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ORIGINAL RESEARCH

High-Mobility Group AT-Hook 1 Served as a Prognosis Biomarker and Associated with Immune Infiltrate in Hepatocellular Carcinoma

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Background: The protein high-mobility group AT-hook 1 (HMGA1) has been demonstrated that modulated cellular proliferation, invasion, and apoptosis with a poor prognosis in miscellaneous carcinomas. However, the mechanism of circumstantial carcinogenesis and association with the immune microenvironment of HMGA1 in hepatocellular carcinoma (HCC) had not been extensively explored.

Methods: The gene expression, clinicopathological correlation, and prognosis analysis were performed in the data obtained from TCGA. The results were further validated by ICGC and GEO database and external validation cohort from Guangxi. The HMGA1 protein expression was further examined in the HPA database. Biological function analyses were conducted by GSEA, STRING database, and Coexpedia online tool. Using TIMER and CIBERSORT method, the relationship between immune infiltrate and HMGA1 was investigated.

Results: In HCC, HMGA1 had much higher transcriptional and proteomic expression than in corresponding paraneoplastic tissue. Patients with high HMGA1 expression had a poor prognosis and unpromising clinicopathological features. High HMGA1 expression was closely related to the cell cycle, tumorigenesis, substance metabolism, and immune processes by regulating complex signaling pathways. Notably, HMGA1 may be associated with TP53 mutational carcinogenesis. Moreover, increased HMGA1 expression may lead to an increase in immune infiltration and a decrease in tumor purity in HCC. CIBERSORT analysis elucidated that the amount of B cell naive, B cell memory, T cells gamma delta, macrophages M2, and mast cell resting decreased when HMGA1 expression was high, whereas T cells follicular helper, macrophages M0, and Dendritic cells resting increased.

Conclusion: In conclusions, HMGA1 is a potent prognostic biomarker and a sign of immune infiltration in HCC, which may be a potential immunotherapy target for HCC.

Keywords: hepatocellular carcinoma, HMGA1, prognostic signature, bioinformatics, immune filtration

Introduction

Hepatocellular carcinoma (HCC), which accounts for 75–85% of all cases, is the predominant type of liver cancer with a highly malignant nature due to its insidious onset, rapid progression, and intrahepatic and distant metastasis.[1,2] Liver cancer is one of the most common malignancies and the second most frequent cause of cancer-associated mortality worldwide. More than 80% of HCC cases are estimated to occur in the countries with low-medium resources, particularly in Eastern Asia and sub-Saharan Africa.[1] An investigation showed that liver cancer was one of the five leading causes

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of years of life lost (YLLs) in 2017 in China, suggesting it had brought an grave burden to society.[3]

Over the past few decades, there has been considerable progress in comprehending the epidemiology, risk factors, and molecular profiles of HCC. On the etiology, HCC arises from infection of chronic hepatitis (B and C) virus and cirrhosis, excessive drinking, metabolic liver diseases, particularly nonalcoholic fatty liver disease (NAFLD), prolonged dietary toxins exposure such as aflatoxin and aristolochic acid, type 2 diabetes mellitus, obesity, and smoking.[4,5] These ongoing risk factors mediate the liver cells damage, chronic inflammation, and further genomic changes. Indeed, HCC can actually be clinically cured with an early diagnosis with a satisfactory prognosis when potentially curative approaches are applicable.[1,6] In recent years, the researchers have made headway on the operative treatment and medication of HCC.[7–11] Immune checkpoint inhibitor (ICI) therapy has become an incrementally utilized treatment modality across the various stages of HCC. This provides a new therapeutic prospect for patients with HCC and underscores the crucial role of the tumor microenvironment (TME) in the development of HCC.[12–15] Unfortunately, due to the complexity of immunotherapy and the genetic heterogeneity, this method took effect only for partial patients.[16] Emerging evidence demonstrated that the change of tumor-induced immune infiltrate cells was a momentous mechanism that carcinomas develop to evade immune surveillance. Meanwhile, tumor immune infiltration carries a clinicopathological significance in predicting prognosis and therapeutic efficacy.[17–19]

HMGA1 is a non-histone nuclear protein as well as a chromatin architectural protein. In contrast to the high expression levels in various carcinomas, HMGA1 expression levels in normal tissue were low or undetectable. Several studies demonstrated that a high level of HMGA1 expression correlated with adverse clinical outcomes and more advanced disease in cancers.[20–23] Previous researches have shown that HMGA1 connected with hepatitis B virus-mediated changes and liver fibrosis.[24,25] Further, it acted as an oncogenic driver of progression and migration with a poor prognosis in HCC.[26,27] Higher HMGA1 mRNA and protein levels were discovered in HCCs with intrahepatic metastases compared to those without intrahepatic metastases, indicating the aggressive nature of HMGA1 in HCC development.[28,29] The reviews concluded that the ability to modulate gene expression and chromatin remodeling led to complex biological activities of HMGA1, resulting in the transformation of different molecular pathways.[23,30–32] Despite this, the exact mechanisms of HMGA1 on hepatocarcinogenesis and its immune relevance remain to be further elucidated to lay the foundation for the next analysis at a deeper level. Therefore, this study comprehensively evaluated the prognosis significance, latent biological functions, and the association with immune infiltrating cells of HMGA1 in HCC.

Materials and Methods

Hepatocellular Carcinoma (HCC) Datasets and Data Processing

The Cancer Genome Atlas (TCGA) database portal (https://portal.gdc.cancer.gov/cart; up to May 15, 2019) was employed to retained data of HCC samples on gene expression (Workflow Type: HT Seq-FPKM), clinical features (Data Type: Clinical Supplement), and genetic mutation. Samples lacking information on survival time, pathological grade, lymphatic, and distant metastasis were excluded. Processed RNA-Seq FPKM data of 374 pathologically verified HCC and 50 adjacent normal tissues were included. In addition, 232 HCC patients were included in The combined International Cancer Genomics Consortium (ICGC) cohort, and the Gene Expression Omnibus (GEO) cohort (GSE14520 datasets) included 212 HCC patients. Their expression and the clinical information was respectively obtained from the ICGC (http://dcc.icgc.org) and GEO database (https://www.ncbi.nlm.nih.gov/geo/). For the Kaplan-Meier survival analysis, the patients were divided into high- and low-HMGA1 expression groups according to the optimal cutoff value figured out by a user-friendly and web-based integrative tool (ESurv, https://www.giantonline.org/).[33]

Differential mRNA Expression, Prognosis Value, and Clinicopathological Correlation Analyses

The differentially expressed HMGA1 in HCC and normal tissues were detected using the Wilcoxon test method. To investigate the prognostic value of HMGA1, the Kaplan–Meier analysis was performed to compare overall survival (OS) time between high- and low-HMGA1 expression groups. Log rank test was used for statistical comparison. The log-rank p-values and the hazard ratios were figured. Univariate Cox proportional hazards regression and multivariate Cox regression analyses were utilized to assess the relationship between HMGA1 and OS time. The receiver operating characteristic (ROC) curve was also implemented. Logistic regression analysis was utilized to figure out whether there are significant

variances in HMGA1 expression under different clinicopathological statuses. The associations between HMGA1 expression and certain clinicopathological parameters were analyzed using the Student's *t*-test.

