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Diagnostic and prognostic value of *WNT* family gene expression in hepatitis B virus-related hepatocellular carcinoma

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Abstract. The aim of the present study was to investigate the diagnostic and prognostic value of Wingless-type MMTV integration site (*WNT*) gene family expression in patients with hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC). The clinical data of the patients and gene expression levels were downloaded from Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA) databases. Receiver operating characteristic curve analysis was used to investigate the diagnostic value of *WNT* genes. Cox proportional hazard regression analysis and Kaplan-Meier survival analysis were performed to evaluate the association of *WNT* gene expression level with overall survival (OS) and recurrence-free survival (RFS). A nomogram was constructed for the prediction of prognosis. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated. Diagnostic receiver operating characteristic curve analysis suggested that *WNT2* had a high diagnostic value, with an area under the curve (AUC) of >0.800 (P<0.0001, AUC=0.810, 95% CI: 0.767-0.852). Survival analysis indicated that the expression level of *WNT1* was significantly associated with OS and RFS (adjusted P=0.033, adjusted HR=0.607, 95% CI: 0.384-0.960; and adjusted P=0.007, adjusted HR=0.592, 95% CI: 0.404-0.868, respectively). In the TCGA validation cohort, we also observed that *WNT2* was significantly differentially expressed between HCC tissues and adjacent non-tumor tissues, and *WNT1* was associated with both the OS and RFS of HCC. Therefore, through the GSE14520 HBV-related HCC cohort we concluded that *WNT2* may serve as a diagnostic biomarker and *WNT1* may serve as a prognostic biomarker. These results may also be extended to TCGA HCC verification cohort.

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Key words: hepatocellular carcinoma, *WNT* family genes, diagnosis, prognosis, biomarker

Introduction

Liver cancer is one of the most common lethal cancers worldwide. It has been reported that liver cancer ranks sixth among the most commonly diagnosed cancers worldwide, and was the fourth major cause of cancer-related deaths in 2018. Global cancer statistics indicate that ~841,000 new cases and 782,000 deaths occur annually (1). Hepatocellular carcinoma (HCC) is the major histological type of primary liver cancer, accounting for 75-85% of all cases. Infection with hepatitis virus [mainly hepatitis B virus (HBV) and hepatitis C virus], aflatoxin exposure, excessive alcohol consumption and tobacco smoking, are considered as the main risk factors for the development of HCC (2,3). In China, the predominant cause of HCC is chronic HBV infection, and it is estimated that ~70% of these patients have an established HBV infection history (4). Although the available therapies for HCC patients have greatly improved over the past decades, the clinical prognosis remains unfavorable, with a 5-year overall survival (OS) rate of ~30% following hepatic resection (2,5). Therefore, it is imperative to identify more sensitive diagnostic and prognostic biomarkers for HCC.

Wingless-type MMTV integration site (*WNT*) genes are a family of 19 genes that modulate both the canonical WNT signal transduction pathway (referred to as β-catenin-dependent) and non-canonical WNT signal transduction pathway (referred to as β-catenin-independent) (6). Previous studies indicated that *WNT* family genes are associated with various tumor biological processes, including cell proliferation (7,8), invasion (8-11), metastasis (12,13) and drug resistance (13-15). In addition, some researchers have reported that aberrant *WNT* expression levels are associated with diagnosis and prognosis prediction for certain tumors. Fu *et al* (16) proved that *WNT2* can activate the WNT/β-catenin signal transduction pathway, which ultimately promotes esophageal cancer cell growth, and *WNT2* enhances cell motility and invasiveness by inducing

epithelial-to-mesenchymal transition. Furthermore, *WNT2* expression level was found to be closely associated with the poor clinical performance status of patients with esophageal squamous cell carc *WNT* inoma.

However, the diagnostic and prognostic value of the *WNT* gene family expression in HBV-related HCC remains unclear. The primary goal of the present study was to investigate this association by collecting data from public databases and performing a series of bioinformatics analyses.

Materials and methods

Data sources. The clinical characteristics of patients with HBV-related HCC and the corresponding *WNT* gene family expression levels were downloaded from the GSE14520 dataset of Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE14520, accessed November 28, 2018) (17,18). The detailed process of GSE14520 genome-wide expression profile dataset processing has been described in our previous article (19). The Cancer Genome Atlas (TCGA) database HCC cohort was used as a validation cohort, and the data processing of RNA sequencing was described in our previous paper (19). The raw RNA sequencing dataset of TCGA was normalized by DESeq (20). The clinical data of these patients in the GSE14520 dataset included age, gender, serum alanine aminotransferase level, serum α-fetoprotein (AFP) level, cirrhosis, main tumor size, tumor number, Barcelona Clinic Liver Cancer (BCLC) stage, tumor-node-metastasis (TNM) stage, survival time, and survival status. The data for mRNA expression level of five *WNT* family genes (*WNT3A*, *WNT8A*, *WNT9A*, *WNT9B* and *WNT10A*) were unavailable in the Gene Expression Omnibus (GEO) database. Therefore, only 14 *WNT* genes were finally analyzed in the present study. As the dataset included in our research was obtained from a public database, the study did not require the approval of an ethics committee.

Bioinformatics analysis of WNT family genes. To explore the potential biological functions and possible pathways of *WNT* family genes, gene enrichment analyses, including Gene Ontology (GO) functional analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, were conducted by applying the Database for Annotation, Visualization, and Integrated Discovery (DAVID) bioinformatics online tool, version 6.8 (https://david.ncifcrf.gov/, accessed December 2, 2018) (21). Statistically, an enrichment P-value of <0.05 was considered to indicate statistically significant differences. In order to further validate the results of enrichment analysis by DAVID, application package Biological Networks Gene Ontology tool (BiNGO) in the Cytoscape software (version 3.7.1) (22) was used to explore the GO terms of *WNT* family genes. Pearson's correlation coefficient was calculated to assess the relevance among *WNT* genes in the co-expression analysis. These data were visualized by the correlation plot package in the R platform (version 3.4.0). *WNT* gene-gene and protein-protein interactions were investigated by using the online resource GeneMANIA (http://genemania.org/, accessed December 6, 2018) (23) and the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) (https://string-db.org/cgi/input.pl, accessed December 6, 2018) (24,25), respectively.

Assessment of diagnostic value. The expression level of *WNT* family genes in tumor tissues and corresponding adjacent non-tumor tissues were compared via t-test statistical analysis. The public online resource Metabolic gEne RApid Visualizer (MERAV) (http://merav.wi.mit.edu/, accessed December 8, 2018) (26) was used to further validate that genes of the *WNT* family were differentially expressed between primary liver cancer tissues and normal liver tissues. Receiver operating characteristic (ROC) curve analysis was selected to determine the diagnostic value of these differentially expressed *WNT* family genes.

Survival analysis. The 212 patients with HBV-related HCC were categorized into high- and low-expression groups based on the median *WNT* gene expression level in tumor tissues. In order to investigate whether the expression level of *WNT* family genes was correlated with prognosis and outcome, Cox proportional hazard regression analysis and Kaplan-Meier survival analysis with log-rank tests were used to evaluate the association between *WNT* family gene expression, OS and recurrence-free survival (RFS).

Joint effects analysis of WNT family genes. Based on the results of multivariate Cox proportional hazard regression analysis and Kaplan-Meier survival analysis, only *WNT1* and *WNT6* were found to be significantly associated with RFS. Therefore, the combined effects of *WNT1* and *WNT6* were analyzed. The combinations were as follows: Low *WNT1* expression and low *WNT6* expression (group 1), low *WNT1* expression and high *WNT6* expression (group 2), high *WNT1* expression and low *WNT6* expression (group 3), and high *WNT1* expression and high *WNT6* expression (group 4).

Prognostic nomogram for survival prediction. All 212 patients with HBV-related HCC in the GSE14520 dataset of the GEO database were identified as the source population for nomogram construction. The variables that were related to prognosis outcome were selected to construct the nomogram, including sex, serum AFP level, cirrhosis, BCLC stage, tumor size and *WNT* gene expression. With each variable being assigned a score, the total point was calculated by summing up the scores of all the variables and located onto the scale. Therefore, the probabilities of the survival outcome could be predicted by drawing a vertical line to the total point.

