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Abstract

Background

HCC is the most common digestive system malignancy, with unclear pathogenesis and low survival rates. AP1M2 is associated with tumor progression, but its role and molecular mechanisms in HCC remain poorly understood and require further investigation.

Methods

We utilized the Gene Expression Omnibus (GEO) and Expression Analysis Interactive Hub (XENA) databases to assess AP1M2 mRNA expression levels in HCC patients. Additionally, we employed the Cancer Genome Atlas (TCGA) database to identify pathways associated with both AP1M2 and HCC development. To evaluate the effect of AP1M2 on hepatocellular Carcinoma cell proliferation and migration, we employed various techniques including EdU, CCK8, Colony formation assay, and Transwell assays. Furthermore, Western blot analysis was conducted to examine the signaling pathways influenced by AP1M2.

Results

AP1M2 expression was significantly increased at the mRNA level in HCC tissues($P < 0.001$). Importantly, overall survival (OS) analysis confirmed the association between higher AP1M2 expression and a poorer prognosis in HCC patients compared to those with lower AP1M2 expression ($P < 0.019$).Multivariate Cox regression analysis showed that AP1M2 was an independent prognostic factor and a valid predictor for HCC patients. Furthermore, GSEA results indicated differential enrichment of lipid, bile acid, metal metabolism, and coagulation processes in HCC samples demonstrating a high AP1M2 expression phenotype. In vitro experiments supported these findings by demonstrating that AP1M2 promotes HCC cell proliferation and migration, while activating the JNK/ERK pathway.

Conclusion

Our findings indicate that AP1M2 expression may serve as a potential molecular marker indicating a poor prognosis for HCC patients. Furthermore, we have demonstrated that AP1M2 significantly influences HCC cell proliferation and migration, with the JNK/ERK signaling pathway playing a key role in AP1M2-mediated regulation in the context of HCC.

Key words Hepatocellular carcinoma, AP1M2,Proliferation, Migration;

Abbreviations

HCC	Hepatocellular Carcinoma
AP1M2	Adaptor-Related Protein Complex 1 Mu-2 Subunit
log_2FC	log_2 Fold Change
GEO	Gene Expression Omnibus
TCGA	The Cancer Genome Atlas
AUC	Areas Under the Curves
ROC	Receiver Operating Characteristic
OS	Overall Survival
ROS	Reactive Oxygen Species
DEGs	Differentially Expressed Genes
PPI	Protein−Protein Interaction
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
KM	Kaplan−Meier
GSEA	Gene Set Enrichment Analysis
mRNA	Messenger RNA

Declarations

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Conflicts of Interest The authors have no conflicts of interest to disclose.

Data Availability The datasets generated and analysed during the current study are available in TCGA (https://portal.gdc.can cer.gov/) and GEO (https://www.ncbi.nlm.nih.gov/geo/). Further inquiries can be directed to the corresponding authors.

Code availability All statistical analyses were conducted by R software version 4.2.1 (www.xiantao.love). Concurrently, Metascape, a gene annotation and analysis resource, and GraphPad Prism (version 8.0.1) also participated in the analysis.

Author's contributions Wang Huan, Xie Xin, Du Minwei and Wang Kuanyuan performed data processing and analysed data. Wang Huan and Xie Xin participated in writing the manuscript. Xingyuan Chen, and Hui Yang, contributed to the discussion and critical evaluation of the manuscript. Hui Yang designed the study. All authors read and approved the manuscript.

Introduction

Hepatocellular Carcinoma（HCC） is a common and widespread malignancy, ranking among the most prevalent types of cancer globally. HCC, accounting for 80-90% of all primary liver cancer cases, is the second leading cause of cancer-related mortality, following lung cancer[1][2]. Unfortunately, a significant proportion of patients diagnosed with HCC are already in advanced stages, making surgical intervention impractical and limiting treatment options to palliative care[3][4]. However the median survival time for these patients receiving palliative treatment is only 6 to 12 months, and the 5-year survival rate is only 10%[5]. Therefore, the identification of innovative and effective molecular-based therapies for HCC patients is of utmost clinical significance.

AP1M2 is a gene that encodes a protein subunit of the heterotetrameric adaptor-related protein complex 1.Being a member of the adaptor complex subunit family, it plays an important role in cell signal transduction by participating in the interaction of tyrosine sorting signals on the cell membrane

surface. Within this complex, AP1M2 serves as a protein subunit and plays a crucial role in cell signal transduction by participating in the sorting signal interaction of tyrosine on the cell membrane surface[6][7]. AP1M2 is of significant clinical relevance as it may influence the tumor environment in patients with invasive breast cancer and potentially serve as a valuable target for early screening and treatment of this disease[8]. Exploring its role could improve the efficiency of early screening and overall survival rates for individuals diagnosed with invasive breast cancer[9]. Studies have shown that the deficiency of AP1M2 in mice leads to the development of chronic colitis, alterations in intestinal carbohydrate metabolism and nutrient transport mechanisms, as well as exacerbation of colitis by promoting the growth of sulfur-reducing and lactic acid-producing bacteria[10]. However, the specific role of AP1M2 in HCC has not been extensively researched.

This study aims to analyze the role of AP1M2 in HCC using bioinformatics methods, investigate its effects on the migration and proliferative phenotypes of HCC cell lines in vitro, and explore the downstream pathways associated with AP1M2. Through these investigations, we aim to uncover the mechanism by which AP1M2 influences HCC and provide a new theoretical basis for the clinical treatment of hepatocellular carcinoma.

Materials and methods

RNA Expression and Data Mining

mRNA expression data from HCC samples was collected from the XENA and GEO databases.. We employed various keywords, including HCC, program name, project ID, HTseq-counts, data category, transcriptome analysis, and data type, for the search. Furthermore, gene expression data was obtained. Clinical and pathological information, such as sex, race, age, coagulation tests, stage, grade, radiotherapy, and primary treatment outcome, were also collected. It is important to note that since this study was conducted using bioinformatics methods and did not involve human subjects directly, no approval from an ethics committee or institutional review board was necessary.

