Expression of *yhwaz* and gene regulation network in hepatocellular carcinoma

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Abstract. The adaptor protein 14-3-3ζ is encoded by the *yhwaz* gene and implicated in a wide range of biological processes. In tumorigenesis, 14-3-3ζ recognizes specific phosphorylation motifs and interacts with hundreds of target proteins and is, thus, involved in the regulation of tumor proliferation, migration and differentiation. In the present study, bioinformatics tools were used to analyze data from The Cancer Genome Atlas and Gene Expression Omnibus databases and the expression of *yhwaz*, and gene regulation networks were identified as potentially relevant in hepatocellular carcinoma (HCC). In HCC, *yhwaz* expression was demonstrated to be upregulated and significantly associated with poor prognosis. Expression levels of microRNAs targeting *yhwaz* were associated with improved prognosis in patients with liver cancer. Gene networks that are regulated by *yhwaz* were found to be involved in cell cycle regulation and tumorigenesis, indicating the potential use of the expression levels of *yhwaz* in liver tissue as predictive biomarkers in patients with liver cancer. In the present study, *yhwaz* was identified as a gene of interest through data mining gene expression databases and its involvement in regulatory networks in HCC was indicated. Therefore, further *in vitro* and *in vivo* studies on the role of *yhwaz* in the carcinogenesis of HCC would be greatly beneficial.

Introduction

Hepatocellular carcinoma (HCC) is a leading cause of cancer-associated mortality, with >700,000 new cases being diagnosed annually throughout the world (1). HCC is the result of complex interactions between genetic and non-genetic host factors, including exposure to environmental chemicals and viruses. In >90% of cases, a primitive chronic liver disease, e.g., cirrhosis, usually develops and creates a procarcinogenic environment in its final stage (2).

The *yhwaz* gene encodes 14-3-3ζ, which belongs to a family of highly conserved dimeric proteins that are considered master regulators of intracellular signaling (3-5). 14-3-3ζ has been shown to interact with numerous protein kinases, enzymes, receptor proteins, structural and cytoskeletal proteins, proteins associated with cell cycle and transcriptional control and proteins involved in apoptosis (6). Upregulated expression of *yhwaz* is frequently observed in several types of cancer (including lung and liver cancer) (7-9). For example, *yhwaz* is upregulated in >40% of cases of breast cancer and is able to activate transforming growth factor-β/SMAD signaling during the epithelial-to-mesenchymal-transition, thus promoting tumor progression (10). 14-3-3ζ interacts with β-catenin, GLI family zinc finger 2, Yes-associated protein 1 and the downstream effectors of the Wnt, Hedgehog and Hippo signaling pathways, all of which are involved to varying degrees in a number of different types of cancer (such as breast cancer or lymphocytic leukemia) (11-14). Therefore, 14-3-3ζ may potentially be used as a prognostic biomarker for the diagnosis of certain types of cancer.

The upregulation of *yhwaz* in patients with HCC has been frequently examined (15) and 14-3-3ζ protein expression levels have been found to be significantly upregulated in hepatoma cell lines. In HCC cell lines with upregulated 14-3-3ζ expression, knockdown of 14-3-3ζ using RNA interference was reported to inhibit cell proliferation by activating the c-jun N-terminal kinase and p38/mitogen-activated protein kinase (MAPK) (16). Additionally, knockdown of 14-3-3ζ was observed to enhance radio-induced apoptosis in liver cancer stem-like cells (17). Wang *et al* (18) demonstrated that 14-3-3ζ was upregulated in the hepatocarcinomatous environment, attenuated the anti-tumor function of tumor-infiltrating T cells and may have partially been transferred from liver cancer cells to T cells via exosomes. A recent study revealed that 14-3-3ζ regulated the stability of heme oxygenase 1, which in turn promoted cancer cell proliferation via activation of STAT3 signaling in HCC (19). Nonetheless, the prognostic roles of *yhwaz* at the mRNA level in HCC remain to be clearly

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elucidated. In the present study, bioinformatics tools were used to analyze the functional effects of *yhwaz* expression in liver cancer. The expression of *yhwaz* was shown to be elevated in liver cancer tissues and cell lines and this upregulation was associated with poor prognosis. Additionally, it was demonstrated that mutations in *yhwaz* may have important implications in the survival rates of patients with liver cancer. By contrast, upregulated expression levels of microRNAs (miRNAs/miRs) that targeted *yhwaz* were associated with improved prognosis. The results demonstrated that the regulatory network of 14-3-3ζ and its interacting proteins serve an important role in the development and prognosis of HCC, suggesting the potential application of 14-3-3ζ as a therapeutic target for liver cancer. Additionally, the results of the present study support additional *in vitro* and *in vivo* studies on the functional effects of 14-3-3ζ expression in HCC.

Materials and methods

Bioinformatics tools and databases. Several bioinformatics tools were used to analyze *yhwaz* expression in patients with HCC. The specific tools that were used are listed in Table I.

Table I. Bioinformatics tools used for analysis in the present study.

Databases	Samples	(Refs.)
HCCDB	Tissues	(20)
TCGA	Tissues	(21)
GTEx	Tissues	(22)
UALCAN	Tissues	(23)
Oncomine	Tissues	(24)
KEGG	N/A	(25)
Kaplan-Meier plotter	Tissues	(26)
cBioPortal	N/A	(27,28)
TargetScan	N/A	(29)
GSEA	N/A	(30,31)
GeneMANIA	N/A	(32)
GEPIA	Tissues	(33)

GEPIA, Gene Expression Profiling Interactive Analysis; HCCDB, Integrative Molecular Database of Hepatocellular Carcinoma; GSEA, Gene Set Enrichment Analysis; N/A, not applicable.

