Journal of Hepatocellular Carcinoma

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

GALNT14 Genetic Variants Harbor Differential Prognostic Values Linking to Distinct Macrophage Cell Types in Hepatocellular Carcinoma

Yu-De Chu[1,*], Pei-Huan Ho[1,2,*], Wei-Ting Chen[1,2,*], Yu-Lin Shih[1], Ming-Wei Lai[2,3], Chao-Wei Hsu[1,2], Chau-Ting Yeh[1,2,4]

[1]Liver Research Center, Linkou Chang Gung Memorial Hospital, Taoyuan, 333, Taiwan; [2]Department of Hepatology and Gastroenterology, Linkou Chang Gung Memorial Hospital, Taoyuan, 333, Taiwan; [3]Division of Pediatric Gastroenterology, Department of Pediatrics, Linkou Chang Gung Memorial Hospital, Taoyuan, 333, Taiwan; [4]Institute of Stem Cell and Translational Cancer Research, Linkou Chang Gung Memorial Hospital, Taoyuan, 333, Taiwan

*These authors contributed equally to this work

Correspondence: Chau-Ting Yeh; Chao-Wei Hsu, Liver Research Center, Linkou Chang Gung Memorial Hospital, No. 5, Fu-Shin Street, Guei-Shan District, Taoyuan, 333, Taiwan, Tel +886-3-3281200 ext.8102, Fax +886-3-3282824, Email chautingy@gmail.com; hsu500331@gmail.com

Purpose: Surgical resection is the primary curative treatment for hepatocellular carcinoma (HCC), while high recurrence rates can limit the prognosis, emphasizing the need for reliable biomarkers. *GALNT14*-rs9679162 is associated with postoperative prognosis and therapeutic responses. However, relying on one single nucleotide polymorphism (SNP) greatly limits its predictive power. This study aims to identify an SNP panel to improve prognosis prediction and explore its role in modulating tumor-infiltrating immune cells (TIICs).

Patients and Methods: We included 345 HCC patients underwent surgical resection: 15 in the exploration cohort and 330 in the validation cohort. Genome-wide association study (GWAS) and PCR-based genotyping identified SNPs in linkage disequilibrium (LD) with rs9679162. The link between *GALNT14* expression and TIICs was analyzed. Prognostic evaluation was performed using Kaplan-Meier survival analysis and Cox proportional hazards models, with statistical significance set at $P < 0.05$.

Results: GWAS identified 39 SNP loci linked to rs9679162 and associated with postoperative prognosis. In the validation cohort, 10 SNPs were selected and categorized into four groups. Eight SNPs showed strong LD with rs9679162 and were significantly associated with recurrence-free survival and metastasis-free survival. The predictive performance of the combined SNP groups surpassed that of rs9679162 alone, with the most effective stratification achieved by combining groups-2+3. Additionally, *GALNT14* expression, linked to the identified genotypes, correlated with M2-macrophage abundance within TIICs.

Conclusion: An SNP panel in LD with rs9679162, particularly from group-2 (rs62140629, rs4952033, rs56284247) and group-3 (rs9679162, rs6752303), serves as a prognostic marker for HCC. GALNT14 expression was associated with M2-macrophages, suggesting an immune-regulatory mechanism.

Keywords: hepatocellular carcinoma, genetic biomarker, SNP panel, personalized medicine, tumor-infiltrating macrophage

Introduction

Hepatocellular carcinoma (HCC) is a leading cause of cancer-related mortality in Southeast Asia.[1,2] Major risk factors include chronic hepatitis B or C virus (HBV and HCV, respectively) infection and alcoholic liver disease.[3] Surgical resection and ablation offer curative potential but are limited to patients with early-stage disease and sufficient liver function.[4] Most HCC cases are diagnosed at advanced stages, necessitating alternative treatments such as transarterial chemoembolization (TACE) for intermediate-stage disease and systemic therapies, including immunotherapy and targeted agents, for advanced disease.[5] Despite these interventions, HCC remains highly recurrent and progressive, underscoring the need for predictive biomarkers.[6–8]

Received: 9 April 2025
Accepted: 26 July 2025
Published: 4 August 2025

Genome-wide association studies (GWAS) have identified germline single nucleotide polymorphisms (SNPs) in the GALNT14 gene as prognostic markers in HCC.[9] Notably, rs9679162, located in intron 5, has been shown to influence treatment outcomes in patients with BCLC stage C HCC.[10,11] The "TT-genotype" correlates with improved responses to chemotherapy and sorafenib, whereas the "GG-genotype" predicts poorer prognosis. Interestingly, in patients treated with Lenvatinib or hepatic arterial infusion chemotherapy, the "GG-genotype" is linked to better therapeutic responses, suggesting a potential role for this SNP in guiding personalized treatment strategies.[12] Moreover, the "TT-genotype" is associated with favorable outcomes in BCLC stage B patients treated with TACE[13] and in BCLC stage A patients undergoing surgical resection.[14] In resected HCC tissues, the "TT-genotype" is linked to lower *GALNT14* expression, while the "GG-genotype" corresponds to higher expression levels. This genotype-dependent expression difference may be mechanistically explained by the impact of rs9679162 on RNA splicing, particularly through its potential role in modulating the production of an exon-6-skipping *GALNT14* mRNA isoform,[14] which may alter the stability or translational efficiency of the transcript. These findings suggest that rs9679162 and related SNPs may regulate GALNT14 expression post-transcriptionally, contributing to interindividual variability in HCC prognosis and treatment response.

These genotype-dependent differences in expression are functionally relevant. GALNT14, an *O*-glycosyltransferase, modifies the *O*-glycosylation of mucin-type proteins, thereby influencing protein function, stability, and cellular localization. Altered GALNT14 activity contributes to tumorigenesis, cancer progression, and drug susceptibility across various malignancies, including breast, ovarian, and HCC, likely through modulation of its transferase activity,[14–17] and downstream signaling pathways.[14,18–20]

Despite these findings, the role of GALNT14 in regulating the tumor immune microenvironment remains largely unexplored, especially in the context of immunotherapy, which has emerged as a first-line treatment for advanced HCC.[21,22] A previous study identified GALNT14 as a hub gene involved in immune cell infiltration, particularly affecting activated dendritic cells and effector memory $CD8^+$ T cells in sepsis patients.[23] In cancer, elevated GALNT14 expression has been associated with poor prognosis and disease progression in osteosarcoma, where it influences immunogenic cell death and modulates responses to chemotherapy and immunotherapy.[24] These findings suggest that GALNT14 may function as an immune-related glycosylation gene.[25] However, its specific contribution to immune cell modulation in HCC remains poorly understood and warrants further investigation.

Since rs9679162 was identified through SNP microarrays, additional prognostic SNPs may remain undiscovered. Moreover, the association between *GALNT14* expression and immune infiltration in HCC is unclear. This study aims to identify SNPs in linkage disequilibrium (LD) with rs9679162, evaluate their prognostic significance in BCLC stage A HCC, and examine *GALNT14* expression and immune infiltration to elucidate its role in HCC immunoregulation.

Materials and Methods

Patient Samples

This study was approved by the Institutional Review Board of Chang Gung Memorial Hospital (approval numbers: 202200367B0, 202200645B0, and 202400881B0), and all experiments were conducted in accordance with relevant guidelines and regulations. A total of 345 HCC patients who underwent surgical resection were retrospectively enrolled. Given the retrospective nature of the study, the requirement for informed consent was waived by the Institutional Review Board of Chang Gung Memorial Hospital. Surgically resected tissue samples were obtained from the Biobank at Chang Gung Memorial Hospital. Among these patients, 15 were included in the exploration cohort, while the remaining 330 constituted the validation cohort. Patient data confidentiality was strictly maintained in accordance with institutional policies, and the study was conducted in full compliance with the principles of the Declaration of Helsinki.

Clinical parameters were retrospectively analyzed, including gender, age, hepatitis B surface antigen (HBsAg) status, anti-HCV antibody status, history of alcoholism, liver cirrhosis status, presence of ascites, histological grade, vascular invasion status, tumor number, largest tumor size (diameter), alpha-fetoprotein levels, albumin levels, bilirubin levels, aspartate transaminase (AST), alanine transaminase (ALT), creatinine levels, prothrombin time, date of hepatic recurrence, date of extrahepatic metastasis, and date of last follow-up or HCC-related death.