Construction and Prediction of the Nomogram

To improve the customization of life expectancy prediction, we developed a nomogram using the rms package in R. The nomogram incorporates the results of our multivariate analysis, including essential clinical features and calibration plots. Calibration and discrimination are commonly used techniques for

assessing model performance. Based on the median risk score, we divided the TCGA HCC data into two groups: the AP1M2 high-risk group and AP1M2 low-risk group. We used the Kaplan-Meier method and a two-sided log-rank test to assess the difference in overall survival (OS) between these high-risk and low-risk groups. Additionally, we created a calibration plot to evaluate the predictive accuracy of the nomogram using the prognostic model.

Gene Set Enrichment Analysis（GSEA）

We utilized GSEA to identify gene sets associated with AP1M2 expression.We divided the samples into high and low expression groups, using them as training sets to explore potential functions and address significant differences in survival. We selected the annotated gene set c2.cp.v7.0 from the msigdb collection as the reference GMT file, utilizing genes as reference gene sets with a false discovery rate <0.25 and an adjusted p-value <0.05, thus considering them as significantly enriched. Multiple permutations of the gene sets were performed for each test. The pathways enriched in each phenotype were ranked using standardized enrichment scores and adjusted p-values.

Enrichment analyses of Gene Ontology and Kyoto Encyclopedia of Genes and Genomes for Identification of Differentially Expressed Genes

We used the Cluster Profiler R package to identify significant differentially expressed genes (DEGs) between HCC and normal samples. [18]. DEGs were identified using an unpaired t-test, with the threshold values set at adjusted $P \leqslant 0.05$ and $|logFC| \geqslant 1.5$, as determined by the Benjamini-Hochberg method. The DEGs then underwent Gene Ontology (GO) analysis, revealing their representation in various functional categories, including biological process, molecular function, and cellular component. For further analysis, we conducted Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis and pathway analysis of the DEGs. We conducted this analysis using the online tools provided by the Database for Annotation, Visualization, and Integrated Discovery (DAVID) at https://david.ncifcrf.gov/. A significance threshold of $P < 0.05$ was used to identify enriched pathways.

Composition of Invasive Immune Cells in HCC

We identified marker genes for 24 immune cells through a literature analysis [19]. We assessed the infiltration of these immune cells in HCC using the single-sample Gene Set Enrichment Analysis (ssGSEA) method. We conducted correlation analysis between AP1M2 and immune cells using the

Spearman correlation method. Additionally, we compared the infiltration of these cells between the high-expression and low-expression AP1M2 samples using the Wilcoxon rank-sum test.

Protein-Protein Interaction Network Construction and Analysis

We constructed the protein-protein interaction (PPI) network using the Search Tool for the Retrieval of Interacting Genes (STRING) database (http://string-db.org/). We applied a significance threshold of >0.4 to the scores.

Cell culture

The human hepatoma cell line 97H, and Hep3B were obtained from the Institute of Cell Research, Chinese Academy of Sciences in Shanghai. The cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Carlsbad, CA, USA), supplemented with 10% FBS, and incubated at 37° C in a humidified incubator with 5% CO2.

CCK8 assay

Cell viability was assessed using the Cell Counting Kit‐8 (CCK8) from Beyotime (Shanghai, China) according to the manufacturer's instructions.Cells were seeded at a density of 5×10^3 cells per well in 100 μL of medium in 96‐well microplates (Corning, NY, USA). After 24 hours of treatment, 10 μL of CCK‐8 reagent was added to each well and incubated for 2 hours at 37° C. Each experiment was performed in triplicate. The absorbance was measured at 450 nm using a microplate reader (Bio‐Rad, Hercules, CA, USA), and the blank samples without cells were used for baseline correction.Cell proliferation was assessed based on the absorbance values obtained.

Colony formation assay

97H and Hep3B cell lines were seeded at a density of 3×10^3 cells per well in DMEM supplemented with 10% FBS in six‐well plates and cultured for 24 hours. After treatment, the cells were resuspended in DMEM supplemented with 10% FBS and cultured in a 5% CO_2, 37° C incubator for 15 days to promote colony formation. The plate was gently washed with cold PBS, and the colonies were fixed with 4% polyformaldehyde solution at room temperature. Then, the colonies were stained with 1% crystal violet solution for 30 minutes at room temperature. Colonies with more than 100 cells were counted under a microscope (Leica Microsystems, Germany). Each experiment was conducted in triplicate.

EdU staining assay

Cancer cell proliferation was assessed using EdU staining according to the following protocol. 97H and Hep3B cell lines were treated with EdU at a concentration of 20 mmol/L for 2 hours. The cells were fixed with 4% paraformaldehyde at room temperature for 20 minutes. Apollo staining, DNA staining, and fluorescence microscopy were performed for photographic preservation. The experiment was independently repeated three times.

Western blot

Total protein was extracted from the cells, and western blot analysis was performed according to the provided instructions. Subsequently, the membranes were incubated with primary antibodies at specified dilutions: AP1M2 (1:1000, proteintech, 10618-1-AP), MMP9 (1:1000, Affinity, #AF5228), E-cadherin(1:1000, CST, #AF0131), CHOP (1:1000, CST, #2895S), β-actin (1:1000,CST,3700S),and c-MYC (1:1000, CST, #13987),ERK(1:1000, Affinity, #AF0155),p-ERK (1:1000, Affinity, #AF1015),JNK(1:1000, Affinity, #AF6318),p-JNK(1:1000, Affinity, #AF3318). Signal detection was performed using the ECL chemiluminescence system.

Statistical analysis

Data are presented as mean ± standard deviation (SD).Differences between groups were analyzed using either the two-tailed paired t-test or the unpaired t-test.A P-value less than 0.05 was considered statistically significant.

Result

The expression of AP1M2 is upregulated in human HCC tissues.

