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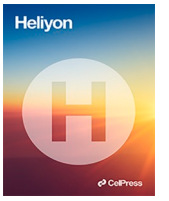

The combined characteristics of cholesterol metabolism and the immune microenvironment may serve as valuable biomarkers for both the prognosis and treatment of hepatocellular carcinoma

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A R T I C L E I N F O

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LIHC
Single-cell sequencing
TIME
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A B S T R A C T

Background: Hepatocellular carcinoma (HCC) being a complex disease, commonly exhibits multifaceted presentations, rendering its treatment challenging and necessitating specific approaches. The tumor immune microenvironment is crucial in cancer treatment, and cholesterol metabolism is a key component that helps cells grow and produce vital metabolites. However, the reprogramming of cholesterol metabolism in the tumor microenvironment (TME) can promote HCC development, and cancer classifiers relating to cholesterol metabolism are currently limited. Despite significant progress, further research is needed to improve early detection, liver function, and treatment options to improve patient outcomes.

Methods: To evaluate the expression abundance of tumor immune microenvironment (TIME) and cholesterol metabolism in 8 types of liver cancer cells, we comprehensively evaluated the immune cell composition, extracellular matrix alterations, and activity of relevant signaling pathways in the TIME through nine liver cancer patients, stromal scoring, immune scoring, tumor purity scoring, immune infiltration analysis, and pathway enrichment. Subsequently, we utilized machine learning techniques to construct prognostic models for both cholesterol metabolism and the tumor immune microenvironment, further exploring the tumor mutation burden, immune infiltration levels, and drug sensitivity in different subtypes of HCC patients.

Results: Our study constructed three cancer screening models to identify HCC patients with high cholesterol metabolism and low TIME, who have a poorer prognosis. On the contrary, patients with low cholesterol metabolism and high TIME often have better prognosis. Furthermore, we identified chemical compounds, such as BPD-00008900, ML323, Doramapimod, and AZD2014, which display better chemotherapy results for high-risk patients in specific sub-groups.

1. Introduction

Liver cancer is a highly lethal malignant tumor in the digestive tract and metabolism, and it is also a significant public health problem worldwide [1–4]. Worldwide, approximately 900,000 individuals are newly diagnosed with cancer every year, with approximately 88 % of liver cancer patients dying [5]. It is projected that the incidence of new cases of liver cancer will increase by 55 % by 2040, yielding a possible diagnosis of 1.4 million individuals that year, nearly 1.3 million of whom will subsequently die from liver cancer [6]. HCC accounts for the majority of liver cancer, usually with poor prognosis and unsatisfactory therapeutic effects

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[7–10]. In all cases of liver cancer, >90 % are HCC, drug suppression and immunotherapy are currently the main treatment methods for HCC [11]. However, chemotherapy can result in drug resistance and poor drug sensitivity, leading to extremely poor prognosis for patients. Therefore, studying the mechanism of HCC occurrence and developing treatment plans is urgent.