Statistical analysis. All statistical analyses were performed with the SPSS software package, version 17.0 (SPSS Inc.). Comparison of *WNT* family gene expression levels between tumor tissues and corresponding adjacent non-tumor tissues was performed using t-tests. The Cox proportional hazards regression model was selected for univariate and multivariate analyses. By applying Kaplan-Meier survival analysis with log-rank test, the association of *WNT* family gene expression levels with OS and RFS time was observed. Vertical scatter plots, ROC curves and survival curves were plotted by GraphPad Prism software, version 7.0 (GraphPad Software,

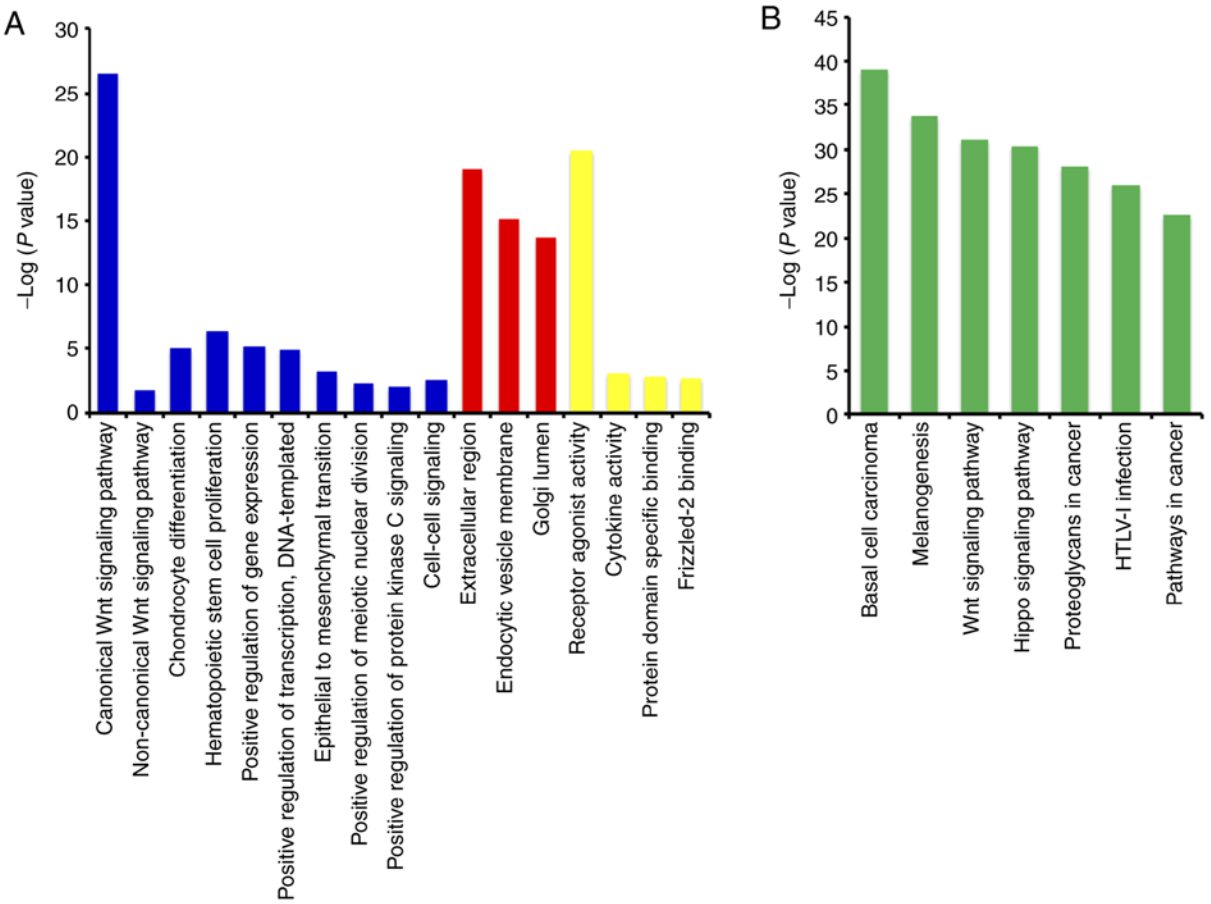

Figure 1. Gene Ontology (GO) functional analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of *WNT* family genes performed by DAVID online tool. (A) GO term enrichment of *WNT* family genes. (B) KEGG pathway enrichment of *WNT* family genes.

Inc.). P<0.05 was considered to indicate statistically significant differences.

Results

Characteristics of patients in the GEO database. In the GSE14520 dataset of the GEO database, there remained 212 patients with HBV-related HCC after excluding patients who had no reported HBV infection or any available survival information. Detailed characteristics of these patients are shown in Table SI. Serum AFP level, cirrhosis, tumor size, BCLC stage and TNM stage were found to be closely associated with OS (P<0.05), whereas sex, cirrhosis, BCLC stage and TNM stage were significantly associated with RFS (P<0.05). The remaining characteristics did not exhibit a significant association with OS or RFS (all P>0.05).

Bioinformatics analysis of WNT family genes. The GO function analysis indicated that *WNT* family genes were mainly enriched in the regulation of cell differentiation, cell proliferation, epithelial-to-mesenchymal transition, and modulation of the WNT signaling pathway (Figs. 1A, S1 and S2, and Table SII). The KEGG pathway analysis suggested that *WNT* family genes were associated with the WNT signaling pathway and other pathways (Fig. 1B and Table SIII). The Pearson's correlation coefficients of *WNT* family genes were calculated and used to assess whether these genes were correlated with each other. As shown in Fig. 2, the *WNT* family genes were correlated to some degree. The gene-gene and protein-protein interaction networks constructed by GeneMANIA and STRING, respectively, indicated that the *WNT* family genes were co-expressed and exhibited extensive homology at the protein level (Fig. 3A and B).

Assessment of diagnostic value. By comparing *WNT* family gene expression levels between tumor tissues and corresponding adjacent non-tumor tissues, a total of 8 *WNT* family genes (*WNT2*, *WNT2B*, *WNT4*, *WNT5A*, *WNT7B*, *WNT10B*, *WNT11* and *WNT16*) were found to be differentially expressed in tumor and non-tumor tissues (Fig. 4) (P<0.05). The online resource MERAV was used to further validate genes of the *WNT* family that were differentially expressed in normal liver tissues and primary liver cancer tissues (Fig. 5). An ROC curve was constructed to further explore the diagnostic value of these 8 differentially expressed genes. As shown in Fig. 6, six *WNT* genes (*WNT2*, *WNT2B*, *WNT5A*, *WNT10B*, *WNT11* and *WNT16*) had a potential prediction value, with all P-values <0.05 and area under the curve (AUC) >0.500; *WNT2* in particular exhibited high accuracy in differentiating HCC tissues from non-tumor tissue (P<0.0001, AUC=0.810, 95% CI: 0.767-0.852).

Survival analysis. The characteristics associated with clinical prognostic outcome, including cirrhosis, tumor size and