The cohort data for this study was obtained from the XENA database, which included 110 GTEx normal cases, 50 TCGA para-cancer cases, and 371 TCGA tumor cases. The histogram illustrates an increased expression of AP1M2 in tumor samples compared to normal samples (Figure 1A). Paired plots were used to verify the expression of AP1M2 in normal and tumor samples, revealing a statistically significant difference (Figure 1B). Furthermore, we confirmed these results by analyzing the sequencing data from the GEO databases GSE50579, GSE101685, and GSE113996, which also showed higher expression of AP1M2 in tumor samples compared to normal samples (Figure 1C). The significant difference in AP1M2

expression between normal and tumor tissues suggests that patients with high AP1M2 mRNA expression are more likely to develop cancer cell metastasis compared to patients with low AP1M2 mRNA expression (Table 1). Receiver operating characteristic analysis showed that AP1M2 expression can be utilized as a differentiating factor, with an area under the curve of 0.654 for distinguishing AP1M2 expressing HCC from normal tissue (Figure 1D).

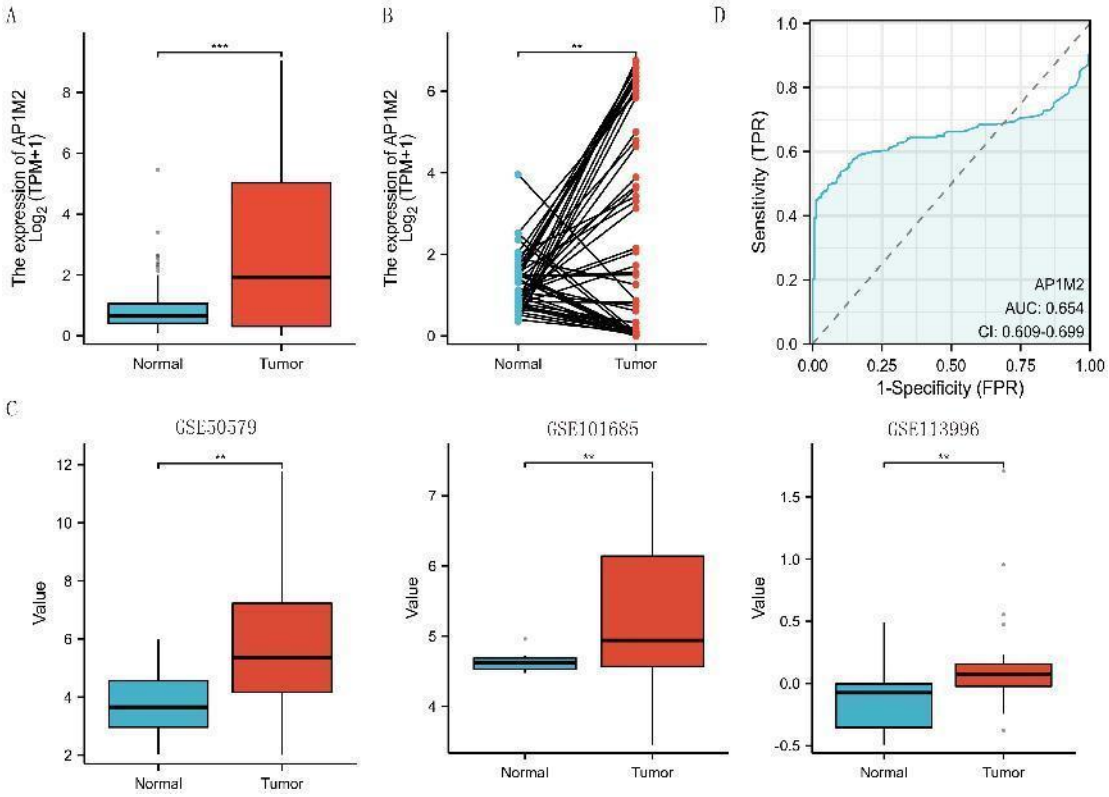

Figure 1．The expression difference of AP1M2 in normal tissue and tumor tissue.

(A)(B)Differential expression of AP1M2 in normal and tumor tissues. (C) The differential expression of AP1M2 between normal and tumor samples was validated using sequencing data from GEO databases GSE50579, GSE101685, and GSE113996.(D) The expression patterns of AP1M2 were analyzed in both normal and HCC tissues.

table1 . Correlation study between AP1M2 expression and clinicopathological features of hepatocellular carcinoma patients

Characteristics	Low expression of AP1M2	High expression of AP1M2	P value
n	187	187	
Pathologic T stage, n (%)			0.157
T1	101 (27.2%)	82 (22.1%)	
T2	42 (11.3%)	53 (14.3%)	
T3	39 (10.5%)	41 (11.1%)	
T4	4 (1.1%)	9 (2.4%)	
Pathologic N stage, n (%)			0.165
N0	133 (51.6%)	121 (46.9%)	
N1	4 (1.6%)	0 (0%)	
Pathologic M stage, n (%)			1.000
M0	135 (49.6%)	133 (48.9%)	
M1	2 (0.7%)	2 (0.7%)	
Pathologic stage, n (%)			0.496
Stage I	92 (26.3%)	81 (23.1%)	
Stage II	38 (10.9%)	49 (14%)	
Stage III	41 (11.7%)	44 (12.6%)	
Stage IV	3 (0.9%)	2 (0.6%)	
Histologic grade, n (%)			< 0.001
G1	44 (11.9%)	11 (3%)	
G2	88 (23.8%)	90 (24.4%)	
G3	46 (12.5%)	78 (21.1%)	
G4	6 (1.6%)	6 (1.6%)	
Gender, n (%)			0.151
Female	67 (17.9%)	54 (14.4%)	
Male	120 (32.1%)	133 (35.6%)	
Fibrosis ishak score, n (%)			0.796
0	41 (19.1%)	34 (15.8%)	

table1 . Correlation study between AP1M2 expression and clinicopathological features of hepatocellular carcinoma patients