DNA Extraction, Whole Genome Sequencing (WGS), and Genotyping

Total DNA was extracted from the retrieved frozen tissues using the DNeasy Blood & Tissue Kit (QIAGEN, Venlo, Netherlands, Cat. No: 69504) following previously described protocols.[26] A total of 1.0 μg of DNA per sample was used for library preparation. Sequencing libraries were generated using the NEBNext® DNA Library Prep Kit (New England Biolabs, Ipswich, MA, USA, Cat. No: E7654) according to the manufacturer's instructions, with indices added to each sample. Genomic DNA was randomly sheared to an average fragment size of 350 bp, followed by end polishing, A-tailing, and ligation with NEBNext adapters for Illumina sequencing (New England Biolabs, Cat. No: E7874). The DNA fragments were then enriched through polymerase chain reaction (PCR) using the probe-specific oligonucleotides. The resulting PCR products were purified using the AMPure XP system, and their size distribution was assessed with an Agilent 2100 Bioanalyzer. Library quantification was performed using real-time PCR. Sequencing was conducted on the NovaSeq 6000 platform (Illumina, San Diego, CA, USA) with paired-end 150 bp reads.

Following sequencing, raw reads underwent quality control filtering to ensure high-quality data for analysis. Reads containing adapter sequences, those with more than 10% undetermined bases, and those in which low-quality bases accounted for more than 50% of the total sequence were removed. The resulting clean reads were then aligned to the human reference genome (GRCh38) and annotated using RefSeq.[27]

Candidate SNPs of interest were genotyped through PCR amplification followed by direct sequencing using primers listed in the Supplementary Material 1.

Database Mining

To explore the correlation between *GALNT14* expression and tumor-infiltrating immune cells, the publicly accessible web portal TISIDB (http://cis.hku.hk/TISIDB/index.php) was utilized.[28] Additionally, to examine the relationship between *GALNT14* expression, candidate immune cell infiltration, and patient prognosis, analyses were conducted using the TIMER2.0 web portal (http://timer.comp-genomics.org) was used.[29,30]

Statistical Analysis

Parametric data were presented as mean ± standard deviation and compared by using a two-sample *t*-test. Dichotomized data were presented as numbers and percentages (%) and compared utilizing Chi-square test. Univariate and following multivariate Cox proportional hazard models were performed to estimate prognosis for clinical factors, and candidate genotypes. In this study, significant factors determined from the univariate analysis could be included for multivariate Cox proportional hazards. The Kaplan–Meier method was performed to estimate the survival probability between the different groups, and the Log rank test was employed. All tests were two-tailed, and the $P < 0.05$ was considered statistically significant. All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) statistics Version 20.

Results

Identifying Postoperative Prognosis-Associated SNP Candidates in LD with rs9679162 in HCC Patients

To determine whether additional SNPs exhibit a stronger correlation with postoperative prognosis in HCC patients, GWAS were conducted using WGS on DNA samples from 15 patients in the exploration cohort. This cohort included eight patients with the "TT-genotype" and seven with the "GG-genotype" at rs9679162. Baseline characteristics did not significantly differ between the two genotype groups, except for postoperative prognosis (Table S1). Notably, significant differences in survival outcomes were observed, with the "TT-genotype" being associated with longer overall survival (OS), recurrence-free survival (RFS), and metastasis-free survival (MFS) compared to the "GG-genotype", with all three parameters showing statistically significant differences ($P < 0.001$).

WGS analysis identified 39 primary SNP loci and 26 insertion-deletion (INDEL) loci that were potentially in LD with rs9679162 and associated with postoperative prognosis in HCC patients (Figure S1). Further examination revealed that, despite the identification of only 39 SNP loci, several of these loci contained overlapping SNPs, resulting in a more

extensive set of candidate SNPs. In particular, locus 3 was found to contain 53 SNPs within the same intron of the *GALNT14* gene (Tables 1 and S2).

Similarly, for the identified INDEL loci, multiple independent variants were detected at certain positions, with locus 1 containing five distinct INDELs within the same intron as rs9679162 in *GALNT14* (Table S3). These findings suggest that genetic variants within *GALNT14* near rs9679162 may similarly influence postoperative prognosis in HCC patients and warrant further investigation.

Validation of SNP Candidates in LD with rs9679162 in HCC Patients

To validate the SNP candidates potentially in LD with rs9679162, a total of 53 SNPs spanning 35.8 kb (from rs57187063 to rs6543594) were analyzed. These SNPs were distributed across several distinct regions (Figure 1A). To assess their LD with rs9679162, a subset of SNPs positioned at the margins or central regions of these blocks was selected for further validation.

Genotyping was conducted for 10 selected candidate SNPs, including rs57187063, rs59315709, rs62140629, rs4952033, rs56284247, rs9679162, rs6752303, rs4952027, rs7601904, and rs6543594, in 330 HCC patients from the validation cohort. LD analysis identified two SNPs, rs57187063 and rs59315709, as having weaker correlations with the remaining SNPs (Figure 1B, group-1). The remaining eight SNPs exhibited stronger LD with rs9679162 and were further categorized into three additional distinct groups based on genotypic correlations. Group-2 included rs62140629, rs4952033, and rs56284247, while group-3 comprised rs9679162 and rs6752303. Group-4 consisted of rs4952027, rs7601904, and rs6543594 (Figure 1A and B).

Further analysis of genotype frequencies at these loci supported the classification of the SNPs into these four distinct groups. Group-1 consisted of rs57187063 and rs59315709, group-2 included rs62140629, rs4952033, and rs56284247, group-3 contained rs9679162 and rs6752303, and group-4 comprised rs4952027, rs7601904, and rs6543594 (Figure 1C and D). These findings suggest distinct patterns of LD among these SNPs, providing further insight into their potential role in postoperative prognosis in HCC patients.

Lacking Association Between SNP Candidates and Postoperative OS in HCC Patients

To determine whether the identified SNP candidates were associated with postoperative prognosis, their correlation with OS, RFS, and MFS was evaluated. Cox proportional hazard analysis for OS revealed that several clinical factors, including the presence of ascites, microvascular invasion, macrovascular invasion, tumor number, tumor size, and bilirubin levels, were significantly associated with survival outcomes (Table S4). Among these, microvascular invasion (hazard ratio [HR]: 2.733, $P < 0.001$), macrovascular invasion (HR: 4.285, $P < 0.001$), and increased tumor number (HR: 1.467, $P < 0.001$) were strongly correlated with poorer OS. Multivariate analysis further confirmed that microvascular invasion (HR: 2.372, $P = 0.003$), tumor number (HR: 1.412, $P = 0.001$), and elevated bilirubin levels (HR: 1.638, $P = 0.006$) remained independent prognostic factors for OS.

However, none of the SNPs, including rs9679162 and its linked variants, demonstrated statistically significant associations with OS in either univariate or multivariate models. Although rs62140629, rs4952027, and rs7601904 exhibited a trend toward significance, they did not reach statistical significance. These findings suggest that clinical factors, particularly vascular invasion and tumor burden, have a greater influence on OS than the examined SNPs.

Association Between SNP Candidates and Postoperative RFS in HCC Patients

Cox proportional hazard analysis identified anti-HCV positivity, microvascular invasion, macrovascular invasion, tumor number, tumor size, and AFP levels as significant prognostic factors for RFS in HCC patients (Table 2). Among these, microvascular invasion (HR: 2.669, $P < 0.001$) and macrovascular invasion (HR: 2.724, $P < 0.001$) were the strongest predictors of recurrence, while elevated AFP levels (HR: 1.003, $P = 0.005$) and higher histological grade (HR: 1.411, $P = 0.001$) were also associated with an increased risk of recurrence. Multivariate analysis further confirmed that anti-HCV positivity (HR: 1.504, $P = 0.011$), microvascular invasion (HR: 2.473, $P < 0.001$), tumor number (HR: 1.231, $P = 0.004$), and AFP levels (HR: 1.003, $P = 0.024$) remained independent predictors of RFS.

Notably, rs4952033 (HR: 1.561, $P = 0.009$) emerged as a significant predictor in multivariate analysis, suggesting its potential role in HCC recurrence. In univariate analysis, several additional SNPs, including rs62140629, rs56284247,

Table 1 Candidate SNPs Associated with Postoperative Prognosis and Potentially in Linkage Disequilibrium with rs9679162 Within the Intron of GALNT14 Gene on Chromosome 2 in Patients with HCC

POS	ID	REF	ALT	"TT" Type			"GG" Type			"TT" Type		"GG" Type		Fisher's exact test P	Chi square P	"TT" Type		"GG" Type		Fisher's exact test P	Chi square P
				REF homo	Hetero	ALT homo	REF homo	Hetero	ALT homo	REF homo	Non-REF homo	REF homo	Non-REF homo			ALT homo	Non-ALT homo	ALT homo	Non-ALT homo		
31244297	rs2216827	A	G	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31244635	rs6543594	A	C	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31244684	rs7601904	A	G	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31244751	rs4952027	T	C	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31245172	rs7579017	G	A	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31245290	rs10206704	T	C	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31245528	rs2194457	A	G	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31245804	rs12473730	G	A	0	1	7	7	0	0	0	8	7	0	<0.001	<0.001	7	1	0	7	0.001	<0.001
31246027	rs56189378	A	C	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31246249	rs10209881	T	C	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31246423	rs10210092	T	C	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31246546	rs10197865	C	T	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31246601	rs10210300	T	C	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31246784	rs10174368	A	G	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31247332	rs6752071	T	C	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31247485	rs6752303	T	C	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31247514	rs9679162	G	T	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31248062	rs6708989	A	G	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31248423	rs2194456	C	T	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31248597	rs2194454	G	C	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31248842	rs4952028	C	T	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31249014	rs5009910	T	C	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31249794	rs58958623	C	T	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31249974	rs7608731	T	C	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31251034	rs10164479	G	C	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31251147	rs10166921	G	A	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31251399	rs4951959	A	G	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31251417	rs4952030	T	C	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31252378	rs2113488	C	T	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31252552	rs2113487	A	G	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31252661	rs7605643	G	A	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31253187	rs10865086	G	A	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31253404	rs7582401	A	C	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31254348	rs1862968	C	T	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31254374	rs1862967	T	C	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31254752	rs1862966	T	C	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001

(*Continued*)

Table 1 (Continued).