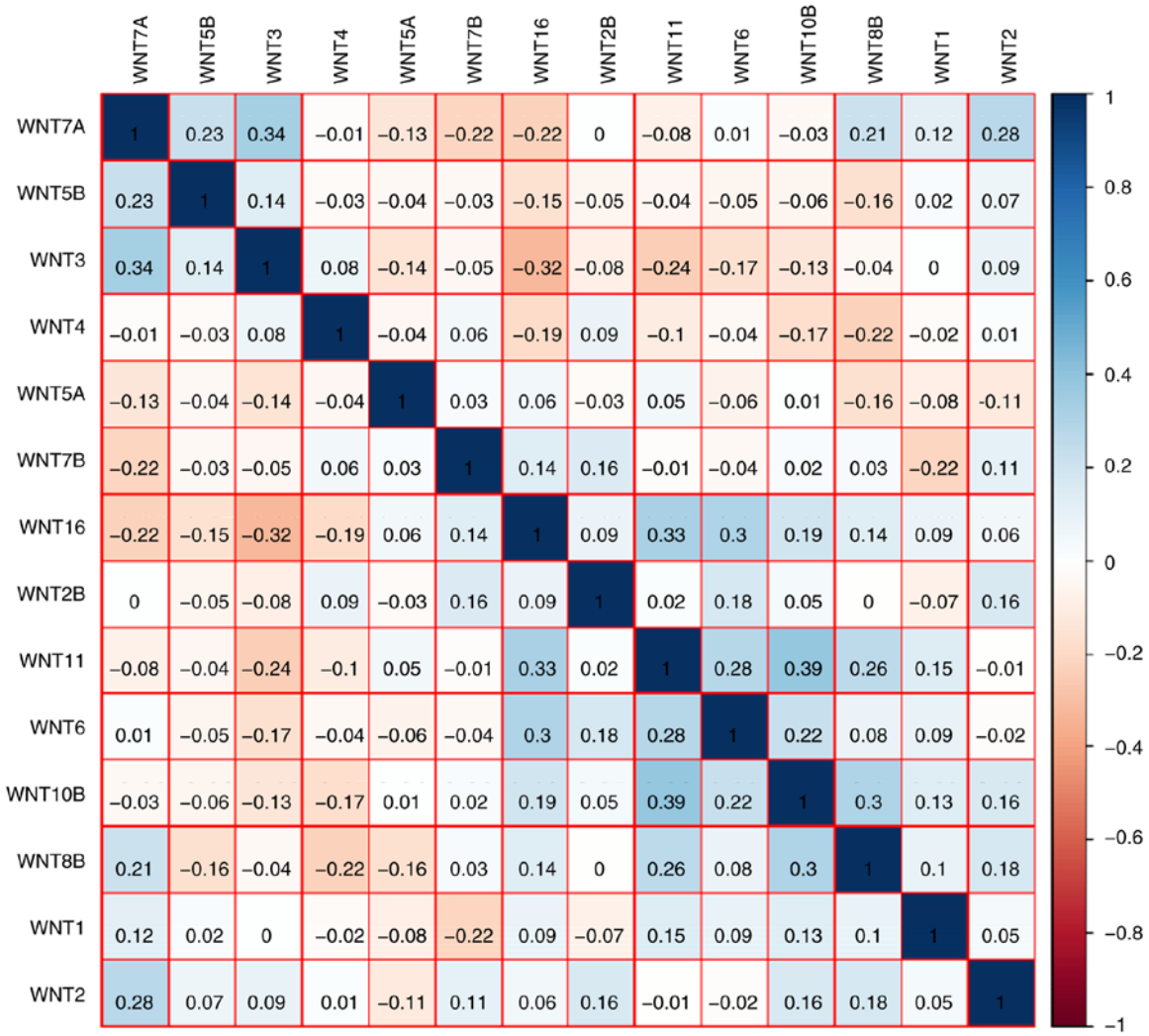

Figure 2. Matrix graph of Pearson's correlations of *WNT* family gene expression levels in the Gene Expression Omnibus database.

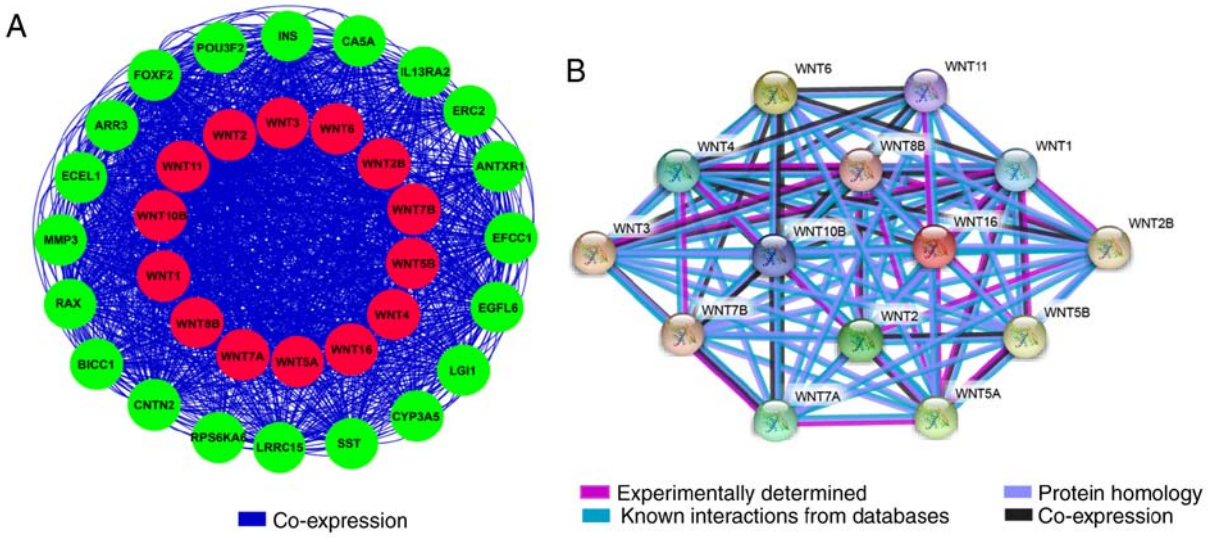

Figure 3. *WNT* family gene-gene and protein-protein interaction networks constructed by GeneMANIA and Search Tool for the Retrieval of Interacting Genes/Proteins databases. (A) Gene-gene interaction network of *WNT* family genes. (B) Protein-protein interaction network of *WNT* family genes.

BCLC stage, were included in the multivariate Cox regression analysis. Following adjustment or these prognosis-related risk factors, the results indicated that the expression level of *WNT1* was significantly associated with OS and RFS in the survival

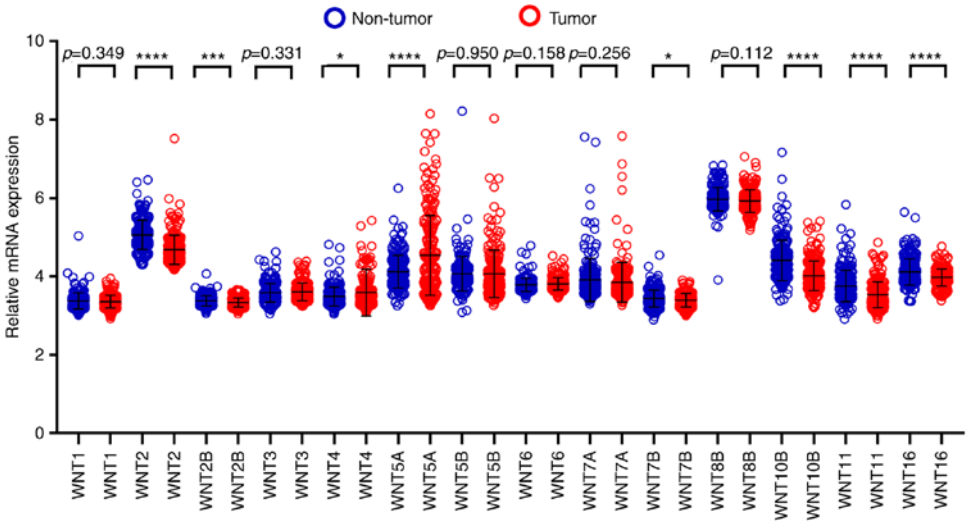

Figure 4. Relative expression of *WNT* family genes in 212 HCC tissues and 204 adjacent tissues in the Gene Expression Omnibus database. *P<0.05, ***P<0.001, ****P<0.0001.