Integrative molecular database of hepatocellular carcinoma (HCCDB). The HCCDB is a database that contains information on HCC expression (20). In the current database release, HCCDB archived 15 public HCC gene expression datasets (HCCDB1, 3, 4, 6-9, 11-18) containing a total of 3917 samples from The Cancer Genome Atlas (TCGA) (21).

Genotype-tissue expression (GTEx). Among the 15 datasets from GTEx (https://www.gtexportal.org) (22), 12 datasets contain both tumor and the adjacent normal samples, whereas only tumor samples are available for the three remaining datasets.

UALCAN. UALCAN is an interactive web resource for studying cancer transcriptome data (23). In particular, 50 healthy individuals and 371 patients with primary HCC were used to analyze the association among *yhwaz* levels and their clinical characteristics.

Oncomine. Oncomine database is a bioinformatics initiative aimed at collecting, standardizing, analyzing, and delivering cancer transcriptome data to the biomedical research community (24). Two microarray datasets *Roessler Liver* (43 samples) and *Roessler Liver 2* (445 samples) were used for analysis in this study. Based on these open source bioinformatics platforms, *yhwaz* expression profiles were obtained for both human HCC tissues and cell lines.

Kyoto encyclopedia of genes and genomes (KEGG) pathway analysis. KEGG (25) is a database resource for understanding high-level functions and utilities of the biological system, such as the cell, the organism and the ecosystem, from molecular-level information, especially large-scale molecular datasets generated by genome sequencing and other high-throughput experimental technologies.

Kaplan-Meier plotter. The Kaplan-Meier plotter (26) with the log-rank test was used to determine disease prognosis, including overall survival (OS) time and post-progression survival (PPS) time. Clinical data from TCGA were used for Kaplan-Meier plotter analysis in order to evaluate the clinical relevance of *yhwaz* mRNA expression levels in HCC. In this project 364 (male, 250; female, 121) HCC RNA-seq samples were used.

CBioPortal. CBioPortal (27,28) can be used to download and analyze large-scale cancer genomics datasets and was used in the present study to analyze mutations in *yhwaz*. In this project 1065 HCC samples were used for analysis.

TargetScan (version 7.1). TargetScan (29) was used to predict the biological targets of miRNAs by searching for the presence of conserved 8mer and 7mer sites that matched the seed region of each miRNA.

Gene set enrichment analysis (GSEA). GSEA (30,31) is a computational method that determines whether an a priori defined set of genes shows statistically significant, concordant differences between two biological states.

GeneMANIA. GeneMANIA (32) is a flexible, user-friendly web interface for constructing protein-protein interaction (PPI) network, generating hypotheses about gene function, analyzing gene lists, and prioritizing genes for functional assays.

Gene expression profiling interactive analysis (GEPIA). GEPIA (33) is a newly developed interactive web server for analyzing the RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from the TCGA and the GTEx projects, using a standard processing pipeline.

Tumor grade and tumor stages. The tumor grade is divided into four subtypes: Grade 1, well differentiated (low grade);