Characteristics	Low expression of AP1M2	High expression of AP1M2	P value
1/2	16 (7.4%)	15 (7%)	
3/4	15 (7%)	13 (6%)	
5&6	38 (17.7%)	43 (20%)	
Vascular invasion, n (%)			0.018
No	114 (35.8%)	94 (29.6%)	
Yes	45 (14.2%)	65 (20.4%)	
Tumor status, n (%)			0.855
Tumor free	101 (28.5%)	101 (28.5%)	
With tumor	75 (21.1%)	78 (22%)	
Age, median (IQR)	59 (51, 68)	63 (54, 69)	0.033
AFP(ng/ml), n (%)			0.564
<= 400	108 (38.6%)	107 (38.2%)	
> 400	30 (10.7%)	35 (12.5%)	
Prothrombin time, n (%)			0.034
<= 4	96 (32.3%)	112 (37.7%)	
> 4	53 (17.8%)	36 (12.1%)	
Race, n (%)			0.941
Asian	81 (22.4%)	79 (21.8%)	
Black or African American	8 (2.2%)	9 (2.5%)	
White	91 (25.1%)	94 (26%)	

Increased AP1M2 expression is associated with a poor prognosis in patients with HCC

Patients with high AP1M2 expression in HCC exhibited a poorer prognosis than those with low AP1M2 expression (Figure 2A). A nomogram was constructed to assess the association between AP1M2 expression and other predictors (Figure 2B). Negative predictors were assigned higher total points in the nomogram. A bias correction line was constructed in the calibration plot to approximate the ideal 45-degree curve, indicating perfect agreement between prediction and observation (Figure 2C). Univariate analysis revealed significant associations between AP1M2 and T stage (hazard ratio [HR] 2.126; 95% CI 1.481-3.052), pathologic M stage (HR 4.077; 95% CI 1.281-12.973), tumor status (HR 2.317; 95% CI 1.590-3.376), and AP1M2 (HR 1.515; 95% CI 1.068-2.149; P = 0.020). The Cox proportional hazards model multivariate analysis identified AP1M2 expression (HR 1.582; 95% CI 1.004-2.492; P = 0.048), tumor status (HR 1.995; 95% CI 1.257-3.168; P = 0.003), pathologic T stage (HR 2.150; 95% CI 1.325-3.489; P = 0.002) as independent prognostic factors for HCC patients (Table 2).

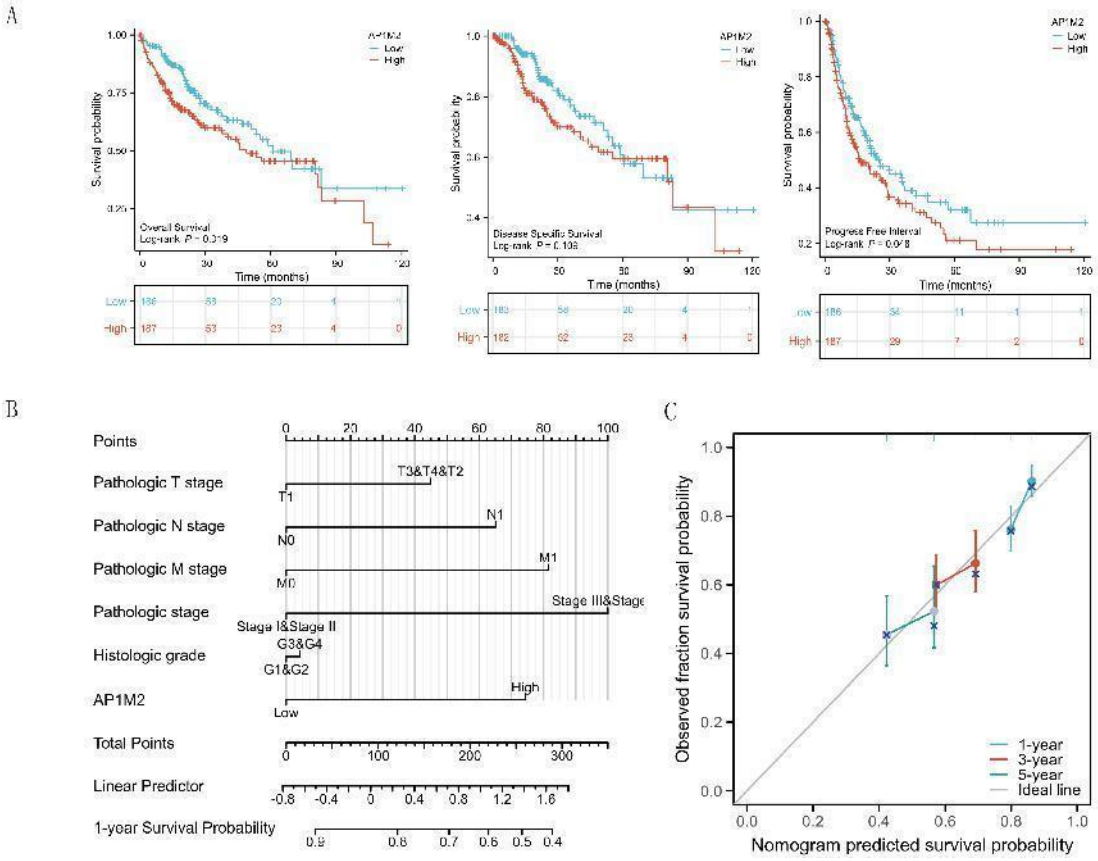

Figure 2．Effect of AP1M2 expression on survival outcome and multivariate examination. (A) The influence of AP1M2 expression levels on the prognosis and survival of hepatocellular carcinoma (HCC) patients. (B) Development of a predictive nomogram that integrates AP1M2 expression and clinical features to predict 1-year survival rates. (C) Calibration curves for precise prediction of survival outcomes.

table2. Univariate and multivariate analysis of the overall survival of patients with hepatocellular carcinoma

