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DDR1 is a Novel Biomarker and Potential Therapeutic Target for the Combination Treatment of Liver Hepatocellular Carcinoma

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Abstract

Aim: This study aimed to investigate the role of discoidin domain receptor tyrosine kinase 1 (DDR1) in liver hepatocellular carcinoma (LIHC) and to evaluate its prognostic value on patient response to combination therapy.

Methods: In the current retrospective study, we examined the protein expression of DDR1 in various cancers by standard immunohistochemical (IHC) methods and evaluated its clinical significance in LIHC personalized treatment. Multiple online databases, including The Cancer Genome Atlas (TCGA), TIMER, GEO, ROC Plotter, and Genomics of Drug Sensitivity in Cancer (GDSC), were used.

Results: DDR1 protein expression was higher in LIHC than in other nine examined cancer types. Additionally, DDR1 exhibited higher expression levels in adjacent normal tissues compared to HBs-positive LIHC tissues. Analysis at single-cell resolution revealed that DDR1 was expressed primarily in epithelial cells but not in stromal and immune cells, and DDR1 expression was lower in HBs-positive LIHC cells in comparison with normal hepatocytes. Correlation of DDR1 upregulation and sorafenib resistance was observed in the patient cohort. Moreover, DDR1 expression positively correlated with the expression of inflammatory response-related genes, ECM-related genes, and collagen formation-related genes, but negatively correlated with the infiltration of $CD8^+$ T cells, NK cells, and dendritic cells in LIHC.

Conclusions: Our findings suggest that DDR1 expression might be induced by collagen production-related cellular events involved in liver injury and repair, and that DDR1 overexpression might contribute to the resistance to LIHC targeted therapy and immunotherapy, highlighting DDR1 as a potential prognostic biomarker and therapeutic target.

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Plain Language Summary

This study aimed to investigate the role of discoidin domain receptor tyrosine kinase 1 (DDR1) in liver hepatocellular carcinoma (LIHC) and to evaluate its prognostic value on patient response to combination therapy. In the current retrospective study, we examined the protein expression of DDR1 in various cancers by standard immunohistochemical (IHC) methods and evaluated its clinical significance in LIHC personalized treatment. Multiple online databases, including The Cancer Genome Atlas (TCGA), TIMER, GEO, ROC Plotter, and Genomics of Drug Sensitivity in Cancer (GDSC), were used. DDR1 protein expression was higher in LIHC than in other nine examined cancer types. Additionally, DDR1 exhibited higher expression levels in adjacent normal tissues compared to HBs-positive LIHC tissues. Analysis at single-cell resolution revealed that DDR1 was expressed primarily in epithelial cells but not stromal cells and immune cells, and DDR1 expression was lower in HBs-positive LIHC cells in comparison with normal hepatocytes. Correlation of DDR1 upregulation and sorafenib resistance was observed in patient cohort. Moreover, DDR1 expression positively correlated with the expression of inflammatory response-related genes, ECM-related genes, and collagen formation-related genes but negatively correlated with the infiltration of CD8 + T cells, NK cells, and dendritic cells in LIHC. Our findings suggest that DDR1 expression might be induced by collagen production-related cellular events involved in liver injury and repair and that DDR1 overexpression might contribute to the resistance to LIHC targeted therapy and immunotherapy, highlighting DDR1 as a potential prognostic biomarker and therapeutic target.

Keywords

liver hepatocellular carcinoma, discoidin domain receptor tyrosine kinase 1, hepatitis B virus, sorafenib, immunotherapy

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Introduction

Liver hepatocellular carcinoma (LIHC) is a prevalent malignant tumor worldwide and is the third leading cause of cancer-related death.[1] Despite substantial progress in cancer detection and treatment options, most patients are diagnosed at advanced stages and missed the opportunity for curative treatment, resulting in the poor clinical outcomes.[2-5] Although targeted therapy and the emerging immunotherapeutic strategies have exhibited encouraging therapeutic efficacy in certain LIHC patients, the majority of patients did not benefit from these therapies due to the apparent heterogeneity and the complex tumor immune microenvironment (TIME) of LIHC.[6,7] Therefore, it is crucial to identify effective predictors to stratify patients for personalized therapy. Discoidin domain receptor tyrosine kinase 1 (DDR1), a receptor activated by fibrillar collagen, the major component of the extracellular matrix (ECM), has been reported to be implicated in the development of numerous invasive cancers.[8,9] However, the specific role of DDR1 in LIHC and its clinical implications have not been fully explored.