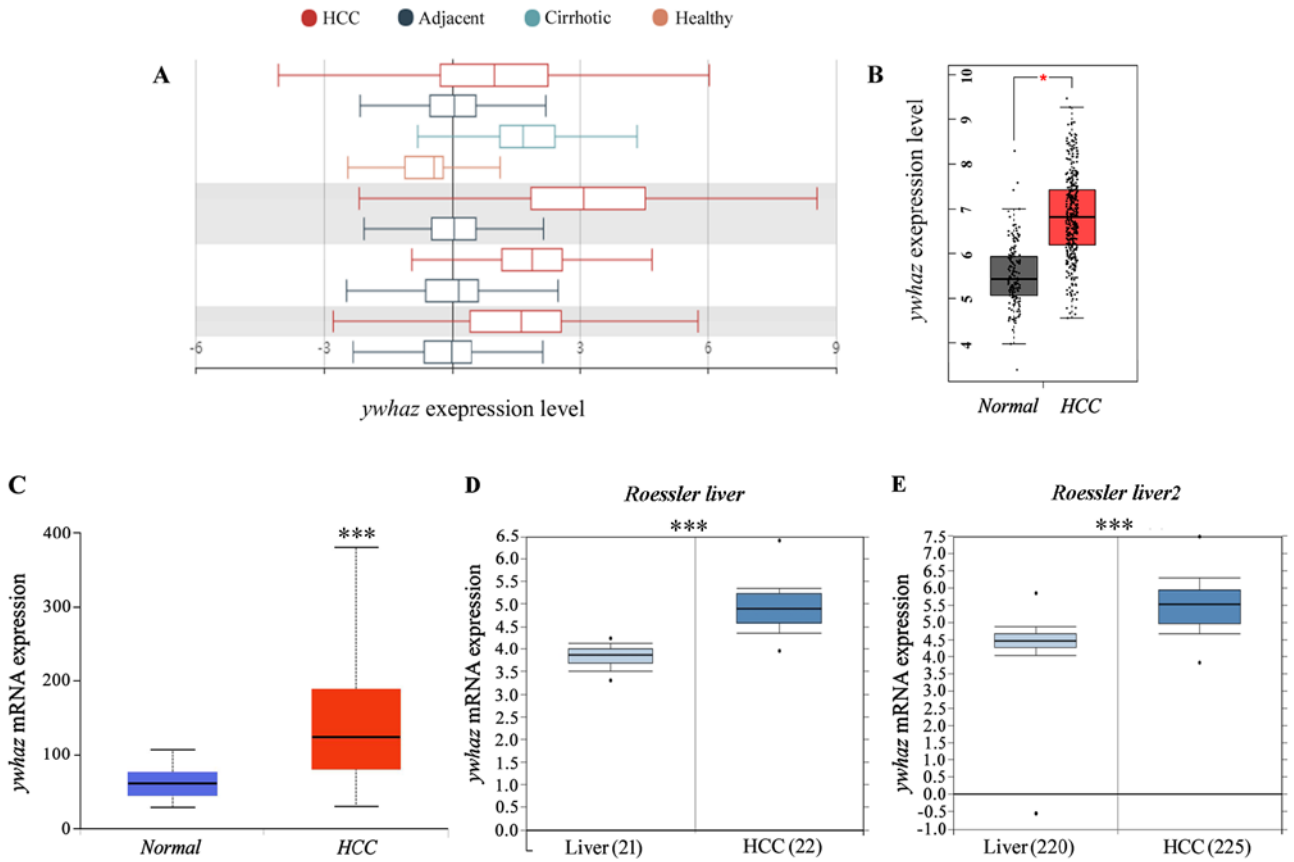

Figure 1. *yhwaz* expression levels in HCC. (A) mRNA expression levels of *yhwaz* in cancer tissues. (B) *yhwaz* expression levels in normal and HCC liver tissues. *P<0.05. (C) RNA expression levels of *yhwaz* in data obtained from UALCAN. ***P<0.001. (D and E) mRNA expression levels of *yhwaz* in HCC tissues and the surrounding normal liver tissues. ***P<0.001. HCC, hepatocellular carcinoma.

grade 2, moderately differentiated (intermediate grade); grade 3, poorly differentiated (high grade); and grade 4, undifferentiated (high grade). The tumor stage class depends on the tumor-node-metastasis staging system (AJCC 8th edition) (34).

Statistical analysis. Data are expressed as mean ± SD and analyzed for significance using GraphPad Prism 6.00 software (IBM Corp.). Difference between two-groups was assessed using Student's t-test. One-way ANOVA followed by Newman-Keuls post hoc testing (95% confidence) was used to determine difference among more than two groups. The survival analysis was illustrated by Kaplan-Meier curves with log-rank test. Univariate and multivariate survival analyses were performed using the likelihood ratio test of the stratified Cox proportional hazards model of SPSS 17.0 (IBM Corp.). P<0.05 was considered to indicate a statistically significant difference.

Results

Yhwaz is upregulated in HCC cell lines and tissues. The results from the analysis of the data in the GTEx portal revealed that *ywhaz* is expressed in multiple organs and tissues (Fig. S1). As *yhwaz* has been reported to affect a number of different types of cancer, such as liver, lung and breast (3), HCCDB, an analytical tool for gene expression profiling, was used to determine *yhwaz* expression in tumor and other tissues. As presented in Fig. 1A and Table II, *yhwaz* expression was significantly higher in tumor tissues compared with tissue adjacent to HCC tissues. Additionally, in patients with cirrhosis, *yhwaz* expression was higher compared with healthy human liver tissues. Liver HCC tissues exhibited upregulated expression levels of *yhwaz* compared with healthy liver tissue (Fig. 1B). In order to investigate the changes in *yhwaz* expression between HCC and adjacent non-tumor tissues, *yhwaz* expression profiles were used from different independent bioinformatics databases. Based on the gene expression profiles obtained from the UALCAN database, *yhwaz* was determined to be upregulated in the majority of HCC tissues (Fig. 1C). To confirm this finding, two microarray datasets from the Oncomine database (24) were analyzed (Fig. 1D and E). *yhwaz* expression levels were significantly increased in HCC tissues compared with in healthy liver tissues. Taken together, these data demonstrate that increased mRNA expression of *yhwaz* contributed to HCC, indicating its potential role in liver cancer.

Yhwaz expression may be a prognostic factor in HCC. Although *yhwaz* has been demonstrated to participate in the development of multiple types of cancer, there are no clear reports on the association between its expression and clinical prognosis in HCC. The prognostic efficacy of the mRNA expression of *ywhaz* in patients with HCC was analyzed using the Kaplan-Meier plotter (26). As presented in Fig. 2A-C and Table II, increased expression of *yhwaz* was found to increase

Table II. Summary of *yhwaz* expression profiles based on HCCDB datasets.

Datasets	P-value	Type	Nums	Mean	SD	IQR
HCCDB3	$7.20x10^{-15}$	HCC	268	10.43	3.269	4.115
		Adjacent	243	8.609	1.632	1.771
		Cirrhotic	40	11.55	1.868	2.095
		Healthy	6	7.790	1.401	1.458
HCCDB4	$7.58x10^{-66}$	HCC	240	8.692	0.5139	0.6828
		Adjacent	193	7.886	0.2552	0.2674
HCCDB6	$2.62x10^{-52}$	HCC	225	9.271	0.8106	0.9875
		Adjacent	220	8.014	0.7027	0.8710
HCCDB18	$5.66x10^{-28}$	HCC	212	6.626	0.8582	1.135
		Adjacent	177	5.774	0.5318	0.5900

HCCDB, Integrative Molecular Database of Hepatocellular Carcinoma; Nums, Number of samples; IQR, interquartile range; SD, standard deviation.

the risk of death and decrease survival probability in female (Fig. 2A) and male (Fig. 2B) patients with HCC alone or all both sex combined (Fig. 2C; Table III). Therefore, *yhwaz* expression analysis was performed with regard to the clinicopathological characteristics of patients. As presented in Fig. 2D, *yhwaz* expression in African-American patients with HCC was significantly higher compared with Caucasian patients. The expression levels of *yhwaz* were shown to increase with tumor grade (Fig. 2E). Based on tumor stages (34), the *ywhaz* expression level was significant higher in patients of all tumor stages compared with healthy individuals, but *yhwaz* expression levels in stage 3 cancer were significantly higher compared with stage 1 ($P<0.01$; Fig. 2F), and there were no other significant difference amongst the other stages. The expression levels of *yhwaz* decreased with age in patients with HCC (Fig. 2G). The weight of patients seemed to have less influence on the expression of *yhwaz*, with the only significant difference observed between normal weight patients and obese patients with HCC (Fig. 2H), and Together, these data suggest that high expression levels of *yhwaz* may predict tumors with increased malignancy.

