family genes was significantly upregulated in LIHC tissues (Figure 1A). *ING* family genes were also significantly upregulated in other cancers, such as gallbladder cancer, head and neck cancer (Figure 1A). The transcriptional level of *ING* family genes was further studied using the UALCAN database[13], and similar results were obtained (Figure 1B), which also showed that mRNA expressions of *ING* family genes was significantly upregulated in LIHC tissues. The gene expression at the protein level is closer to the original manifestation of the disease. Therefore, the protein expression data of LIHC were studied using CPTAC (Figure 1C), which showed that protein expression of *ING1/3/4/5* was significantly upregulated in LIHC tissues. This further verified the reliability of these results.

Clinicopathological parameters of ING family genes in LIHC

After comprehensive research of the expression patterns of various *ING* family genes, we further studied the correlation between mRNA expression of *ING* family genes and clinical pathologic characteristics such as sex, age, individual cancer stage, race, body weight, and *TP53* mutation in LIHC patients. There was a significant difference in the expression of *ING2/3/5* between stages 1 and 3, and a significant difference in expression of *ING3* between stages 2 and 3 (Figure 2A). Between normal tissues and different pathological stages of LIHC, there were significant differences in the expression of *ING* family genes. There was no significant difference in the expression of *ING* family members among different races in LIHC (Figure 2B). There was no significant sex difference in expression of *ING* family members in patients with LIHC (Figure 2C). There was a significant difference in the expression of *ING5* between normal weight and obese patients with LIHC (Figure 2D). There was a significant difference in the expression of *ING5* between patients aged 41-60 and 61-80 years, and between ages 61-80 and 81-100 years (Figure 2E). The expression of *ING2/3/4/5* was significantly different between patients with nonmutated and mutated *TP53* (Figure 2F).

The protein expression data of LIHC from the CPTAC database were used to analyze the relationship between the expression of *ING* family members and the sex and age of patients (Figure 2G and H). There was no significant sex difference in the protein expression of *ING* family members in LIHC patients. There was a significant difference in the expression of *ING5* between patients aged 21-40 and 41-60 years, and between 21-40 and 81-100 years. Expression of *ING5*


A
ING1 Expression Level (log2 TPM)
ING2 Expression Level (log2 TPM)
ING3 Expression Level (log2 TPM)
ING4 Expression Level (log2 TPM)
ING5 Expression Level (log2 TPM)
ACC.Tumor
BLCA.Tumor
BLCA.Normal
BRCA.Tumor
BRCA.Normal
BRCA−Basal.Tumor
BRCA−Her2.Tumor
BRCA−LumA.Tumor
BRCA−LumB.Tumor
CESC.Tumor
CESC.Normal
CHOL.Tumor
CHOL.Normal
COAD.Tumor
COAD.Normal
DLBC.Tumor
ESCA.Tumor
ESCA.Normal
GBM.Tumor
GBM.Normal
HNSC.Tumor
HNSC.Normal
HNSC−HPV+.Tumor
HNSC−HPV−.Tumor
KICH.Tumor
KICH.Normal
KIRC.Tumor
KIRC.Normal
KIRP.Tumor
KIRP.Normal
LAML.Tumor
LGG.Tumor
LIHC.Tumor
LIHC.Normal
LUAD.Tumor
LUAD.Normal
LUSC.Tumor
LUSC.Normal
MESO.Tumor
OV.Tumor
PAAD.Tumor
PAAD.Normal
PCPG.Tumor
PCPG.Normal
PRAD.Tumor
PRAD.Normal
READ.Tumor
READ.Normal
SARC.Tumor
SKCM.Tumor
SKCM.Metastasis
STAD.Tumor
STAD.Normal
TGCT.Tumor
THCA.Tumor
THCA.Normal
THYM.Tumor
UCEC.Tumor
UCEC.Normal
UCS.Tumor
UVM.Tumor

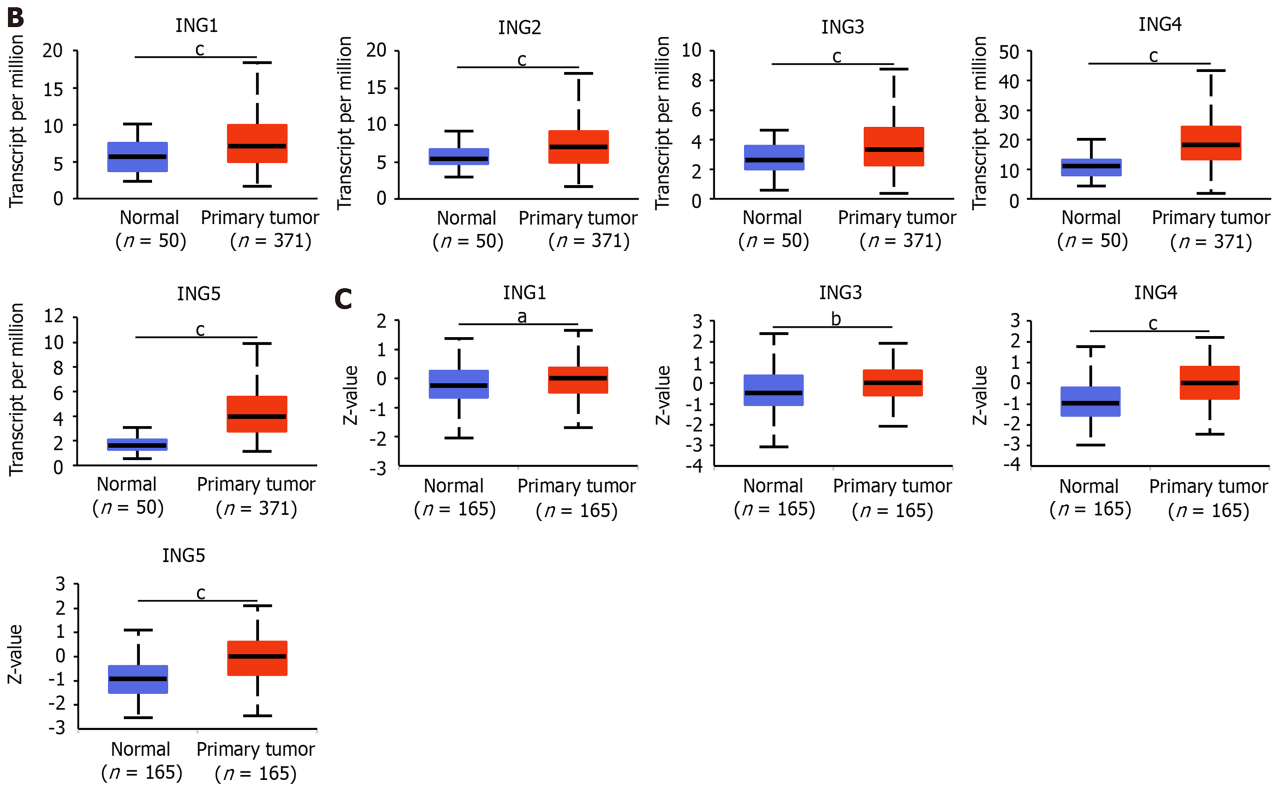


Figure 1 Differential expression of *ING* family genes in normal and cancer tissues. A: Expression of *ING* family genes in different tumor types using Tumor Immune Estimation Resource 2.0 (data from The Cancer Genome Atlas); B: Expression of *ING* family genes in liver cancer (LIHC) using the University of Alabama at Birmingham Cancer database; C: Protein expression of *ING* family members in normal and primary LIHC tissues based on the Clinical proteomic tumor analysis consortium dataset. [a]*P* < 0.05; [b]*P* < 0.01; [c]*P* < 0.001.

also differed significantly between patients aged 21-40 and 61-80 years. This study also evaluated the correlation between different *ING* family genes by analyzing their mRNA expression, showing that there was a significant positive correlation among all genes (Figure 2I).

Genetic alteration analysis of ING family genes in LIHC

The genetic changes in the *ING* family genes of LIHC patients were studied using the cBioPortal website. *ING* family genes were altered in 47 samples from 366 patients with LIHC, accounting for a 13% alteration rate (Figure 3). According to TCGA data, the genetic change percentages of *ING1-5* in LIHC were 5%, 4%, 2.7%, 0.8%, and 1.1%, respectively (Figure 3).