POS	ID	REF	ALT	"TT" Type			"GG" Type			"TT" Type		"GG" Type		Fisher's exact test P	Chi square P	"TT" Type		"GG" Type		Fisher's exact test P	Chi square P
				REF homo	Hetero	ALT homo	REF homo	Hetero	ALT homo	REF homo	Non-REF homo	REF homo	Non-REF homo			ALT homo	Non-ALT homo	ALT homo	Non-ALT homo		
31255144	rs12712300	G	A	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31256520	rs1862965	T	G	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31256579	rs1862964	A	C	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31256937	rs6752662	C	T	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31258266	rs2365191	T	C	8	0	0	0	0	7	8	0	0	7	<0.001	<0.001	0	8	7	0	<0.001	<0.001
31259918	rs4952031	C	A	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31260242	rs4952032	C	G	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31260660	rs2161835	G	A	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31260833	rs10192472	T	A	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31261249	rs7565923	C	T	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31262903	rs10210124	A	G	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31263433	rs9308914	C	A	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31265588	rs56284247	T	C	8	0	0	0	0	7	8	0	0	7	<0.001	<0.001	0	8	7	0	<0.001	<0.001
31265650	rs4952033	C	T	0	0	8	7	0	0	0	8	7	0	<0.001	<0.001	8	0	0	7	<0.001	<0.001
31265665	rs62140629	C	A	8	0	0	0	0	7	8	0	0	7	<0.001	<0.001	0	8	7	0	<0.001	<0.001
31280259	rs59315709	T	C	8	0	0	0	1	6	8	0	0	7	<0.001	<0.001	0	8	6	1	0.001	<0.001
31280386	rs57187063	C	A	8	0	0	0	2	5	8	0	0	7	<0.001	<0.001	0	8	5	2	0.007	0.003

Abbreviations: "TT" type, rs9679162 "TT-genotype"; "GG" type, rs9679162 "GG-genotype"; Chrm, chromosome; POS, position; ID, Reference SNP (rs) ID; REF, reference sequence; ALT, alteration sequence; Hetero, heterozygote; REF homo, reference homozygote; ALT homo, alteration homozygote; Non-REF homo, non-reference homozygote; Non-ALT homo, non-alteration homozygote.

https://doi.org/10.2147/JHC.S530180

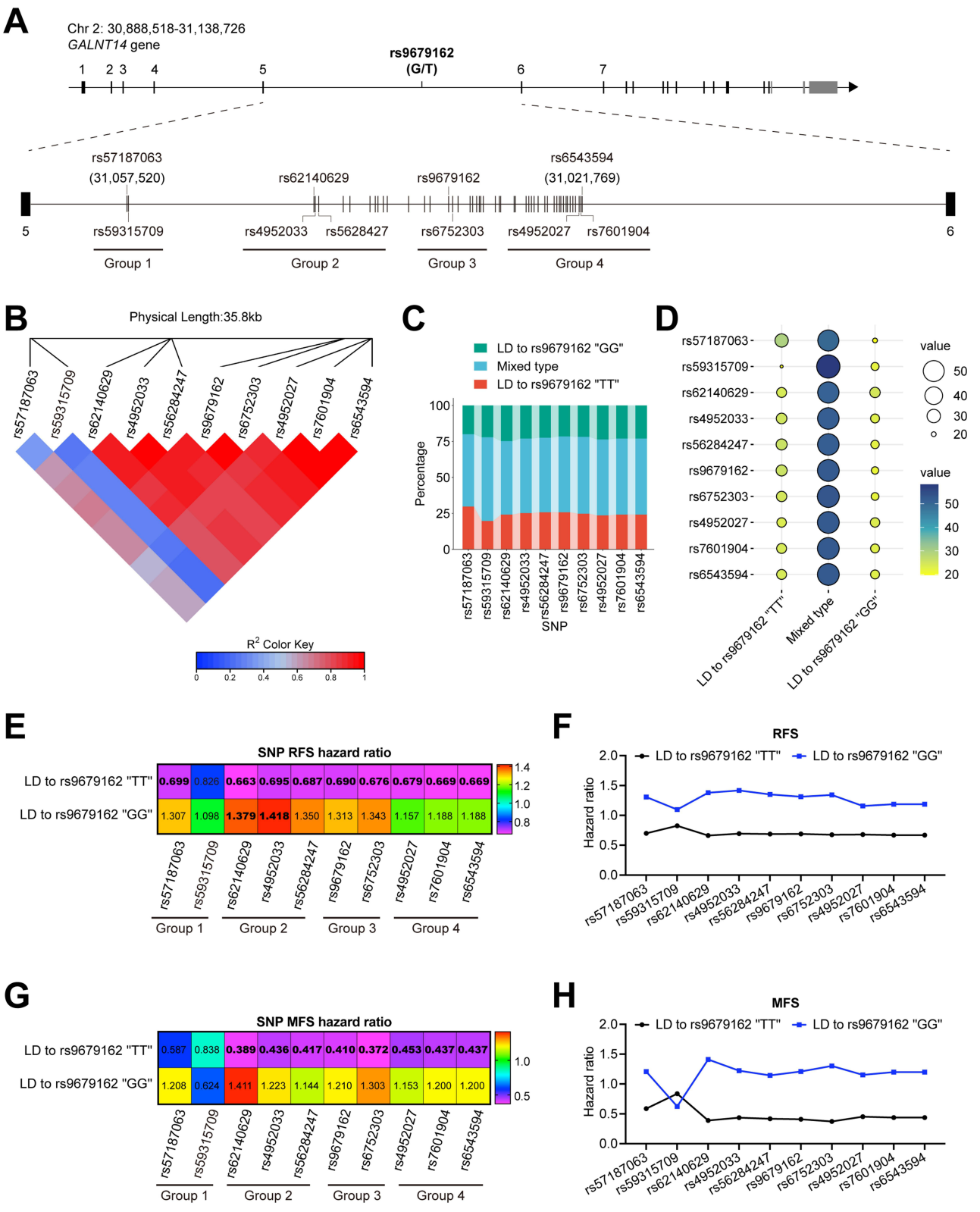

Figure 1 Identification of candidate SNPs in linkage disequilibrium with rs9679162 within *GALNT14* and their association with postoperative prognosis in HCC patients. (**A**) Genomic structure of *GALNT14* and the locations of the identified SNP candidates. (**B**) Heatmap representation of SNP candidates in linkage disequilibrium with rs9679162 in patients from the validation cohort. (**C** and **D**) Genotypic distribution of individual SNP candidates within the validation cohort. Hazard ratios of individual SNPs for recurrence-free survival (RFS), represented as a heatmap (**E**) and peak chart (**F**). Hazard ratios of individual SNPs for metastasis-free survival (MFS), represented as a heatmap (**G**) and peak chart (**H**). Bolded values in (**E**) and (**G**) represent those with significant P values (<0.05) in the Cox proportional hazards analysis.

https://doi.org/10.2147/JHC.S530180

Table 2 Cox Proportional Hazard Analysis for Association Between Clinical Factors or Genotypes and Recurrence-Free Survival in 330 HCC Patients