Characteristics	Total(N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Pathologic T stage	370		**< 0.001**		
T1	183	Reference		Reference	
T3&T4&T2	187	2.126 (1.481 - 3.052)	**< 0.001**	2.150 (1.325 - 3.489)	**0.002**
Pathologic N stage	258		0.375		
N0	254	Reference			
N1	4	2.029 (0.497 - 8.281)	0.324		
Pathologic M stage	272		0.050		
M0	268	Reference		Reference	
M1	4	4.077 (1.281 - 12.973)	**0.017**	1.762 (0.424 - 7.326)	0.436
Histologic grade	368		0.637		
G1&G2	233	Reference			
G3&G4	135	1.091 (0.761 - 1.564)	0.636		
Residual tumor	344		0.203		
R0	326	Reference			
R1&R2	18	1.604 (0.812 - 3.169)	0.174		
Age	373		0.293		
<= 60	177	Reference			
> 60	196	1.205 (0.850 - 1.708)	0.295		
Gender	373		0.204		
Female	121	Reference			
Male	252	0.793 (0.557 - 1.130)	0.200		
AFP(ng/ml)	279		0.773		
<= 400	215	Reference			
> 400	64	1.075 (0.658 - 1.759)	0.772		
Albumin(g/dl)	299		0.665		

table2. Univariate and multivariate analysis of the overall survival of patients with hepatocellular carcinoma

Characteristics	Total(N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
< 3.5	69	Reference			
>= 3.5	230	0.897 (0.549 - 1.464)	0.662		
Prothrombin time	296		0.178		
<= 4	207	Reference			
> 4	89	1.335 (0.881 - 2.023)	0.174		
Fibrosis ishak score	214		0.319		
0	75	Reference			
1/2&3/4&5&6	139	0.772 (0.465 - 1.281)	0.316		
Vascular invasion	317		0.169		
No	208	Reference			
Yes	109	1.344 (0.887 - 2.035)	0.163		
Tumor status	354		**< 0.001**		
Tumor free	202	Reference		Reference	
With tumor	152	2.317 (1.590 - 3.376)	**< 0.001**	1.995 (1.257 - 3.168)	**0.003**
AP1M2	373		**0.019**		
Low	186	Reference		Reference	
High	187	1.515 (1.068 - 2.149)	**0.020**	1.582 (1.004 - 2.492)	**0.048**

AP1M2 potentially functions as an oncogene in HCC by activating multiple signaling pathways.

We conducted RNAseq gene expression analysis to compare the gene expression profiles between the high and low expression groups of AP1M2, aiming to elucidate the role of AP1M2 in the occurrence and development of hepatocellular carcinoma (HCC). For the analysis of TCGA database data, we employed the cluster Profiler package in R language, using a cutoff of corrected p-value < 0.05 and log2FC > 1.5. Consequently, we identified a total of 612 DEGs, including 518 up-regulated genes and 94 down-regulated genes, and visualized their expression patterns in a volcano plot (Figure 3A). To gain a deeper understanding of the biological pathways involved in HCC pathogenesis stratified by AP1M2 expression levels, we performed gene set enrichment analysis (GSEA). The GSEA enrichment map revealed the top five significantly enriched pathways in patients with high AP1M2 expression, which encompassed fatty acid metabolism, metal ions, coagulation function, amino acid metabolism, and metallothionein-binding metal (Figure 3B).This suggests that AP1M2 may play a carcinogenic role in HCC by activating multiple signaling pathways.Additionally, we presented a graph illustrating the AP1M2 and its potential co-expressed gene network among the DEGs related to AP1M2 (Figure 3C).

