Significance of HMMR Overexpression in Hepatocellular Carcinoma

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Research Article

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Abstract

Background: Hyaluronan Mediated Motility Receptor (HMMR), as one of the key surface binding proteins of HA, is up-regulated in many tumors. What's more, the expression level of HMMR is usually correlate with tumor progression and prognosis. However, the relationship between the expression of HMMR and the prognosis and immune infiltration of hepatocellular carcinoma (HCC) is still unclear.

Methods: We analyzed the expression level of HMMR by TIMER database, GEO database and GEPIA database. The correlation between the HMMR expression and tumor prognosis was analyzed via the Kaplan-Meier plots. The TIMER database and GEPIA database were used to study the relationship between HMMR expression and immune infiltration. GO and KEGG enrichment analysis were used to explore the potential biological functions of HMMR.

Results: HMMR expression was significantly higher in several human cancers, including HCC, than in corresponding normal tissues. High HMMR expression associated with poorer overall survival, disease-specific survival, progression-free survival and relapse-free survival in HCC patients. HMMR showed strong correlation with tumor-infiltrating B cells, CD4 + and CD8 + T cells, macrophages, neutrophils, and dendritic cells. Several immune marker genes expression, including CD86, IRF5, CD11b, KIR2DL4, CD11c, IFN- γ , STAT3, STAT5B, and CTLA4, have markedly positive correlations with HMMR expression. Enrichment analysis found that HMMR is mainly involved in cell cycle, DNA replication and repair, PLK1 pathway, E2F pathway, ATR pathway and AURORA B pathway.

Conclusions: HMMR is a potential prognostic biomarker that influence tumor progression and correlated with tumor immune cells infiltration in HCC.

Introduction

Hepatocellular carcinoma is one of the most malignant tumors, and its incidence rate is increasing year by year [1]. The main factors include liver cirrhosis, viral hepatitis, aflatoxin and chemical carcinogens, among which chronic hepatitis virus infection is the most important factor [2–3]. Only about 20% of patients with HCC are in the early stages at the initial diagnosis and have the chance for surgery [4]. Most of the patients can't be completely cured because of complications and distant metastasis [5–8]. In addition, HCC is prone to recrudescence, which further shortens the survival time of patients. Therefore, it is particularly important to further explore the occurrence and development mechanism of liver cancer and find a new treatment model.

Previous studies have shown that hyaluronic acid (HA) is related to the growth, metastasis and anti-apoptosis of a variety of tumors [9–10]. As one of the key surface binding proteins of HA, HMMR expression level is related to the cell cycle, mainly peaking in the late G2 and early mitosis [11]. Consistently, HMMR expression is low in most healthy tissues but is elevated in proliferative tissues, such as the testis, spleen, placenta, thymus [12–13] and tumor tissues, such as lung cancer [14], breast cancer [15], Endometrial carcinoma [16], rectal cancer [17], Multiple myeloma and prostatic cancer [18–19].

Besides, HMMR expression is usually related to tumor progression and metastasis [20–22]. This study aims to investigate the role of HMMR in the development of hepatocellular carcinoma

Materials And Methods

HMMR expression level analysis

The expression mRNA levels of mRNA HMMR in cell lines were obtained from the HPA (<https://www.proteinatlas.org/>), which contains the human transcriptomic and proteomic data in cells, tissues and organs from human normal or pathological tissues via RNA sequencing (RNA-Seq) analysis [23]. The HMMR expression in several cancers including HCC were identified from the TIMER database (<http://timer.cistrome.org/>), an online analysis website for The Cancer Genome Atlas (TCGA) [24], and the GEPIA database (<http://gepia.cancer-pku.cn/>), an online analysis website for The Cancer Genome Atlas (TCGA) and Gene Tissue Expression (GTEx) databases [25]. The correlation between HMMR expression and clinicopathological features of HCC patients was analyzed by UALCAN (<http://ualcan.path.uab.edu/index.html>) [26].