The current study aims to investigate the expression patterns of DDR1 in LIHC and to evaluate its predictive value on patient response to combination therapy, facilitating the development of personalized therapeutic strategies for LIHC patients.

Materials and Methods

Immunohistochemical Staining

In the current retrospective study, we employed a standard immunohistochemistry (IHC) method as previously described to evaluate the protein expression level of DDR1 across various cancer types.[10] The sample collection procedure was approved by the ethics committee of Changzheng Hospital (2016SL018), and written informed consent was obtained from all study participants. Briefly, cancer tissues were fixed in formalin and embedded in paraffin for serial sectioning at a thickness of 5 μm before DDR1 immunostaining. Immunoblotting was performed using a primary antibody (Cell Signaling Technology, USA) and a secondary antibody (anti-rabbit) following the manufacturer's instructions. The assessment of immunostaining was conducted objectively with consideration of both the proportion of cells exhibiting positive staining and the intensity of the staining itself via the use of ImageScope software (Aperio Technologies, Inc.).[11] The reporting of this study conforms to remark guidelines.[12]

Specifically, we assessed ten different cancer types, namely, liver cancer, prostate cancer, kidney cancer, bile duct cancer, pancreatic cancer, colon cancer, stomach cancer, bladder cancer, lymphoma, and melanoma. For each cancer type, we analyzed a total of five cases to ensure a robust representation of DDR1 expression patterns.

Single-Cell Analysis

To elucidate the expression and distribution of DDR1 at the single-cell level, we downloaded single-cell RNA-seq data for HBsAg-positive liver cancer from the GEO database (GSE202642)[13] and analyzed the data via the "Seurat" and "SingleR" packages.[14] The proportions of mitochondrial genes were calculated by the PercentageFeatureSet function, and cells with a percentage of mitochondrial genes greater

than 20% were excluded from further analysis. Finally, a total of 88 448 cells were included for further analysis. To normalize the gene expression levels in cells, the "NormalizeData" and "ScaleData" functions were used, and the "FindVariableFeatures" function was applied to identify the top 2000 highly variable genes. To cluster and visualize the cell subpopulations, the "RunPCA", "FindNeighbors", "FindClusters" (resolution = 0.4), and "RunTSNE" functions were applied. Moreover, the "FindAllMarkers" function was used to identify the marker genes of each cluster. The "SingleR" function and known markers from the literature were used to assign cell types. A heatmap of the expression of the top 5 marker genes in the identified clusters was generated via the "DoHeatmap" function in Seurat. Additionally, the expression and distribution of DDR1 across various cell types in the HBsAg-negative LIHC dataset (GSE166635) were explored via the TISCH database.[15]

Associations Between DDR1 Expression and the sorafenib Response in Patients with LIHC

To assess the predictive potential of DDR1 expression for identifying patients who may benefit from sorafenib treatment, we used the ROC Plotter database.[16] This online tool is capable of correlating gene expression with drug response on the basis of transcriptome-level data from a diverse array of cancer cell models. Our analysis involved an examination of the DDR1 expression level in relation to sorafenib sensitivity, leveraging the ROC plot functionality within the ROC Plotter database for analysis of in vitro data. This approach allowed us to compare DDR1 expression patterns between cell lines that are responsive to sorafenib (n = 3) and those that are resistant (n = 6), thereby assessing the potential of DDR1 as a biomarker for therapeutic stratification.