Immune cell infiltration of ING family genes in LIHC

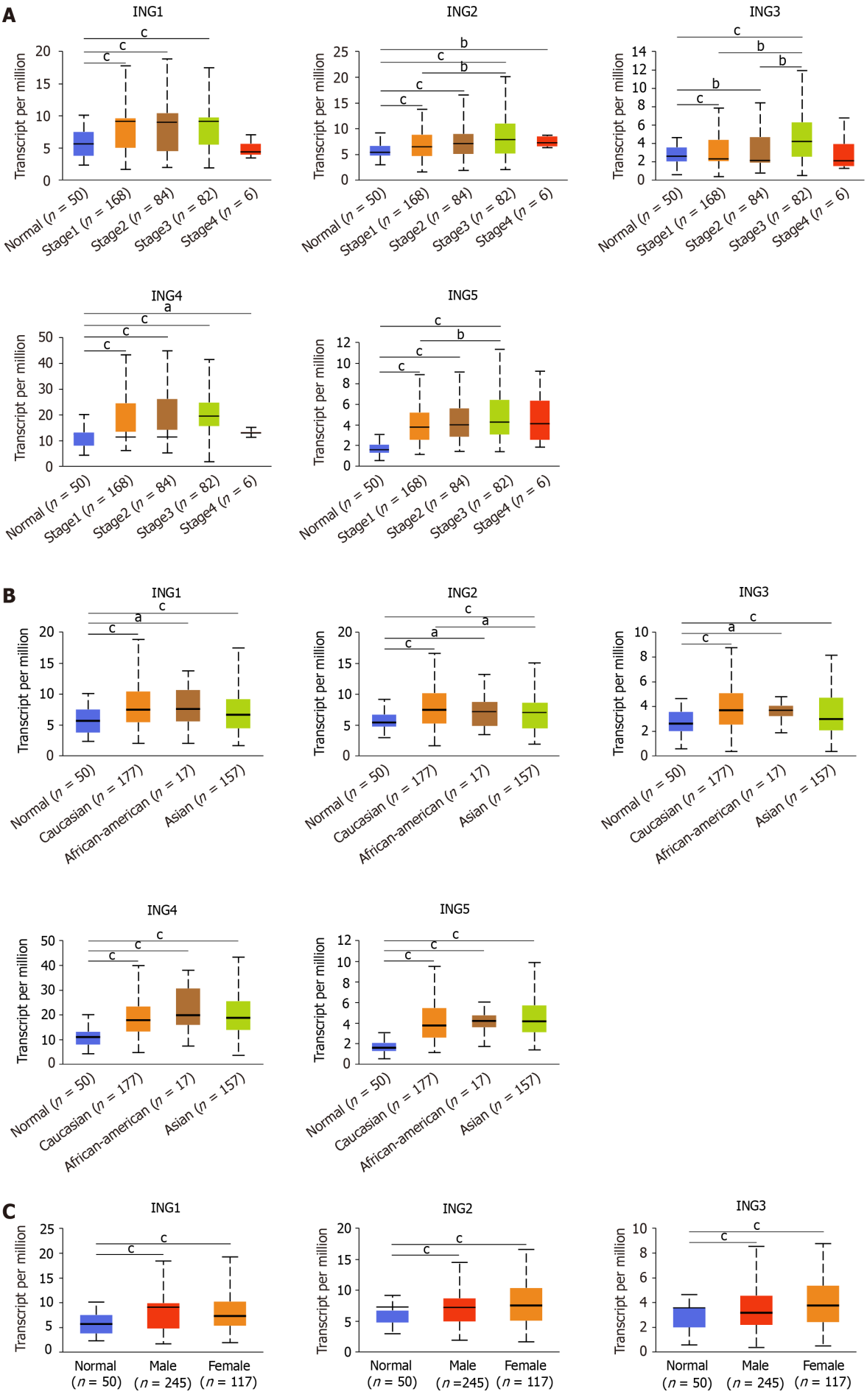
Emerging evidence supports the correlation between immune cell levels and the occurrence and progression of multiple types of tumors[19,20]. We explored the relationship between different *ING* members and immune cell infiltration levels in LIHC using TIMER2, which will contribute to the monitoring of immune therapy response in LIHC and the exploration of immune infiltration mechanisms. The expression of *ING1-4* was significantly positively related to neutrophils and CD8+ T cells (Figure 4). Besides, the expression of *ING1-5* was significantly positively related to cancer-associated fibroblasts, B cells, macrophages and myeloid dendritic cells. In addition, the expression of *ING2/3/5* was significantly positively related to CD4+ T cells. Supplementary Figure 1 shows the results in detail.

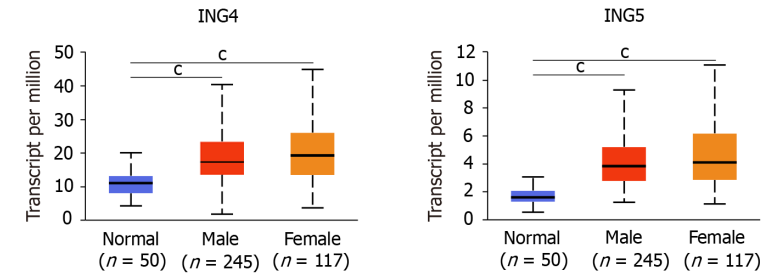
Correlation between expression of ING family genes and immune checkpoints in LIHC

Immunotherapy based on cytotoxic T lymphocyte-associated antigen (CTLA)-4 and PD-1/PD ligand (PD-L)1 has emerged as a new pillar of LIHC treatment[21,22]. We determined the effect of *ING* gene family expression on LIHC immunotherapy (Figure 5). Expression of *ING2/3/5* in patients with LIHC was significantly positively related to CTLA-4, PD-1, and PD-L1. Expression of *ING4* in LIHC was significantly positively related to PD-L1 and PD-1. Expression of *ING1* was significantly positively related to PD-L1. These results suggest that positive expression of *ING* family genes may be a better predictor of immunotherapy response than negative expression. Supplementary Figure 2 shows the results in detail.

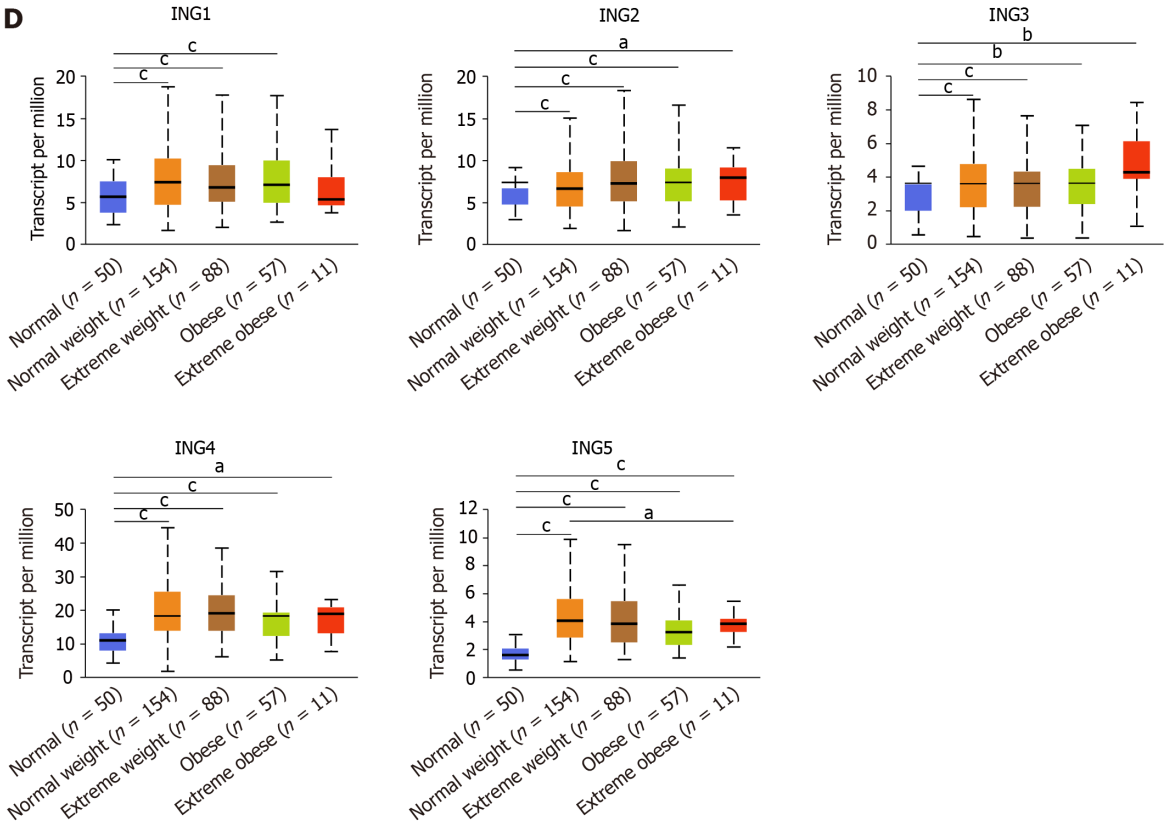
Survival analysis of ING family genes in LIHC