Biological Function Analysis

To gain insight into the biological processes associated with the HMGA1 regulatory network that could underlie HCC development, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed based on normalized RNA-Seq data obtained from the TCGA dataset using Gene Set Enrichment Analysis (GSEA). The count of permutations was set to 1000. Functional categories with a false discovery rate (FDR) < 0.050 and a nominal p-value < 0.050 were regarded as statistically significant pathways. Furthermore, we explored the STRING (search tool for recurring instances of neighboring genes) website (https://string-db.org/) to construct a protein-protein interaction (PPI) network among HMGA1 and the neighboring 10 genes significantly associated with it. An interaction score of 0.4 was set as a cut-off value.[34] The HMGA1 co-expression network was assessed utilizing Coexpedia online tools (http://www.coexpedia.org/) and was reconstructed by Cytoscape.[35] To examine the possible mechanism by which HMGA1 acts on the p53 signaling pathway, Pearson correlation analyses were performed on HMGA1 and some TP53-related genes respectively.

Immune Infiltrates Analysis

TIMER (Tumor Immune Estimation Resource) is a comprehensive resource, based on statistical computation known as deconvolution,[36] which is used to perform interactive analysis of the associations between the abundance of immune infiltrates and gene expression level (https://cistrome.shinyapps.io/timer/).[37] Using TIMER, the abundance of six tumor-infiltrating immune cells (TIICs), including B cells, CD4 T cells, CD8 T cells, macrophages, neutrophils, and dendritic cells as well as infiltrate purity was approximated. Besides, CIBERSORT is an analytical tool that estimates the abundance of member cell types in a mixed cell population using gene expression data (http://cibersort.stanford.edu/).[38] Through the CIBERSORT (Cell type Identification through the Estimating Relative Subsets of RNA Transcripts) algorithm, we evaluated the immune response of 22 TIICs in HCC based on the TCGA dataset and then evaluated their abundance between the high and low HMGA1 expression groups. A p-value < 0.05 makes known the statistical significance of deconvolution outcomes.

Collection and Reverse Transcription-Quantitative PCR (RT-qPCR) of HCC Samples in the Guangxi Cohort

From 2017 to 2018, surgical biopsies were collected from 29 patients who underwent curative hepatic resections for HCC at The First Affiliated Hospital of Guangxi Medical University (Guangxi, China) without the preoperative therapies. OS information was retrieved through electronic medical records or telephone follow-up. All patients enrolled signed informed consent forms to donate their tissue samples for biomedical research, which was authorized by the ethics committee of The First Affiliated Hospital of Guangxi Medical University [Approval Number: 2021 (KY-E-032)]. In our previous study, the way of specimen preservation and the specific procedures of RNA extraction and reverse transcription-quantitative PCR had been mentioned.[39] The sequence of primers was as following: GAPDH, forward GTCAGCCGCATCTTCTTT, reverse CGCCCAATAC GACCAAAT. HMGA1, forward AAACC AAGGGGCAGACCCAA, reverse CTGTGTA GTGTGGTGGTGAGG. Using the $2^{-\Delta\Delta Ct}$ method, the expression levels of HMGA1 were estimated to conduct validation analysis.

Validation of Differential Expression, Prognosis Value of HMGA1

The differential expression in HCC vs para-carcinoma tissues and prognosis value of HMGA1 were further validated by investigating the data from ICGC, GSE14520, and Guangxi cohorts. Furthermore, the HPA database (https://www.proteinatlas.org) was used to exam the protein expression of HMGA1 and to accurately assess protein localization.

Statistical Analysis

Unless otherwise noted, all analyses were performed using R software (version 3.6.3) and p-value < 0.05 was considered to be significant.

Result

Discrepant mRNA and Protein Expression of HMGA1

The HMGA1 expression levels were significantly up-regulated in HCC tissues in comparison with the non-tumor controls (Figure 1A, P < 0.001). Such result was further verified by mining Guangxi (Figure 1B, P < 0.001), ICGC

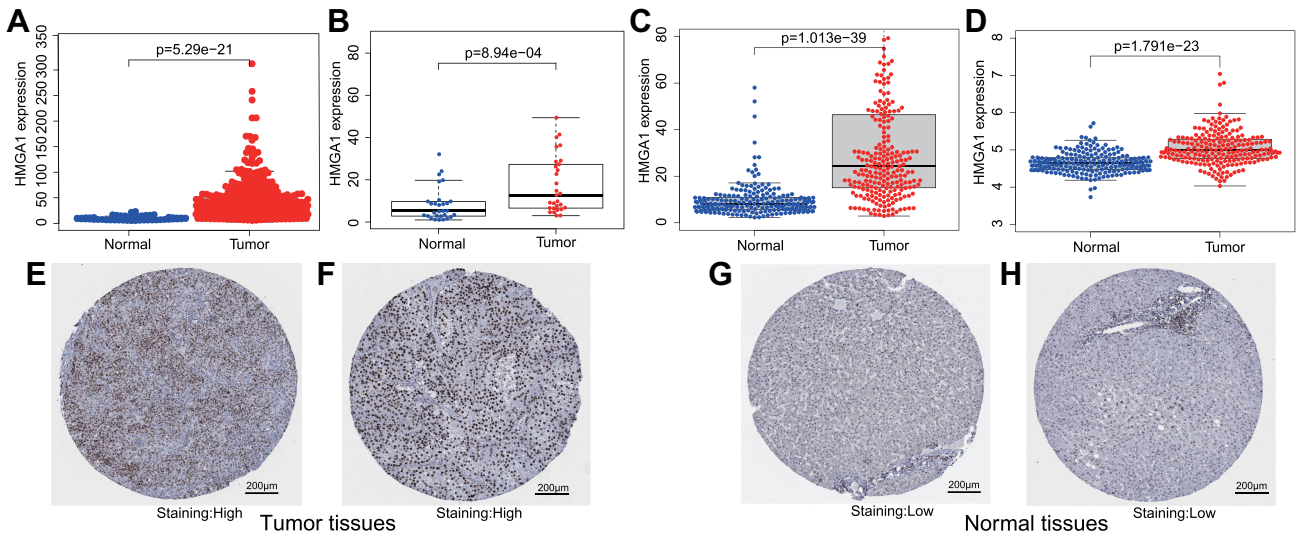

Figure 1 HMGA1 mRNA expression levels in normal and HCC tissues, as obtained from the (**A**) TCGA, (**B**) ICGC, (**C**) GEO, and (**D**) Guangxi cohort. Representative immunohistochemical staining images of HMGA1 protein in HCC specimens (**E** and **F**) and in normal liver tissues (**G** and **H**), which taken from the open HPA dataset.
Abbreviations: HMGA1, high-mobility group AT-hook 1; HCC, hepatocellular carcinoma; TCGA, The Cancer Genome Atlas; ICGC, International Cancer Genomics Consortium; GEO, Gene Expression Omnibus; HPA, The Human Protein Atlas.