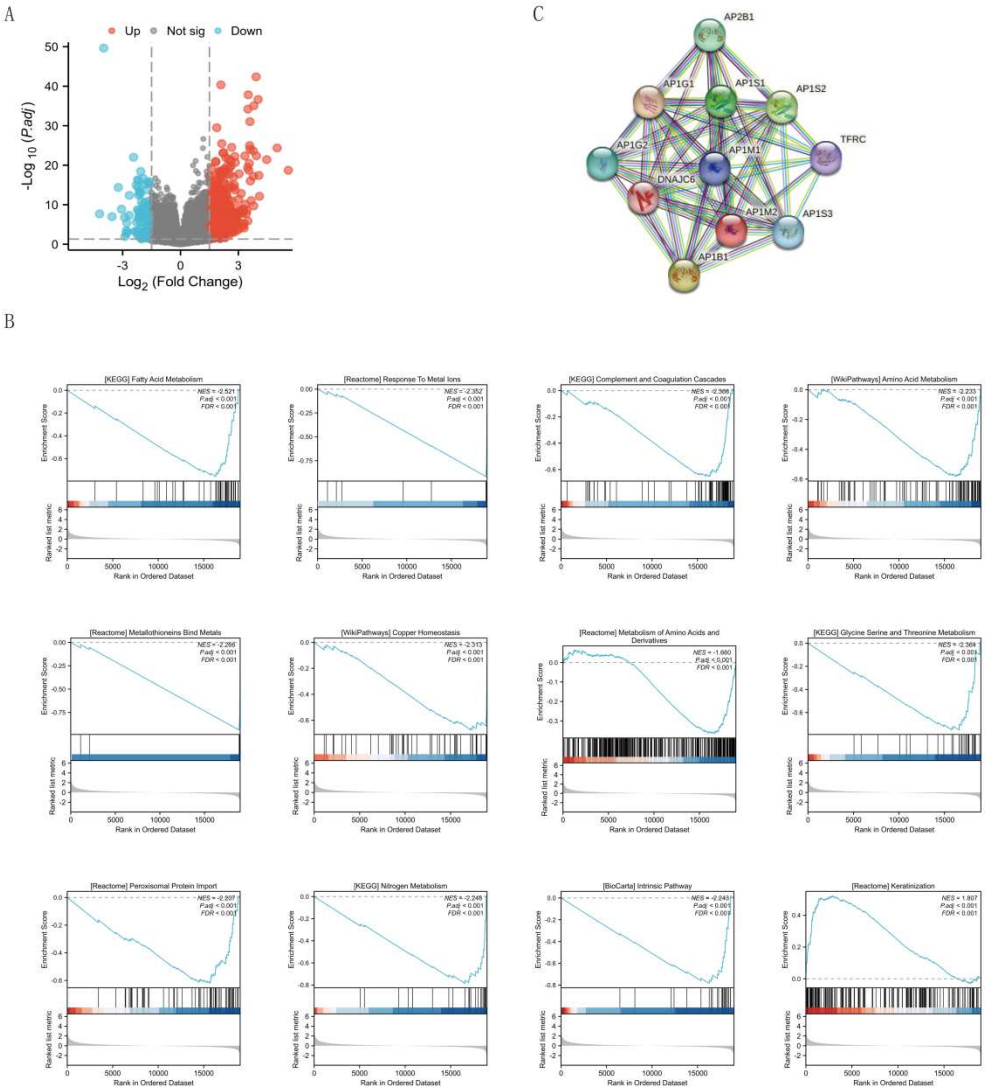

Figure 3．GSEA identified AP1M2-related signaling pathways

(A)Comparative analysis of AP1M2 expression in high and low expression groups within the volcanic atlas. (B) GSEA enrichment map to identify pathways enriched in patients with high AP1M2 expression. (C) Co-expression gene network map highlighting the potential interactions between AP1M2 and other differentially expressed genes (DEGs) associated with AP1M2.

GO and KEGG enrichment analysis of differential genes

We performed GO function and KEGG pathway enrichment analysis on the differentially expressed genes to gain further insights. In terms of biological processes, the differentially expressed genes were mainly enriched in stress response to metal ions, inorganic compound metabolism, and stress response to copper ions. In the "molecular function" category, the differentially expressed genes exhibited significant enrichment in transferase activity involving sulfur-containing groups and sulfotransferase activity. Additionally, the KEGG analysis revealed that the DEGs were predominantly enriched in Mineral absorption, Bile secretion, Pentose and glucuronate metabolism, and Ascorbate and aldarate metabolism, along with other signaling pathways. Previous studies have suggested that the proliferation and migration of HCC cells are influenced by lipid, bile acid, and mineral metabolism, which play crucial roles in the occurrence and development of HCC. Therefore, we plan to verify these mechanisms in future experiments (Figure 4).

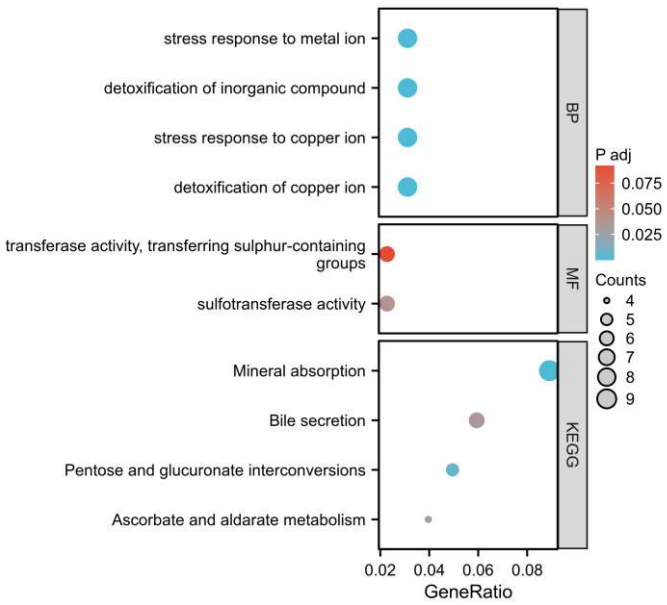

Figure 4．GO and KEGG enrichment analysis of differential genes

Enrichment analysis was conducted to investigate the functional implications of the differentially expressed gene AP1M2, employing the GO and KEGG databases.

Elevated AP1M2 expression could potentially impact the immune response in HCC

The Spearman correlation analysis was performed to examine the association between AP1M2 expression and the enrichment of immune cells using ssGSEA. The results revealed a positive correlation between AP1M2 expression and the presence of CD56+ NK cells, Th2 cells, and macrophages. Conversely, a negative correlation was observed with Th17 cells, CD8+ T cells, and eosinophils (Figure 5A, B). These findings indicate that AP1M2 may influence the immune response in liver cancer. However, further investigation is needed to understand the precise immunophenotype and underlying mechanisms.

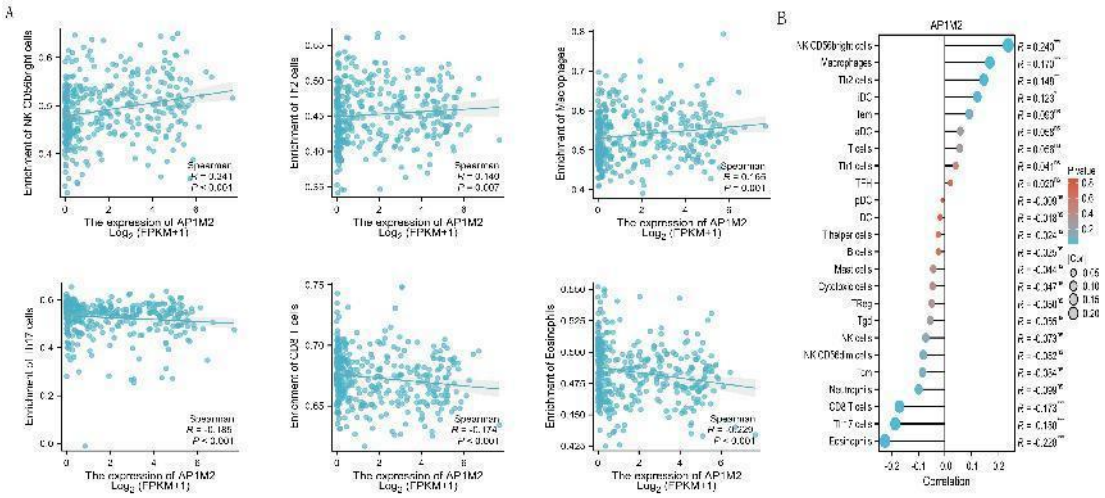

Figure 5．Correlation between AP1M2 expression and immune infiltration

(A)The correlation between AP1M2 expression and immune cell enrichment was assessed using Spearman correlation analysis. (B) The association between AP1M2 expression and immune cell infiltration.