To investigate whether expression of *ING* family genes was related to prognosis of LIHC, we explored the prognostic potential of *ING* family genes in cancer using GEPIA2 tool. We were using two prognostic indicators, OS and DFS, to evaluate the prognostic potential of *ING* family genes. In OS analysis, high expression of *ING1/5* indicated poorer survival

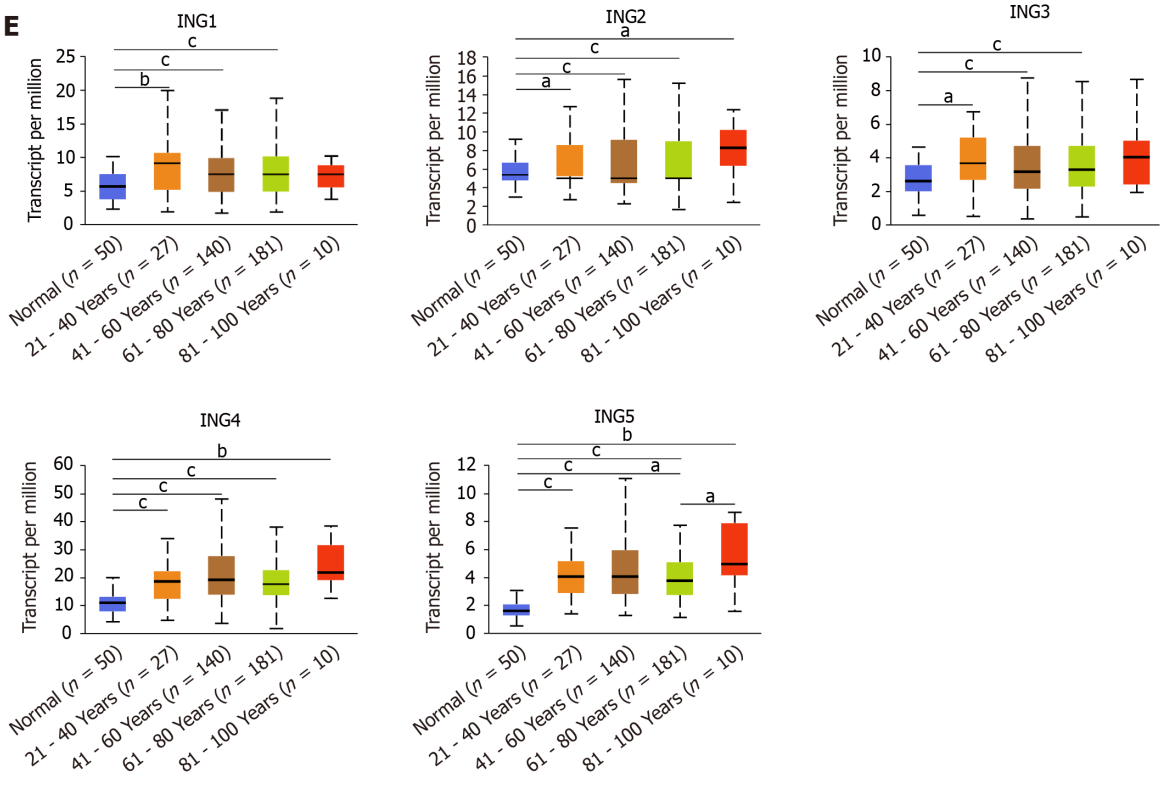

A
ING1
ING2
ING3
ING4
ING5
Transcript per million
Normal (n = 50)
Stage1 (n = 168)
Stage2 (n = 84)
Stage3 (n = 82)
Stage4 (n = 6)
B
ING1
ING2
ING3
ING4
ING5
Transcript per million
Normal (n = 50)
Caucasian (n = 177)
African-american (n = 17)
Asian (n = 157)
C
ING1
ING2
ING3
Transcript per million
Normal (n = 50)
Male (n = 245)
Female (n = 117)


ING4
Transcript per million
Normal (n = 50)
Male (n = 245)
Female (n = 117)
ING5
Transcript per million
Normal (n = 50)
Male (n = 245)
Female (n = 117)

D


ING1
ING2
ING3
ING4
ING5
Transcript per million
Normal (n = 50)
Normal weight (n = 154)
Extreme weight (n = 88)
Obese (n = 57)
Extreme obese (n = 11)

E


ING1
ING2
ING3
ING4
ING5
Transcript per million
Normal (n = 50)
21 - 40 Years (n = 27)
41 - 60 Years (n = 140)
61 - 80 Years (n = 181)
81 - 100 Years (n = 10)

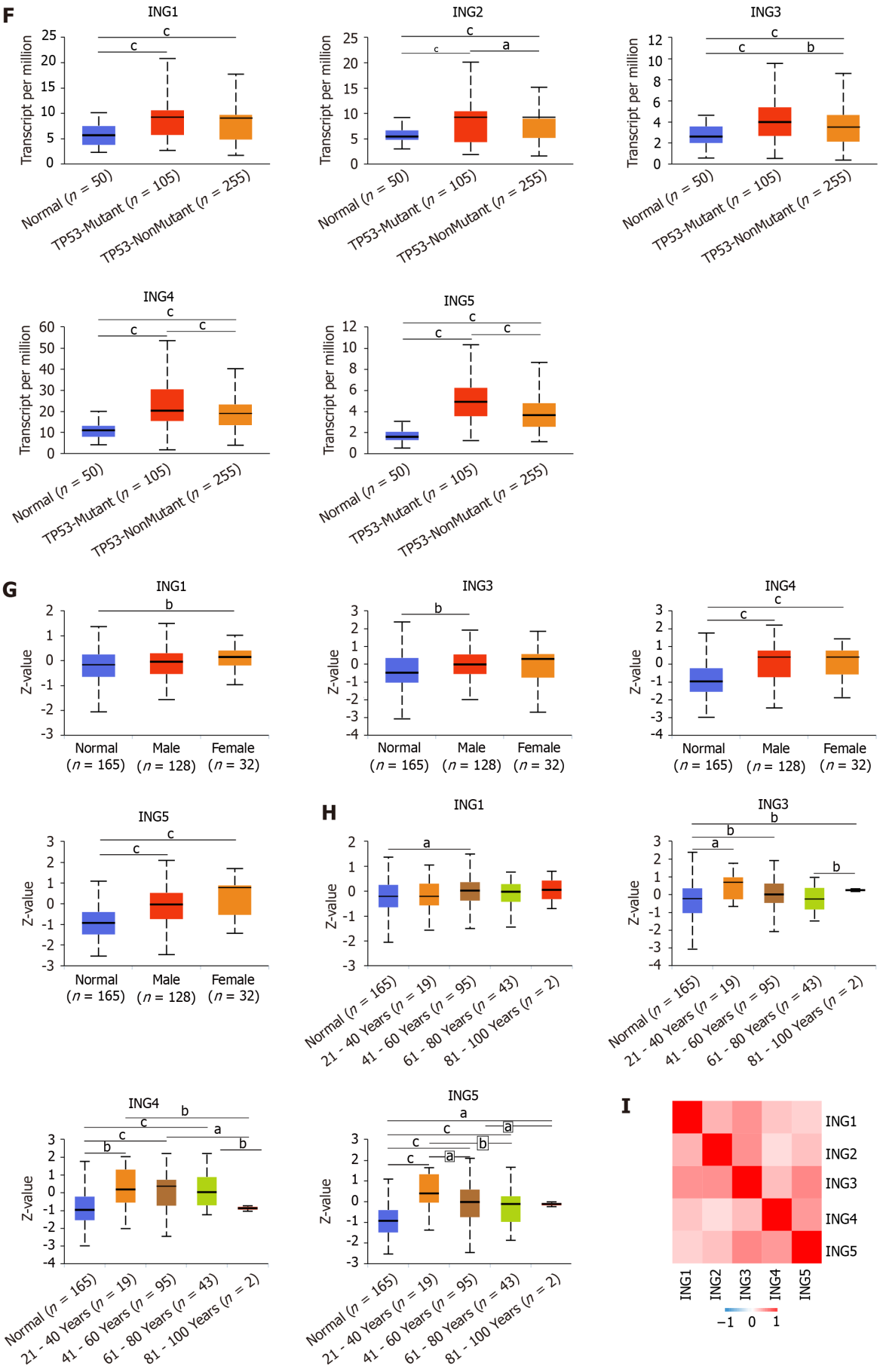


Figure 2 The relationships between the mRNA/protein expression of *ING* family members and clinicopathological parameters in liver cancer. A: Between mRNA expression of *ING* family members and individual cancer stages; B: Between mRNA expression of *ING* family members and race; C:

Between mRNA expression of *ING* family members and patient sex; D: Between mRNA expression of *ING* family members and patient body weight; E: Between mRNA expression of *ING* family members and patient age; F: Between mRNA expression of *ING* family members and TP53 mutation status; G: Between protein expression of *ING* family members and patient sex; H: Between protein expression of *ING* family members and patient age; I: Correlation of mRNA expression in different *ING* family members. $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$.