Yhwaz mutations affect the outcome of patients with HCC. Mutations in a gene may not always affect the function of the coded proteins. In the cBioPortal data sets, based on the TCGA, there were 8 studies (35-40) on HCC, and mutations, amplification and deep deletions were observed in the *yhwaz* sequence in these datasets (Fig. 3A). *yhwaz* was mutated or otherwise altered in 98 (7.00%) of the 1,487 patients. These alterations were amplified in 90 cases (6.00%), deep deletion in 2 cases (0.13%) and mutations in 6 cases (0.40%). Therefore, amplification was the most common type of *yhwaz* alteration in HCC. The 6 mutations identified are shown in Table IV, and the single nucleotide polymorphisms seen were S207I, N108S, R127G and C94S. The biological interaction network of *yhwaz* was next identified in HCC. The tab Network in cBioPortal was used to show *yhwaz* neighboring genes that were altered with a frequency >15% (Fig. 3B; Table V). The neighbor genes of *yhwaz* with highest frequency of alterations were CRTC2 (38.1%), SDC2 (34.4%) and F11R (29.2%). OS and disease-free survival were significantly decreased in patients with *yhwaz* alterations compared with individuals without alterations (Fig. 3D), suggesting that alterations in *yhwaz* may affected the survival of patients with HCC.

Table III. Association between *yhwaz* expression and sex in patients with hepatocellular carcinoma.

Sex	Gene IDs	HR	95% CI	P-value
Female	7534	5.20	2.05-13.19	0.00011
Male	7534	2.02	1.29-3.16	0.0016
All	7534	1.74	0.95-3.20	0.07

HR, hazard ratio; CI, confidence interval.

Increased expression of miRNAs that target yhwaz are associated with improved survival time in patients with HCC. miR-22 has been reported to target *yhwaz* and inhibit its expression in HCC cells (41). miRNAs that targeted *yhwaz* were predicted using TargetScan. As presented in Fig. 4A, miR-22 and miR-1/miR-206 were predicted to exhibit a higher probability of binding with *yhwaz*. miR-451 negatively regulated the expression of *yhwaz* through binding with the 3'untranslated region of *yhwaz* (42). miR-155 and miR-140 were also predicted to exhibit a high probability of binding with *yhwaz*. For reasons not yet known, the survival curves for miR-140-3p (Fig. 4G) exhibited a late stage crossover. The most mentioned miRNAs that were predicted to bind with yhwaz, were associated with improved survival time in patients with HCC when expression was upregulated (Fig. 4B-G; Table VI). Together, these data show that miRNAs that were predicted to target *yhwaz* were associated with improved survival time in patients with HCC.

KEGG pathway analysis of co-expression genes correlated with yhwaz and its networks of kinase and miRNA targets in HCC. To further examine the targets of *yhwaz* in HCC, the KEGG pathway analysis was used to determine the kinase and