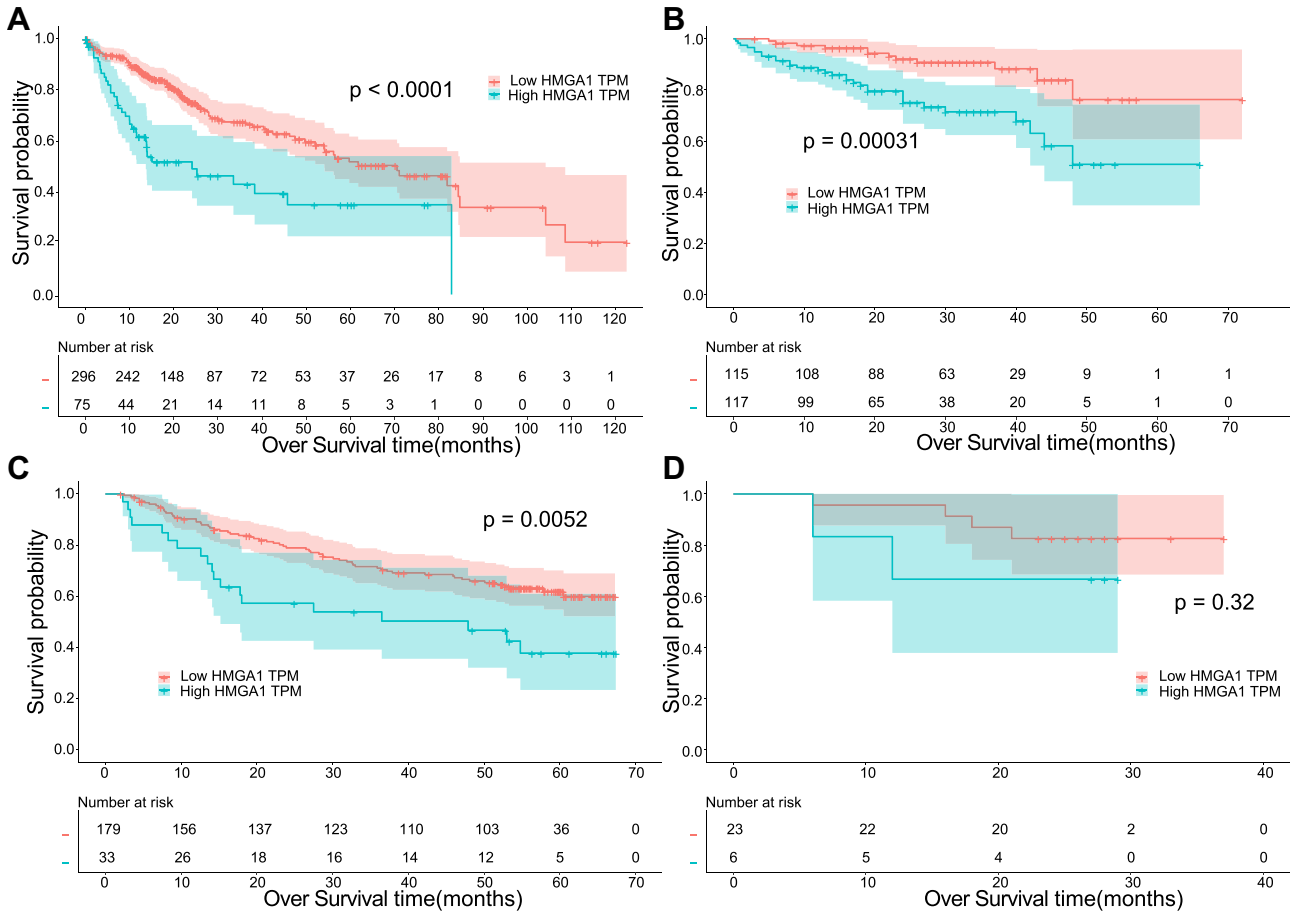

Figure 2 Kaplan-Meier analysis of HMGA1 and OS time of HCC in the (**A**) TCGA, (**B**) ICGC, (**C**) GEO, and (**D**) Guangxi cohorts.
Abbreviations: HMGA1, high-mobility group AT-hook 1; HCC, hepatocellular carcinoma; OS, overall survival; TCGA, The Cancer Genome Atlas; ICGC, International Cancer Genomics Consortium; GEO, Gene Expression Omnibus.

(Figure 1C, P < 0.001), and GSE14520 (Figure 1D, P < 0.001) cohorts. Consistent with the mRNA expression analysis, the proteins encoded by HMGA1 were strongly positive in HCCs (Figure 1E and F), while were low-expression in normal liver tissues in the human Protein Profiles (Figure 1G and H). Besides, HMGA1 protein was mainly localized to nuclei.

Prognosis Value and Clinicopathological Correlation of HMGA1

In the TCGA cohort, the augmented expression of HMGA1 corresponded with worse OS at the optimal cut-off point of 54.73 (Figure 2A, P < 0.001). Consistently, the enhanced mRNA expression of HMGA1 linked to the poor OS in the ICGC (Figure 2B, P < 0.05) and GSE14520 cohorts (Figure 2C, P < 0.05). Nevertheless, survival analysis was not statistically significant in Guangxi cohort, which may be due an insufficient sample size and adequate follow-up time (Figure 2D). The connections between HMGA1 expression and clinical information in patients with HCC were analyzed (Table 1). As for cancer status, HMGA1 upregulation expressions were prone to be found in the HCCs with poorer clinicopathological features, including histological grade, T stage, and pathological stage (Figure 3A–C, all P < 0.001).

Table 1 Association Between Clinicopathological Features of Patients and HMGA1 Expression in the Cancer Genome Atlas

Clinicopathological Parameters	Cases(n)	HMGA1 Expression Levels	T	P-value
Age				
<60 years	125	43.01	1.342	0.181
≥60 years	110	32.00		
Gender				
Male	161	39.48	−0.234	0.815
Female	74	38.22		
Stage				
I+II	163	35.65	−2.081	0.039*
III+IV	72	46.85		
Grade				
G1+G2	132	32.57	−2.858	0.005*
G3+G4	103	47.43		
T stage				
T1+T2	167	35.68	−1.800	0.075
T3+T4	68	47.44		
N stage				
N0	231	38.58	0.252	0.801
N1	4	19.81		
M stage				
M0	231	38.61	0.195	0.845
M1	4	13.68		

Note: *Statistically significant.