(Figure 6A). DFS analysis showed that high expression of *ING5* was related to poor prognosis (Figure 6B). The remaining *ING* family members were unrelated to survival time. Therefore, mRNA expression of *ING1/5* was significantly related to prognosis of LIHC and could serve as a useful predictive biomarker. Finally, based on comprehensive analysis of the expression, clinicopathological parameters, and prognostic potential of *ING* family genes, we identified that *ING1/5* may play important roles in the occurrence and progression of LIHC.

Upstream miRNAs prediction of ING1

ncRNAs refer to RNA that do not translate to proteins in the transcriptome but regulate gene expression at diversified levels, such as transcription, chromatin structure editing and RNA splicing[23]. To study whether *ING1* is regulated by ncRNAs, we used the ENCORI database to predict upstream miRNAs that may bind to *ING1*. Finally, three miRNAs were identified (hsa-miR-193a-3p, hsa-miR-193b-3p, and hsa-miR-214-3p). The results showed that hsa-miR-214-3p negatively regulated *ING1* expression with *r* and *P* value of -0.143 and 5.80×10^{-3}, respectively. The overall idea of miRNA and mRNA binding analysis was to find target genes and target miRNAs according to the negative correlation between miRNA expression and target gene expression in view of the targeting relationship between miRNA and mRNA[24]. hsa-miR-214-3p was identified as an upstream miRNA that might bind to *ING1*. We further explored whether hsa-miR-214-3p was involved in the regulation of *ING1* expression in LIHC. hsa-miR-214-3p was significantly downregulated in LIHC with $P = 2.11 \times 10^{-15}$ (Figure 7A). We further explored the correlation between hsa-miR-214-3p miRNA expression and clinical pathological characteristics of LIHC. Expression of hsa-miR-214-3p in different pathological stages of LIHC was lower than that in normal tissues, and the same results were shown for other clinicopathological parameters (Figure 7B-G). These results indicated that the regulation of *ING1* expression by hsa-miR-214-3p plays a function in the tumorigenesis of LIHC.

Upstream lncRNAs prediction of hsa-miR-214-3p

Using the ENCORI database, the upstream lncRNAs of hsa-miR-214-3p were explored. The differential expression of these lncRNAs in cancer and normal tissues was evaluated using the TCGA database. Only two lncRNAs were finally identified, namely U91328.1 and HCG17. Compared with normal tissue samples, their expression in LIHC was significantly upregulated (Figure 8A). The analysis showed that expression of U91328.1 and HCG17 in normal tissues was lower than that in LIHC tissues at different pathological stages (Figure 8B), and the similar results were found for other clinicopathological parameters (Figure 8B). According to the theory of competing endogenous RNA (ceRNA), lncRNAs can regulate the transcription of target genes by competing with shared miRNAs as ceRNAs[24,25]. Therefore, expression of lncRNA and mRNA must be positively correlated, while expression of miRNA and lncRNA must be negatively correlated. Subsequently, the ENCORI database was used to explore pairwise correlations between mRNAs, lncRNAs and miRNAs to determine collinearity (Figure 8C). Finally, U91328.1 and HCG17 were identified as direct targets of hsa-miR-214-3p.

ING1-related partners functional analysis

To further explore the molecular mechanism of *ING1* in the occurrence of LIHC, we screened for targeted *ING1* binding proteins and *ING1* expression related genes, and conducted a series of functional analyses. Fifty *ING1* binding proteins were obtained using the STRING database[17]. The interaction networks of these proteins were visualized in Figure 9A. Using GEPIA2[14] combined with TCGA LIHC expression data, the top 100 genes related to *ING1* expression were obtained. The results indicated that expression of *ING1* was positively correlated with expression of *TUBGCP3* ($r = 0.66$), *CARKD* ($r = 0.61$), *ABHD13* ($r = 0.61$), *CUL4A* ($r = 0.60$), and *CREBBP* ($r = 0.48$) (all $P < 0.001$, Figure 9B). An intersection of the two obtained gene sets was taken, and a common gene, *CREBBP*, was found (Figure 9C). Previous studies have confirmed that *CREBBP* gene is associated with various cancers (*e.g.*, colorectal and gastric cancer)[26-28]. Therefore, we infer that *CREBBP* plays a functional role in LIHC.

Functional analysis was performed on all genes in these two datasets. The gene annotation results showed that these genes were mainly correlated with chromatin organization (GO:0006325), regulation of stem cell population maintenance (GO:2000036), nuclear factor-kappaB binding (GO:0051059), p53 binding (GO:0002039), chromosome organization (GO:0051276), programmed cell death (GO:0012501), and transcription factor binding (GO:0008134) (Figure 9D). Pathway annotation showed that these genes were mainly concentrated in 19 pathways (Figure 9E), including hepatitis B (hsa05161), Wnt signaling pathway (hsa04310), Notch signaling pathway (hsa04330) and pathways in cancer (hsa05200). These biological processes, molecular functions and pathways all play vital roles in cancers.

DISCUSSION

LIHC is the leading cause of death from cancer worldwide[29]. At present, the main methods to treat LIHC are radiotherapy, chemotherapy, surgery and liver transplantation[30]. Although immunotherapy has made significant

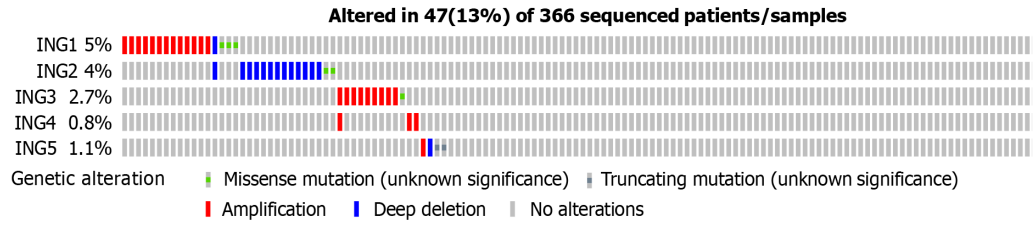


Figure 3 Genetic alterations of *ING* family genes in patients with liver cancer.

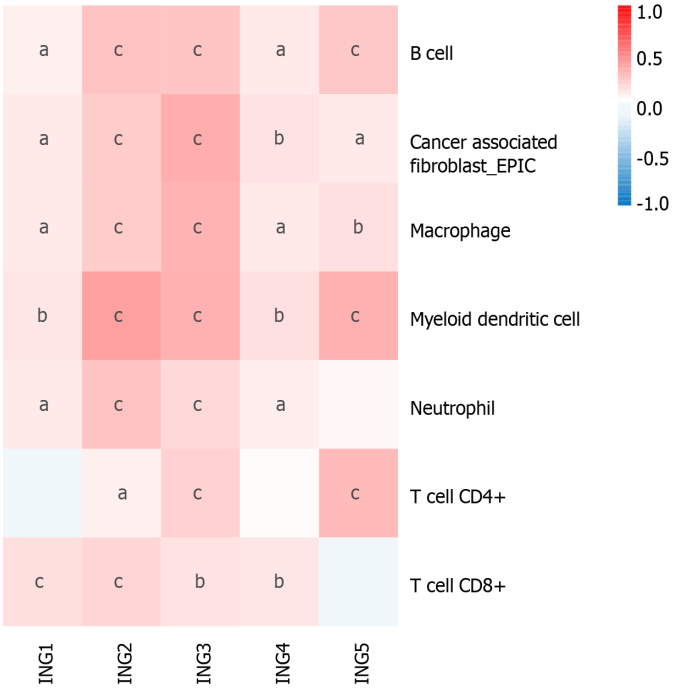


Figure 4 Correlation between expression of *ING* family genes and B cells, cancer-associated fibroblasts, myeloid dendritic cells, neutrophils, CD4+ T cells, CD8+ T cells, and macrophage infiltration levels in liver cancer. [a]*P* < 0.05; [b]*P* < 0.01; [c]*P* < 0.001.