To further investigate the clinical significance of DDR1 in LIHC, we enrolled 265 LIHC patients from the Eastern Hepatobiliary Surgery Hospital (EHBH) and evaluated DDR1 protein expression in both cancerous and adjacent normal liver tissues via immunohistochemistry. Ethical approval was obtained from the ethics committee of EHBH, and paired t tests were used to compare the DDR1 expression levels between LIHC tissues and adjacent normal tissues. Using the "maxstat" (maximally selected rank statistics with several *P* value approximations, version 0.7-25) R package, we calculated the cutoff value of DDR1 expression (cutoff = 0.25) among the 265 LIHC patients and separated them into the DDR1-high (n = 92) and DDR1-low (n = 173) subgroups. Given that hepatitis B virus (HBV) infection is a major risk factor for LIHC and that the majority of the enrolled LIHC patients were HBsAg positive (86.8%), we explored the association between DDR1 expression and overall survival (OS) in the sorafenib-treated (n = 91) and nontreated (n = 139) subgroups of the 230 HBsAg-positive patients. All methods were performed in accordance with the relevant guidelines and regulations.

Associations Between DDR1 Expression and Sensitivity to Immunotherapy in Patients with LIHC

Building upon previous research indicating that DDR1 activation by collagen can promote collagen fiber alignment, impede immune cell infiltration, and contribute to immune exclusion in the context of breast cancer,[9] we conducted a comprehensive analysis to explore the relationship between DDR1 expression and sensitivity to immunotherapy in patients with LIHC. We compiled a list of genes implicated in the inflammatory response, extracellular matrix (ECM), and collagen formation from established pathways (Table S2)[17] and analyzed their transcript levels in LIHC patients via the Gene Set Variation Analysis (GSVA) package in R software, with the method parameter set to 'ssgsea'. This approach facilitated the assessment of gene set expression scores on the basis of individual gene expression measurements.

We subsequently performed Spearman correlation analysis to determine the correlation between DDR1 expression and the gene set scores for the above pathways in LIHC patients. Furthermore, we investigated the associations between the DDR1 expression level and the infiltration scores of immune cells, including $CD8^+$ T cells, NK cells, and dendritic cells, within the tumor microenvironment of LIHC. Additionally, given the significant association between immune checkpoint molecules and immunotherapy efficacy in cancer,[18] we examined the correlation between DDR1 expression and the expression of seven immune checkpoint molecules in LIHC. In addition, the association between DDR1 expression and the Tumor Immune Dysfunction and Exclusion (TIDE) score was evaluated using the TIDE algorithm.[19]

Associations between DDR1 Expression and the Response to Combined Targeted Therapy and Immunotherapy in Patients with LIHC

A total of six patients diagnosed with advanced LIHC were included in this study to assess the clinical relevance of DDR1 expression in guiding combined treatment with immunotherapy and targeted therapy in patients with LIHC. All patients were treated with a PD-1 inhibitor plus lenvatinib followed by surgical resection, and the treatment response was evaluated. Among the six patients, one had progressive disease (PD), two had stable disease (SD), and three had a partial response (PR). IHC staining was performed to determine the level of DDR1 protein expression in the patients using standard protocols. Based on the previously identified cutoff value for DDR1 expression, patients with a DDR1 level lower than the cutoff were classified as DDR1-low, whereas those with a higher level were classified as DDR1-high.

Results and Discussion

DDR1 Expression Profiling Across Cancer Types and its Clinical Significance in LIHC Patients

Previous studies have reported that DDR1 is upregulated in liver cancer tissues compared to adjacent normal tissues.[4,8,9,20] Overexpression of DDR1 has been shown to be positively correlated with tumor grade and the poor prognosis of LIHC patients. However, the expression profile of DDR1 in LIHC and its clinical significance in predicting patient responses to targeted therapy and immunotherapy have not been reported to date. We aimed to fill this knowledge gap by investigating the associations between DDR1 expression and various immunological parameters, as well as the potential of DDR1 as a predictive biomarker for the response to LIHC treatment in patients.

We first used a standard IHC method to evaluate DDR1 expression in various cancer types and found that DDR1 protein level was higher in LIHC than any of other nine cancer types, including prostate cancer, kidney cancer, bile duct cancer, pancreatic cancer, colon cancer, stomach cancer, bladder cancer, lymphoma, and melanoma (Figure 1(A), Figure 1, Table S1). Interestingly, greater DDR1 expression was detected in adjacent normal tissues than in liver cancer tissues from HBsAg-positive LIHC patients (Figure 1(B), Table S3). It is accepted that HBV infection is a major causative factor of LIHC, and patients with HBV infection always present with hepatic injury and subsequent fibrosis, which usually results in the induction of DDR1 expression. In our study, 86.8% of LIHC patients (230/265) were infected with HBV, which could at least partially explain the higher level of DDR1 in their adjacent normal liver tissues.