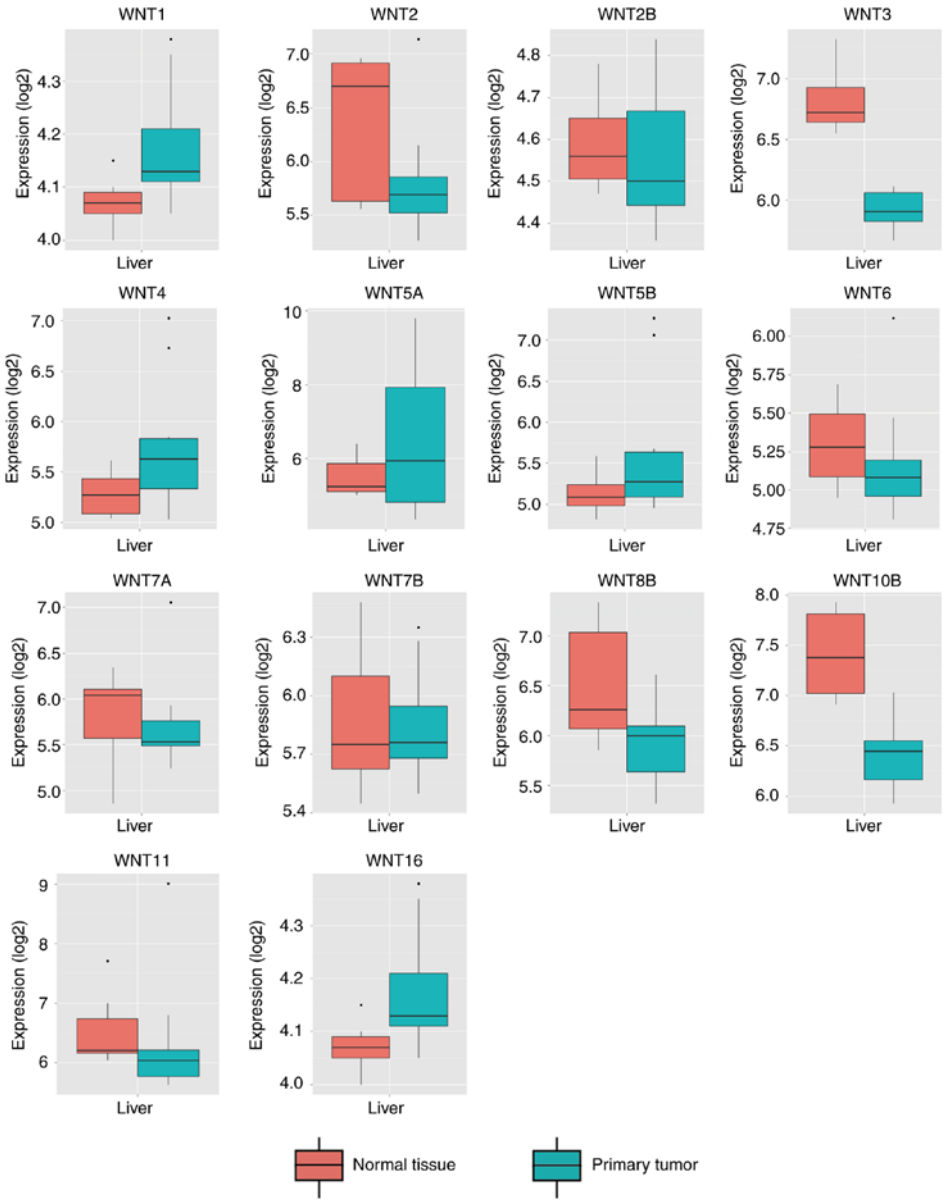

Figure 5. Expression levels of *WNT* family genes in normal liver and primary liver cancer tissues, as analyzed by MERAV.

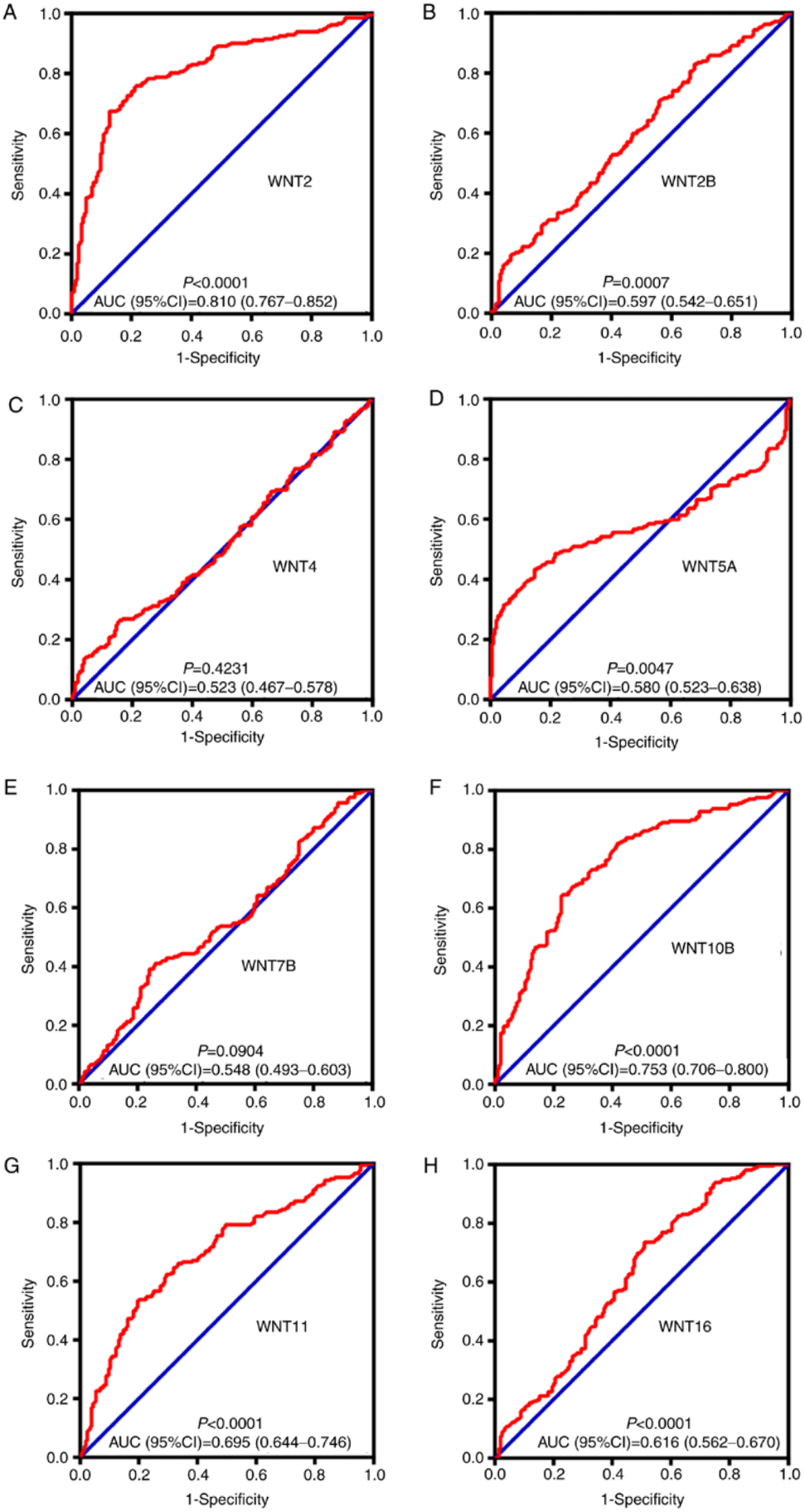

Figure 6. The receiver operating characteristics (ROC) curves of *WNT* family genes in distinguish HBV-related HCC tumor tissues and adjacent non-tumor tissues in GES14520. ROC curves of (A) *WNT2*; (B) *WNT2B*; (C) *WNT4*; (D) *WNT5A*; (E) *WNT7B*; (F) *WNT10B*; (G) *WNT11*; and (H) *WNT16*. AUC, area under the ROC curve; HBV, hepatitis B virus; HCC, hepatocellular carcinoma.

Table I. Prognostic values of *WNT* gene expression in HBV-related HCC of the GSE14520 cohort.