Clinical Parameters	Univariate Analysis		Multivariate Analysis	
	Hazard Ratio (95% CI)	P	Hazard Ratio (95% CI)	P
Gender, male =1	1.228 (0.862–1.749)	0.256		
Age, per year increase	1.001 (0.990–1.012)	0.891		
Anti-HCV, positive = 1	1.423 (1.049–1.930)	**0.024**	1.504 (1.098–2.059)	**0.011**
HBsAg, positive = 1	0.876 (0.652–1.177)	0.380		
Alcoholism, yes = 1	1.322 (0.928–1.884)	0.122		
Cirrhosis, yes = 1	1.115 (0.830–1.498)	0.469		
Ascites, yes = 1	1.469 (0.851–2.536)	0.167		
Microvascular invasion, yes = 1	2.669 (1.971–3.613)	**<0.001**	2.473 (1.810–3.380)	**<0.001**
Macrovascular invasion, yes = 1	2.724 (1.576–4.710)	**<0.001**		
Capsule, yes = 1	1.409 (1.005–1.976)	**0.046**	1.500 (1.063–2.115)	**0.021**
Grade, per grade increase	1.411 (1.159–1.717)	**0.001**		
Tumor number, per number increase	1.246 (1.088–1.426)	**0.001**	1.231 (1.067–1.419)	**0.004**
Largest tumor size, per cm increase	1.061 (1.022–1.102)	**0.002**		
AFP, per 1000 ng/mL increase	1.003 (1.001–1.006)	**0.005**	1.003 (1.000–1.005)	**0.024**
Albumin, per g/dL increase	0.756 (0.586–0.976)	**0.032**		
Bilirubin, per mg/dL increase	0.954 (0.722–1.259)	0.737		
Prothrombin time, per sec increase	0.980 (0.893–1.076)	0.672		
Creatinine, per mg/dL increase	1.066 (0.958–1.187)	0.240		
AST, per U/L increase	1.002 (1.001–1.003)	**0.001**	1.002 (1.001–1.003)	**0.002**
ALT, per U/L increase	1.002 (1.001–1.003)	**0.002**		
Rs57187063, CC = 1	0.699 (0.502–0.974)	**0.035**		
Rs57187063, AA = 1	1.307 (0.928–1.840)	0.125		
Rs59315709, TT = 1	0.826 (0.566–1.206)	0.322		
Rs59315709, CC = 1	1.098 (0.782–1.541)	0.588		
Rs62140629, CC = 1	0.663 (0.462–0.952)	**0.026**		
Rs62140629, AA = 1	1.379 (1.004–1.896)	**0.047**		
Rs4952033, TT = 1	0.695 (0.488–0.991)	**0.044**		
Rs4952033, CC = 1	1.418 (1.027–1.960)	**0.034**	1.561 (1.119–2.176)	**0.009**
Rs56284247, TT = 1	0.687 (0.484–0.977)	**0.036**		
Rs56284247, CC = 1	1.350 (0.973–1.874)	0.072		
Rs9679162, TT = 1	0.690 (0.487–0.977)	**0.037**		
Rs9679162, GG = 1	1.313 (0.940–1.834)	0.110		
Rs6752303, CC = 1	0.676 (0.474–0.963)	**0.030**		
Rs6752303, TT = 1	1.343 (0.964–1.871)	0.082		
Rs4952027, CC = 1	0.679 (0.473–0.975)	**0.036**		
Rs4952027, TT = 1	1.157 (0.832–1.608)	0.386		
Rs7601904, GG = 1	0.669 (0.468–0.957)	**0.028**		
Rs7601904, AA = 1	1.188 (0.852–1.655)	0.310		
Rs6543594, CC = 1	0.669 (0.468–0.957)	**0.028**		
Rs6543594, AA = 1	1.188 (0.852–1.655)	0.310		

Note: Bold values indicate statistical significance P < 0.05.
Abbreviations: AFP, alpha-fetoprotein; AST, aspartate transaminase; ALT, alanine transaminase; CI, confidence interval.

rs9679162, rs6752303, rs4952027, rs7601904, and rs6543594, were significantly associated with RFS, with carriers of the alternative alleles exhibiting a lower risk of recurrence. These findings suggest that genetic variants in LD with rs9679162 may contribute to recurrence risk in HCC patients.

A summary of HR values from univariate analysis further demonstrated that SNP candidates in LD with the rs9679162 "TT-genotype", including rs62140629, rs4952033, rs56284247, rs9679162, rs6752303, rs4952027,

rs7601904, and rs6543594, consistently exhibited low and statistically significant HRs. In contrast, when analyzing the reverse genotype linked to the rs9679162 "GG-genotype", only rs62140629 and rs4952033 showed significant associations, with rs4952033 displaying the highest HR, highlighting its potential as a key prognostic marker (Figure 1E and F).

Further analysis of individual SNPs using Kaplan-Meier survival curves confirmed the association of these variants with RFS (Figure S2). Among SNPs in LD with the rs9679162 "TT-genotype", rs62140629 exhibited the strongest association with RFS, while rs9679162 and rs6752303 also maintained statistical significance. Conversely, when considering SNPs linked to the rs9679162 "GG-genotype", only rs62140629 and rs4952033 showed significant associations, with rs4952033 demonstrating the strongest correlation with RFS.

Association Between SNP Candidates and Postoperative MFS in HCC Patients

For MFS, Cox analysis identified microvascular invasion (HR: 3.673, P < 0.001) and macrovascular invasion (HR: 5.508, P < 0.001) as the strongest predictors of metastasis (Table 3). Additionally, tumor size (HR: 1.189, P < 0.001) and AFP levels (HR: 1.006, P < 0.001) were associated with a higher risk of metastasis. Multivariate analysis further confirmed that microvascular

Table 3 Cox Proportional Hazard Analysis for Association Between Clinical Factors or Genotypes and Metastasis-Free Survival in 330 HCC Patients

Clinical Parameters	Univariate Analysis		Multivariate Analysis	
	Hazard Ratio (95% CI)	P	Hazard Ratio (95% CI)	P
Gender, male =1	1.342 (0.717–2.513)	0.358		
Age, per year increase	0.988 (0.970–1.006)	0.177		
Anti-HCV, positive = 1	0.492 (0.257–0.941)	**0.032**		
HBsAg, positive = 1	1.132 (0.676–1.895)	0.636		
Alcoholism, yes = 1	1.351 (0.747–2.446)	0.320		
Cirrhosis, yes = 1	0.725 (0.446–1.179)	0.195		
Ascites, yes = 1	1.110 (0.403–3.054)	0.840		
Microvascular invasion, yes = 1	3.673 (2.251–5.994)	**<0.001**	1.881 (1.081–3.273)	**0.025**
Macrovascular invasion, yes = 1	5.508 (2.869–10.575)	**<0.001**	2.470 (1.197–5.098)	**0.014**
Capsule, yes = 1	0.696 (0.420–1.155)	0.161		
Grade, per grade increase	1.402 (1.000–1.966)	**0.050**		
Tumor number, per number increase	1.478 (1.237–1.766)	**<0.001**	1.289 (1.065–1.560)	**0.009**
Largest tumor size, per cm increase	1.189 (1.132–1.249)	**<0.001**	1.124 (1.062–1.189)	**<0.001**
AFP, per 1000 ng/mL increase	1.006 (1.004–1.008)	**<0.001**	1.004 (1.002–1.006)	**<0.001**
Albumin, per g/dL increase	0.870 (0.563–1.345)	0.532		
Bilirubin, per mg/dL increase	1.408 (0.990–2.002)	0.057		
Prothrombin time, per sec increase	0.955 (0.802–1.136)	0.600		
Creatinine, per mg/dL increase	0.744 (0.436–1.270)	0.278		
AST, per U/L increase	1.001 (0.999–1.004)	0.369		
ALT, per U/L increase	0.999 (0.995–1.003)	0.655		
Rs57187063, CC = 1	0.587 (0.324–1.061)	0.078		
Rs57187063, AA = 1	1.208 (0.678–2.154)	0.521		
Rs59315709, TT = 1	0.838 (0.446–1.576)	0.584		
Rs59315709, CC = 1	0.624 (0.318–1.225)	0.170		
Rs62140629, CC = 1	0.389 (0.186–0.817)	**0.013**		
Rs62140629, AA = 1	1.411 (0.833–2.391)	0.201		
Rs4952033, TT = 1	0.436 (0.216–0.883)	**0.021**		
Rs4952033, CC = 1	1.223 (0.703–2.128)	0.476		
Rs56284247, TT = 1	0.417 (0.206–0.844)	**0.015**		
Rs56284247, CC = 1	1.144 (0.650–2.014)	0.640		
Rs9679162, TT = 1	0.410 (0.203–0.829)	**0.013**		

(*Continued*)

Table 3 (Continued).