DDR1 Expression Pattern in LIHC at the Single-Cell Resolution

To elucidate the expression and distribution of DDR1 at the single-cell level, we analyzed single-cell RNA sequencing data from HBsAg-positive LIHC patients (GSE202642). After a series of standard data quality control procedures, 88 448 cells were included in the analysis (Figure S2), and we found that DDR1 was expressed primarily in epithelial cells but not stromal cells and immune cells (GSE166635), and DDR1 level was lower in HBs-positive LIHC cells than that in normal hepatocytes (GSE202642) (Figure 1(C)–(E)).

Correlation of DDR1 Expression and sorafenib Response in LIHC Patients

Sorafenib is one of the most common used first-line targeted drugs for advanced LIHC in clinic, but only the minority of patients exhibited positive response to sorafenib therapy.[6] To explore the predictive value of DDR1 expression in the response of LIHC to sorafenib treatment, we compared DDR1 expression between sorafenib responders and nonresponders utilizing the ROC Plotter database. Our data revealed a much higher level of *DDR1* expression in sorafenib nonresponder group than in sorafenib responder group, suggesting that *DDR1* overexpression might contribute to sorafenib resistance (Figure 1(F)). To verify the correlation between DDR1 expression and the response to sorafenib in patients with LIHC, we examined the DDR1 protein level in the LIHC tissues of patients treated with or without sorafenib. Interestingly, we found that DDR1 expression did not correlate with OS in LIHC patients who were not treated with sorafenib (n = 139) (Figure 1(G)), whereas LIHC patients with high DDR1 had worse OS than those patients with low DDR1 levels upon sorafenib treatment (Figure 1(H)). These results suggest that high DDR1 expression might lead to sorafenib resistance and that DDR1 could be a potential biomarker for predicting the response to targeted therapy in LIHC. Sorafenib is a multityrosine kinase inhibitor, whereas DDR1 is not one of the target of sorafenib. Therefore, overexpression of DDR1 could compensate the activation of those sorafenib-inhibited kinases, which explains, at least partially, the mechanism underlying DDR1-mediated sorafenib resistance.

Association Between DDR1 Levels with Immune Evasion and Therapy in LIHC

Currently, immunotherapy has strikingly improved cancer therapy and has been widely used in LIHC treatment.[7] Sufficient infiltration and activation of immune cells in the tumor microenvironment are the basis for the success of cancer immunotherapy. Most recently, Sun et al reported that DDR1 could promote collagen fiber alignment and contribute to immune exclusion.[8] They found that high DDR1 expression was inversely associated with $CD8^+$ T cell infiltration, which facilitated the immune escape of tumor cells and immunotherapy resistance in human breast cancer.[9] In our study, we observed the positive correlations between the *DDR1* expression and the levels of inflammatory response-related genes, ECM-related genes, and collagen formation-related genes in LIHC, suggesting that DDR1 expression could be activated by collagen production-related cellular events involved in liver injury and repair (Figure 1(I), Figure 3(A)–(C)). We also found the negative correlations between *DDR1* expression and the infiltration of $CD8^+$ T cells, NK cells, and dendritic cells (Figure 1(I), Figure S3(D)), consistent with the observation that DDR1 can bind collagen and promote the alignment of collagen fibers, obstructing immune cell infiltration in breast cancer. In addition, we found that *DDR1* expression was positively correlated with the expression of seven typical immune checkpoint molecules and the TIDE score in LIHC (Figure 1(I), Figure 3(E) and (F)). Taken together, these results suggest that high DDR1 expression might contribute to immunotherapy resistance and that