Interestingly, HMGA1 expression showed a monotone increasing from histological grade I to IV, pathological stage I to III, and T1 stage to T3 stage. Nevertheless, HMGA1 expression in pathological stage IV or T4 stage is not the highest. This may be attributed to the cause that operation should not be considered as a major treatment measure for advanced-stage patients. Logistic regression analysis demonstrated that T stage (II and III vs I), stage (II and III vs I) and grade (II and III vs I) were significantly associated with increased HMGA1 expression level in HCC (Table 2). Furthermore, a notable discovery indicated that HCCs with TP53 mutations had higher HMGA1 expression levels in the TCGA (Figure 3D, P < 0.001). Univariate analysis indicated that pathological stage, T and M stage were markedly correlated with OS (Table 3, P < 0.05). Following that, multivariate analysis indicated that HMGA1 overexpression remained as an independent predictive marker of OS (Table 2, Figure 3E, P < 0.001). Meanwhile, HMGA1 expression showed a promising prognostic power as the ROC curve illustrated that the area under the curve (AUC) of HMGA1 expression for predicting OS was 0.622 (Figure 3F).

Biological Function of HMGA1

According to the results of GO terms enrichment analysis, upregulated HMGA1 was significantly concentrated in "RNA catabolic process, mRNA binding, cell cycle G2/M phase transition, and mRNA processing" and downregulated HMGA1 was enriched in "cellular amino acid catabolic process, alpha-amino acid catabolic process, organic acid catabolic process, protein activation cascade, and complement activation alternative pathway" (Figure 4A). KEGG pathway analysis elucidated that the upregulated HMGA1 was mainly involved in "DNA replication, pathway in cancer, cell cycle, MAPK signaling pathway, NOTCH signaling pathway, VEGF signaling pathway, p53 signaling pathway, and WNT signaling pathway", while "complement and coagulation cascades, tryptophan metabolism, PPAR signaling pathway, fatty acid metabolism, and primary bile acid biosynthesis" were enriched in downregulated HMGA1 (Figure 4B). Moreover, a PPI network constructed from the STRING database showed that TP53, LMNB1, RB1, RPS6KB1, EP400, HMGCR, INSIG1, HMGA2, CEBPB, and C6orf1 proteins were significantly related to HMGA1 protein (Figure 4C). The HMGA1 co-expression network was analyzed and created by Coexpedia, in which heterogeneous nuclear ribonucleoprotein U-like 1 (hnRPUL1) carried the greatest correlation with the highest edges' LLS

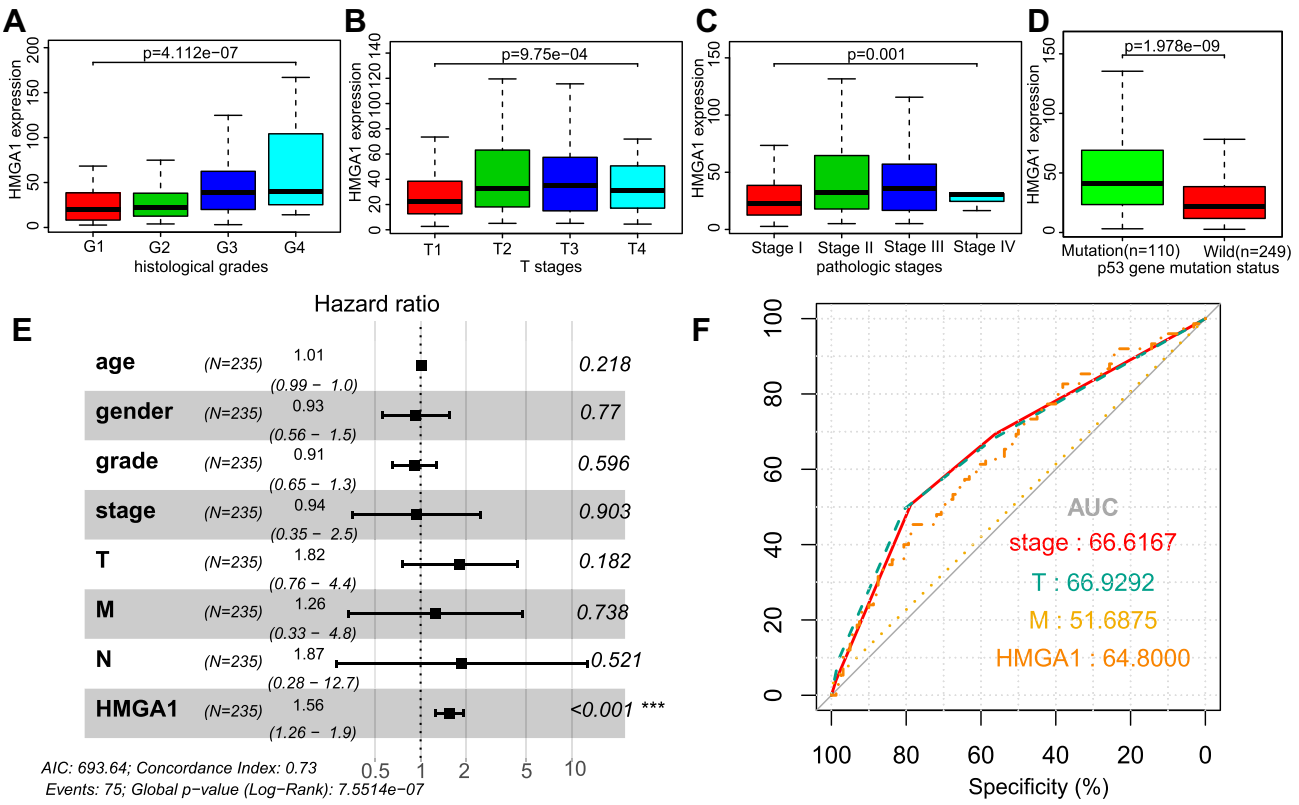

Figure 3 Boxplot showing expression of HMGA1 correlate with different histological grades (**A**), T stages (**B**), pathologic stages (**C**), and p53 gene mutation status (**D**) in HCC. The *t*-test was used to estimate the significance of the difference in gene expression levels between groups. The application value of HMGA1 in HCC clinical parameters. (**E**) Forest plot illustrated the results of multivariate Cox regression analyses. ***Stands for $P < 0.001$. (**F**) ROC curves of pathologic stage, T stage, M stage, and HMGA1 expression.

Abbreviations: HMGA1, high-mobility group AT-hook 1; HCC, hepatocellular carcinoma; ROC, Receiver operating characteristic; T stage, Tumor stage; M stage, Metastasis stage; AUC, Area under Curve.