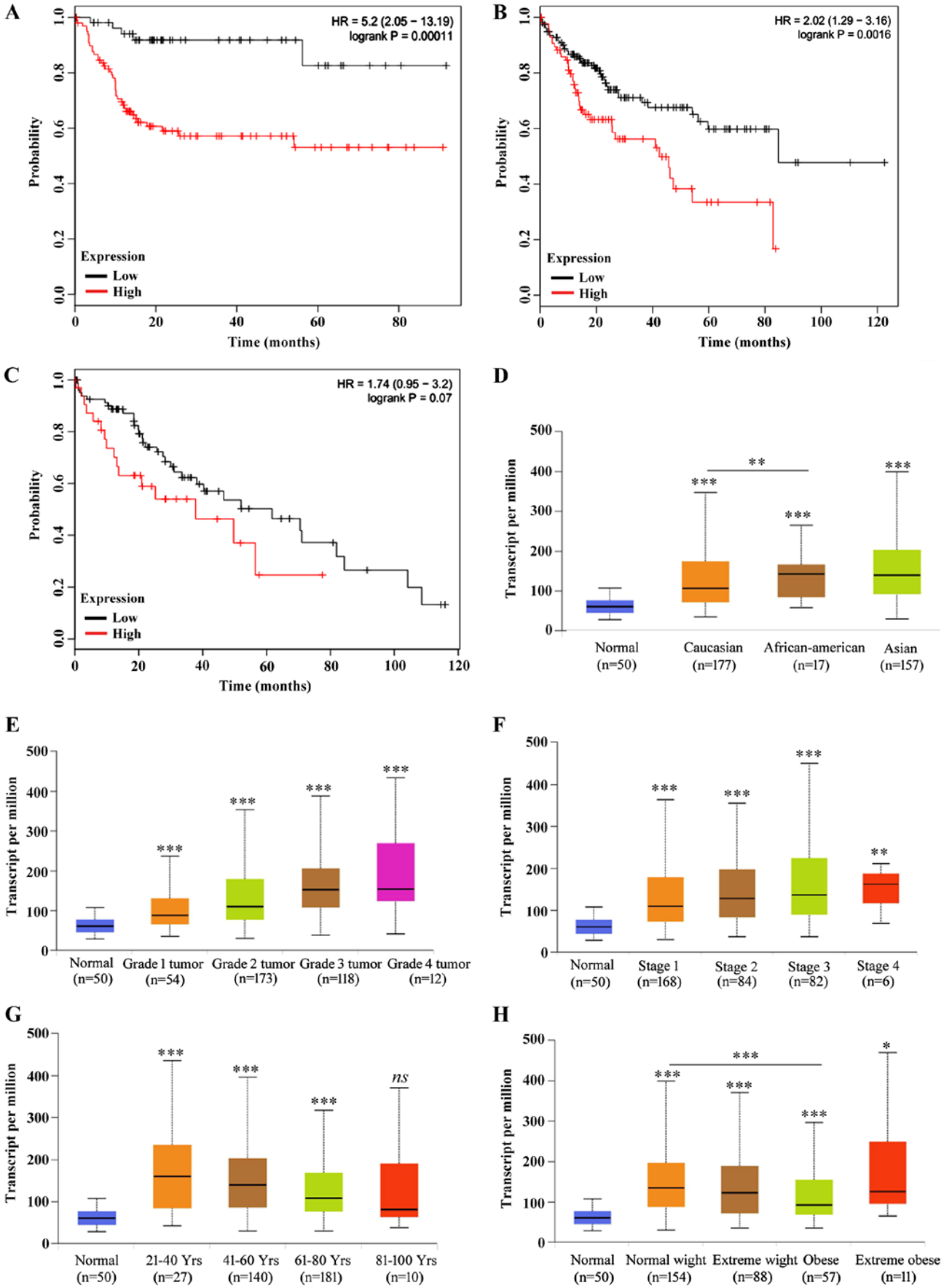

Figure 2. Association between *yhwaz* expression and clinical characteristics of patients with HCC. (A-C) Kaplan-Meier analysis of OS time and PPS time in patients with HCC based on *yhwaz* expression. (D-H) TCGA clinical data and UALCAN were used to categorize patients with HCC according to the expression levels of *yhwaz*. Data are presented as the mean ± standard error of the mean. *P<0.05, **P<0.01, ***P<0.001 vs. normal. Grade 1, well differentiated (low grade); Grade 2, moderately differentiated (intermediate grade); Grade 3 poorly differentiated (high grade); Grade 4 undifferentiated (high grade); HCC, hepatocellular carcinoma; OS, overall survival; PPS, post-progression survival; TCGA, The Cancer Genome Atlas.

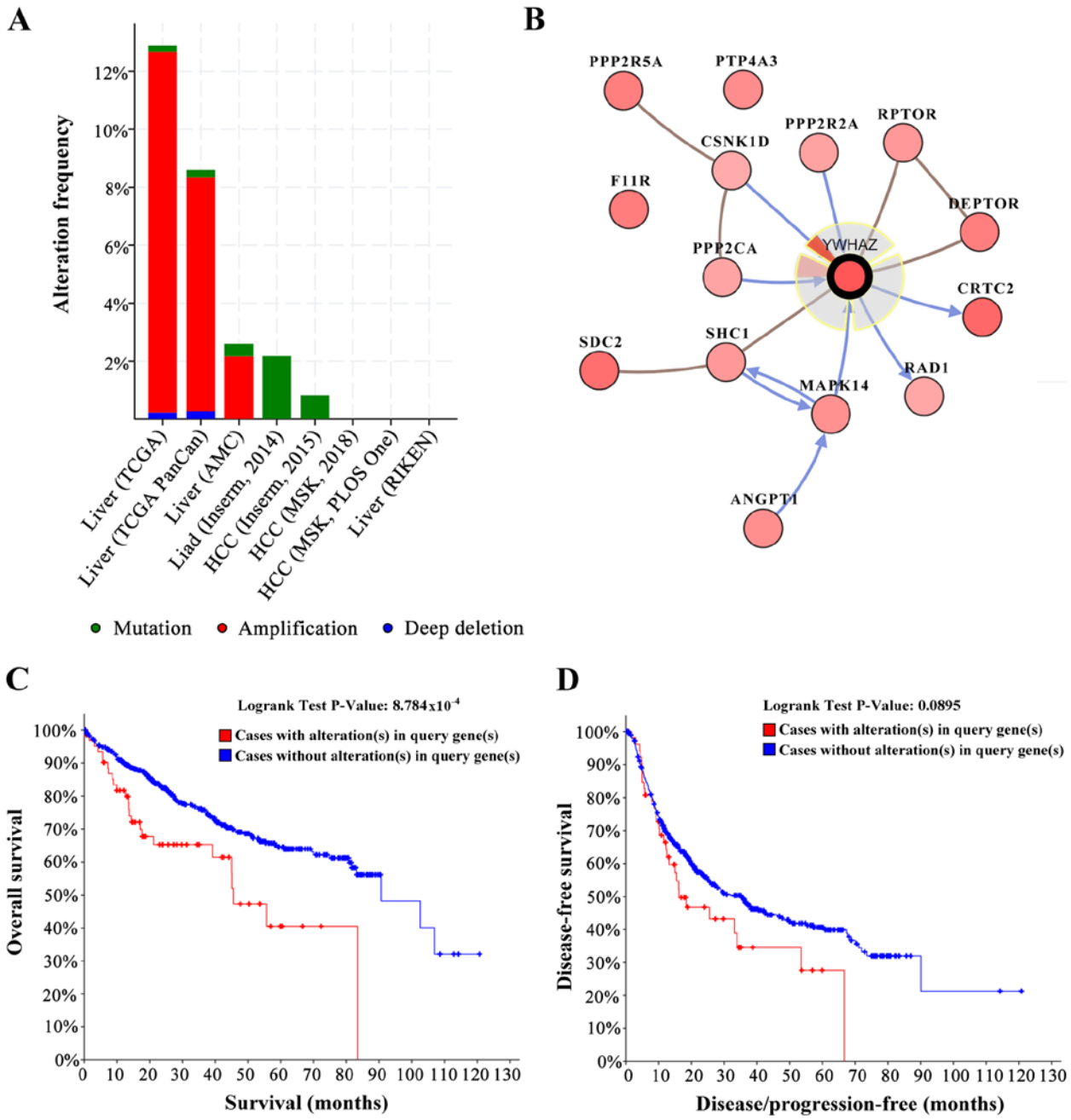

Figure 3. *yhwaz* mutation types in HCC. (A) Mutation types in patients with HCC. (B) Network view of the *yhwaz* neighborhood in LIHC. *yhwaz* seed genes (indicated with a different colour), and all the other genes were automatically identified as altered in LIHC. Darker red indicates increased frequency of alteration in LIHC. The interaction types are derived from BioPAX: Blue connections indicate that the first protein controls a reaction that changes the state of the second protein; red connections indicate proteins are members of the same complex. (C and D) Overall survival time and disease-free survival time of patients with HCC with alterations in the *yhwaz* gene. BioPAX, Biological Pathway Exchange; HCC, hepatocellular carcinoma; LIHC, liver HCC.