Clinical Parameters	Univariate Analysis		Multivariate Analysis	
	Hazard Ratio (95% CI)	P	Hazard Ratio (95% CI)	P
Rs9679162, GG = 1	1.210 (0.687–2.130)	0.509		
Rs6752303, CC = 1	0.372 (0.178–0.781)	**0.009**	0.403 (0.188–0.865)	**0.020**
Rs6752303, TT = 1	1.303 (0.749–2.268)	0.349		
Rs4952027, CC = 1	0.453 (0.224–0.918)	**0.028**		
Rs4952027, TT = 1	1.153 (0.663–2.006)	0.615		
Rs7601904, GG = 1	0.437 (0.216–0.885)	**0.021**		
Rs7601904, AA = 1	1.200 (0.690–2.088)	0.519		
Rs6543594, CC = 1	0.437 (0.216–0.885)	**0.021**		
Rs6543594, AA = 1	1.200 (0.690–2.088)	0.519		

Note: Bold values indicate statistical significance $P < 0.05$.
Abbreviations: AFP, alpha-fetoprotein; AST, aspartate transaminase, ALT, alanine transaminase, CI, confidence interval.

invasion (HR: 1.881, P = 0.025), macrovascular invasion (HR: 2.470, P = 0.014), tumor number (HR: 1.289, P = 0.009), tumor size (HR: 1.124, P < 0.001), and AFP levels (HR: 1.004, P < 0.001) remained independent predictors of MFS. Among the SNPs, rs6752303 (HR: 0.403, P = 0.020) was identified as a significant protective factor in multivariate analysis. In univariate analysis, several SNPs, including rs62140629, rs4952033, rs56284247, rs9679162, rs4952027, rs7601904, and rs6543594, were all associated with reduced metastasis risk, suggesting their potential role in predicting disease progression.

A summary of HR values from univariate analysis further demonstrated that SNP candidates in LD with the rs9679162 "TT-genotype", including rs62140629, rs4952033, rs56284247, rs9679162, rs6752303, rs4952027, rs7601904, and rs6543594, consistently exhibited low and statistically significant HRs for MFS. In contrast, when analyzing the reverse genotype linked to the rs9679162 "GG-genotype", none of these SNPs showed a significant association (Figure 1G and H).

Further analysis of individual SNPs using Kaplan-Meier survival curves confirmed their association with MFS (Figure S3). Among SNPs in LD with the rs9679162 "TT-genotype", rs62140629 (P = 0.001) exhibited the strongest association with MFS, while rs9679162 (P = 0.010) and rs6752303 (P = 0.007) also maintained statistical significance. Conversely, none of the SNPs linked to the rs9679162 "GG-genotype" demonstrated a significant association with MFS.

Improved Predictability of Postoperative Prognosis in HCC Patients Through SNP Combination

To determine whether specific SNP genotypes, particularly those in groups-2 to −4, could serve as more effective genetic biomarkers for postoperative prognosis in HCC, we assessed various combinations of SNPs within each group and across groups. As shown in Figure 2A, when SNPs within each group were analyzed individually, all groups demonstrated improved stratification for RFS and MFS when patients were categorized based on LD with the rs9679162 "TT-genotype" and "non-TT-genotypes" (P = 0.016 for group-2, P = 0.012 for group-3, and P = 0.014 for group-4 in RFS; P = 0.004 for group-2, P = 0.004 for group-3, and P = 0.009 for group-4 in MFS). These findings indicate that using a single SNP genotype alone may not be the most effective approach for predicting postoperative prognosis in HCC.

Subsequent analyses assessing combinations of SNPs from different groups revealed that the combination of SNPs from groups-2+3 provided the most effective patient stratification for RFS (P = 0.007) and MFS (P = 0.004), outperforming the combinations of groups-3+4 (P = 0.018 for RFS, P = 0.006 for MFS) and groups-2+4 (P = 0.021 for RFS, P = 0.010 for MFS) (Figure 2B). Interestingly, further combining SNPs from all three groups-2+3+4, while still significant, did not enhance patient stratification beyond the combination of groups-2+3 alone (P = 0.021 for RFS, P = 0.010 for MFS) (Figure 2C).

These findings suggest that while individual SNP genotypes within the *GALNT14* gene exhibit prognostic significance, they may not provide optimal patient stratification compared to SNP combinations. Notably, the combination of SNPs from groups-2+3, comprising a total of five SNPs, demonstrated the strongest predictive capacity for postoperative prognosis in HCC patients.

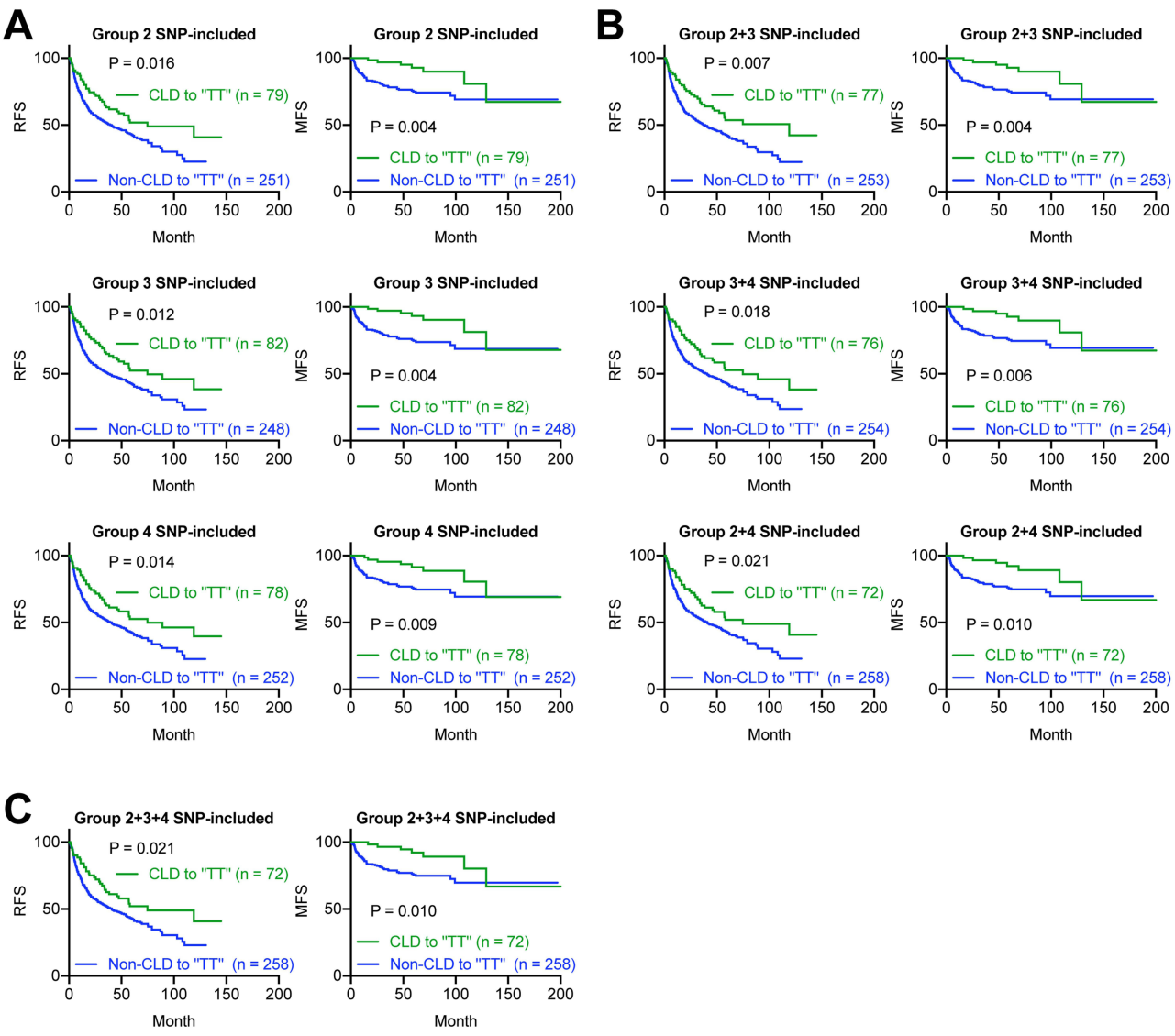

Figure 2 Improved stratification of postoperative prognosis in HCC patients through SNP group combinations. Kaplan-Meier analysis assessing the impact of SNP combinations on postoperative prognosis. (**A**) Prognostic stratification using SNPs within each individual group. (**B**) Prognostic stratification using combined SNPs from groups-2+3, groups-3+4, and groups-2+4. (**C**) Prognostic stratification using SNPs from all three groups (2+3+4). P values were determined using the Log rank test. CLD, complete linkage disequilibrium with rs9679162; non-CLD, non-complete linkage disequilibrium with rs9679162.

GALNT14 Expression Links to HCC-Infiltrating M2-Macrophages and Influences Prognosis

Previous studies have demonstrated that SNP genotypes, including rs9679162 and rs17010547, are significantly associated with *GALNT14* expression at both the mRNA and protein levels.[14,31] These findings suggest that the prognostic relevance of these SNPs within the *GALNT14* gene may be attributed to their regulatory effects on *GALNT14* expression in HCC. However, despite this evidence, the precise role of *GALNT14* in HCC development and progression remains largely unclear, particularly in the context of tumor-infiltrating immune cells.