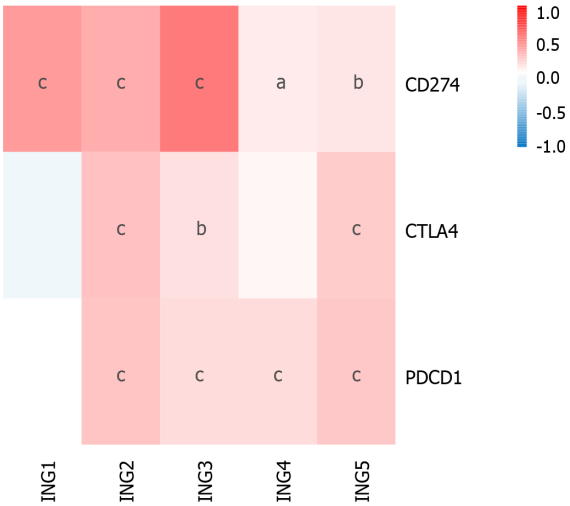


Figure 5 Correlation between expression of *ING1* and immune checkpoint genes (*PDCD1*, *CTLA4* and *CD274*) in liver cancer. [a]*P* < 0.05; [b]*P* < 0.01; [c]*P* < 0.001.

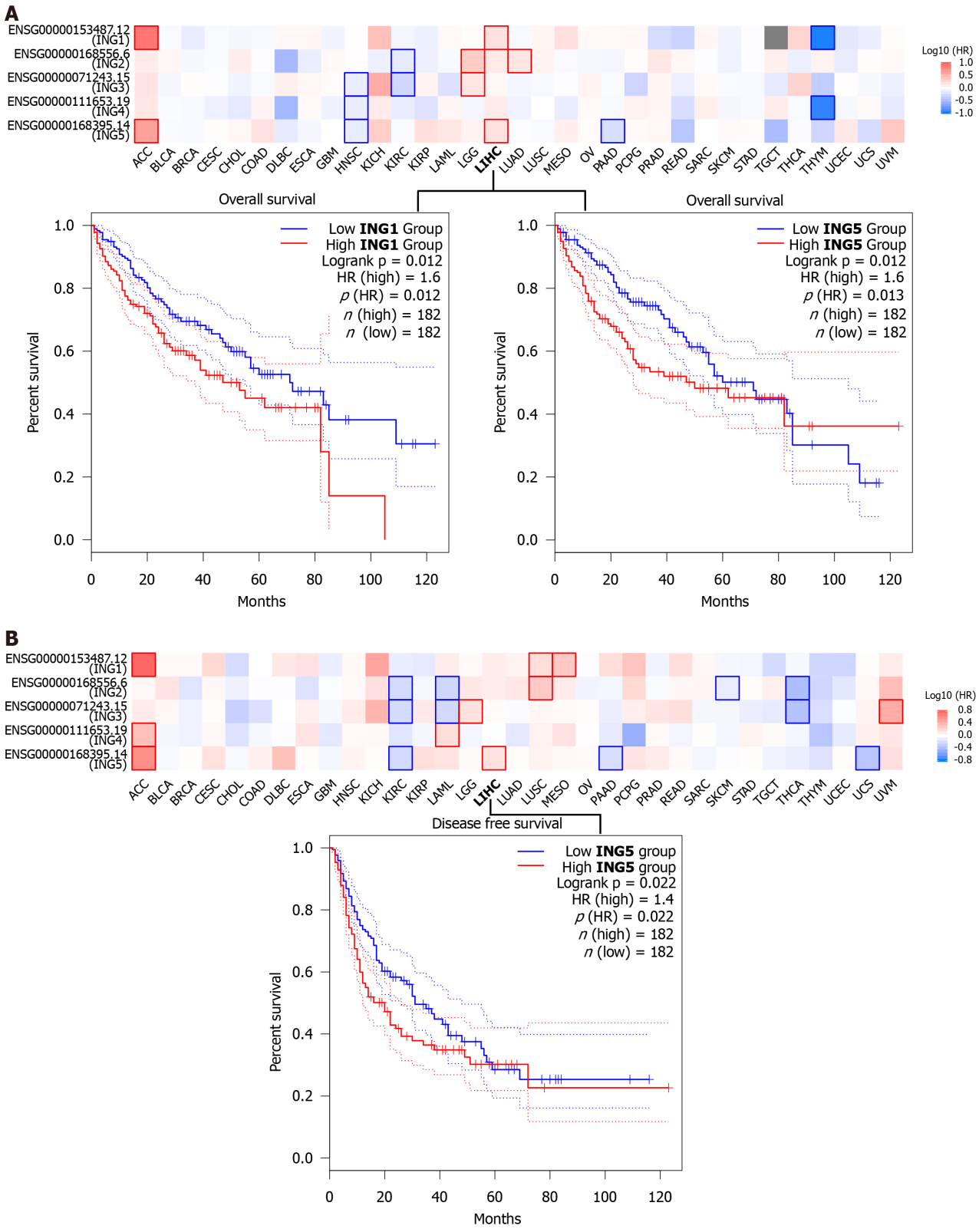


Figure 6 Correlation between expression of *ING* family genes and prognosis of cancers in The Cancer Genome Atlas using the GEPIA2 tool. A: Overall survival analysis; B: Disease-free survival analysis. The survival chart and Kaplan-Meier curve with positive results were shown. HR: Hazard ratio.

progress, such as PD-1 targeted therapy, prognosis of LIHC remains poor[31]. Therefore, it is urgent to explore the pathogenesis of LIHC and find new therapeutic and prognostic biomarkers. Based on several large public databases, we investigated the expression profile, clinical relationships, prognostic significance, and immune infiltration of *ING* family genes in LIHC.

mRNA/protein expression of different *ING* family genes in LIHC was analyzed in different databases. The mRNA expression of *ING* family genes was significantly upregulated in LIHC tissues and in other cancers, such as gallbladder