miRNA target networks of associated gene sets generated by Gene Set Enrichment Analysis (GSEA) (30,31). KEGG pathway analysis showed enrichment in tumor necrosis factor, proteoglycans in cancer and miRNAs in cancer pathways (Fig. 5A). The miRNA-target network was associated with miR-149 (GAG CCAG), miR-133A/miR-133B (GGGACCA) and miR-296 (GGGGCCC). The most significant target networks were the kinase-target networks associated primarily with the MAPK2, Rho Associated Coiled-Coil Containing Protein Kinase 1 and Polo-like Kinase 1 (Table VII). The protein-protein interaction network constructed by GeneMANIA (32) revealed associations amongst genes for MAPKAPK2 and miRNA-149. The GSEA for MAPK2 is responsible primarily for regulating the cell cycle, kinase activity and the immune response (Fig. 5B), and the GSEA for miRNA-527 (Fig. 5C) was involved primarily in the regulation of cell activation, kinase activity and leukocyte activation.

Discussion

The 14-3-3 family of proteins serve various roles in signaling and interact with several protein partners, including their function as adaptors that stimulate protein-protein interactions (3,41). Among the 14-3-3 proteins, the 14-3-3ζ isotype is

Table IV. Known mutations in the *yhwaz* gene.

Sample ID	Protein change	Type of mutation	Copy no.	COSMIC	Allele frequency (T)	No. of mutations
CHC1915T	*N108S*	Missense		1		39
CHC703T	*R127G*	Missense				104
CHC1915T	*N108S*	Missense		1		36
H093892	*S207I*	Missense	Diploid		0.04	74
TCGA-DD-A115-01	*C94S*	Missense	ShallowDel		0.08	66
TCGA-DD-A115-01	*C94S*	Missense	ShallowDel		0.07	80

Table V. Type and frequency of *yhwaz* neighbor gene alterations in patients with hepatocellular carcinoma.

Gene symbol	Amplification	Homozygous deletion	Upregulation	Downregulation	Mutation	Total alteration
YWHAZ	15.3	0.3	23.6	0	0.3	30.8
AGPT1	16.7	0.3	7.5	0	1.4	23.1
CRTC2	12.2	0	34.2	0	0.8	38.1
CSNK1D	6.9	0.6	11.1	0.6	0	15.8
DEPTOR	17.5	0.3	14.7	0	0.3	28.3
F11R	11.4	0	24.2	0	0.3	29.2
MAPK14	3.1	0.3	20.3	1.1	0.6	22.5
NTRK1	11.9	0	1.9	0	1.1	15.0
PPP2CA	0.6	0	13.9	3.1	0.6	17.5
PPP2R2A	0	6.1	2.5	9.7	0.8	18.1
PPP2R5A	8.9	0	24.2	0	0	28.3
PTP4A3	16.4	0.3	11.7	0	0.8	23.9
SDC2	15.3	0.3	21.9	2.5	0.3	34.4
SHC1	13.6	0	8.9	0	0.8	20.6

Table VI. Association between *yhwaz* targeting miRNAs and survival time in patients with hepatocellular carcinoma.

miRNAs	Gene IDs	HR	95% CI	P-value
miR-22	Has-miR-22	0.47	0.32-0.67	$2.6x10^{-5}$
miR-1	has-miR-1	0.6	0.41-0.87	0.0072
miR−451	has-miR-451	0.68	0.36-1.28	0.23
miR−155	has-miR-155	0.51	0.28-0.91	0.021
miR−206	has-miR-206	0.37	0.24-0.59	$1.2x10^{-5}$
miR−140	has-miR-140-3p	0.74	0.45-1.21	0.23

miRNA/miR, microRNA; hsa, homosapien; HR, hazard ratio; CI, confidence interval.

one of the most studied members of this family (42). 14-3-3ζ interacts with numerous key cellular proteins involved in tumor development and progression (43). The importance of 14-3-3ζ in the development and progression of cancer has been demonstrated in a number of different types of cancer (44). In the present study, bioinformatics analysis was used to determine the prognostic value of *yhwaz* in liver cancer, and the results revealed that *yhwaz* expression was upregulated in HCC tissues and cell lines. Upregulated levels of *yhwaz* were significantly associated with a poor prognosis. Mutations in *yhwaz* significantly affected the survival time of patients with HCC, and miRNAs that targeted *yhwaz* were associated with outcomes of HCC.