To investigate the potential correlation between *GALNT14* expression and HCC-infiltrating immune cells, we analyzed various immune cell types and found that most exhibited little to no association with *GALNT14* expression (Figure S4). However, three immune cell populations, macrophages (rho = 0.280), follicular helper T cells (rho = 0.268), and eosinophils (rho = 0.267), showed a positive correlation with *GALNT14* expression (Figure 3A). In contrast, memory B cells were negatively associated with *GALNT14* expression (Figure 3B), though the correlation strength was moderate.

https://doi.org/10.2147/JHC.S530180

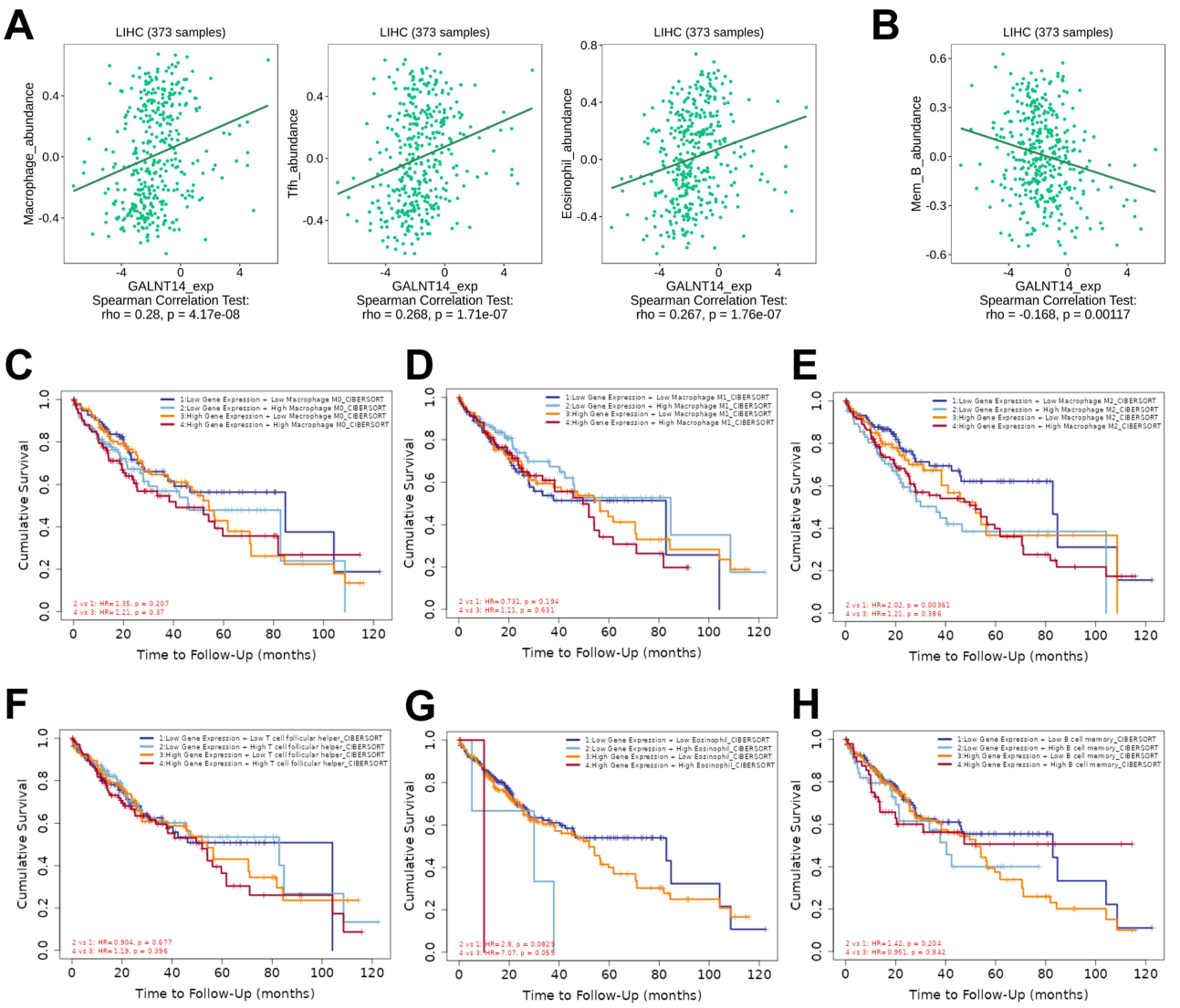

Figure 3 Association between *GALNT14* expression and HCC-infiltrating M2-macrophages in relation to prognosis. Correlation analysis between *GALNT14* mRNA expression and HCC-infiltrating immune cells in (**A**) positive correlations and (**B**) negative correlation. Results were obtained from the TISIDB database (http://cis.hku.hk/TISIDB/index.php). Prognostic impact of combined *GALNT14* expression and immune cell abundance, including (**C**) M0-macrophages, (**D**) M1-macrophages, (**E**) M2-macrophages, (**F**) follicular T helper cells, (**G**) eosinophils, and (**H**) memory B cells. Results were obtained from the TIMER2.0 database (http://timer.comp-genomics.org). P values were determined using the Log rank test.

To further assess the relationship between *GALNT14* expression and these tumor-infiltrating immune cells in HCC prognosis, we examined their combined impact on patient stratification. Given the distinct functional roles of macrophage subtypes, we separately analyzed M0-, M1-, and M2-macrophages in relation to *GALNT14* expression. Neither M0- nor M1-macrophages showed a significant effect on patient prognosis when analyzed alongside *GALNT14* expression (Figure 3C and D). However, a notable association was observed for M2-macrophages, where low *GALNT14* expression combined with low M2-macrophage abundance was associated with the best survival outcomes in HCC patients (Figure 3E). In contrast, no significant prognostic association was observed when *GALNT14* expression was analyzed in combination with follicular helper T cells (Figure 3F), eosinophils (Figure 3G), or memory B cells (Figure 3H).

These findings suggest that *GALNT14* expression may influence HCC prognosis through its association with tumor-infiltrating M2-macrophages, highlighting a potential immunoregulatory mechanism in HCC progression.

Discussion

Surgical resection remains the primary curative treatment for HCC patients;[32] however, the high rates of recurrence and disease progression pose significant challenges. Consequently, the identification of reliable biomarkers for predicting HCC relapse and progression remains an unmet clinical need.[33] Such biomarkers may provide insight into the underlying mechanisms driving HCC development and progression.[6] In this study, through GWAS analyzing LD between patients with favorable prognosis carrying the rs9679162 "TT-genotype" and those with poor prognosis carrying the rs9679162 "GG-genotype", we identified a panel of SNP candidates within the *GALNT14* gene. These SNPs were classified into four groups, and their contributions to postoperative prognosis, including RFS and MFS, were subsequently validated (Figure 1). Given that a single SNP genotype may not be the most effective prognostic biomarker,[34] we further evaluated SNP combinations and found that combining SNPs from groups-2+3 significantly improved stratification for post-operative prognosis (Figure 2). These findings suggest that a combined SNP panel could serve as a predictive biomarker for postoperative survival, recurrence, and metastasis in HCC patients.

Previous studies have demonstrated that rs9679162 genotypes are associated with GALNT14 expression at both the mRNA and protein levels in HCC tissues, contributing to hepatocarcinogenesis, tumor progression, and drug resistance by modulating cellular *O*-glycosylation.[14] Specifically, the "TT" genotype is linked to lower GALNT14 expression, while the "GG" genotype correlates with higher expression, potentially through the modulation of exon-6-skipping alternative splicing isoforms.[14] Given this well-established association, the current study did not replicate expression analyses but instead focused on evaluating the broader prognostic significance of rs9679162-linked SNPs and their potential immunological implications in HCC. However, the role of GALNT14 in regulating tumor-infiltrating immune cells remains largely unexplored, particularly in the context of immunotherapy, which has emerged as a first-line treatment for advanced HCC.[21,22] Understanding the molecular mechanisms governing immune cell infiltration is therefore critical. A previous study identified GALNT14 as a key hub gene involved in immune cell infiltration, particularly in regulating activated dendritic cells and effector memory $CD8^+$ T cells in sepsis patients.[23] In cancer, elevated GALNT14 expression has been linked to poor prognosis and disease progression in osteosarcoma, where it modulates immunogenic cell death and influences responses to chemotherapy and immunotherapy.[24] This suggests that GALNT14 may function as an immune-related glycosylation gene.[25] However, its role in immune cell modulation in HCC remains poorly understood.