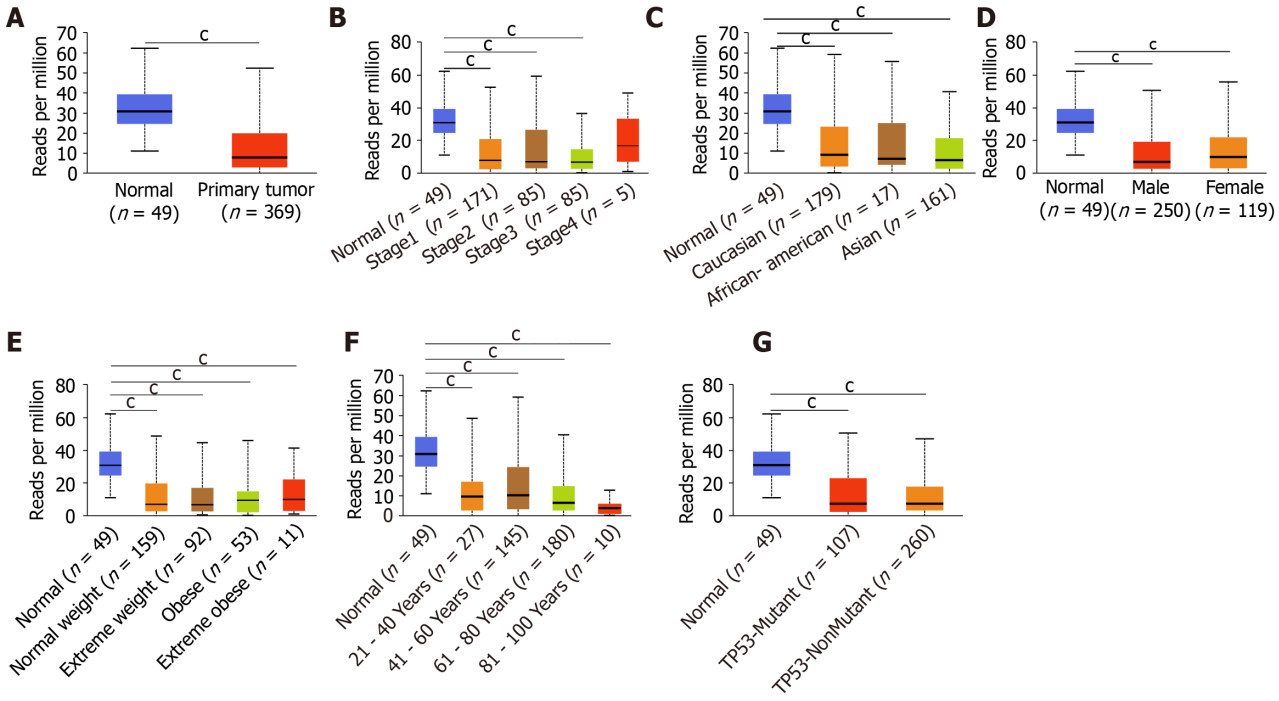


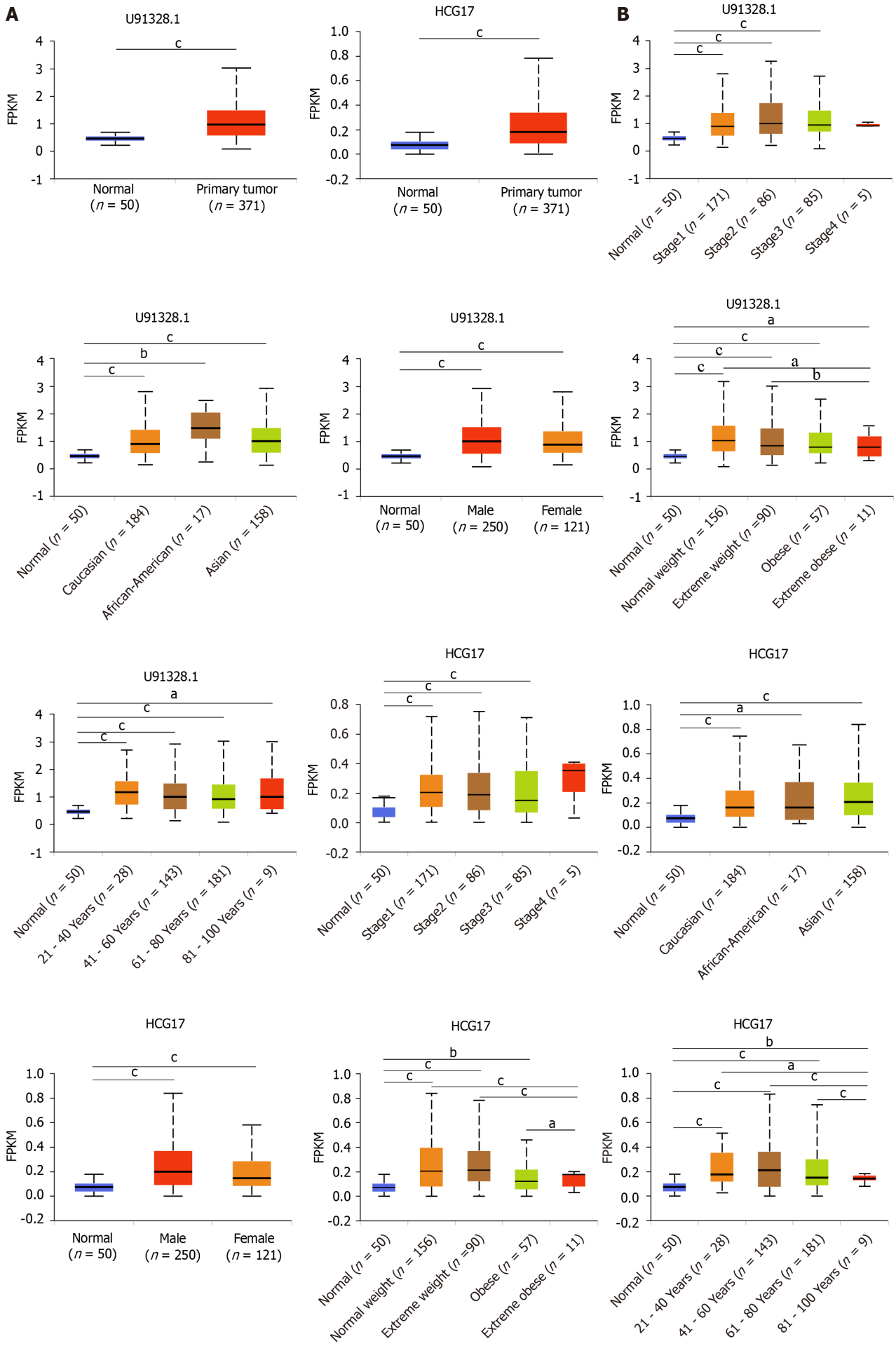
Figure 7 Relationships between hsa-miR-214-3p expression and clinicopathological parameters in liver cancer. A: Differential expression of hsa-miR-214-3p between normal and cancer tissue; B: Between expression of hsa-miR-214-3p and individual cancer stages; C: Between expression of hsa-miR-214-3p and patient race; D: Between expression of hsa-miR-214-3p and patient gender; E: Between expression of hsa-miR-214-3p and patient weight; F: Between expression of hsa-miR-214-3p and patient age; G: Between expression of hsa-miR-214-3p and *TP53* mutation status. °$P < 0.001$.

cancer, head and neck cancer. Similar results were obtained using the UALCAN database to further explore the transcription levels of *ING* family genes. Analysis of protein expression data showed that the *ING1/3/4/5* proteins were significantly upregulated in LIHC tissue, verifying the reliability of the results. After comprehensive analysis of expression patterns of *ING* family genes, we further studied the correlation between mRNA expression of *ING* family genes and clinical pathologic characteristics in LIHC. Using the LIHC protein expression data in the CPTAC database, we further analyzed the correlation between expression of *ING* members and the sex and age of patients. We also evaluated the correlation between different *ING* family genes by analyzing their mRNA expression. There was significant positive correlation among all genes. There is increasing evidence for the important role of *ING* family genes in different malignancies[8,10,11]. It is reported that the *ING* family genes (*ING1-5*) were identified as tumor suppressor genes[6,7]. Our analysis combined with previous studies suggests that *ING1-5* may be biomarker of LIHC.

The genetic alterations of *ING* family genes in LIHC were explored. The *ING* family genes were altered in 47 samples from 366 patients with LIHC, accounting for a 13% alteration rate. According to TCGA data, the percentage genetic change of *ING1-5* in LIHC were 5%, 4%, 2.7%, 0.8%, and 1.1%, respectively. Immunotherapy has emerged as a promising strategy in LIHC. Tumor-infiltrating immune cells in the tumor microenvironment have a great influence on the responsiveness and effectiveness of such treatments[32]. Therefore, we further investigated the correlation between expression of *ING* family genes and tumor-infiltrating immune cell infiltration in LIHC tissues. *ING1-4* were significantly positively correlated with CD8+ T cells and neutrophils. *ING1-5* were significantly positively correlated with cancer-associated fibroblasts, macrophages, B cells and myeloid dendritic cells. *ING2/3/5* were significantly positively correlated with CD4+ T cells. The function of *ING* family genes and their roles in the occurrence and development of LIHC require further explored through clinical and experimental research.

In recent years, immune checkpoint inhibitors (such as CTLA-4, PD-1, and PD-L1 inhibitors) have shown significant therapeutic effects in the clinical treatment of various solid tumors[22]. However, only a small percentage of cancer patients have shown significant benefits from such drugs[33]. Thus, there is a need to accurately identify patients who could benefit from immune checkpoint inhibitor therapy. To determine the influence of expression of *ING* family genes on LIHC immunotherapy, we studied the correlation between gene expression and CTLA-4, PD-L1 and PD-1. *ING2/3/5* expression was significantly positively correlated with PD-1, PD-L1, and CTLA-4. *ING4* expression was significantly positively correlated with PD-1 and PD-L1. *ING1* expression was significantly positively correlated with PD-L1. Therefore, these results indicate that positive expression of *ING* family genes is more predictive of immune therapy response than negative expression. The potential mechanism of the correlation between *ING* family genes and immune checkpoint inhibitors needs to be further explored.