The Gene Expression Omnibus (GEO) database analysis

HMMR expression profiles matrix files from GSE54236 based on platform GPL6480 (including 81 HCC samples and 80 adjacent noncancerous samples), GSE76427 based on platform GPL10558 (including 115 HCC samples and 52 adjacent noncancerous samples), and GSE14520 based on platform GPL3921 (including 225 HCC samples and 220 adjacent noncancerous samples) were downloaded from the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>) [27].

Kaplan-Meier survival curve analysis

The correlation between HMMR expression and tumor prognosis in liver, gastric, breast, lung, and ovarian cancers were analyzed by the Kaplan-Meier plots (<http://www.kmplot.com/analysis/>), an online analysis website for survival analysis [28].

Tumor immune infiltration analysis

Using TIMER database and GEPIA database, we investigated the correlation between HMMR expression and immune infiltration levels in HCC. At the same time, we further explored the relationship between immune infiltration and prognosis of HCC patients.

Functional and pathway enrichment analysis

The top 200 differential genes in LIHC, including top 100 over-expression genes and top 100 under-expression genes, obtained from GEPIA database were analyzed by using tools in Metascape (<https://metascape.org/>) to study KEGG and GO annotation [29].

Statistical analysis

Statistical analysis was performed using SPSS Statistics 26.0. Two-sample Student's t-test was used if the values in each group were normally distributed; otherwise, the Mann–Whitney U test was used to analyze the expression of HMMR in normal and tumor samples. $p < 0.05$ was considered as statistically difference.

Results

HMMR overexpression in hepatoma cell line

To explore the significance of HMMR expression in liver cancer cells, we analyzed HMMR expression based on the RNA-Seq data obtained from cell lines by HPA databases. The results showed that HMMR was significantly overexpressed in hepatoma cell line compared with normal hepatocyte line (Fig. 1).

Expression of HMMR in hepatocellular carcinoma and other tumors

Using the TIMER and GEPIA databases, we subsequently tested the HMMR expression in different human tumor samples. Compared with the corresponding normal tissues, HMMR is highly expressed in a variety of tumor tissues, including bladder cancer, breast cancer, cholangiocarcinoma, colorectal cancer, pancreatic cancer, gastric cancer, lung cancer, hepatocellular carcinoma and so on (Fig. 2A). Similar results were obtained from GEPIA database in a variety of tumors, except in acute myeloid leukemia and testicular germ cell tumors (Fig. 2B, 2C). To verify the above results, the gene expression profile matrix files from GSE54236 based on platform GPL6480, GSE76427 based on platform GPL10558 and GSE14520 based on platform GPL3921 were downloaded from the Gene Expression Omnibus (GEO) database. In the above three datasets, the expression of HMMR was significantly overexpressed compared with corresponding normal tissues in HCC (Fig. 2D, 2E, 2F). Further analysis indicated that HMMR expression level was significantly correlated to tumor stage and grade, as well as TP53 gene status by UALCAN database (Fig. 3A-3D).

Prognostic values of HMMR in hepatocellular carcinoma and other cancers

In addition, we explored the prognostic significance of HMMR expression in human cancers by using the Kaplan-Meier plotter database. The results show that higher HMMR expression was associated with poorer prognosis in HCC (OS: HR=2.29(1.62-3.24), $P=1.3e-06$; PFS: HR=1.92(1.43-2.59), $P=1.2e-05$; RFS: HR=1.99(1.42-2.78), $P=3.8e-05$; DSS: HR=2.56(1.64-3.98), $P=1.7e-05$; Fig. 4A-4D), breast cancer (OS: HR=1.38(1.14-1.67), $P=0.0073$; PPS: HR=1.33(1.05-1.67), $P=0.017$; RFS: HR=1.68(1.51-1.86), $P<1e-16$; DMFS: HR=1.68(1.44-1.97), $P=5.4e-11$; Fig. 4E-4H), gastric cancer (OS: HR=0.56(0.46-0.69), $P=1.2e-08$; RFS: HR=2.45(1.2-4.99), $P=0.011$; Fig. 4I-4J), lung cancer (OS: HR=1.44(1.27-1.64), $P=1.7e-08$; FP: HR=1.38(1.14-1.68), $P=0.00086$; Fig. 4K, 4M), ovarian cancer (OS: 1.29(1.13-1.47), $P=0.00017$; PFS:

HR=1.24(1.08-1.43), P=0.0028; Fig. 4N-4O). However, the expression of HMMR was not related to PPS in lung cancer and ovarian cancer (Fig. 4L, 4P).

Correlation between HMMR expression and prognosis of HCC patients under different clinical characteristics

Subsequently, we analyzed the relationship between the expression of HMMR and the prognosis of patients with liver cancer under different clinical characteristics by using the Kaplan Meier plotter database. The results show that high HMMR expression correlated with poorer prognosis in stage1+2 (OS: HR=2.74(1.68-4.47), P=2.4e-05; PFS: HR=1.97(1.31-2.94), P=0.00081; Fig. 5A, 5C), stage3+4 (OS: HR=2.77(1.23-6.25), P=0.011; Fig. 5B), grade1 (OS: HR=6.5(2.17-19.48), P=0.00022; Figure 5E), grade2 (OS: HR=2.05(1.22-3.45), P=0.0056; PFS: 2.74(1.74-4.3), P=5.4e-06; Fig. 5F, 5I), grade 3 (OS: 2.71(1.47-5.01), P=0.00093; PFS: HR=1.93(1.15-3.25), P=0.012; Fig. 5G, 5J), AJCC_T1 (OS: HR=2.61(1.42-4.8), P=0.0013; PFS: HR=1.77(1.03-3.03), P=0.035; Fig. 5K, 5N), AJCC_T2 (OS: HR=3.14(1.09-9.09), P=0.026; PFS: HR=2.18(1.25-3.81), P=0.0048; Fig. 5L, 5O), AJCC_T3 (OS: HR=2.39(1.09-5.23), P=0.025; Figure 5M), male (OS: HR=2.53(1.62-3.94), P=2.1e-05; PFS: HR=2.32(1.62-3.34), P=2.3e-05; Fig. 5Q, 5S), female (OS: HR=2.2(1.25-3.85), P=0.0048; PFS: HR=1.85(1.03-3.32), P=0.038; Fig. 5R, 5T), drinker (OS: HR=2.74(1.25-6.02), P=0.0092; PFS: HR=2.91(1.74-4.89), P=2.3e-05; Figure 5U, 5W), non-alcoholics (OS: HR=2.74(1.64-4.15), P=2.8e-05; PFS: HR=1.78(1.18-2.69), P=0.0053; Fig. 5V, 5X), hepatitis patients(OS: HR=3.0(1.56-5.76), P=0.00052; PFS: HR=1.91(1.2-3.04), P=0.0053; Fig. 5Y, 5AA), non-hepatitis patients(OS: HR=2.81(1.76-4.49), P=6.8e-06; PFS: 2.77(1.74-4.41), P=8.2e-06; Fig. 5Z, 5AB). However, the expression of HMMR did not correlate with PFS in stage3+4(PFS: HR=1.67(0.98-2.84), P=0.055; Fig. 5D), grade1(PFS: HR=1.91(0.81-4.52), P=0.14; Fig. 5H) and AJCC_T3(PFS: HR=1.72(0.98-3.04), P=0.058; Fig. 5P). These results explain that prognostic significance of HMMR expression in HCC patients based on their clinical factors.