(log-likelihood score) of 9.276 (Figure 4D). Furthermore, Pearson correlation analyses uncovered that HMGA1 was significant positive correlation to TP53 (R=0.4), RB1 (R=0.17), CCNB1 (R=0.54), PAK1 (R=0.42), CDK1 (R=0.37), CDK2 (R=0.34), CDK4 (R=0.55), E2F1 (R=0.22), E2F2 (R=0.39), HNRNPUL1 (R=0.45), while was negatively correlated to GADD45A (R = - 0.23) (Figure 5A–L, all P < 0.001).

Table 2 Association Between HMGA1 Expression and Clinicopathological Features (Logistic Regression)

Clinical Characteristic	Total(N)	(OR) in HMGA1 Expression	P-value
Age	370	0.822(0.545–1.237)	0.348
Stage (II vs I)	258	2.103(1.247–3.581)	0.006*
Stage (III vs I)	256	2.387(1.407–4.095)	0.001*
Stage (IV vs I)	176	2.272(0.367–17.587)	0.376
Grade (II vs I)	234	0.414(0.213–0.776)	0.007*
Grade (III vs I)	206	0.359(0.179–0.698)	0.003*
Grade (IV vs I)	67	0.320(0.084–1.140)	0.081
Tumor status (II vs I)	275	2.496(1.503–4.192)	<0.001*
Tumor status (III vs I)	261	2.323(1.363–4.006)	0.002*
Tumor status (IV vs I)	194	1.808(0.578–5.824)	0.304
Lymph node (II vs I)	256	1.000(0.118–8.439)	1.000
Distant metastasis (II vs I)	300	3.045(0.384–61.996)	0.338

Note: *Statistically significant.
Abbreviation: OR, odds ratio.

Immune Cells Infiltration and HMGA1

In TIMER, increased HMGA1 expression resulted in a rise in immune infiltration level, including infiltrated B cells, CD4+, CD8+ T cells, neutrophils, macrophages, and dendritic cells (DCs) in HCC, as well as a reduction in infiltrating purity (Figure 6A, all P < 0.001). Notably, macrophages had the highest correlation coefficient among these immune infiltrate cells. Moreover, CIBERSORT analysis elucidated that the amount of B cell naive, B cell memory, T cells gamma delta, Macrophages M2, and mast cell resting decreased when HMGA1 expression was high, whereas T cells follicular helper, Macrophages M0, and Dendritic cells resting increased (Figure 6B, P < 0.05). The resulting heat map manifested that there was a weak to moderate correlation among diverse TIICs subpopulations (Figure 7).

Table 3 Univariate Cox Proportional Hazards Regression and Multivariate Cox Regression Analyses of HMGA1 and Clinical Features in HCC

Variable	Univariate Analysis			Multivariate Analysis		
	HR	95% CI	P-value	HR	95% CI	P-value
Age	1.005	0.987–1.023	0.591	1.012	0.993–1.032	0.218
Gender	0.780	0.487–1.249	0.301	0.927	0.556–1.545	0.770
Grade	1.017	0.746–1.387	0.914	0.913	0.652–1.279	0.596
Stage	1.865	1.456–2.388	<0.001*	0.941	0.353–2.508	0.903
T stage	1.804	1.434–2.270	<0.001*	1.816	0.756–4.360	0.182
N stage	2.022	0.494–8.276	0.328	1.872	0.275–12.743	0.521
M stage	3.850	1.207–12.281	0.023 *	1.256	0.331–4.758	0.738
HMGA1	1.571	1.285–1.922	<0.001*	1.558	1.257–1.922	<0.001*

Note: *P<0.05 was considered statistically significant.
Abbreviations: HR, hazard ratio; CI, confidence interval.

Discussion

Several studies have elucidated that high mobility group A1 (HMGA1), an important member of the HMGA family, played a central role in the pathogenesis and progression of diverse malignant tumors.[23,40] HMGA1 is overexpressed during embryogenesis but at relatively low levels in normal adult tissues, implying that HMGA1 may act as a critical molecular switch to form transcriptional networks that