Tumors are complex ecosystems that contain not only malignant cells but also immune components [12–15]. There are many studies proving the close relationship between TIME and tumor development, recurrence, and metastasis [16]. As rapidly proliferating cells, malignant cells in HCC accelerate cholesterol metabolism to meet the needs of membrane biogenesis and other functions [17]. Cholesterol metabolism provides energy for the growth of HCC and promotes cell proliferation, while also providing necessary substrates for the biosynthesis of cell membranes [18–21]. Therefore, blocking cholesterol consumption or transport hampers tumor growth and invasion in various cancers [18,22–26]. In the tumor microenvironment, cholesterol metabolism seems to be associated with immune function as well [20,27–32]. The biosynthesis of cholesterol is jointly completed by SREBP2 and its regulated genes, which are significantly upregulated under lipid or oxygen limiting conditions, promoting tumor growth [32]. Meanwhile, elevated cholesterol metabolism can lead to a sharp decrease in CD8+T cells in the tumor microenvironment, while protecting tumor cells from lipid peroxidation damage caused by elevated oxidative stress environment [33]. It is noteworthy that cholesterol and its metabolites demonstrate contrasting anticancer effects in tumors. In the TME, the high level of the cholesterol oxidized derivative, oxysterol, inhibits T cell-mediated antitumor immunity via LXR activation [34–36]. However, in T cells with strong immune capacity and most lymphocytes, the upregulation of cholesterol biosynthesis or uptake enhances T cell-mediated antitumor functions. Although there are currently some studies on the role of cholesterol and its related product synthesis in diseases, most of them focus on the entire liver cancer tissue or liver cancer cells without in-depth quantitative analysis of specific cell types in the TME [37–39]. Additionally, there is a lack of literature comparing the immune metabolism levels between different types of liver cancer cell subtypes, which is crucial for understanding the occurrence and metastasis of liver cancer. Our study aims to explore the role and relationship of cholesterol metabolism in the tumor immune microenvironment of liver cancer by analyzing differences in immune and metabolic levels among different types of liver cancer cells. In addition, there are many macrophages in the tumor microenvironment, which not only affect immunity but also affect tumor progression [40–43]. Macrophages will choose two completely different ways of differentiation in different situations. M1 macrophages have always been considered as anti-tumor, while M2 polarized macrophages, usually considered as Tumor-associated macrophages, contribute to many tumors promoting cancer results through angiogenesis and Lymphatic vessel regulation, immunosuppression, hypoxia induction, tumor cell proliferation, and metastasis [44]. Therefore, it is necessary to study TAMs. Targeted TAMs are currently one of the targeted immunotherapy methods, with more advantages compared to direct drug therapy and less damage to internal organs and the human body. However, the main drawbacks of this method are still reduced drug resistance and chemotherapy sensitivity [45–48], which needs to be addressed in future liver cancer treatments. However, tumor heterogeneity, macrophage polarization, and cholesterol metabolism are all important factors contributing to drug resistance in tumors, which is crucial for the treatment of liver cancer patients in the future.

In this study, we delved into the relationship between the immune microenvironment of liver cancer tumors and cholesterol metabolism. We compared the immune levels, cellular communication levels, and differentiation trajectories of eight types of HCC. We also compared the tumor mutation burden and drug sensitivity of liver cancer with different levels of cholesterol metabolism and immunity, and selected the most sensitive chemotherapy drug to reduce the side effects and tolerance of cancer patients during chemotherapy.

2. Materials and methods

2.1. Model construction and evaluation

We utilized previously established computational methods for modeling inflammation in breast cancer to construct tumor immune microenvironment models, cholesterol metabolism models, and combined models. We calculated the sum of the products of risk coefficients (Supplementary Table 1) and the expression levels of hub genes for tumor immune microenvironment, cholesterol metabolism, and the combined model, respectively [49].

2.2. Immune infiltration analysis

We used Cibersort software to evaluate the expression level and abundance of each immune cell throughout the entire patient. Then, classify the risk scores based on the integration of the immune microenvironment and cholesterol metabolism, and use the Wilcoxon rank sum test for difference analysis and p-value calculation.

2.3. Data processing

We selected data from nine HCC patients in GSE125449, analyzed and compared the immune levels of different types of cells using ESTIMATE, and displayed the corresponding scores of each type of cell in the form of heat maps. Afterward, we used addModuleScore to score various signal pathways and represented their activation status through color depth. Differentially expressed genes for immune scores and cholesterol metabolism in single-cell sequencing data were analyzed using the Seurat R package (Supplementary Table 2). The use of the oncoPredict R software package for drug sensitivity screening yielded results, which are provided in Supplementary Table 3. Genes with $|\log2FoldChange|>1$ and p-value <0.05 were selected. The workflow for data analysis is illustrated in Fig. 1.

3. Results

3.1. The existence of significant tumor heterogeneity in liver cancer cells

We are very interested in understanding whether there is tumor heterogeneity in 9 types of HCC tissues, as it is crucial to gain a deeper understanding of the causes and mechanisms of HCC occurrence from the perspective of cell composition types. Through analyzing single-cell sequencing data from nine liver cancer patients, we discovered the existence of significant tumor heterogeneity in liver cancer (Fig. 2A). Specifically, we identified seven types, including immune cells and malignant liver cancer cells, with clear boundaries after clustering (Fig. 2B). Therefore, studying the mechanism of TIME in HCC can provide direction and ideas for personalized treatment. We utilized the ESTIMATE software, which has many advantages, to score nine cases of liver cancer cells. Our results indicated that a higher stromal score is closely related to cancer-associated fibroblasts (CAF) and tumor-associated macrophages (TAM) and that this relationship may contribute to higher rates of metastasis and invasion (Fig. 2C). Meanwhile, the tumor purity was found to be the highest in Malignant cells, tumor endothelial cells (TEC), and CAF. This finding suggests that the tumor purity score can be used to classify and predict malignant tumor cells and immune cells well (Fig. 2D). In contrast, the immune scores and ESTIMATE scores were found to be very low in these cell types, indicating a negative correlation between malignancy and immune/ESTIMATE scores (Fig. 2E and F). We found that tumor purity accounted for the highest proportion of Malignant cells and immune score accounted for the highest proportion of immune cells. (Fig. 2G). In short, there is a significant tumor heterogeneity in liver cancer, and a more in-depth study of their differences has vital importance for the treatment of HCC patients.