14-3-3ζ has been suggested to be a potential prognostic marker and therapeutic target in a number of different types of cancer (43). 14-3-3ζ overexpression increases Akt phosphorylation and further increased hypoxia-inducible factor-1α expression in HCC cells (45,46). In the present study,

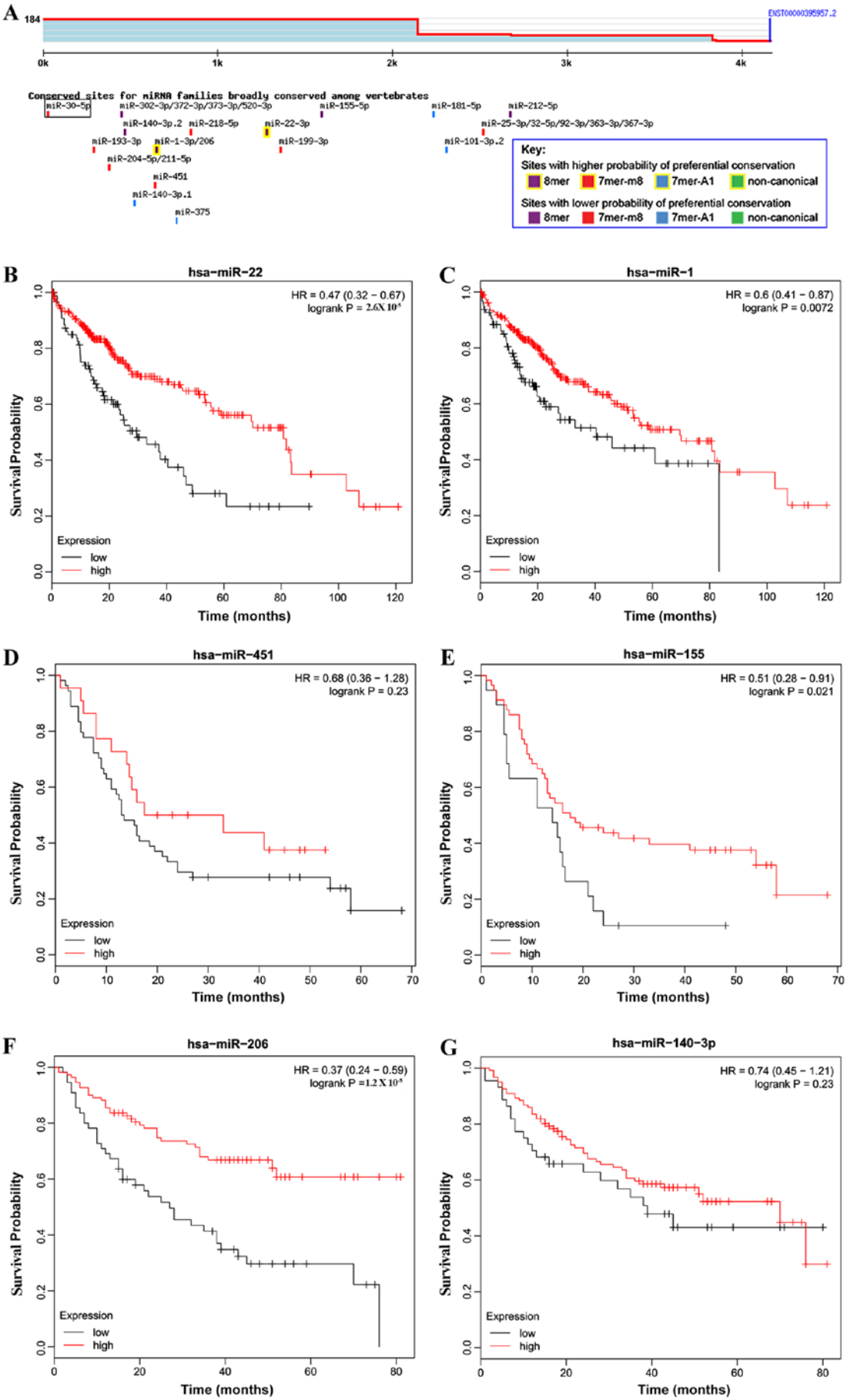

Figure 4. miRNAs targeting *yhwaz* and the effect of miRNA expression levels on survival in patients with HCC. (A) miRNAs predicted to target *yhwaz*. (B-G) Kaplan-Meier analysis of survival times in patients with HCC based on miR-22, miR-1, miR-451, miR-155, miR-206 and miR-104 expression, all of which were predicted to target *yhwaz*. HCC, hepatocellular carcinoma; miRNA/miR, microRNA; OS, overall survival; PPS, post-progression survival.

Table VII. Kinase and miRNA target networks of *yhwaz* in hepatocellular carcinoma.

Enriched category	Geneset	Leading EdgeNum	FDR
miRNA target	GAGCCAG, MIR-149	46	0.039256
	GGGACCA, MIR-133A, MIR-133B	52	0.038166
	GGGGCCC, MIR-296	21	0.037802
Kinase target	Kinase_MAPKAPK2	12	0.043584
	Kinase_ROCK1	19	0.035556
	Kinase_PLK1	41	0.030585

miRNA, microRNA; FDR, false discovery rate.