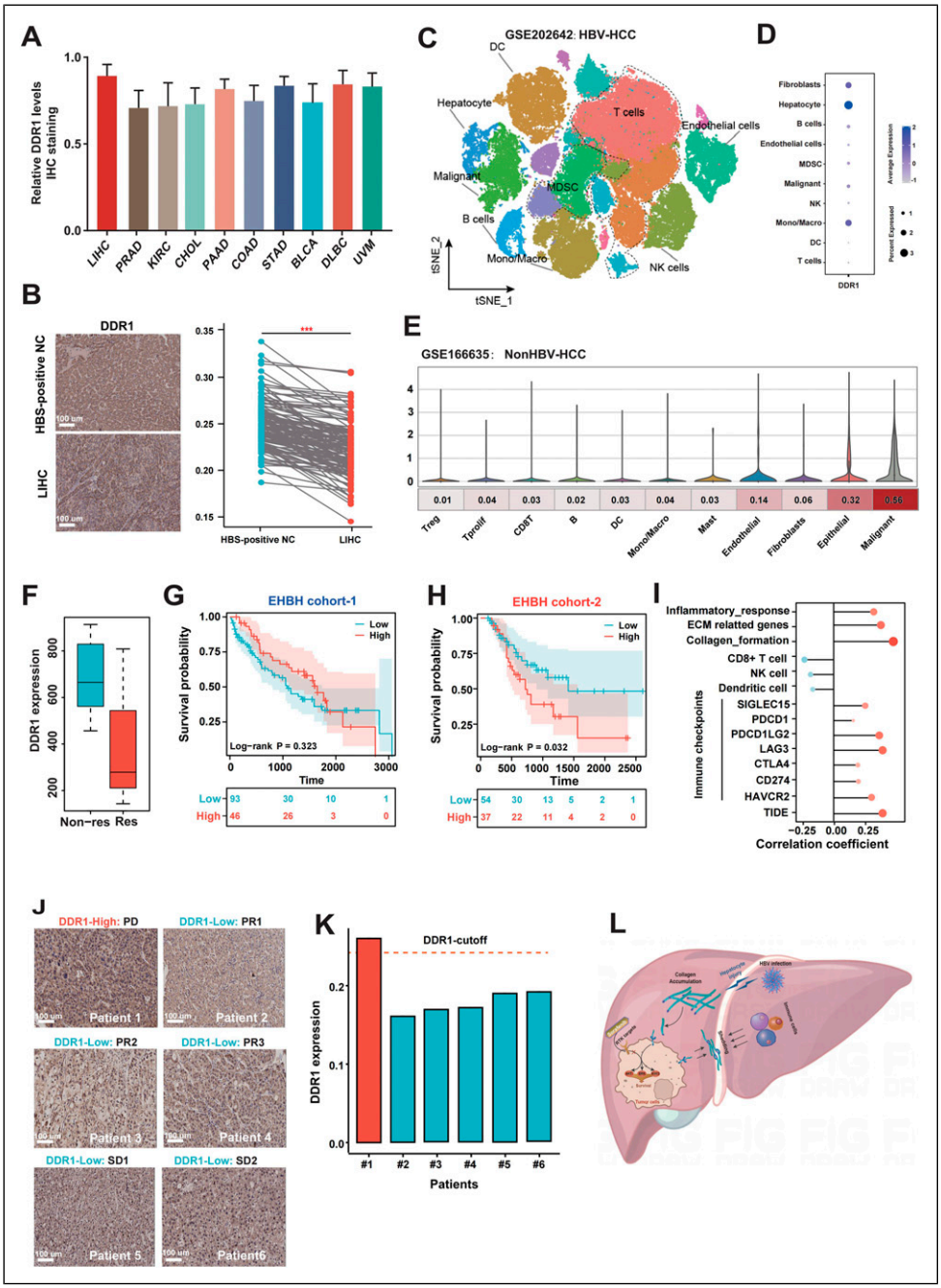

Figure 1. Genetic function and clinical significance of DDR1 expression in LIHC. (A) Protein expression of DDR1 across cancers. All values are presented as the means ± SDs. (B) Comparison of DDR1 protein expression between LIHC tissues and adjacent normal tissues. (C) The t-distributed stochastic neighbor embedding (t-SNE) plot of 88 448 single-cell transcriptomes in the HBsAg-positive LIHC dataset (GSE202642). Cell types were color-coded and annotated post hoc based on their transcriptional profile identities. (D) Distribution of DDR1 in various cell types. (E) Distribution of DDR1 across various cell types in the HBsAg-negative LIHC dataset (GSE166635). (F) Association between DDR1 expression and the response to sorafenib in liver cancer cell lines based on data from the GDSC2 dataset. (G) Kaplan–Meier curves of OS for the DDR1-high and DDR1-low groups of HBsAg-positive and untreated patients with LIHC from the clinical cohort. (H) Kaplan–Meier curves of OS for the DDR1-high and DDR1-low groups of HBsAg-positive and sorafenib-treated patients with LIHC from the clinical cohort. (I) Correlations between DDR1 expression and relevant parameters. (J) Protein expression of DDR1 in LIHC tissues from 6 patients treated with lenvatinib plus a PD-1 inhibitor-, including patients with progressive disease (PD) (n = 1), stable disease (SD) (n = 2), and a partial response (PR) (n = 3). (K) IHC staining of DDR1 in LIHC tissues from patients treated with lenvatinib plus a PD-1 inhibitor. LIHC: liver hepatocellular carcinoma; PRAD: prostate cancer; KIRC: kidney clear cell carcinoma; CHOL: bile duct cancer; PAAD: pancreatic cancer; COAD: colon cancer; STAD: stomach cancer; BLCA: bladder cancer; DLBC: large B-cell lymphoma; UVM: ocular melanoma; OS: overall survival; ***$P < 0.001$.

DDR1 could serve as a potential biomarker for predicting the response to immunotherapy in LIHC patients.

Predictive Value of DDR1 for Personalized LIHC Combination Therapy