		OS						RFS					
Gene expression	Patient no.	No. of events	MST (months)	Crude HR (95% CI)	Crude P-value	Adjusted HR (95% CI)	Adjusted P-value[a]	No. of events	MRT (months)	Crude HR (95% CI)	Crude P-value	Adjusted HR (95% CI)	Adjusted P-value[a]
WNT1													**0.007**
Low	106	49	NA	1		1		66	27	1		1	
High	106	33	NA	0.575 (0.370-0.895)	0.014	0.607 (0.384-0.960)	**0.033**	50	57	0.588 (0.406-0.850)	0.005	0.592 (0.404-0.868)	
WNT2													0.444
Low	106	43	NA	1		1		58	40	1		1	
High	106	39	NA	0.872 (0.565-1.345)	0.534	0.784 (0.503-1.222)	0.282	58	46	0.935 (0.650-1.346)	0.718	0.865 (0.597-1.254)	
WNT2B													0.091
Low	106	38	NA	1		1		54	46	1		1	
High	106	44	NA	1.178 (0.763-1.818)	0.461	1.344 (0.862-2.093)	0.192	62	37	1.215 (0.844-1.751)	0.295	1.378 (0.950-1.997)	
WNT3													0.622
Low	106	37	NA	1		1		56	51	1		1	
High	106	45	NA	1.306 (0.845-2.018)	0.230	1.279 (0.814-2.008)	0.285	60	41	1.116 (0.775-1.606)	0.556	1.099 (0.754-1.601)	
WNT4													0.113
Low	106	39	NA	1		1		63	35	1		1	
High	106	43	NA	1.229 (0.796-1.896)	0.352	1.116 (0.720-1.730)	0.624	53	53	0.828 (0.575-1.194)	0.313	0.742 (0.512-1.074)	
WNT5A													0.663
Low	106	45	NA	1		1		62	36	1		1	
High	106	37	NA	0.847 (0.549-1.309)	0.456	0.915 (0.591-1.417)	0.690	54	51	0.875 (0.607-1.260)	0.473	0.922 (0.638-1.331)	
WNT5B													0.641
Low	106	40	NA	1		1		64	46	1		1	
High	106	42	NA	1.068 (0.693-1.647)	0.766	1.328 (0.848-2.078)	0.215	52	43	0.803 (0.557-1.158)	0.240	0.915 (0.630-1.329)	
WNT6													**0.033**
Low	106	48	60	1		1		66	30	1		1	
High	106	34	NA	0.662 (0.426-1.027)	0.066	0.756 (0.483-1.184)	0.222	50	57	0.644 (0.446-0.931)	0.019	0.665 (0.457-0.968)	

Table I. Continued.

		OS						RFS					
Gene expression	Patient no.	No. of events	MST (months)	Crude HR (95% CI)	Crude P-value	Adjusted HR (95% CI)	Adjusted P-value[a]	No. of events	MRT (months)	Crude HR (95% CI)	Crude P-value	Adjusted HR (95% CI)	Adjusted P-value[a]
WNT7A													0.180
Low	106	43	NA	1		1		62	41	1		1	
High	106	39	NA	0.776 (0.503-1.198)	0.253	0.764 (0.492-1.185)	0.229	54	48	0.757 (0.526-1.091)	0.136	0.778 (0.539-1.123)	
WNT7B													0.522
Low	106	36	NA	1		1		53	54	1		1	
High	106	46	60	1.421 (0.918-2.200)	0.115	1.057 (0.670-1.667)	0.812	63	30	1.390 (0.963-2.004)	0.078	1.132 (0.775-1.655)	
WNT8B													0.721
Low	106	38	NA	1		1		57	48	1		1	
High	106	44	NA	1.192 (0.772-1.840)	0.428	0.904 (0.580-1.407)	0.654	59	37	1.103 (0.766-1.588)	0.597	0.935 (0.646-1.354)	
WNT10B													0.079
Low	106	46	60	1		1		64	30	1		1	
High	106	36	NA	0.704 (0.455-1.090)	0.115	0.691 (0.445-1.0750)	0.101	52	57	0.703 (0.487-1.014)	0.060	0.717 (0.495-1.039)	
WNT11													0.371
Low	106	47	60	1		1		63	35	1		1	
High	106	35	NA	0.685 (0.442-1.061)	0.090	0.740 (0.474-1.156)	0.186	53	54	0.744 (0.515-1.073)	0.113	0.843 (0.581-1.225)	
WNT16													0.349
Low	106	40	NA	1		1		60	37	1		1	
High	106	42	NA	0.974 (0.632-1.503)	0.906	0.950 (0.611-1.477)	0.819	56	46	0.835 (0.580-1.202)	0.332	0.838 (0.580-1.212)	

[a]Adjusted for cirrhosis, tumor size and BCLC stage. Bold print indicates statistical significance. HBV, hepatitis B virus; HCC, hepatocellular carcinoma; OS, overall survival; RFS, recurrence-free survival; MST, median survival time; MRT, median recurrence time; HR, hazard ratio; CI, confidence interval; NA, not available; BCLC, Barcelona Clinic Liver Cancer.

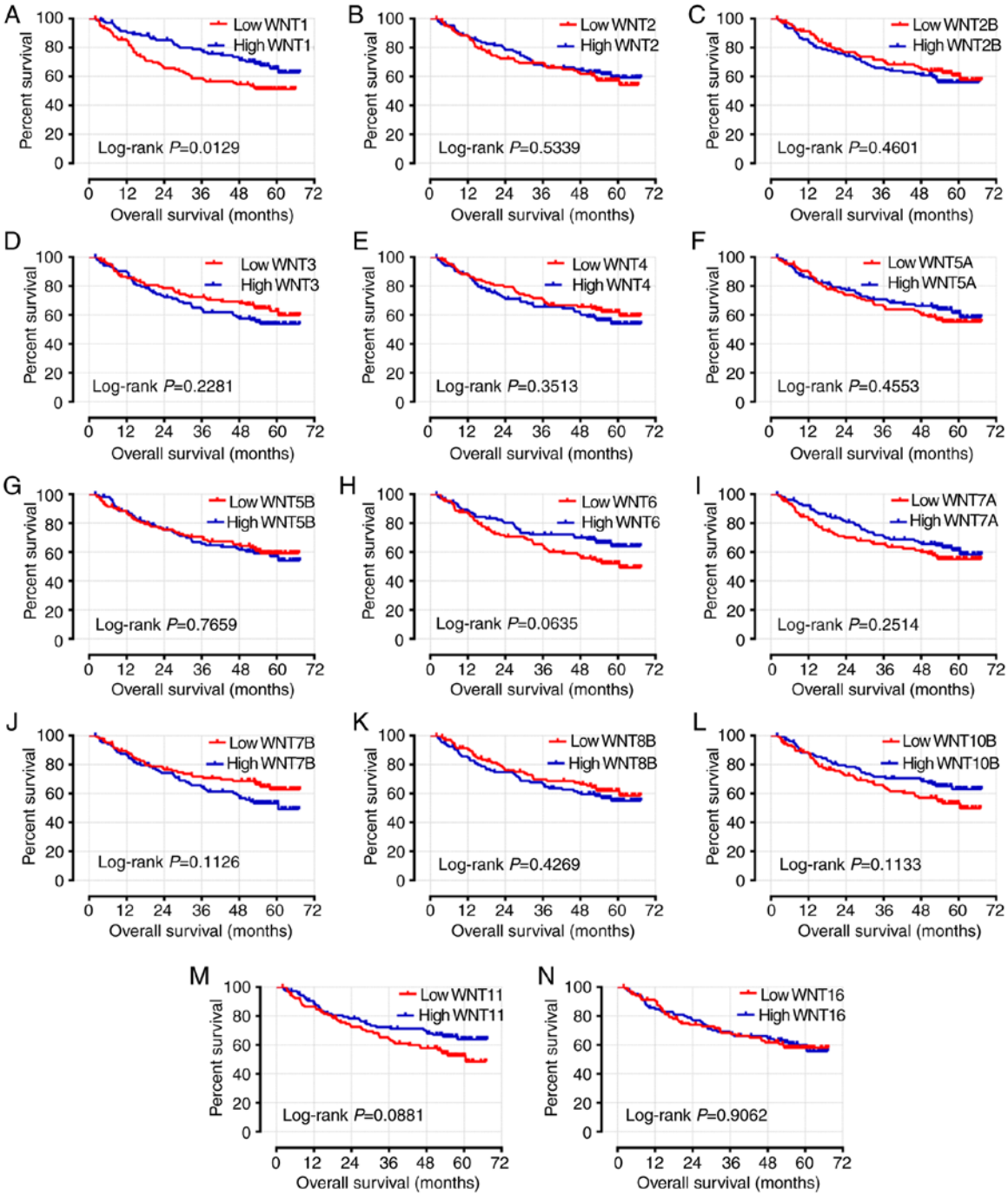

Figure 7. Kaplan-Meier survival curves for *WNT* genes in HBV-related HCC of GES14520. OS stratified by (A) *WNT1*; (B) *WNT2*; (C) *WNT2B*; (D) *WNT3*; (E) *WNT4*; (F) *WNT5A*; (G) *WNT5B*; (H) *WNT6*; (I) *WNT7A*; (J) *WNT7B*; (K) *WNT8B*; (L) *WNT10B*; (M) *WNT11*; and (N) *WNT16*. HBV, hepatitis B virus; HCC, hepatocellular carcinoma; OS, overall survival.

analysis (adjusted P=0.033, adjusted HR=0.607, 95% CI: 0.384-0.960 and adjusted P=0.007, adjusted HR=0.592, 95% CI: 0.404-0.868, respectively) (Table I; Figs. 7A and 8A). The expression level of *WNT6* was closely associated with RFS (adjusted P=0.033, adjusted HR=0.665, 95% CI: 0.457-0.968), but *WNT6* did not exhibit a significant association with OS (P>0.05) (Table I; Figs. 7H and 8H).