Correlation between immune infiltration and expression of HMMR in HCC

Tumor infiltrating lymphocyte is reported to be an independent factor for multiple tumor site healing [30]. We used the TIMER database to analyze the correlation between HMMR expression and immune infiltration levels in HCC. The results showed that several immune cell infiltration levels seemed to associate with altered HMMR gene copy numbers, including CD4+T cells, macrophages, and neutrophil in HCC (Fig. 6A). Then, we also found that HMMR expression has markedly positively correlated with infiltrating levels of B cell, CD4+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells in HCC (Fig. 6B). Finally, we further evaluated the impact of immune infiltration on the prognosis of HCC patients. The results indicated that high levels of CD4+ T cells, macrophages, and neutrophils were associated with poor prognosis of HCC patients within two years of survival (Fig. 6C). Subsequently, we further explored the relationship between HMMR expression and immune marker gene expression in HCC using the TIMER database [31], the results showed that several marker genes expression, including CD86, IRF5, CD11b, KIRIDL4, CD11c, IFN- γ , STAT3, STAT5B, CTLA4, TIM-3, have markedly positive correlations

with HMMR expression (Table. 1). We also investigated the correlation between HMMR expression and the above immune marker genes in the GEPIA database in HCC, the results similar to TIMER database (Table 2). These results suggested that the level of immune infiltration was affected by the expression of HMMR.

Functional enrichment analysis

The top 200 differential genes in LIHC, including top 100 over-expression genes and top 100 under-expression genes, obtained from GEPIA database, were analyzed by using tools in Metascape to study KEGG and GO annotation (Supplement file1). The top 20 clusters of enriched sets are shown in Fig. 7. The results indicated that enrichment terms related to HMMR were mainly enriched in “cell cycle,” “meiotic cell cycle,” “DNA replication and repair,” “PLK1 pathway,” “E2F pathway,” “ATR pathway,” and “AURORA B pathway,” in HCC.

Table 1
Correlation analysis between HMMR and relate genes and markers of immune cells in TIMER.

Description	Gene markers	HCC			
		None		Purity	
		Core	P	Core	P
CD8+ T cell	CD8A	0.139	7.49e-3	0.232	1.36e-5
	CD8B	0.096	0.063	0.184	5.90e-4
T cell (general)	CD3D	0.154	3.02e-3	0.257	1.30e-6
	CD3E	0.12	0.021	0.251	2.36e-6
	CD2	0.121	0.020	0.246	3.62e-6
B cell	CD19	0.204	7.26e-5	0.271	3.31e-7
	CD79A	0.065	0.212	0.155	3.83e-3
Monocyte	CD86	0.266	2.25e-7	0.412	1.44e-15
	CD115	0.138	7.97e-3	0.27	3.59e-7
TAM	CCL2	0.023	0.656	0.13	0.016
	CD68	0.186	3.12e-4	0.268	4.16e-7
	IL10	0.223	1.39e-5	0.328	4.20e-10
M1 Macrophage	IRF5	0.407	2.88e-16	0.411	1.75e-15
	COX2	0.071	0.171	0.197	2.38e-4
M2 Macrophage	CD163	0.153	3.14e-3	0.261	8.48e-7
	VSIG4	0.138	7.78 e-3	0.254	1.79e-6
	MS4A4A	0.142	6.12e-3	0.271	3.29e-7
Neutrophils	CD66b	0.096	0.066	0.12	0.026
	CD11b	0.333	6.06e-11	0.439	1.16e-17
	CCR7	0.045	0.385	0.162	2.60e-3
Natural killer cell	KIR2DL1	-0.023	0.656	-0.036	0.510
	KIR2DL3	0.184	3.81e-4	0.226	3.19e-5
	KIR2DL4	0.202	9.20e-5	0.23	1.59e-5
	KIR3DL1	0.068	0.189	0.085	0.116