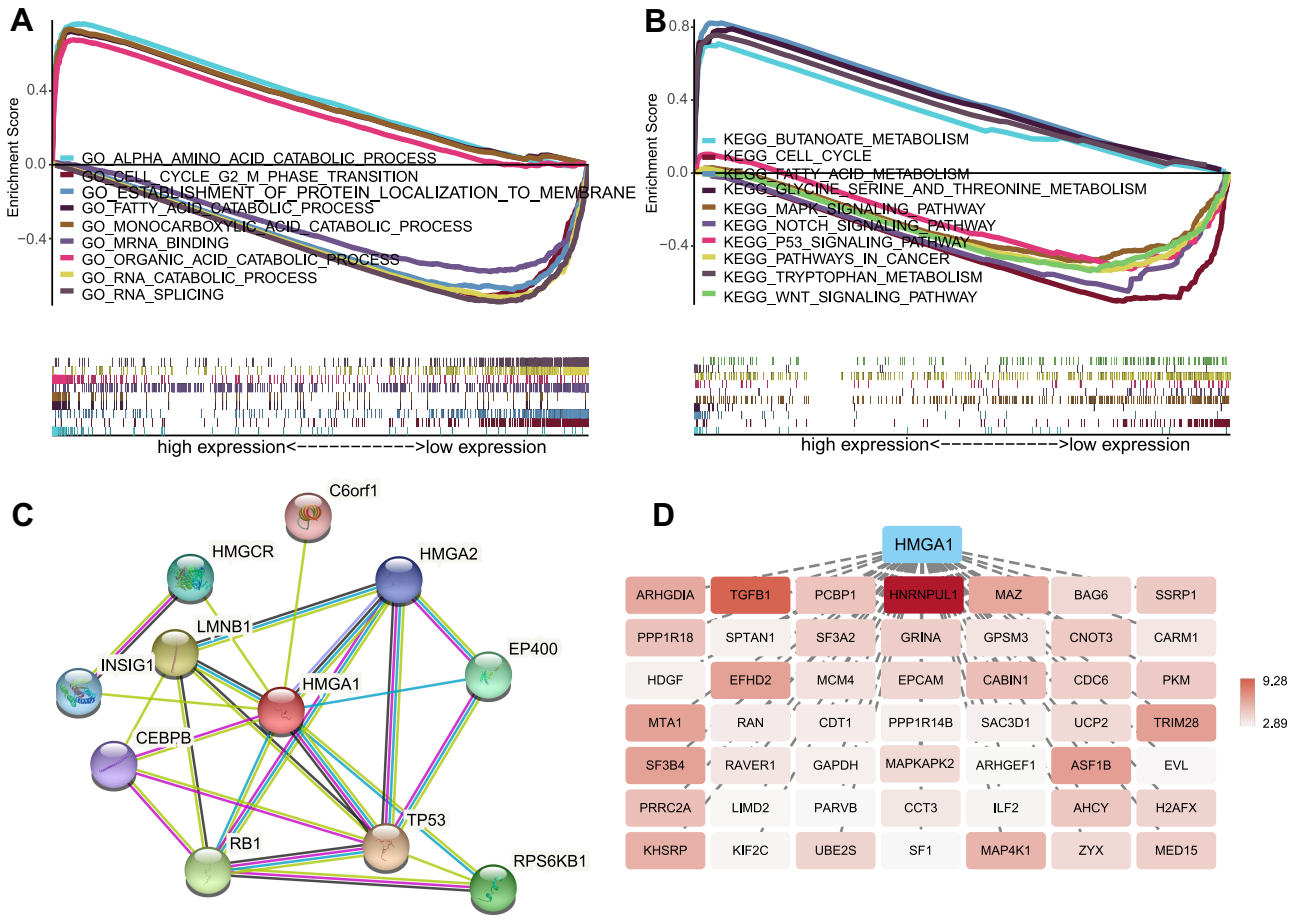

Figure 4 Significantly enriched GO annotations (**A**) and KEGG pathways (**B**) of HMGA1 in HCC based on TCGA database. (**C**) PPI analysis of HMGA1 by STRING. (**D**) HMGA1 co-expression network was inferred from Coexpedia and deep hue indicated a higher degree of association with HMGA1.
Abbreviations: GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; PPI, Protein-protein interaction; HMGA1, high-mobility group AT-hook 1; TCGA, The Cancer Genome Atlas; STRING, search tool for recurring instances of neighboring genes.

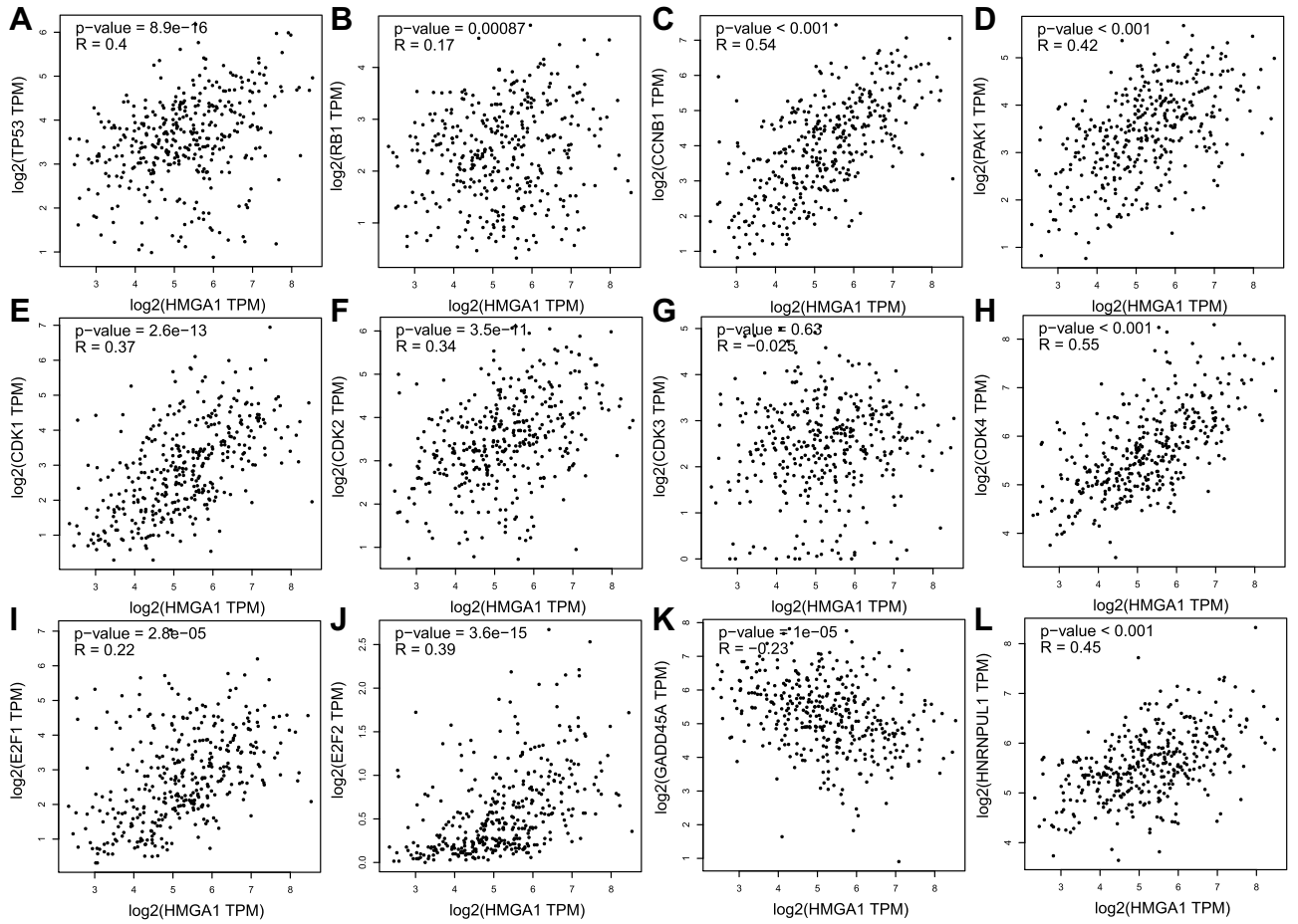

Figure 5 HMGA1 mRNA expression was respectively significant positive correlation to the mRNA expressions of TP53 (R=0.4, P<0.001), RB1 (R=0.17, P<0.001), CCNB1 (R=0.54, P<0.001), PAK1 (R=0.42, P<0.001), CDK1 (R=0.37, P<0.001), CDK2 (R=0.34, P<0.001), CDK4 (R=0.55, P<0.001), E2F1 (R=0.22, P<0.001), E2F2 (R=0.39, P<0.001), HNRNPUL1 (R=0.45, P<0.001), while was significant negative correlation to GADD45A (R=−0.23, P<0.001) (**A**–**L**).

Abbreviations: HMGA1, high-mobility group AT-hook 1; TP53, tumor protein p53; RB1, RB Transcriptional Corepressor 1; CCNB1, Cyclin B1; PAK1, P21 (RAC1) Activated Kinase 1; CDK1, Cyclin Dependent Kinase 1; CDK2, Cyclin Dependent Kinase 2; CDK4, Cyclin Dependent Kinase 4; E2F1, E2F Transcription Factor 1; HNRNPUL1, Heterogeneous Nuclear Ribonucleoprotein U Like 1; GADD45A, Growth Arrest and DNA Damage Inducible Alpha.