AP1M2 promotes the proliferation and migration of hepatocellular carcinoma cells and activates the JNK/ERK pathway

To investigate the in vitro role of AP1M2, we conducted cell proliferation and migration assays using Hep3B and 97H cells. Our findings revealed that knockdown of AP1M2 resulted in a decrease in the proliferation ability of Hep3B and 97H cells, as demonstrated by colony formation assay, EdU, and CCK8 assays (Figure 6A, B, C, and D). Additionally, the transwell assay showed a reduction in the migration ability of Hep3B and 97H cells after AP1M2 knockdown (Figure 6E). To elucidate the downstream pathways through which AP1M2 influences proliferation and migration phenotypes, we performed a western blot analysis. The results demonstrated decreased phosphorylation levels of JNK and ERK proteins, as well as reduced protein expression levels of CHOP, E-cadherin, c-MYC, and MMP9, which are associated with proliferation and migration (Figure 6F).

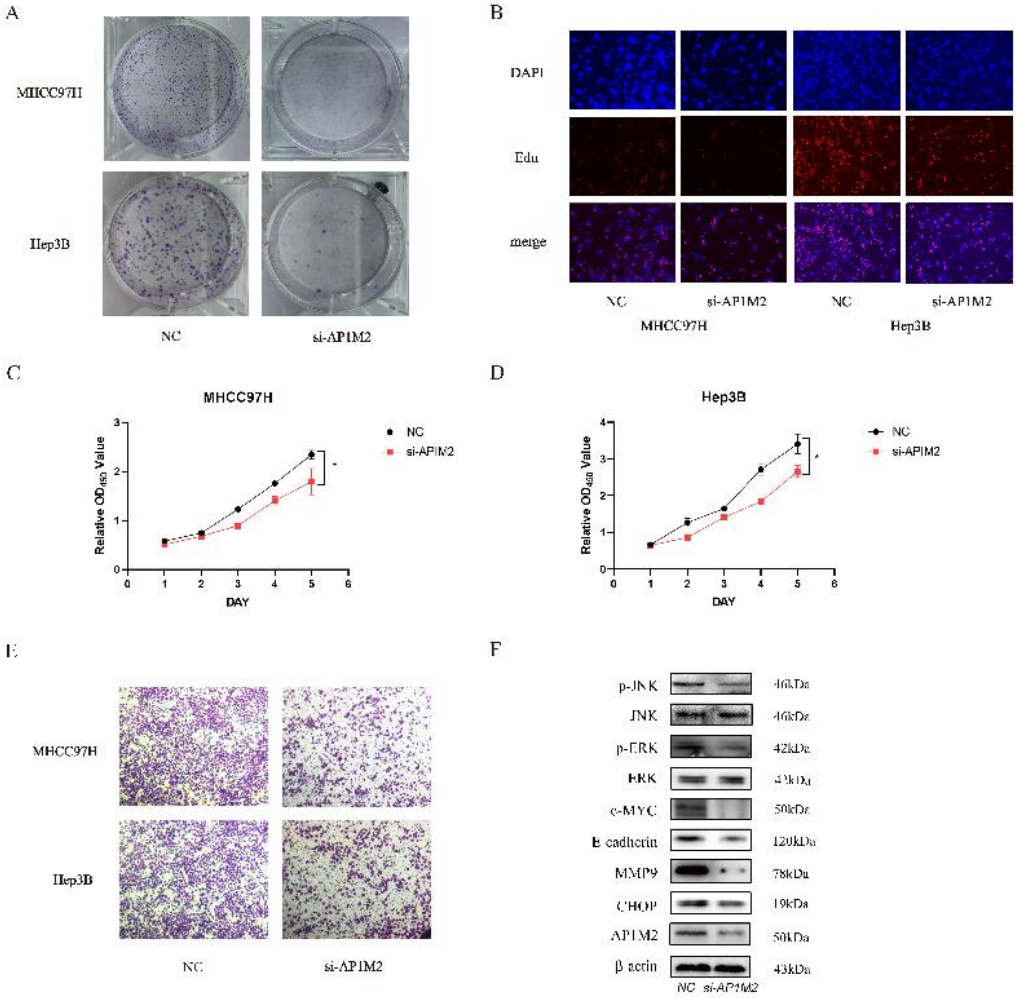

Figure 6．AP1M2 enhances the growth and movement of liver cancer cells and triggers the JNK/ERK pathway

(A) Hep3B and MHCC97H cells were plated and divided into two groups: the NC group and the si-AP1M2 group. (B) Hep3B and MHCC97H cells were stained with Edu and DAPI, respectively, and then divided into the NC group and the si-AP1M2 group. (C) (D) Hep3B and MHCC97H cells were subjected to a CCK8 assay, and the absorbance curve was determined by incubation for 2 hours over a period of 5 days. (E) A Transwell assay was performed using Hep3B and MHCC97H cells to assess cell migration capacity.(F) A Western blot assay was performed to identify the downstream signaling pathway involved in AP1M2-mediated proliferation and migration phenotypes.