Description	Gene markers	HCC			
		None		Purity	
		Core	P	Core	P
	KIR3DL2	0.057	0.270	0.09	0.093
	KIR3DL3	0.067	0.199	0.059	0.278
	KIR2DS4	0.076	0.142	0.073	0.177
Dendritic cell	HLA-DPB1	0.139	7.42e-3	0.251	2.46e-6
	HLA-DQB1	0.111	0.033	0.215	5.66e-6
	HLA-DRA	0.198	1.28 e-4	0.321	1.05e-9
	HLA-DPA1	0.168	1.22e-3	0.166	1.99e-3
	BDCA-1	0.066	0.204	0.162	2.55e-3
	BDCA-4	0.195	1.50e-4	0.227	2.01e-5
	CD11c	0.31	1.26e-9	0.437	1.72e-17
Th1	T-bet	0.05	0.341	0.14	9.11e-3
	STAT4	0.179	5.46e-4	0.239	6.93e-6
	STAT1	0.372	1.83e-13	0.412	1.41e-15
	IFN- γ	0.219	2.11e-5	0.296	2.20e-8
	TNF- α	0.241	2.7e-6	0.366	2.26e-12
Th2	GATA3	0.135	9.13e-3	0.255	1.64e-6
	STAT6	0.192	2.04e-4	0.171	1.40e-3
	STAT5A	0.235	4.76e-6	0.286	6.69e-8
	IL13	0.13	0.012	0.133	0.013
Tfh	BCL6	0.207	6.07e-5	0.202	1.59e-4
	IL21	0.161	1.92e-3	0.199	2.06e-4
Th17	STAT3	0.195	1.57e-4	0.232	1.30e-5
	IL17A	0.109	0.035	0.115	0.033
Treg	FOXP3	0.271	1.14e-7	0.354	1.24e-11
	CCR8	0.418	4.04e-17	0.507	5.97e-24

Description	Gene markers	HCC			
		None		Purity	
		Core	P	Core	P
	STAT5B	0.355	2.72e-12	0.34	9.07e-11
	TGFβ	0.174	7.74e-4	0.257	1.37e-5
T cell exhaustion	PD-1	0.196	1.54e-4	0.27	3.60e-7
	CTLA4	0.244	1.99e-6	0.348	3.06e-11
	LAG3	0.205	7.06e-5	0.244	4.37e-6
	TIM-3	0.281	4.33e-8	0.428	7.80e-17
	GZMB	0.048	0.355	0.116	0.031

Table 2
Correlation analysis between HMMR and marker genes of immune cells in GEPIA.

Description	Gene markers	HCC			
		Tumor		Normal	
		R	P	R	P
Monocyte	CD86	0.23	5.2e-6	0.063	0.66
M1 Macrophage	IRF5	0.3	3.1e-9	0.031	0.83
Neutrophils	CD11b	0.38	8.8e-14	0.22	0.12
Natural killer cell	KIR2DL4	0.22	2e-5	0.23	0.11
Dendritic cell	CD11c	0.2	8.5e-5	-0.015	0.92
Th1	IFN-γ	0.2	8.3e-5	0.22	0.13
Th17	STAT3	0.35	6.4e-12	-0.11	0.43
Treg	STAT5B	0.31	1.4e-9	-0.065	0.65
T cell exhaustion	CTLA4	0.22	2.2e-5	0.3	0.035
	TIM-3	0.076	0.15	0.02	0.89

Discussion

Hyaluronan Mediated Motility Receptor (HMMR) is a centrosome and microtubule-associated protein that regulates cell growth. Previous studies report that HMMR expression was overexpressed in various types of cancers, including lung cancer [14], breast cancer [15], Endometrial carcinoma [16], rectal cancer [17], Multiple myeloma and prostatic cancer [18–19]. In the current study, we first explored HMMR expression in liver cancer cell line and normal liver cell line using HPA datasets. Subsequently, we studied the expression of HMMR in HCC and paired normal tissues by using TIMER database, GEPIA database and GEO database. All results indicated that HMMR expression was up-regulated in hepatocellular carcinoma compared with the corresponding normal tissues, Subgroup analysis showed that HMMR expression level was significantly correlated to tumor stage, tumor grade, and TP53 gene status in HCC by UALCAN database, which indicated that HMMR expression might play a critical role in tumor initiation and development and may be regulated by TP53.