In this study, we identified a moderate correlation between *GALNT14* expression and tumor-infiltrating macrophages, follicular helper T cells, and eosinophils (Figure 3A and B). Further prognostic analysis integrating *GALNT14* expression with immune cell abundance revealed that M2-macrophages, but not M0- or M1-macrophages, were significantly associated with HCC prognosis. Specifically, lower *GALNT14* expression combined with lower M2-macrophage abundance was associated with the most favorable survival outcomes (Figure 3E). This finding suggests that *GALNT14* may promote M2-macrophage infiltration into HCC tumors, potentially facilitating a pro-tumorigenic microenvironment through the secretion of cytokines and chemokines.[35,36] In contrast, no significant prognostic association was observed when *GALNT14* expression was analyzed alongside follicular helper T cells, eosinophils, or memory B cells (Figure 3F–H).

Previous studies have demonstrated that GALNT14 expression is associated with the levels of TGF-β and may directly interact with the TGF-β signaling pathway in various cancer types.[15,37,38] This suggests that GALNT14-mediated O-glycosylation plays a significant role in carcinogenesis, tumor progression, and drug resistance.[39] Importantly, TGF-β has recently been recognized as a critical factor driving the polarization of tumor-associated macrophages toward the M2 phenotype, which contributes to tumor progression.[40,41] Therefore, it is plausible that elevated GALNT14 expression promotes the enrichment of tumor-infiltrating M2 macrophages by enhancing TGF-β signaling and facilitating M2 polarization. This mechanism may further accelerate hepatocarcinogenesis and disease progression, resulting in poor clinical outcomes. Nonetheless, the precise molecular interactions and causal relationships underlying GALNT14's influence on macrophage polarization require further experimental investigation.

Beyond its established role in regulating *O*-glycosylation to promote cancer cell proliferation, migration, invasion, and drug resistance,[15–20,42] *GALNT14* has recently been implicated in ferroptosis regulation. Ferroptosis, a non-apoptotic

form of cell death characterized by intracellular iron accumulation, free radical production, lipid peroxidation, and activation of cell death effectors, ultimately leads to plasma membrane rupture.[43] *GALNT14* has been reported as a key regulator of ferroptosis in multiple conditions, including bronchopulmonary dysplasia,[44–46] polycystic ovary syndrome,[47] bladder cancer,[48] and ovarian cancer, where it modulates EGFR glycosylation and disrupts the mTOR pathway.[49] These findings suggest that elevated *GALNT14* expression may contribute to tumorigenesis and disease progression not only by promoting cancer cell proliferation, migration, and drug resistance but also by regulating ferroptosis sensitivity.

This study provides the first comprehensive analysis of a combined SNP panel in linkage disequilibrium with rs9679162 (located within the GALNT14 intron) as a potential postoperative prognostic biomarker for HCC patients, along with its possible role in regulating tumor-infiltrating immune cells. While the relatively large cohort of 345 patients and the use of appropriate statistical methods (such as Kaplan-Meier survival analysis and Cox proportional hazards models) provide adequate statistical power and methodological rigor, several limitations should be acknowledged. The retrospective and single-center design may introduce selection bias and limit the generalizability of the findings. Moreover, the follow-up duration may be insufficient for evaluating very long-term postoperative outcomes. Although the GWAS-based exploration cohort included only 15 patients (8 with the TT genotype and 7 with the GG genotype), which may limit the statistical power for identifying additional SNPs in linkage with rs9679162, this potential weakness was addressed by validating the findings in a larger independent cohort of 330 patients. In addition, while a link between GALNT14 expression and M2 macrophages was identified, the underlying molecular mechanisms remain unclear and warrant further investigation. Future multicenter, prospective studies with longer follow-up periods and mechanistic analyses are needed to validate these findings and clarify the clinical utility of this SNP panel in predicting HCC prognosis.

Conclusion

In conclusion, in this study, GWAS analysis comparing HCC patients with rs9679162 "TT-genotype" and "GG-genotype" identified a panel of SNPs in LD with rs9679162 that can serve as postoperative prognostic markers, particularly those classified into group-2 (rs62140629, rs4952033, and rs56284247) and group-3 (rs9679162 and rs6752303). Additionally, we demonstrated that *GALNT14* expression may influence HCC prognosis through its association with tumor-infiltrating M2-macrophages, highlighting a potential immunoregulatory mechanism in HCC progression.

Abbreviations

ALT, Alanine transaminase; AST, Aspartate transaminase; BCLC, Barcelona Clinic Liver Cancer; GALNT14, Polypeptide N-acetylgalactosaminyltransferase 14; GalNAc-T, N-acetylgalactosaminyltransferase; GWAS, Genome-wide association study; HBsAg, Hepatitis B surface antigen; HBV, Hepatitis B virus; HCC, Hepatocellular carcinoma; HR, Hazard ratio; INDEL, Insertion-deletion; LD, Linkage disequilibrium; MFS, Metastasis-free survival; OS, Overall survival; RFS, Recurrence-free survival; SNP, Single nucleotide polymorphism; TACE, Transarterial chemoembolization.

Acknowledgments

This study was supported by grants from National Science and Technology Council, Taiwan (113-2314-B-182A-143-) to CTY, and Linkou Chang Gung Memorial Hospital, Taiwan (CMRPG3M0363 to CWH, and CMRPG3M1263, CIRPG3L0013, and CORPG3P0411 to CTY). The authors would like to thank all the members, especially Ms. Chi-Yun Sun and Yu-Ru Liang, in Liver Research Center for the technical support.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Clark T, Maximin S, Meier J, et al. Hepatocellular carcinoma: review of epidemiology, screening, imaging diagnosis, response assessment, and treatment. review. *Curr Probl Diagn Radiol*. 2015;44(6):479–486. doi:10.1067/j.cpradiol.2015.04.004
2. Siegel RL, Miller KD, Wagle NS, et al. Cancer statistics, 2023. *Ca a Cancer J Clinicians*. 2023;73(1):17–48. doi:10.3322/caac.21763