Joint effects analysis of WNT family genes. As shown above, the multivariate Cox regression analysis and Kaplan-Meier survival analysis demonstrated that only *WNT1* and *WNT6* were significantly associated with RFS. Joint effects survival analysis for *WNT1* and *WNT6* was performed following adjustment for cirrhosis, tumor size and BCLC stage. The results suggested that group 4 (high *WNT1* expression and high *WNT6* expression) had the longest RFS, whereas group 1 (low *WNT1* expression and low *WNT6* expression) had the shortest RFS (Table II and Fig. 9). Therefore, patients with a high expression of both *WNT1* and *WNT6* are expected to have a longer RFS.

Prognostic nomogram for survival prediction. The prognostic risk factors that may predict the outcome of survival, including

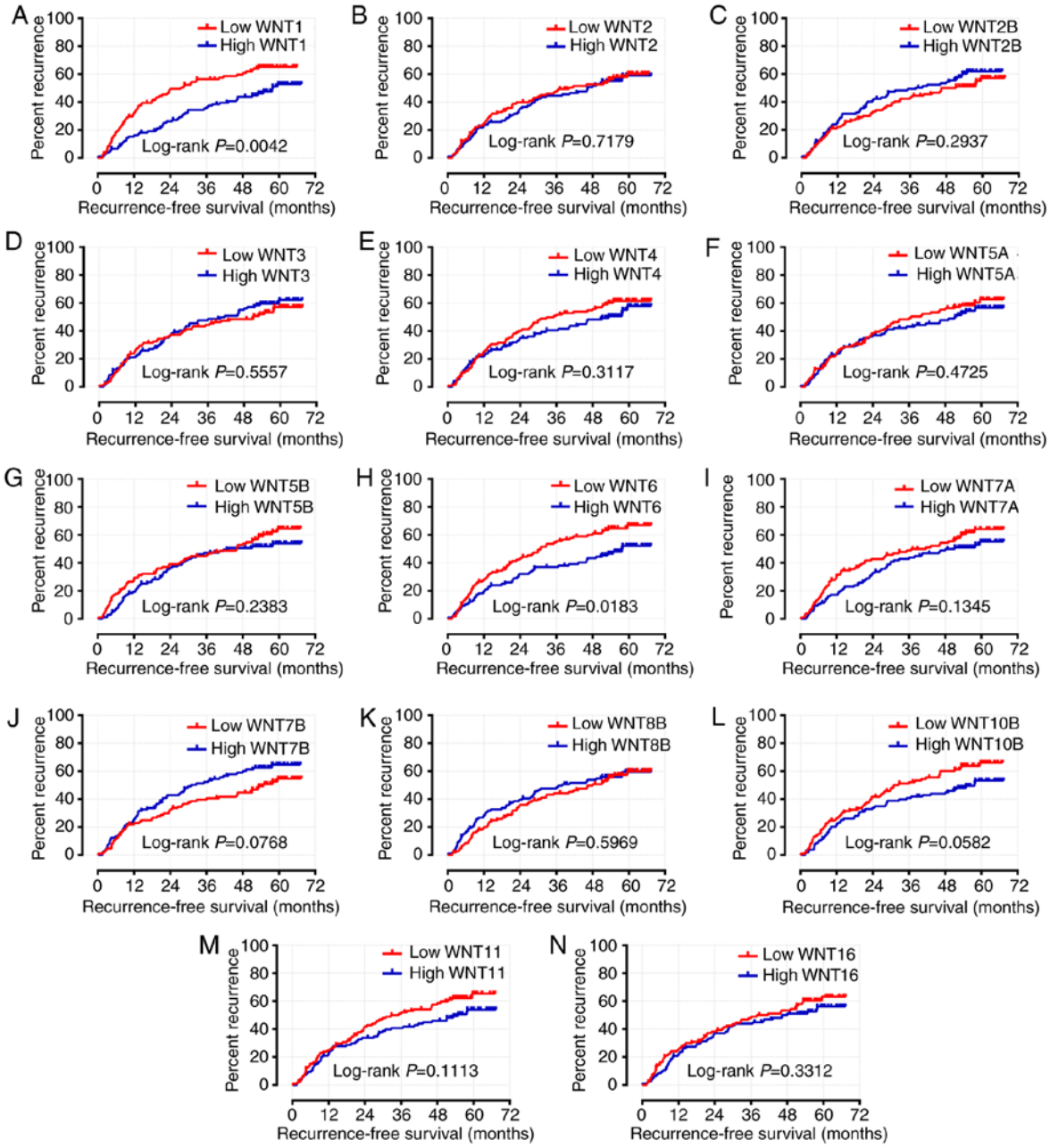

Figure 8. Kaplan-Meier survival curves for *WNT* genes in HBV-related HCC of GES14520. RFS stratified by (A) *WNT1*; (B) *WNT2*; (C) *WNT2B*; (D) *WNT3*; (E) *WNT4*; (F) *WNT5A*; (G) *WNT5B*; (H) *WNT6*; (I) *WNT7A*; (J) *WNT7B*; (K) *WNT8B*; (L) *WNT10B*; (M) *WNT11*; and (N) *WNT16*. HBV, hepatitis B virus; HCC, hepatocellular carcinoma; RFS, recurrence-free survival.

sex, serum AFP level, cirrhosis, BCLC stage, tumor size and *WNT* family gene expression, were selected to construct the nomogram, which can provide an individualized prognosis prediction. For the 212 patients with HBV-related HCC, nomogram analysis was performed for the probabilities of 1-, 2-, 3-, 4- and 5-year OS (Fig. 10A) and RFS (Fig. 10B). As shown in the nomogram, the expression level of *WNT1* and *WNT6* contributed to a certain extent to the patients' clinical prognosis outcome.

TCGA validation cohort. A total of 374 tumor tissues and 50 adjacent non-tumor tissues were included in the present study. Among those, 370 HCC patients with prognostic information were included in the survival analysis. The expression distribution of the *WNT* family genes between HCC tumor tissues and adjacent non-tumor tissues were calculated by DESeq and are shown in Table III. *WNT2* was shown to be significantly differentially expressed between HCC and adjacent non-tumor tissues in the TCGA cohort (Table III, Fig. 11A and B). The clinical characteristics of HCC patients in the TCGA cohort are shown in Table SIV. The survival analysis results of the *WNT* family genes are shown in Table IV. *WNT*1 was found to be associated with both the OS and RFS of HCC in the TCGA cohort (Table IV, Fig. 11C and D).

Discussion

The aim of the present study was to investigate the diagnostic and prognostic values of *WNT* family gene expression in

Table II. Joint effects analysis for the combination of *WNT1* and *WNT6*.

Group	*WNT1* expression	*WNT6* expression	Patients (n=212)	MRT (months)	Crude HR (95% CI)	Crude P-value	Adjusted HR (95% CI)	Adjusted P-value[a]
1	Low	Low	56	18	NA	0.001	NA	P<0.001
2	Low	High	50	49	0.524 (0.318-0.862)	0.011	0.457 (0.276-0.755)	0.002
3	High	Low	50	51	0.478 (0.290-0.787)	0.004	0.413 (0.250-0.683)	0.001
4	High	High	56	NA	0.401 (0.243-0.662)	P<0.001	0.405 (0.240-0.684)	0.001

[a]Adjusted for cirrhosis, tumor size and BCLC stage. MRT, median recurrence time; HR, hazard ratio; CI, confidence interval; NA, not available; BCLC, Barcelona Clinic Liver Cancer.

Table III. Expression distribution of *WNT* family genes between tumor tissue and adjacent non-tumor tissue in the TCGA validation cohort.