We conducted a survival analysis to explore the prognostic potential of *ING* family genes. In OS analysis, high expression of *ING1/5* indicated poorer survival in LIHC patients. In DFS analysis, high expression of *ING5* was related to poor prognosis in LIHC patients. Therefore, the mRNA expression of *ING1/5* was significantly related to the prognosis of LIHC and could serve as a useful predictive biomarker. Finally, based on comprehensive analysis of the expression, clinical clinicopathological parameters, and prognostic potential of *ING* family genes, we identified that *ING1/5* may play


A
U91328.1
FPKM
Normal (n = 50)
Primary tumor (n = 371)
HCG17
Normal (n = 50)
Primary tumor (n = 371)
B
U91328.1
Normal (n = 50)
Stage1 (n = 171)
Stage2 (n = 86)
Stage3 (n = 85)
Stage4 (n = 5)
U91328.1
Normal (n = 50)
Caucasian (n = 184)
African-American (n = 17)
Asian (n = 158)
U91328.1
Normal (n = 50)
Male (n = 250)
Female (n = 121)
U91328.1
Normal (n = 50)
Normal weight (n = 156)
Extreme weight (n =90)
Obese (n = 57)
Extreme obese (n = 11)
U91328.1
Normal (n = 50)
21 - 40 Years (n = 28)
41 - 60 Years (n = 143)
61 - 80 Years (n = 181)
81 - 100 Years (n = 9)
HCG17
Normal (n = 50)
Stage1 (n = 171)
Stage2 (n = 86)
Stage3 (n = 85)
Stage4 (n = 5)
HCG17
Normal (n = 50)
Caucasian (n = 184)
African-American (n = 17)
Asian (n = 158)
HCG17
Normal (n = 50)
Male (n = 250)
Female (n = 121)
HCG17
Normal (n = 50)
Normal weight (n = 156)
Extreme weight (n =90)
Obese (n = 57)
Extreme obese (n = 11)
HCG17
Normal (n = 50)
21 - 40 Years (n = 28)
41 - 60 Years (n = 143)
61 - 80 Years (n = 181)
81 - 100 Years (n = 9)

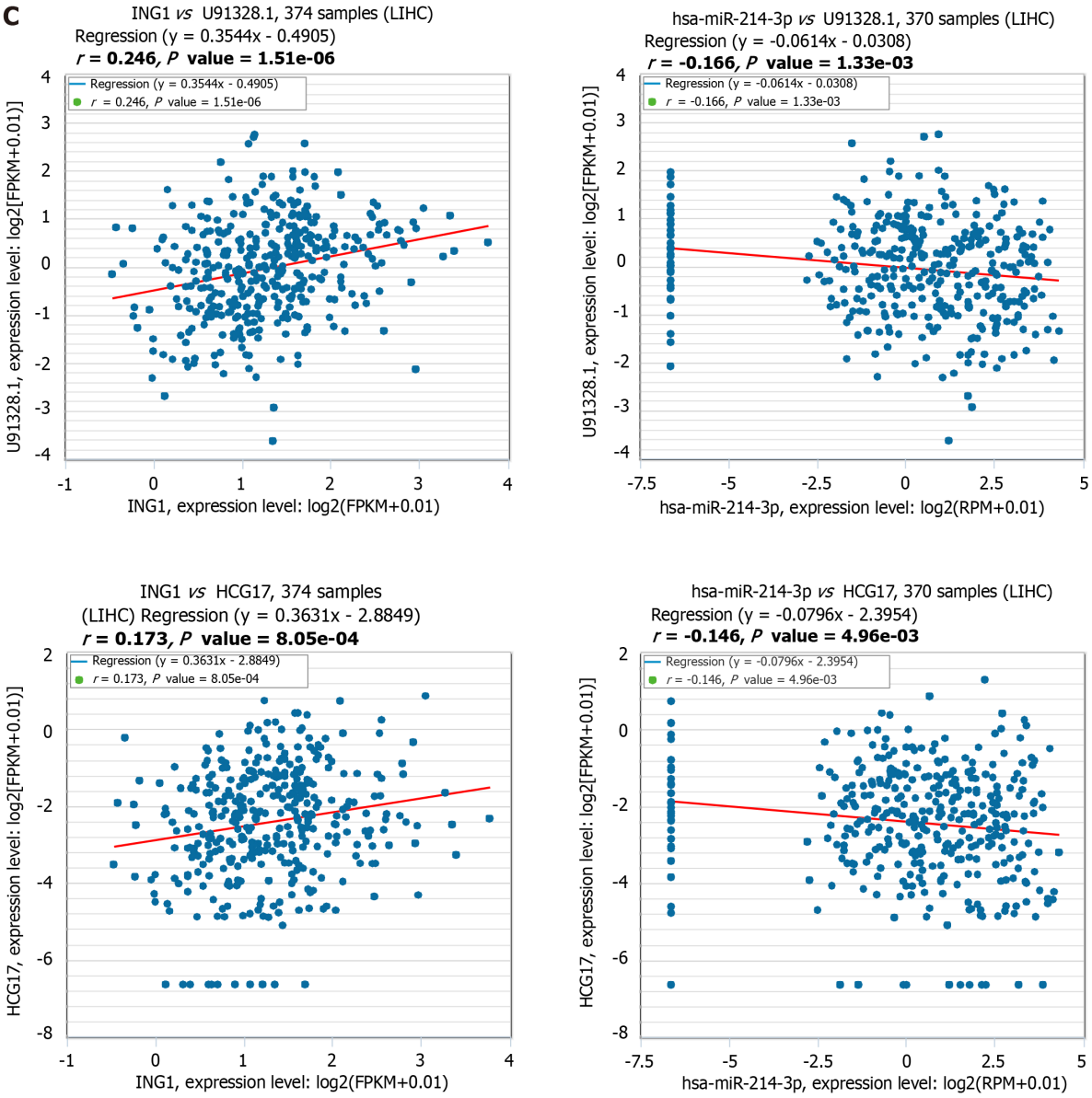
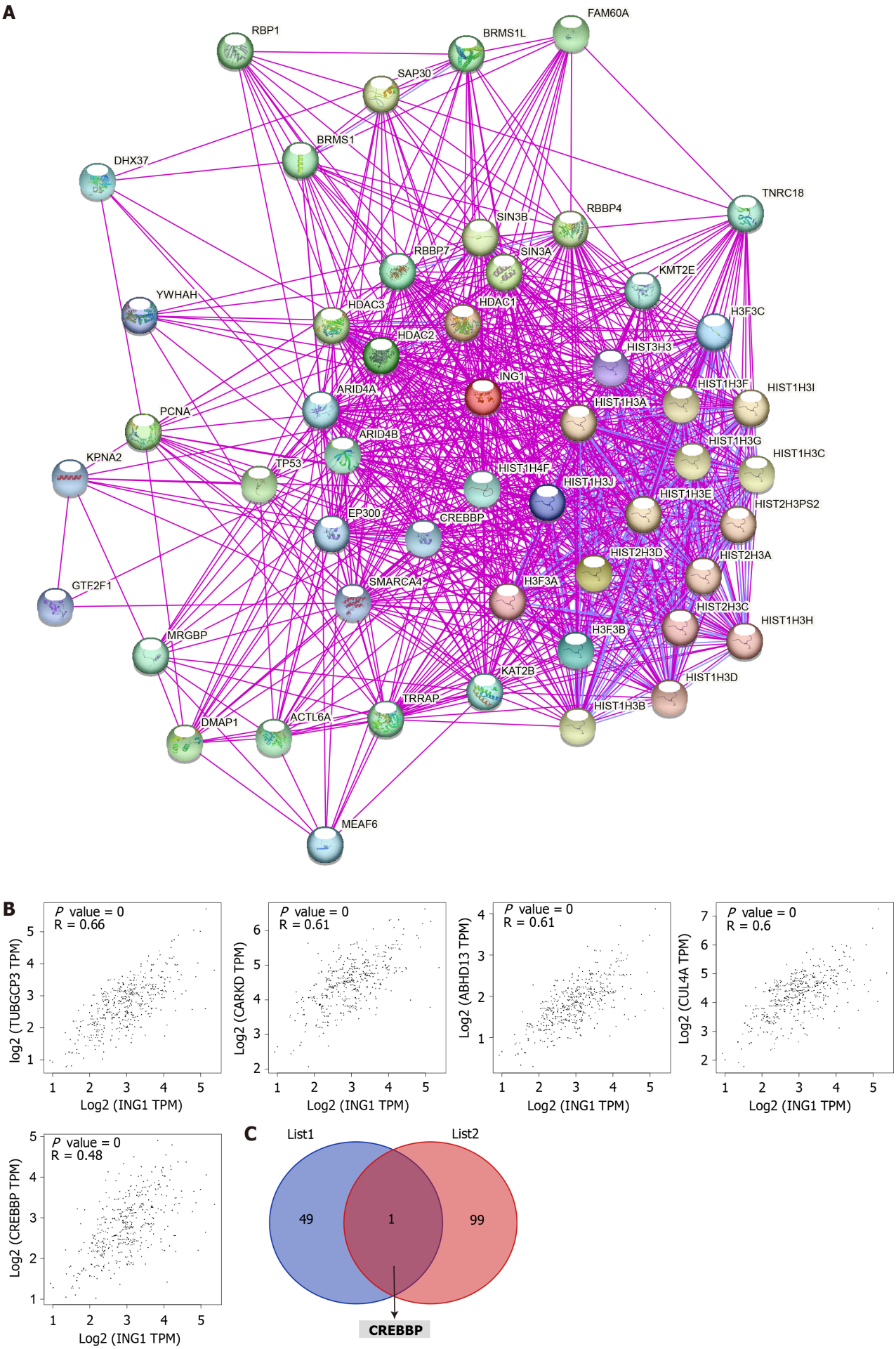


Figure 8 Upstream long noncoding RNAs prediction analysis of hsa-miR-214-3p. A: Differential expression of two long noncoding RNAs (lncRNAs) in cancer and normal tissues; B: Expression of two lncRNAs in other clinicopathological parameters; C: Correlation analysis between lncRNAs and *ING1* or between hsa-miR-214-3p and lncRNAs in liver cancer. [a]*P* < 0.05; [b]*P* < 0.01; [c]*P* < 0.001. LIHC: Liver cancer.

vital roles in the occurrence and progression of LIHC.