Discussion

In this study, we evaluated the expression levels of AP1M2 in patients with hepatocellular carcinoma (HCC) using both the Gene Expression Omnibus (GEO) database and The Cancer Genome Atlas (TCGA) database. Our objective was to investigate the correlation between AP1M2 and clinicopathological features, as well as the overall survival of HCC patients. Additionally, we examined the impact of AP1M2 on patient survival. To gain further insights into the molecular mechanism underlying AP1M2 in HCC development, we analyzed the protein interactions of AP1M2. Furthermore, we conducted in vitro cell experiments to investigate the regulatory function of AP1M2 during HCC development.Our findings revealed a significant increase in AP1M2 expression levels not only in HCC tumor samples but also in various other tumor types, indicating its potential as a valuable target for the development of diagnostic strategies for HCC patients.

Histological grading of hepatocellular carcinoma enables the prediction of key factors such as survival, recurrence risk, and treatment response[11].In general, a higher histological grade corresponds to a greater degree of malignancy and a poorer prognosis[12]. The expression of AP1M2 in HCC demonstrates a potential correlation with histological grade, indicating that AP1M2 might serve as a predictive marker in assessing the malignancy and prognosis of HCC. The prognosis of HCC is commonly influenced by multiple factors, including tumor characteristics, treatment modalities, and patient-specific factors[13]. Survival rate analysis provides a valuable means to assess the influence of these factors on the overall survival of individuals diagnosed with hepatocellular carcinoma[14]. Our analysis of survival rates reveals a significant correlation between AP1M2 expression levels and the prognosis of patients with HCC. Specifically, patients exhibiting high AP1M2 expression exhibit a more unfavorable prognosis compared to those with low AP1M2 expression. Our study conducted a univariate logistic regression analysis to investigate the association between AP1M2 expression and two clinical parameters: vascular invasion and prothrombin time. The results indicated a significant relationship between AP1M2 expression and both vascular invasion and prothrombin time. Vascular invasion greatly impacts the prognosis of hepatocellular carcinoma by elevating the likelihood of tumor dissemination and recurrence[15]. Prolonged prothrombin time serves as an indicator not only of liver function but also of potential implications for the progression and prognosis of hepatocellular carcinoma[16][17][18]. Our findings suggest that elevated AP1M2 expression may serve as a potential marker for prolonged coagulation time, indicating compromised liver function, as well as a potential indicator of distant metastasis and dissemination of HCC cells. Further research is required to establish the clinical

significance of AP1M2 in hepatocellular carcinoma.

Through the application of GSEA analysis on liver cancer data from TCGA, we investigated the potential involvement of AP1M2 in hepatocellular carcinoma (HCC)-related signaling pathways. The analysis revealed the top five differentially enriched phenotypes, namely fatty acid metabolism, metalions, coagulation function, amino acid metabolism, and metallothionein-binding metal. Additionally, GO and KEGG analysis indicated significant enrichment in copper metabolism, pentose and glucuronate metabolism, ascorbate and aldarate metabolism, retinol metabolism, and bile acid metabolism. Previous studies have reported that increased fatty acid synthesis can promote the growth and proliferation of hepatocellular carcinoma cells, while abnormal fatty acid oxidation may disrupt energy metabolism and inhibit hepatocellular carcinoma cell proliferation[19][20][21]. Moreover, enhanced glucose uptake, activation of glycolytic pathways, and increased gluconeogenesis provide hepatocellular carcinoma with sufficient energy and building blocks for growth[22][23][24]. Furthermore, hepatocellular carcinoma cells in HCC patients exhibit enhanced uptake and utilization of copper, and elevated copper levels can potentially promote tumor growth and metastasis through involvement in angiogenesis [25][26][27]. By participating in abnormal changes in metabolism, coagulation function, angiogenesis, and invasion, AP1M2 may promote the progression of hepatocellular carcinoma.

Previous studies have shown that hepatocellular carcinoma (HCC) is correlated with decreased NK cell activity and numbers, leading to immune evasion by tumors and suppression of anti-tumor immune responses[28][29][30]. The activation and increased presence of TH2 cells can potentially weaken the cytotoxic effect of NK cells and additionally impair tumor immune surveillance[31][32][33]. Eosinophils have the ability to secrete various cytokines and mediators, such as interleukin-5, eosinophil protein, and eosinophil peroxidase. These secretions could potentially contribute to the proliferation, invasion, and metastasis of hepatocellular carcinoma cells[34][35]. It is worth noting that AP1M2 expression exhibits a positive correlation with CD56+ NK cells and a negative correlation with eosinophils, suggesting that AP1M2 may affect the immune response in hepatocellular carcinoma through these cellular components. However, additional experiments are required to explore the specific immunophenotype and underlying mechanisms.

However, the specific role of AP1M2 in hepatocellular carcinoma (HCC) remains unclear. In order to address this gap, we performed experiments on 97H and Hep3B cells to investigate the phenotypic

effects of AP1M2 in HCC.Our results showed that suppressing AP1M2 expression resulted in decreased proliferation and migration capabilities of both 97H and Hep3B cells..Moreover, Western blot analysis revealed that downregulating AP1M2 expression suppressed the activation of the JNK/ERK pathway. These in vitro findings corroborated the observed correlation between AP1M2 and clinical features identified in the preceding bioinformatics analysis, emphasizing the role of AP1M2 in cell proliferation, migration, and the JNK/ERK pathway.

This study employed an open database platform, which may have inherent limitations that need to be acknowledged. To address these limitations, it is crucial to augment our dataset with supplementary clinical data. Furthermore, additional evidence is needed to establish the correlation between the JNK/ERK signaling pathway and AP1M2. We plan to investigate these aspects in future experiments, which will enable us to acquire a more profound understanding of these relationships.

Conclusions

In summary, the expression of AP1M2 holds promise as a valuable molecular marker for predicting an unfavorable prognosis in HCC.Moreover, AP1M2 has been demonstrated to impact HCC cell proliferation and migration, with the JNK/ERK signaling pathway potentially serving as a vital mediator of AP1M2's effects. However, additional experimental investigations are necessary to achieve a comprehensive understanding of the biological effects and underlying mechanisms through which AP1M2 operates in HCC.

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