remain primitive, poorly differentiated, and stem-like state in both cancer and normal development.[41–43] Amounting studies proved that the suppression of oncogenic HMGA1 significantly inhibited some cancer cell proliferation and migration, providing a novel clue to probe into anti-cancer approaches.[21,29,32,44,45] Furthermore, HMGA1 is up-regulated in a significant proportion of HCC, and its overexpression is associated with a poor prognosis. A research in vitro models ulteriorly elucidated that HMGA1 overexpression may act a pivotal role in cell viability and migration and promote anchorage-independent growth to induce transformation in liver cancer cell lines.[26,27] Consistently, our study demonstrated that HMGA1 expression was amplified in HCC and high-expression HMGA1 was closely associated with advanced pathological stage, unpromising T stage, and poor pathological differentiation. Accordingly, oncogenic properties and aggressive malignancy of HMGA1 were uncovered. Microtubule Interacting and Trafficking Domain containing 1 (MITD1) can serve as a novel predictor for human HCC prognosis, which had been demonstrated. By contrast, HMGA1 had a better prognostic evaluation effectiveness with a higher AUC.[46]

Recent studies had identified many frequently mutated genes in HCC genomic alterations, including TERT promoter, TP53 (tumor protein P53), and CTNNB1 (b-catenin) and so on, wherein TP53, a crucial cancer-driver mutation gene, accounted for about 30% of cases.[1] As a tumor suppressor gene, TP53 activates its protein expression to regulate its downstream genes and subsequently inhibit malignant transformation by inducing cell cycle arrest, apoptosis, DNA repair, and senescence.[47,48] Its mutation is significantly associated with the advanced

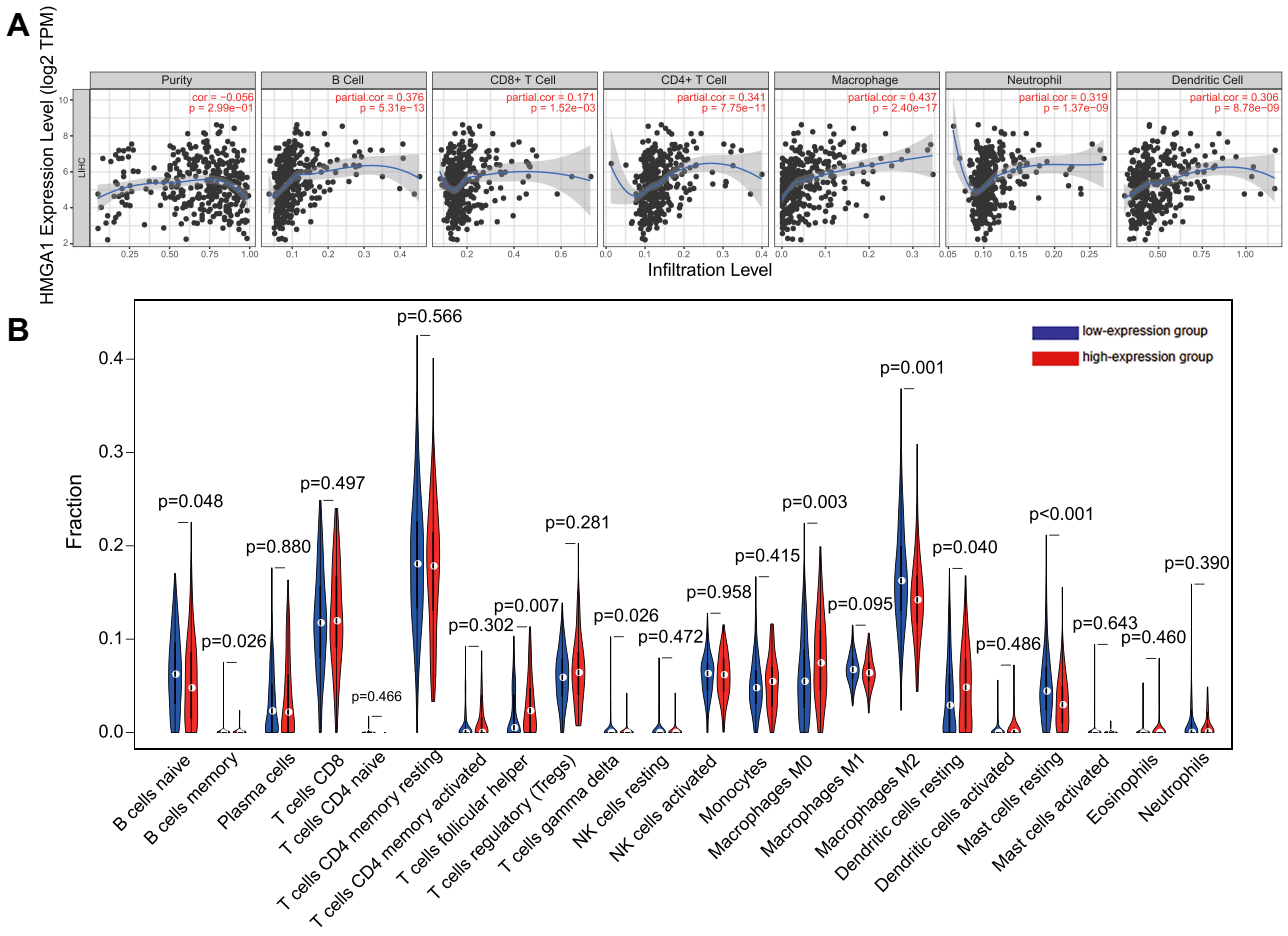

Figure 6 The relationship between HMGA1 and immune invasion in hepatocellular carcinoma. (**A**) Relationship between the abundance of immune cells and HMGA1 expression in HCC (TIMER). (**B**) Different proportions of sorts of immune cells in high- and low-HMGA1 groups in HCC specimens (CIBERSORT).
Abbreviations: HMGA1, high-mobility group AT-hook 1; HCC, hepatocellular carcinoma; TIMER, Tumor Immune Estimation Resource; CIBERSORT, Cell type Identification through the Estimating Relative Subsets of RNA Transcripts.

clinical stage, poor prognosis, and occurrence of liver cancer.[49,50] HMGA1 regulated the p53 signaling pathway at the transcriptional level by regulating the balance between symmetric and asymmetric divisions in cancer stem cells and suppressing apoptosis, according to the new studies.[31,42,51] Besides, hnRPUL1 was demonstrated to interact directly with p53, inhibit p53 transcriptional activity in response to UV radiation, and play a role in DNA damage response, especially in the process of alcohol-related cirrhosis.[52,53] Additionally, substantial epidemiological and experimental evidence had revealed that HMGA1 may regulate carcinogenesis and development of cancer by activating the Wnt/β-catenin pathway.[20,22,30,54] Xian et al uncovered that such activating pattern conformed to a feed-forward amplification loop, whereby Wnt induces HMGA1, which in turn, enhances signaling.[54] Moreover, β-catenin activation promotes immune evasion and resistance to anti-PD-1 therapy in HCC.[55] Our study demonstrated that high expression level HMGA1 phenotype closely linked with several cancer-related pathways (e.g. cell cycle, MAPK signaling pathway, NOTCH signaling pathway, VEGF signaling pathway, p53 signaling pathway, and WNT signaling pathway). Furthermore, HCC patients with TP53 dysregulation had higher HMGA1 expression than non-mutations, and HMGA1 was weakly - moderately correlated with TP53 downstream genes and hnRPUL1. Accordingly, it can be inferred that HMGA1 may exert an important effect on TP53 mutational carcinogenesis. These outcomes may explain why HMGA1 functions as an oncogene and its overexpression portends an aggressive malignancy of cancers.