Gene	Log_2 (fold change)	P-value	FDR
WNT1	1.84421242	0.946083391	0.999996922
WNT2	-1.769264955	0.002852902	0.022026272
WNT2B	1.360412766	0.373384363	0.65436372
WNT3	0.066523683	0.810682922	0.928938592
WNT4	1.309792732	0.026270116	0.120328643
WNT5A	0.650962704	0.073901092	0.249424709
WNT5B	-0.101243468	0.644473376	0.842930166
WNT6	2.792018889	0.42971413	0.703074675
WNT7A	0.13572947	1	1
WNT7B	1.071320247	0.324934599	0.610626696
WNT8B	1.918325871	0.070391904	0.241649148
WNT10B	1.121445938	0.298549628	0.582917358
WNT11	-1.50622528	0.000105087	0.001433939
WNT16	-0.077860553	0.822321775	0.934493001

TCGA, The Cancer Genome Atlas; FDR, false discovery rate.

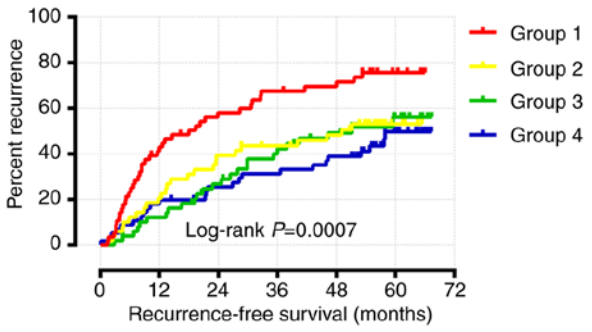

Figure 9. Joint effects analysis for the combination of *WNT1* and *WNT6*.

HBV-related HCC established on public databases and a series of bioinformatics analyses. The results suggested that *WNT2* may serve as potential diagnostic biomarker for HBV-related HCC. In addition, we demonstrated that the expression level of *WNT1* was significantly associated with clinical prognostic outcome, with patients with a higher expression level of *WNT1* expected to have a better prognostic outcome. Therefore, it may be concluded that *WNT1* may serve as potential prognostic biomarker for patients with HBV-related HCC.

Previous studies confirmed that the WNT signaling pathway plays a crucial role in numerous physiological and pathological processes, including the regeneration of hair and skin (27), the repair of liver and lung after injury (28,29), hematopoiesis (30,31) and neurogenesis (32). Furthermore, other studies have demonstrated that the aberrant regulation of WNT signaling may contribute to various diseases, including cancer (6,33-35), osteoporosis (36,37), fibrosis (38-40), autoimmune diseases (41-43), neurological diseases (44,45), and disorders of endocrine function (46-48). The WNT signaling pathway has been shown to either promote or inhibit cancer biological progression in a cancer stage- and type-specific manner (6). *WNT* family genes, as the most important component of the WNT signaling pathway, participate in the initiation and progression of various cancers, such as esophageal carcinoma (16), gastric cancer (49), pancreatic cancer (50), prostate cancer (51), ovarian cancer (52,53), and leukemia (54). Numerous studies have reported that *WNT* family genes may regulate cell proliferation (50,54), differentiation, epithelial-to-mesenchymal

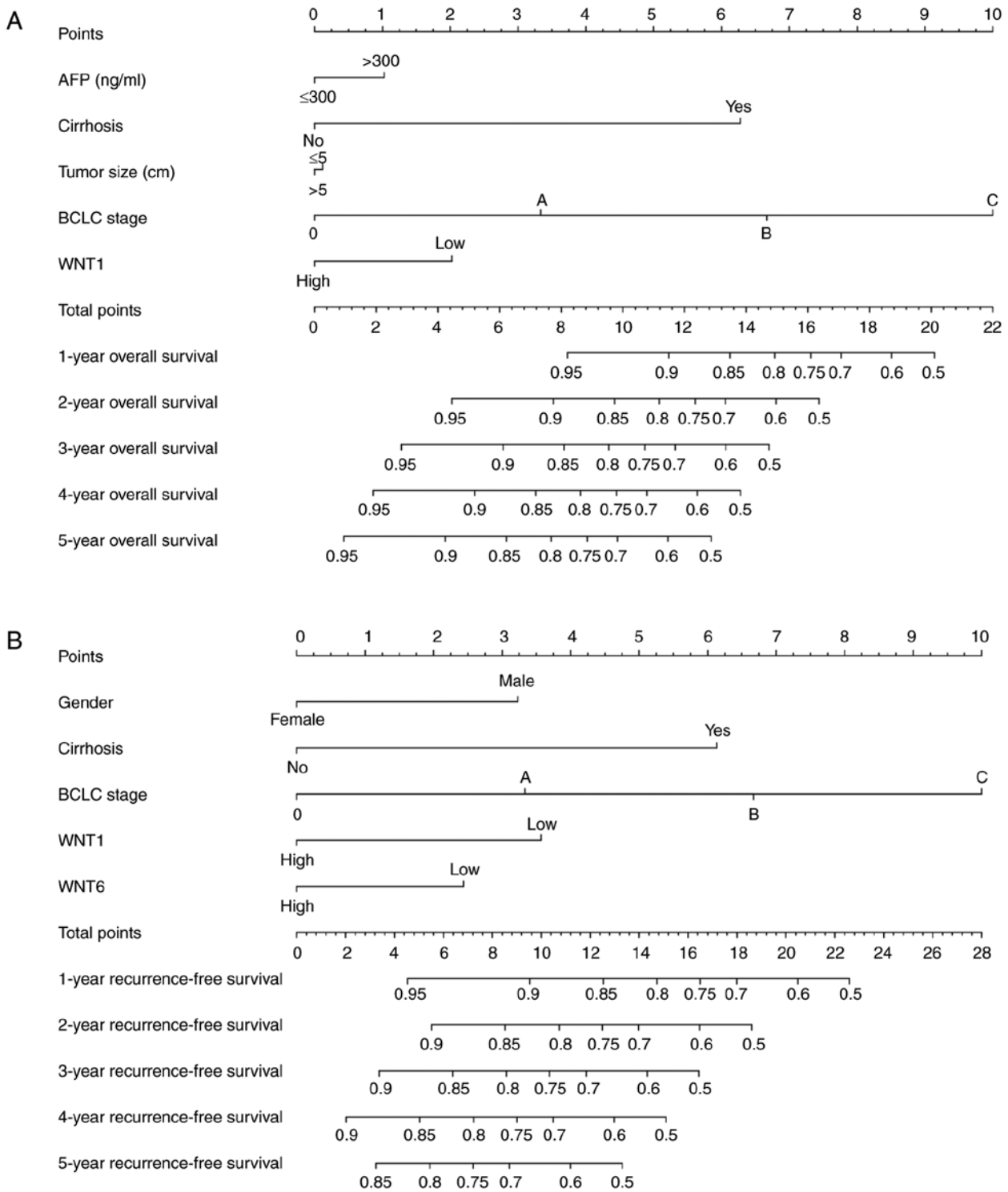

Figure 10. Prognostic nomogram for survival prediction. (A) Nomogram for overall survival; (B) nomogram for recurrence-free survival. AFP, α-fetoprotein; BCLC, Barcelona Clinic Liver Cancer.

transition (7,12) and WNT signaling (9,16). The conclusions of those studies were consistent with the results of gene function enrichment analysis in DAVID.

Early discoveries confirmed that *WNT* family genes may serve as potential diagnostic biomarkers for certain types of cancer. Sin *et al* selected RNA sequencing as a discovery method for specific RNA markers in bladder cancer, and found that *WNT5A* detection was a valuable complementary strategy in cystoscopy that may reduce unnecessary diagnostic procedures for bladder cancer (55). Jiang *et al* had reported that *WNT6* may serve as a diagnostic biomarker for osteosarcoma, with an AUC of 0.854, a specificity of 88.4% and a sensitivity of 77.8% (56). Based on these previous studies, it may be hypothesized that *WNTs* may also predict HCC. To test this hypothesis and evaluate the diagnostic value of *WNT* genes, an ROC curve was constructed, and the analysis suggested that *WNT2* may serve as potential diagnostic biomarker for patients with HBV-related HCC.