The current literature mainly focuses on the key functions and roles of the regulatory ncRNAs in the occurrence and progression of cancer. To identify the upstream regulatory miRNA of *ING1*, seven prediction tools were introduced to predict the possible binding miRNA of *ING1*. Three upstream miRNAs of *ING1* were identified. By differential expression and mRNA-miRNA correlation analyses, hsa-miR-214-3p was discovered as the upstream miRNA of *ING1* that affected progression of LIHC. Upstream lncRNAs of the hsa-miR-214-3p/ING1 axis were also identified. Through expression and correlation analyses, U91328.1 and HCG17, which were the two most promising upregulated lncRNAs of hsa-miR-214-3p, were identified. Compared with normal tissue samples, their expression was significantly upregulated in LIHC. The analyses results showed that expression of U91328.1 and HCG17 in LIHC at different pathological stages was higher than that in normal tissue samples, and the similar results were found for other clinicopathological parameters. According to the ceRNA theory, expression of lncRNA and mRNA must be positively correlated, while the expression of miRNA and lncRNA must be negatively correlated. Subsequently, the ENCORI database was used to explore pairwise correlations between mRNAs, lncRNAs and miRNAs to determine collinearity. Finally, U91328.1 and HCG17 were identified as direct targets of hsa-miR-214-3p. The *ING1*/hsa-miR-214-3p/ U91328.1 or HCG17 axis was discovered as a potential regulatory pathway.


A
FAM60A
RBP1
BRMS1L
SAP30
BRMS1
DHX37
TNRC18
RBBP4
SIN3B
RBBP7
SIN3A
KMT2E
YWHAH
HDAC3
HDAC1
H3F3C
HDAC2
HIST3H3
ING1
HIST1H3F
HIST1H3I
ARID4A
HIST1H3A
PCNA
ARID4B
HIST1H3G
HIST1H3C
KPNA2
TP53
HIST1H4F
HIST1H3J
HIST1H3E
HIST2H3PS2
EP300
CREBBP
HIST2H3D
HIST2H3A
GTF2F1
SMARCA4
H3F3A
HIST2H3C
HIST1H3H
MRGBP
H3F3B
KAT2B
HIST1H3D
TRRAP
HIST1H3B
DMAP1
ACTL6A
MEAF6
B
P value = 0
R = 0.66
log2 (TUBGCP3 TPM)
Log2 (ING1 TPM)
P value = 0
R = 0.61
Log2 (CARKD TPM)
Log2 (ING1 TPM)
P value = 0
R = 0.61
Log2 (ABHD13 TPM)
Log2 (ING1 TPM)
P value = 0
R = 0.6
Log2 (CUL4A TPM)
Log2 (ING1 TPM)
P value = 0
R = 0.48
Log2 (CREBBP TPM)
Log2 (ING1 TPM)
C
List1
List2
49
1
99
CREBBP

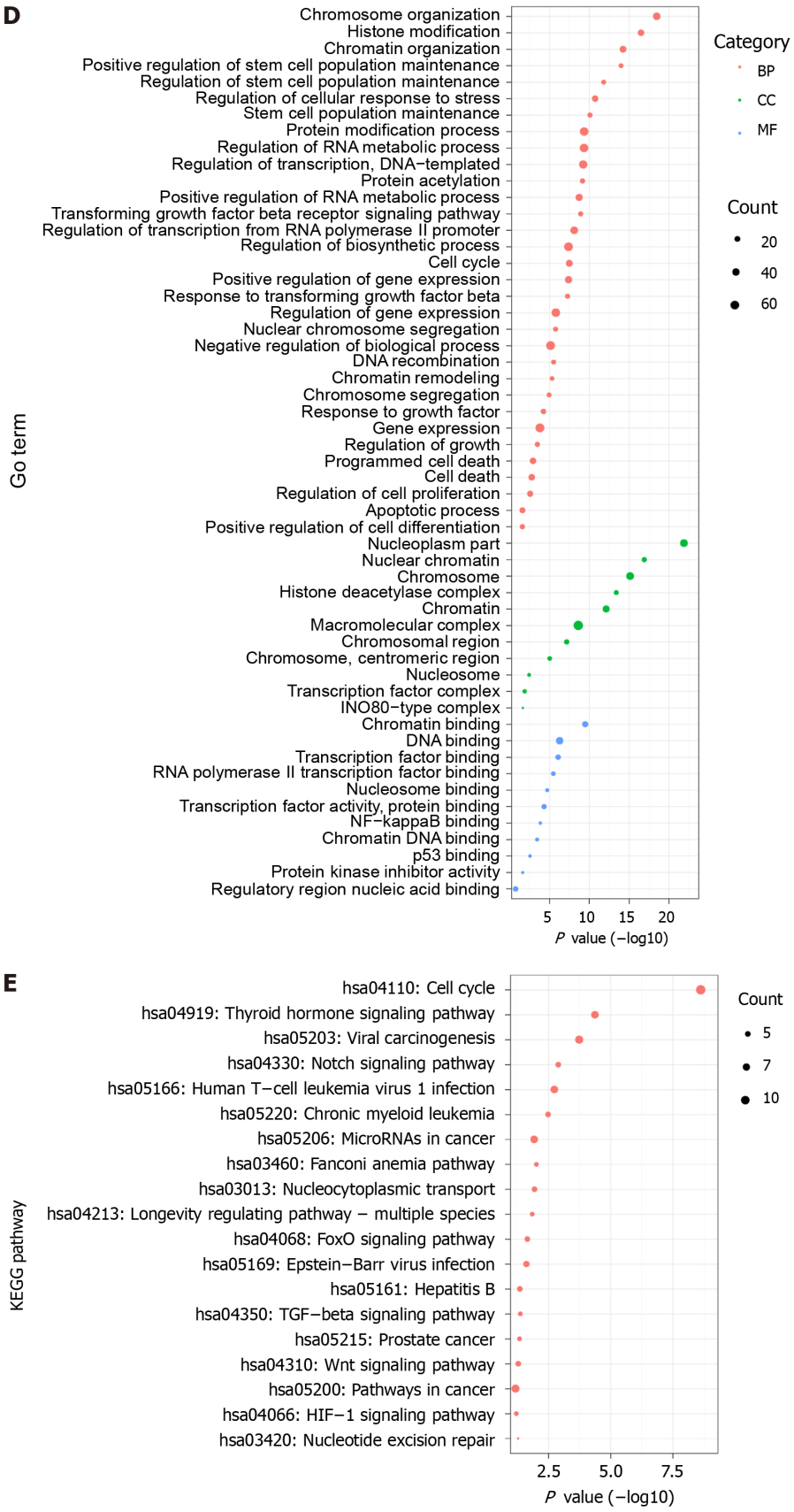


Figure 9 Functional analysis of *ING1*-related genes. A: ING1 binding protein using STRING database; B: Expression correlation between *ING1* and selected target genes, including *TUBGCP3*, *CARKD*, *ABHD13*, *CUL4A* and *CREBBP*; C: Intersection analysis of the binding genes and associated genes of *ING1*. List 1: ING1 binding proteins; List 2: Top 100 genes associated with *ING1* expression; D: Gene Ontology analysis based on the *ING1*-binding and interaction genes; E: Kyoto Encyclopedia of Genes and Genomes pathway analysis based on the *ING1*-binding and interaction genes. BP: Biological processes; CC: Cellular components; MF: Molecular functions; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes.

CONCLUSION

This research studied the expression of *ING* family genes in LIHC, showing that *ING* members were highly expressed in LIHC, and *ING1/5* were related to poor prognosis. An ncRNA-mediated regulatory mechanism of *ING1* in occurrence and development of LIHC (*ING1*/hsa-miR-214-3p/U91328.1 or HCG17) was constructed. We found that the expression of *ING* family genes was related to immune cell infiltration and to the expression of immune checkpoint genes. Our findings provide new insights into diagnosis, treatment, and prognosis of LIHC.

FOOTNOTES

Author contributions: Liu SC collected and analyzed the data, conceptualized, designed, drafted and revised the manuscript, and approved the final version of the manuscript.

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