As a crucial part of TME, the cellular composition of immune infiltration is tightly regulated by the various chemokines, which modulate tumor immunity and the biological phenotype of the tumors and further impact tumor progression,

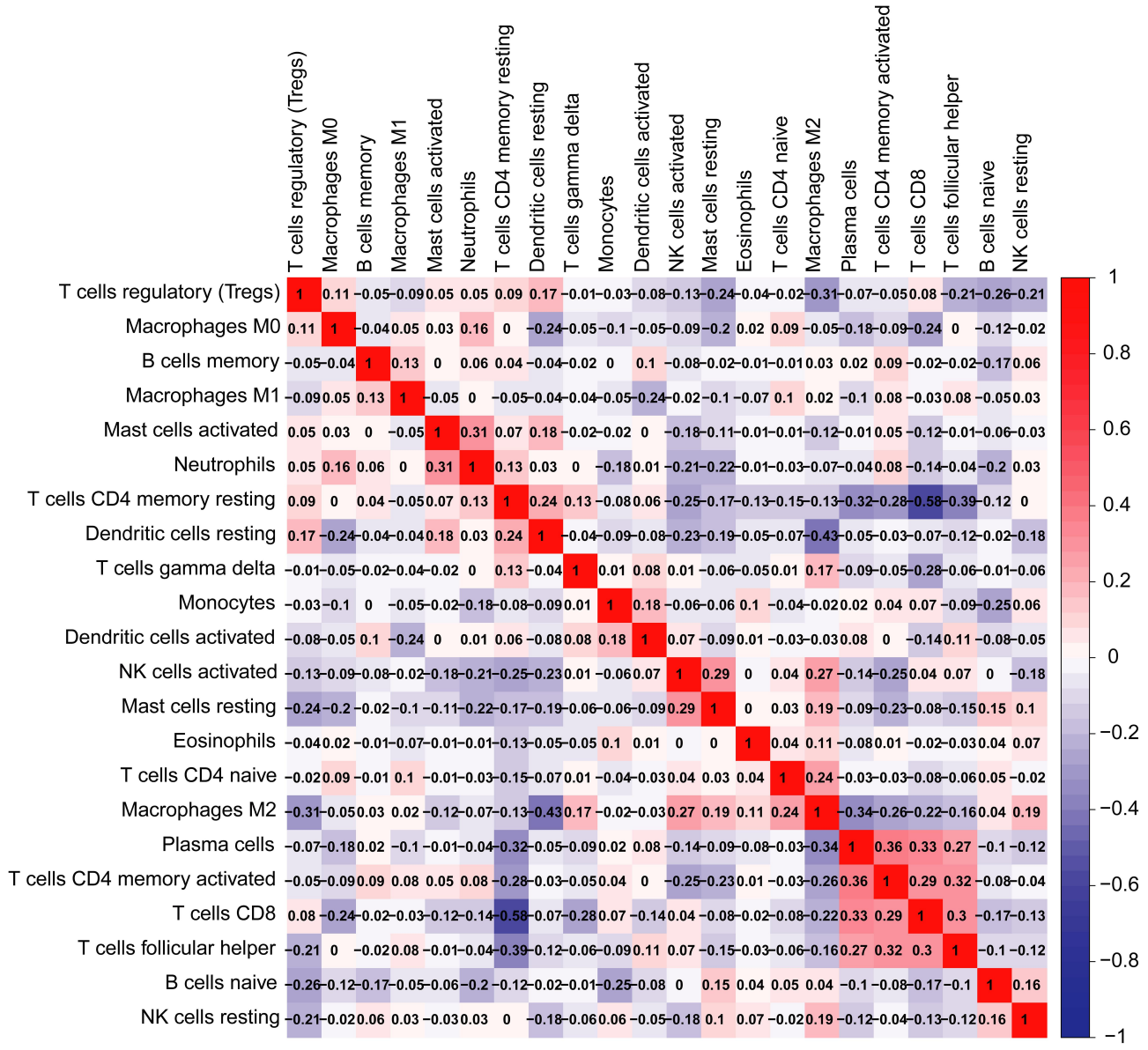

Figure 7 Resulting heat map visualizing the correlation matrix of diverse tumor-infiltrating immune cells subpopulations. Each tile indicates coefficients calculated by Pearson's correlation test.

therapy, and prognosis.[1,12–14,36] These days, emerging insights into tumor biology suggested that successful therapeutics in the future depended on rescuing T cells from the homing and dysfunction state. Tumor-associated macrophages (TAM) function as a vital mediator of tissue homeostasis. Macrophages M1 activity inhibits cell proliferation and causes tissue damage, while M2 activity promotes cell proliferation and tissue repair. Adjuvant immunotherapy targeting macrophages was considered beneficial for HCC patients.[56–59] Most cancers, including HCC, overexpress inhibitory ligands to evade the immune response by dampening T cell function, thus contributing to cancer progression. A study proved that by activating HMGA1-dependent signaling pathways including the PI3K/Akt and MEK/ERK pathways, PD-L1 maintained colorectal cancer stem cells and promoted the expansion of cancer.[60] The results of this study demonstrated that HMGA1 immune infiltration in HCC. Remarkably, the amount of M0 macrophages displayed a significant rise in the high HMGA1 expression phenotype, while Macrophages M2 decreased. Accordingly, it was speculated that HMGA1 may exert dual influences on tumor-infiltrating immune system, namely recruitment and management of tumor-infiltrating cells and helping the immune evade by regulating TAM via activating of the relevant signal pathways.

Conclusions

In conclusion, HCC with a high HMGA1 expression level may be the one with malignant nature and dismal prognosis. Moreover, excess levels of HMGA1 have a crucial role in the regulation of immune infiltrating cells and may be an accomplice to help the immune escape in HCC. However, precise approaches by which HMGA1 with multifaceted functions promotes hepatocarcinogenesis and links with liver cirrhosis or viral hepatitis need further investigation.

Data Sharing Statement

All raw online RNA sequencing dataset and clinical information of HCC patients, which were included in the current study, can be downloaded from the TCGA (https://portal.gdc.cancer.gov/), ICGC (http://dcc.icgc.org), and GEO database (https://www.ncbi.nlm.nih.gov/geo/). The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Statement

The present study was approved by the Ethics Committee of The First Affiliated Hospital of Guangxi Medical University [Approval Number: 2021 (KY-E-032)] and all patients provided written informed consent. This trial was conducted in accordance with the ethical principles in the Declaration of Helsinki.

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Disclosure

The authors declare that they have no competing interests.

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