Table IV. Survival analysis results of the *WNT* family genes in the TCGA validation cohort.

	RFS				OS			
Gene	P-value	HR	Low 95% CI	High 95% CI	P-value	HR	Low 95% CI	High 95% CI
WNT1	0.004872	0.5585569	0.37237755	0.83782122	0.038128301	0.67054664	0.459578646	0.978358776
WNT2	0.165437	0.74242449	0.48738676	1.13091729	0.463552265	1.15543359	0.78518841	1.700262972
WNT2B	0.172666	0.75604408	0.5058112	1.13007116	0.949318195	0.9878679	0.678014074	1.439325557
WNT3	0.111201	1.39315603	0.92641981	2.09503695	0.23053025	0.7955497	0.547382364	1.156228935
WNT4	0.038296	0.65324005	0.43663377	0.97730085	0.308986119	0.82048053	0.560425379	1.201209516
WNT5A	0.25247	0.79158048	0.53045358	1.18125258	0.823749524	0.95871339	0.661532981	1.389396128
WNT5B	0.421213	0.84734859	0.56593122	1.26870474	0.701474468	0.92888443	0.637015977	1.354481386
WNT6	0.571806	0.88958796	0.5929931	1.33452943	0.640628505	1.09289911	0.752662101	1.586938493
WNT7A	0.000396	0.47050498	0.31004378	0.71401184	0.668569853	0.92232626	0.636972232	1.335514626
WNT7B	0.156162	0.73979971	0.4877709	1.12205056	0.874887781	1.0304464	0.709391742	1.496803142
WNT8B	0.36875	0.83403019	0.56144901	1.23894839	0.230696394	0.79551932	0.547254355	1.156411068
WNT10B	0.052198	0.6701836	0.44743955	1.00381395	0.23391077	0.79061532	0.536982043	1.16404746
WNT11	0.012024	0.59538341	0.397232	0.89237879	0.084433724	0.71556062	0.489264738	1.046523402
WNT16	0.039333	0.65216604	0.43430059	0.97932296	0.457153569	1.15299749	0.792227421	1.678057559

TCGA, The Cancer Genome Atlas; RFS, recurrence-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval.

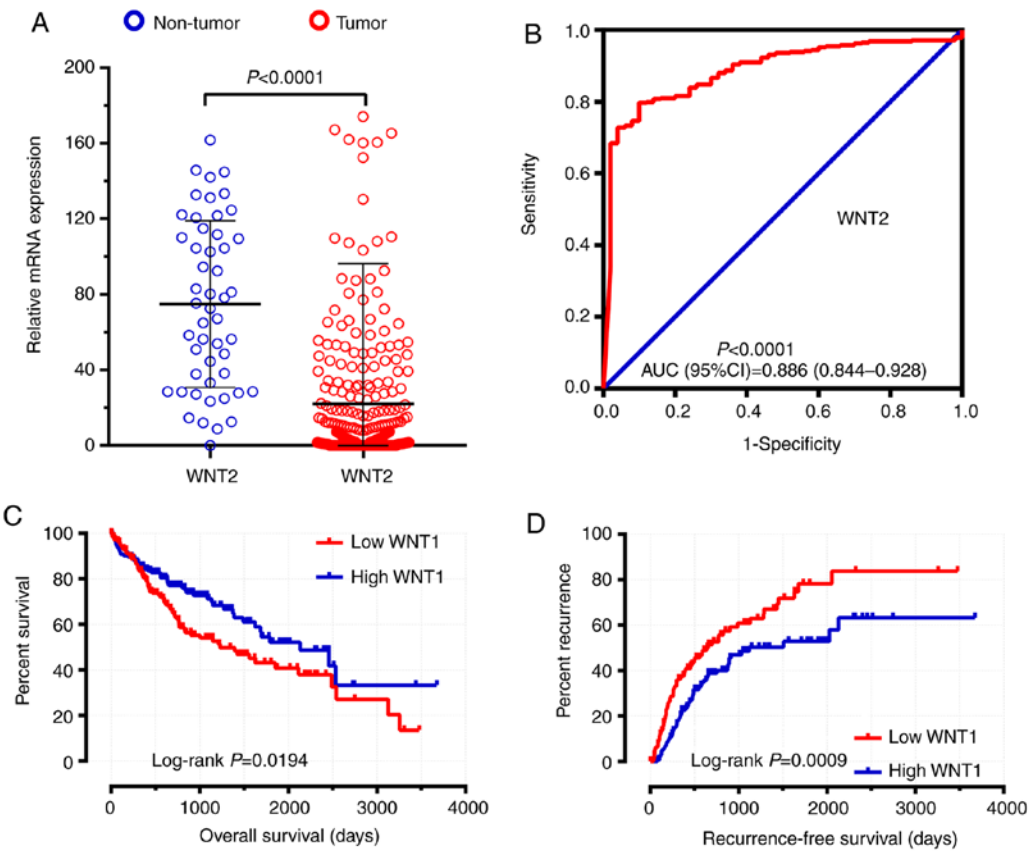

Figure 11. Diagnostic and prognostic values of *WNT* genes in TCGA validation cohort. (A) the relative expression of *WNT2* in HCC tumor tissues and non-tumor tissues; (B) ROC curves of *WNT2*; (C) Kaplan-Meier survival curves of *WNT1* for OS; (D) Kaplan-Meier survival curves of *WNT1* for RFS. TCGA, The Cancer Genome Atlas; HCC, hepatocellular carcinoma; ROC, receiver operating characteristics; AUC, area under the ROC curve; OS, overall survival; RFS, recurrence-free survival.

In addition, we also investigated the prognostic prediction ability of *WNT* family genes. The results demonstrated that the expression level of *WNT1* was associated with OS and RFS, with patients exhibiting a higher expression level of *WNT1* having a better prognostic outcome. Therefore, *WNT1* may serve as potential prognostic biomarker for HBV-related HCC. It has been reported that *WNT* family genes may predict the prognostic outcome in several types of cancer. As previously reported, *WNT1* expression may be one of the mechanisms underlying WNT/β-catenin signaling pathway activation in non-small cell lung cancer, and aberrant *WNT1* expression level was found to be a predictor of adverse prognosis (57). Shi *et al* reported that the *WNT2B* genetic variant may be a biomarker for the outcome of patients with cutaneous melanoma (58). Jiang *et al* observed that high expression of *WNT6* was a predictor of poor survival of osteosarcoma (56). Numerous studies have demonstrated that the expression level of *WNT5A* is associated with prognostic outcome and may serve as a prognostic biomarker in hepatocellular carcinoma (59), gallbladder carcinoma (60) and medulloblastoma (61). Based on these early discoveries, a prognostic predictive function for *WNT* family genes in HBV-related HCC has been confirmed in the present study.

We herein explored the diagnostic and prognostic value of *WNT* family gene expression in HBV-related HCC by collecting data from public databases and performing a series of bioinformatics analyses, with the aim of identifying more sensitive biomarkers and design a novel strategy for HCC diagnosis and treatment. There were certain limitations to the present study that must be addressed. First, the data were obtained from a public database and the sample size was limited; therefore, a larger population and multi-centered clinical studies are required to increase the credibility of our conclusions. Second, complete clinical parameters must be included to better evaluate the association between *WNT* family genes and HCC prognosis. Third, further functional validation and clinical trials are required to reveal the underlying molecular mechanism. Finally, although we explored the diagnostic and prognostic value at the mRNA level, the protein level was not investigated in the present study. Therefore, a comprehensive research design is required to check the consistency between mRNA and protein expression.

In conclusion, the findings of the present study demonstrated that *WNT2* may serve as diagnostic biomarker and *WNT1* may serve as prognostic biomarker for patients with HBV-related HCC in the GSE14520 cohort. Furthermore, through verification of the TCGA cohort, the diagnostic value of *WNT2* and the prognostic value of *WNT1* may be further validated and generalized to HCC patients. Therefore, our results require further confirmation.

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Availability of data and materials

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

Authors' contributions

QH and XY designed this study. XW, XL, CH, TY, CY, GL, BH, KH, GZ, ZL, XZ, HS, LS, YG, XS, TP and XY conducted this study and analyzed the data. QH wrote the manuscript and XY revised the manuscript. All the authors have read and approved the final version of the manuscript for publication and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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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