Currently, the combination of targeted therapy and immunotherapy has become one of the most common strategies in the treatment of advanced LIHC.[7] To further evaluate the clinical significance of DDR1 in the combination therapy of advanced LIHC, we enrolled six LIHC patients treated with lenvatinib plus a PD-1 inhibitor: one patient with progressive disease (PD), two patients with stable disease (SD), and three patients with a partial response (PR). As shown in Figure 1(J) and (K), we found that patients with the worst clinical outcome (PD) presented with a higher level of DDR1 than those with better outcomes (SD and PR), suggesting that DDR1 might be a potential biomarker for predicting the response to combination therapy in LIHC patients (Figure 1(L)), which is worthy of further validation in larger clinical cohorts in future.

Conclusions

The current study revealed that DDR1 expression could be activated by collagen production-related cellular events involved in liver injury and repair, and the upregulated DDR1 might mediate the resistance to targeted therapy and immunotherapy in LIHC. Therefore, we propose that DDR1 might be a novel biomarker for the personalized combination treatment in LIHC patients.

Appendix

Abbreviations

LIHC	Liver hepatocellular carcinoma
TIME	Tumor immune microenvironment
TCGA	The Cancer Genome Atlas
TIMER	Tumor Immune Estimation Resource
DDR1	Discoidin domain receptor tyrosine kinase 1
ECM	extracellular matrix
IHC	immunohistochemical
EHBH	Eastern Hepatobiliary Surgery Hospital
HBV	hepatitis B virus
TIDE	Tumor Immune Dysfunction and Exclusion
PD	progressive disease
SD	stable disease
PR	partial response
OS	overall survival

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Author Contributions

All authors searched the literature, designed the study, interpreted the findings and revised the manuscript. TXL, and HH carried out the data management and statistical analysis and drafted the manuscript. TXL, HH, YHS, DTH, WFS, and BFN helped with cohort identification and data management. All authors approved the final draft of the manuscript. All authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. DTH, WFS, and BFN contributed to the critical revision of the manuscript. BFN performed project administration duties.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical Statement

Ethical Approval

All participants were enrolled from the Eastern Hepatobiliary Surgery Hospital (EHBH), and the sample collection procedure was approved by the ethics committee of Changzheng Hospital (2016SL018). Written informed consent was obtained from all study participants.