3. Janevska D, Chaloska-Ivanova V, Janevski V. Hepatocellular carcinoma: risk factors, diagnosis and treatment. *Open Access Maced J Med Sci*. 2015;3(4):732–736. doi:10.3889/oamjms.2015.111
4. Pang TC, Lam VW. Surgical management of hepatocellular carcinoma. Review. *World J Hepatol*. 2015;7(2):245–252. doi:10.4254/wjh.v7.i2.245
5. Pascual S, Herrera I, Irurzun J. New advances in hepatocellular carcinoma. Review. *World J Hepatol*. 2016;8(9):421–438. doi:10.4254/wjh.v8.i9.421
6. Chan YT, Zhang C, Wu J, et al. Biomarkers for diagnosis and therapeutic options in hepatocellular carcinoma. *Mol Cancer*. 2024;23(1):189. doi:10.1186/s12943-024-02101-z
7. Aasarey R, Yadav K, Kashyap BK, et al. Role of immunological cells in hepatocellular carcinoma disease and associated pathways. *ACS Pharmacol Transl Sci*. 2023;6(12):1801–1816. doi:10.1021/acsptsci.3c00216
8. Li YK, Wu S, Wu YS, et al. Portal venous and hepatic arterial coefficients predict post-hepatectomy overall and recurrence-free survival in patients with hepatocellular carcinoma: a retrospective study. *J Hepatocell Carcinoma*. 2024;11:1389–1402. doi:10.2147/JHC.S462168
9. Liang K-H, Lin -C-C, Yeh C-T. GALNT14 SNP as a potential predictor of response to combination chemotherapy using 5-FU, mitoxantrone and cisplatin in advanced HCC. *Pharmacogenomics*. 2011;12(7):1061–1073. doi:10.2217/pgs.11.43
10. Lin WR, Hsu CW, Chen YC, et al. GALNT14 genotype, alpha-fetoprotein and therapeutic side effects predict post-chemotherapy survival in patients with advanced hepatocellular carcinoma. *Mol Clin Oncol*. 2014;2(4):630–640. doi:10.3892/mco.2014.294
11. Yeh CT, Liang KH, Lin CC, et al. A single nucleotide polymorphism on the GALNT14 gene as an effective predictor of response to chemotherapy in advanced hepatocellular carcinoma. *Int J Cancer*. 2014;134(5):1214–1224. doi:10.1002/ijc.28439
12. Lin CL, Chien RN, Chen LW, et al. Rs9679162 genotype predicts prognosis of real-world advanced hepatocellular carcinoma treated by sorafenib. *Cancer Biomark*. 2023;36(3):251–266. doi:10.3233/CBM-220042
13. Liang K-H, Lin C-L, Chen S-F, et al. GALNT14 genotype effectively predicts the therapeutic response in unresectable hepatocellular carcinoma treated with transcatheter arterial chemoembolization. *Pharmacogenomics*. 2016;17(4):353–366. doi:10.2217/pgs.15.179
14. Chu YD, Fan TC, Lai MW, et al. GALNT14-mediated O-glycosylation on PHB2 serine-161 enhances cell growth, migration and drug resistance by activating IGF1R cascade in hepatoma cells. *Cell Death Dis*. 2022;13(11):956. doi:10.1038/s41419-022-05419-y
15. Song KH, Park MS, Nandu TS, et al. GALNT14 promotes lung-specific breast cancer metastasis by modulating self-renewal and interaction with the lung microenvironment. *Nat Commun*. 2016;7(1):13796. doi:10.1038/ncomms13796
16. Tian H, Zuo T, Wang X, et al. GALNT14 mediates tumor invasion and migration in breast cancer cell MCF-7. *Mol Carcinogenesis*. 2014;54 (10):1159–1171. doi:10.1002/mc.22186
17. Shan J, Liu Y, Wang Y, et al. GALNT14 involves the regulation of multidrug resistance in breast cancer cells. *Transl Oncol*. 2018;11(3):786–793. doi:10.1016/j.tranon.2018.04.003
18. Wang R, Yu C, Zhao D, et al. The mucin-type glycosylating enzyme polypeptide N-acetylgalactosaminyltransferase 14 promotes the migration of ovarian cancer by modifying mucin 13. *Oncol Rep*. 2013;30(2):667–676. doi:10.3892/or.2013.2493
19. Zuo T, Shan J, Liu Y, et al. EFEMP2 mediates GALNT14-dependent breast cancer cell invasion. *Transl Oncol*. 2018;11(2):346–352. doi:10.1016/j.tranon.2018.01.021
20. Wagner KW, Punnoose EA, Januario T, et al. Death-receptor O-glycosylation controls tumor-cell sensitivity to the proapoptotic ligand Apo2L/TRAIL. *Nat Med*. 2007;13(9):1070–1077. doi:10.1038/nm1627
21. Fu Y, Liu S, Zeng S, et al. From bench to bed: the tumor immune microenvironment and current immunotherapeutic strategies for hepatocellular carcinoma. *J Exp Clin Cancer Res*. 2019;38(1):396. doi:10.1186/s13046-019-1396-4
22. Budhu A, Pehrsson EC, He A, et al. Tumor biology and immune infiltration define primary liver cancer subsets linked to overall survival after immunotherapy. *Cell Rep Med*. 2023;4(6):101052. doi:10.1016/j.xcrm.2023.101052
23. Ma W, Liu X, Yu R, et al. Exploring the relationship between sepsis and Golgi apparatus dysfunction: bioinformatics insights and diagnostic marker discovery. *Front Genet*. 2025;16:1483493. doi:10.3389/fgene.2025.1483493
24. Liu Z, Liu B, Feng C, et al. Molecular characterization of immunogenic cell death indicates prognosis and tumor microenvironment infiltration in osteosarcoma. *Front Immunol*. 2022;13:1071636.
25. Wang W, Jiao Y, Du X, et al. Immune-related glycosylation genes based classification predicts prognosis and therapy options of osteosarcoma. *Gene*. 2025;933:148985. doi:10.1016/j.gene.2024.148985
26. Chu YD, Liu HF, Chen YC, et al. WWOX-rs13338697 genotype predicts therapeutic efficacy of ADI-PEG 20 for patients with advanced hepatocellular carcinoma. *Front Oncol*. 2022;12:996820. doi:10.3389/fonc.2022.996820
27. O'Leary NA, Wright MW, Brister JR, et al. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. *Nucleic Acids Res*. 2016;44:D733–45.
28. Ru B, Wong CN, Tong Y, et al. TISIDB: an integrated repository portal for tumor-immune system interactions. *Bioinformatics*. 2019;35 (20):4200–4202. doi:10.1093/bioinformatics/btz210
29. Li T, Fu J, Zeng Z, et al. TIMER2.0 for analysis of tumor-infiltrating immune cells. *Nucleic Acids Res*. 2020;48(W1):W509–w514. doi:10.1093/nar/gkaa407
30. Li T, Fan J, Wang B, et al. TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells. *Cancer Res*. 2017;77(21):e108–e110. doi:10.1158/0008-5472.CAN-17-0307
31. Zhan S, Qiu M, Wei X, et al. Potentially functional genetic variants in ferroptosis-related CREB3 and GALNT14 genes predict survival of hepatitis B virus-related hepatocellular carcinoma. *Cancer Med*. 2024;13(1):e6848. doi:10.1002/cam4.6848
32. Llovet JM, Kelley RK, Villanueva A, et al. Hepatocellular carcinoma. *Nat Rev Dis Primers*. 2021;7(1):6. doi:10.1038/s41572-020-00240-3
33. Wang QB, Li J, Zhang ZJ, et al. The effectiveness and safety of therapies for hepatocellular carcinoma with tumor thrombus in the hepatic vein, inferior vena cave and/or right atrium: a systematic review and single-arm meta-analysis. *Expert Rev Anticancer Ther*. 2025;25(5):561–570. doi:10.1080/14737140.2025.2489651
34. Hassan MM, Li D, Han Y, et al. Genome-wide association study identifies high-impact susceptibility loci for HCC in North America. *Hepatology*. 2024;80(1):87–101. doi:10.1097/HEP.0000000000000800
35. Li D, Zhang T, Guo Y, et al. Biological impact and therapeutic implication of tumor-associated macrophages in hepatocellular carcinoma. *Cell Death Dis*. 2024;15(7):498. doi:10.1038/s41419-024-06888-z
36. Zheng H, Peng X, Yang S, et al. Targeting tumor-associated macrophages in hepatocellular carcinoma: biology, strategy, and immunotherapy. *Cell Death Discov*. 2023;9(1):65. doi:10.1038/s41420-023-01356-7

37. Huanna T, Tao Z, Xiangfei W, et al. GALNT14 mediates tumor invasion and migration in breast cancer cell MCF-7. *Mol Carcinog*. 2015;54 (10):1159–1171.
38. Xi Q, Ma J. Polypeptide N-Acetylgalactosaminyltransferase 14 (GALNT14) as a chemosensitivity-related biomarker for osteosarcoma. *J Oncol*. 2023;2023:1083423. doi:10.1155/2023/1083423
39. Zhang J, Ten Dijke P, Wuhrer M, et al. Role of glycosylation in TGF-β signaling and epithelial-to-mesenchymal transition in cancer. *Protein Cell*. 2021;12(2):89–106. doi:10.1007/s13238-020-00741-7
40. Zhang F, Wang H, Wang X, et al. TGF-β induces M2-like macrophage polarization via SNAIL-mediated suppression of a pro-inflammatory phenotype. *Oncotarget*. 2016;7(32):52294–52306. doi:10.18632/oncotarget.10561
41. Gratchev A. TGF-β signalling in tumour associated macrophages. *Immunobiology*. 2017;222(1):75–81. doi:10.1016/j.imbio.2015.11.016
42. Wang Y, Chen H. Protein glycosylation alterations in hepatocellular carcinoma: function and clinical implications. *Oncogene*. 2023;42 (24):1970–1979. doi:10.1038/s41388-023-02702-w
43. Ajoolabady A, Tang D, Kroemer G, et al. Ferroptosis in hepatocellular carcinoma: mechanisms and targeted therapy. *Br J Cancer*. 2023;128 (2):190–205. doi:10.1038/s41416-022-01998-x
44. Luo Y, Zhang Z, Xi S, et al. Bioinformatics analyses and experimental validation of ferroptosis-related genes in bronchopulmonary dysplasia pathogenesis. *PLoS One*. 2024;19(6):e0291583. doi:10.1371/journal.pone.0291583
45. Hu Z, Liu C, Mao Y, et al. Integration of transcriptomics reveals ferroptosis-related signatures and immune cell infiltration in bronchopulmonary dysplasia. *Heliyon*. 2023;9(10):e21093. doi:10.1016/j.heliyon.2023.e21093
46. Ma X, Tao Z, Chen L, et al. Genetic analysis of potential biomarkers and therapeutic targets associated with ferroptosis from bronchopulmonary dysplasia. *Medicine*. 2023;102(29):e34371. doi:10.1097/MD.0000000000034371
47. Lin S, Jin X, Gu H, et al. Relationships of ferroptosis-related genes with the pathogenesis in polycystic ovary syndrome. *Front Med*. 2023;10:1120693. doi:10.3389/fmed.2023.1120693
48. Liu X, Qiu Z, Zhang X, et al. Generalized machine learning based on multi-omics data to profile the effect of ferroptosis pathway on prognosis and immunotherapy response in patients with bladder cancer. *Environ Toxicol*. 2024;39(2):680–694. doi:10.1002/tox.23949
49. Li HW, Liu MB, Jiang X, et al. GALNT14 regulates ferroptosis and apoptosis of ovarian cancer through the EGFR/mTOR pathway. *Future Oncol*. 2022;18(2):149–161. doi:10.2217/fon-2021-0883