Recently, a growing number of evidence suggests that HMMR high expression of HMMR is associated with poor prognosis in a variety of tumor types, such as gastric cancer [32], head and neck squamous cell carcinoma [33], lung cancer [14], breast cancer [15] and so on. In our study, Kaplan-Meier Plotter analyses showed that high HMMR expression correlated with worse prognosis in liver, lung, breast, gastric, ovarian cancer. That is consistent with previous literature reports. Therefore, HMMR may be a potential predictor of HCC prognosis.

Additionally, we also explore the relationship between HMMR expression and infiltration status of immune cells in HCC. The result showed that several immune cell infiltration levels seemed to associate with altered HMMR gene copy numbers, including CD4+T cells, macrophages, and neutrophil, HMMR expression has markedly positively correlated with infiltrating levels of B cell, CD4+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells. We also found that high infiltration levels of immune cell, especially CD4+ T cells, macrophages and neutrophils, were associated with poor prognosis within two years of survival in HCC. This suggests that HMMR plays an important role in regulating tumor immunity, and therefore influences HCC prognosis. Finally, we found in both TIMER and GEPIA databases that several marker genes expression, including CD86, IRF5, CD11b, KIR2DL4, CD11c, IFN- γ , STAT3, STAT5B, CTLA4, have markedly positive correlations with HMMR expression.

As a HA receptor, HMMR can play a role in a variety of biological functions that lead to the development of tumors [34]. Enrichment analysis found that HMMR is mainly involved in cell cycle, DNA replication and repair, PLK1 pathway, E2F pathway, ATR pathway and AURORA B pathway, which is consistent with previous research [34–37], PLK1 pathway, E2F pathway, ATR pathway and AURORA B pathway, the role of these pathways in initiation and development of HCC has been reported in previous studies [38–43].

In summary, our study elaborated that relationship between HMMR and HCC, and HMMR is a potential independent prognostic biomarker for HCC. In order to validate these results, we intend to establish *in vivo*, *in vitro* and animal experiments in follow-up studies.

Declarations

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Statements

The data in this study are all from TCGA and GEO. The results in this study are in whole or part based upon data generated by the TCGA and GEO Research Network

Author Contributions

Z.Z.X. designed the study; Z.W.D. analyzed the data and wrote the paper. All authors have read and approved the manuscript.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Availability of Data and Materials

Dataset supporting our findings is available, at the following website: www.ncbi.nlm.nih.gov/geo/.

All data generated or analyzed during this study are available from the corresponding author on reasonable request.

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Figures

Figure 1

HMMR expression in normal and tumor cell lines.

Figure 2

HMMR expression in different types of human cancers. (A) The expression level of HMMR in different human cancer tissues from the TCGA database in TIMER. (B). The expression level of HMMR in different human cancer tissues from the TCGA database in GEPIA. (C). HMMR expression in HCC and normal tissues analyzed by GEPIA. (D, E, F). The expression level of HMMR in HCC from the GEO database (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

Figure 3

Relationship between expression of HMMR and clinicopathological characteristics in HCC (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

Figure 4

Kaplan-Meier survival curve analysis of the prognostic significance of high and low expression of HMHR in different types of human cancers using the Kaplan-Meier plotter database (A-P)

Figure 5

Kaplan-Meier survival curve analysis of the prognostic significance of high and low expression of HMHR in different clinicopathological factors of HCC by Kaplan-Meier plotter database (A-AB)

Figure 6

Correlation analysis of HMMR expression and infiltration levels of immune cells in HCC tissues using the TIMER database. (A) Association between immune cell infiltration levels and HMMR gene copy numbers. (B) Correlation of HMMR expression with immune infiltration level in HCC. (C) Kaplan-Meier plots were used to analyze the immune infiltration and overall survival rate of HCC. * $p < 0.05$, ** $p < 0.01$.

Figure 7

Functional enrichment of HMMR in HCC.

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