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Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Supplemental Material

Supplemental material for this article is available online.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394-424.
2. Chen Y, Chen H, Chen P. Catalase expression is an independent prognostic marker in liver hepatocellular carcinoma. *Oncologie*. 2024;26(1):79-90.
3. Lin X, Tian C, Pan F, Wang R. A novel immune-associated prognostic signature based on the immune cell infiltration analysis for hepatocellular carcinoma. *Oncologie*. 2024;26(1):91-103.

4. Pan Y, Han M, Zhang X, et al. Discoidin domain receptor 1 promotes hepatocellular carcinoma progression through modulation of SLC1A5 and the mTORC1 signaling pathway. *Cell Oncol*. 2022;45(1):163-178.
5. Ye C, Zhang X, Chen X, et al. Multiple novel hepatocellular carcinoma signature genes are commonly controlled by the master pluripotency factor OCT4. *Cell Oncol*. 2020;43(2):279-295.
6. Reiss KA, Yu S, Mamtani R, et al. Starting Dose of sorafenib for the treatment of hepatocellular carcinoma: A retrospective, Multi-Institutional study. *J Clin Oncol*. 2017;35(31):3575-3581.
7. Riley RS, June CH, Langer R, Mitchell MJ. Delivery technologies for cancer immunotherapy. *Nat Rev Drug Discov*. 2019;18(3):175-196.
8. Rammal H, Saby C, Magnien K, et al. Discoidin domain receptors: potential Actors and targets in cancer. *Front Pharmacol*. 2016;7:55.
9. Sun X, Wu B, Chiang HC, et al. Tumour DDR1 promotes collagen fibre alignment to instigate immune exclusion. *Nature*. 2021;599(7886):673-678.
10. Oh SH, Swiderska-Syn M, Jewell ML, Premont RT, Diehl AM. Liver regeneration requires Yap1-TGFβ-dependent epithelial-mesenchymal transition in hepatocytes. *J Hepatol*. 2018;69(2):359-367.
11. Fu J, Chen Y, Cao J, et al. p28GANK overexpression accelerates hepatocellular carcinoma invasiveness and metastasis via phosphoinositol 3-kinase/AKT/hypoxia-inducible factor-1α pathways. *Hepatology*. 2011;53(1):181-192.
12. Mcshane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM, Statistics Subcommittee of the NCI-EORTC Working Group on Cancer Diagnostics. REporting recommendations for tumour MARKer prognostic studies (REMARK). *Br J Cancer*. 2005;93(4):387-391.
13. Zhu GQ, Tang Z, Huang R et al. CD36(+) cancer-associated fibroblasts provide immunosuppressive microenvironment for hepatocellular carcinoma via secretion of macrophage migration inhibitory factor. *CELL DISCOV*. 2023;9(1):25.
14. Sharma A, Seow J, Dutertre CA, et al. Onco-fetal reprogramming of endothelial cells drives immunosuppressive macrophages in hepatocellular carcinoma. *Cell*. 2020;183(2):377-394.
15. Sun D, Wang J, Han Y, et al. TISCH: a comprehensive web resource enabling interactive single-cell transcriptome visualization of tumor microenvironment. *Nucleic Acids Res*. 2021;49(D1):D1420-D1430.
16. Tibor FJ, Gyorffy B. A unified platform enabling biomarker ranking and validation for 1562 drugs using transcriptomic data of 1250 cancer cell lines. *COMPUT STRUCT BIOTEC*. 2022;20:2885-2894.
17. Wei J, Huang K, Chen Z, et al. Characterization of Glycolysis-associated molecules in the tumor microenvironment revealed by Pan-cancer tissues and Lung cancer single cell data. *Cancers*. 2020;12(7):8.
18. Wang Z, Wang Y, Gao P, Ding J. Immune checkpoint inhibitor resistance in hepatocellular carcinoma. *Cancer Lett*. 2023;555:216038.
19. Jiang P, Gu S, Pan D, et al. Signatures of T cell dysfunction and exclusion predict cancer immunotherapy response. *Nat Med*. 2018;24(10):1550-1558.
20. Gong H, Xu HM, Zhang DK. Focusing on discoidin domain receptors in premalignant and malignant liver diseases. *Front Oncol*. 2023;13:1123638.