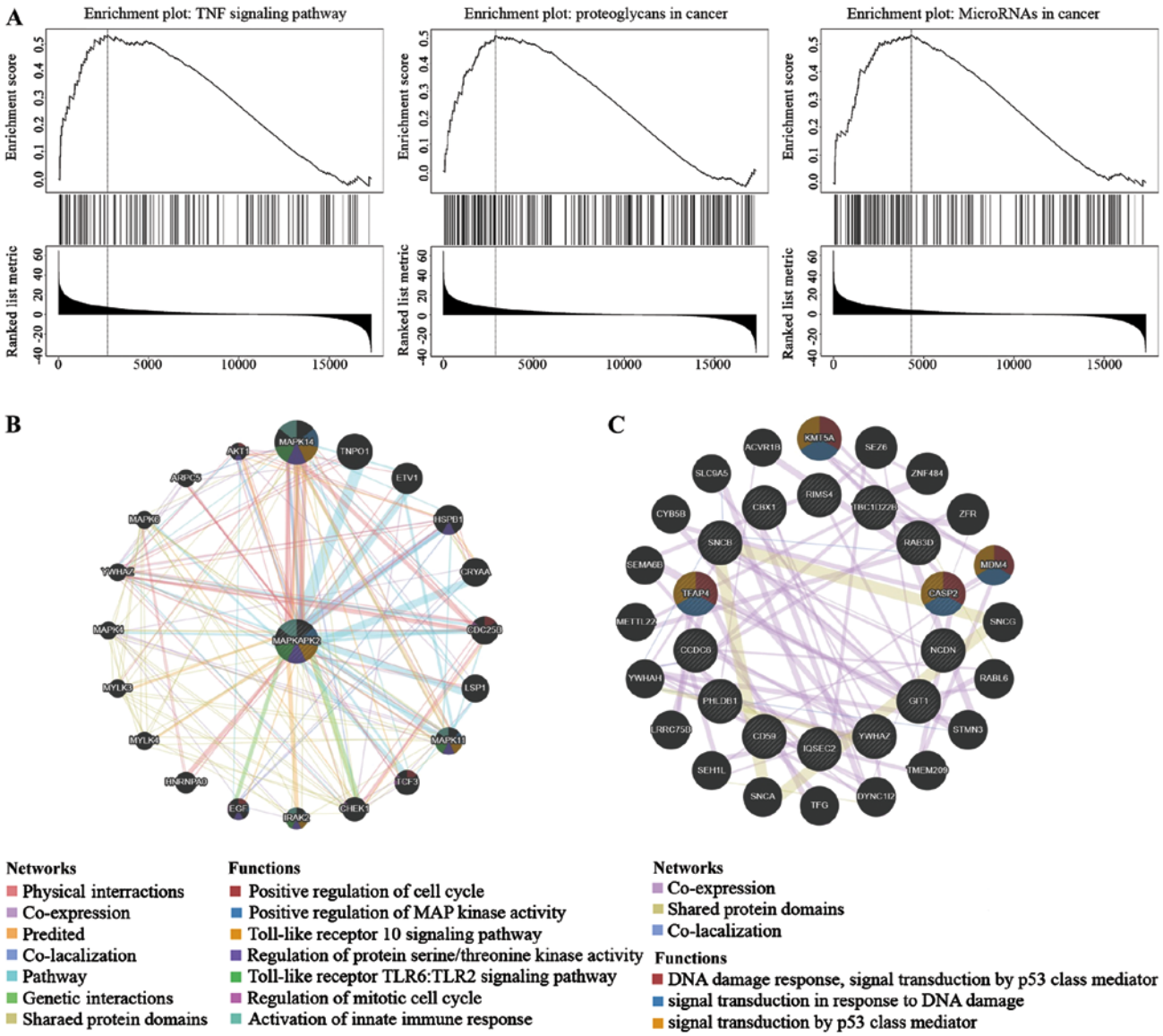

Figure 5. Enrichment analysis of *yhwaz* functional networks in HCC. (A) KEGG analysis of *yhwaz* co-expression genes in HCC. (B and C) Protein-protein interaction network and functional analysis of gene sets that were enriched in the target network of MAPKAPK2 kinases and miR-527. Different colors of the network edge indicate the bioinformatics methods applied: Co-expression, website prediction, pathway, physical interactions and co-localization. The different colors of the network nodes indicate the biological functions of the set of enrichment genes. HCC, hepatocellular carcinoma; KEGG, Kyoto Encyclopedia of Genes and Genomes; MAPKAPK2, mitogen-activated protein kinase-activated protein kinase 2; TNF, tumor necrosis factor.

yhwaz expression was upregulated in the major and different sub-types of HCC. 14-3-3ζ has been hypothesized to bind with the hepatitis B virus (HBV) protein X (HBx) and maintain its protein stability in HCC cells. In cancer cells where 14-3-3ζ

was silenced, the cells exhibited decreased migratory and invasive capacities, and this was accompanied by decreased expression of HBx (47). Together, these data suggest that upregulated expression of 14-3-3ζ may increase the risk of HBV infection.

14-3-3ζ targets and affects the phosphorylation of a number of proteins, a number of which are involved in tumor-promoting processes, such as regulation of autophagy and tumor suppressor pathways (48). Thus, alterations in *yhwaz* expression may result in cancer of the liver through regulation of these pathways and processes. The most common type of alteration observed in HCC was amplification, which was observed in 6% of cases. Multiple *yhwaz* mutations have been observed in patients with liver cancer, the majority of which are primarily phosphorylation sites. p-AKT is the one of the targets of 14-3-3ζ, upregulation of both 14-3-3ζ. p-Akt in patients with HCC predicts a poor prognosis, and 14-3-3ζ triggers activation of the Akt signaling pathway, thus contributing to the development of HCC (49). However, in patients with upregulated expression of miRNAs that target *ywhaz*, prognosis was predicted to be improved compared with patients with lower expression levels of the same miRNAs. Enrichment analysis of *yhwaz* co-expression genes determined that the majority of the interacting genes were involved in processes associated with cancer progression, and *yhwaz* target GSEA may assist in identifying important networks of target kinases and miRNAs.

The present study used the online tools to perform target gene analysis on tumor data from public databases. At the same time, the limitation is that transcriptome sequencing can only detect static mutations; it does not directly provide information on protein activity or expression levels. These issues should be addressed in subsequent research using molecular biology techniques. The other one limitation is that the LIHC samples contained a relatively small number of stage 4 patients, but the clinical reality is that most patients with HCC are diagnosed for the first time when the disease progresses, and thus, the prognosis is extremely poor. Therefore, the results of the present study should be validated in clinical samples including a full coverage of different ethnic groups and HCC stages.

The present study provided theoretical evidence of the importance of *yhwaz* expression in hepatocarcinogenesis and highlighted its potential as a marker in HCC. Based on the results of the present study, it may be hypothesized that targeting 14-3-3ζ in patients with liver cancer may effectively improve the survival rates and prognosis of patients.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YL, LS and XY designed and conducted the experiments. YL and LS wrote and revised the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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