3.2. The cholesterol metabolism signaling pathway is significantly upregulated in HCC malignant cells

In order to better evaluate the immune infiltration level of each type of cell, we define cells with high immune scores as high immune microenvironments and those with low immune scores as low immune microenvironments for differential analysis. We have found that many cancer-related signaling pathways, metabolic signaling pathways, and chemical carcinogenesis-related signaling pathways are enriched (Fig. 3A). Interestingly, among the enriched signaling pathways, the cholesterol metabolism signaling pathway is significantly upregulated in the malignant cells (Fig. 3B and C). Cell communication refers to the process by which cells exchange information through various signal transduction mechanisms. These signals can take the form of chemical substances or interactions between cell surface receptors, among others. Effective communication between cells is crucial for the normal functioning of biological systems, as it regulates essential physiological processes including cell growth, differentiation, secretion, and apoptosis. Cell-to-cell communication serves as a vital mechanism in various physiological events such as growth and development, immune response, neural signaling, and metabolic regulation. Studying the mechanisms of cell communication can help to better understand the mechanisms of liver cancer. We found that there are numerous close contacts among eight types of cells in liver cancer (Fig. 3D left), among which the connections between malignant cells and T cells/TAM cells are the closest (Fig. 3D right). It is worth noting that we found cell communication only exists between CAF, HPC-like, and Malignant cells with the other seven types of cells among the eight types of cells. This indicates that these three types of cells may collaborate to promote cell proliferation and resist other types of immune cells during the development of HCC (Fig. 3E). The above results indicate that understanding the mechanism of malignant cell hyperactivity in liver cancer is crucial, and cholesterol metabolism may be an important breakthrough.

3.3. A high tumor immune microenvironment and low cholesterol metabolism are more favorable for the survival of patients

Given the important role of tumor immune microenvironment and cholesterol metabolism in cancer, we constructed a screening classifier for liver cancer based on the immune microenvironment and cholesterol metabolism. The tumor immune microenvironment

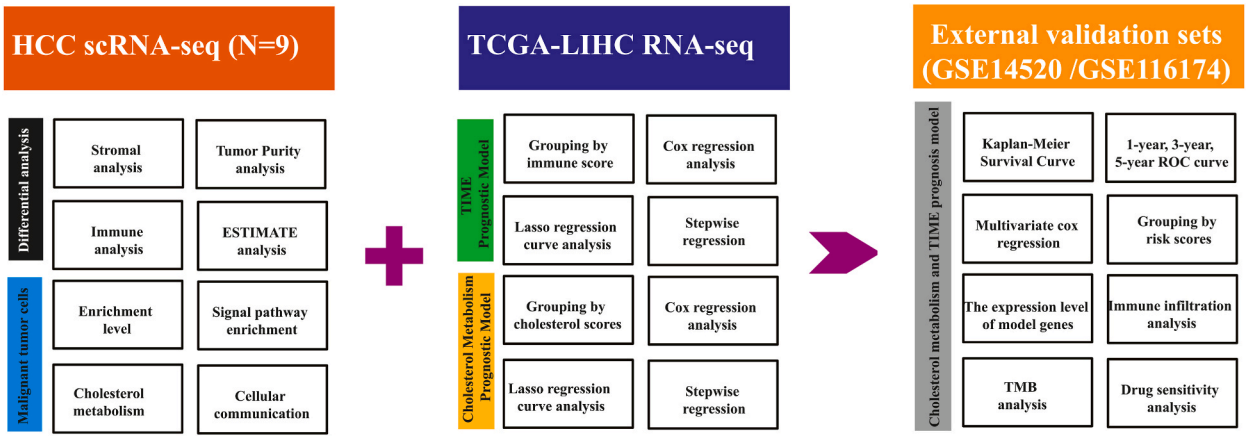

Fig. 1. Workflow.

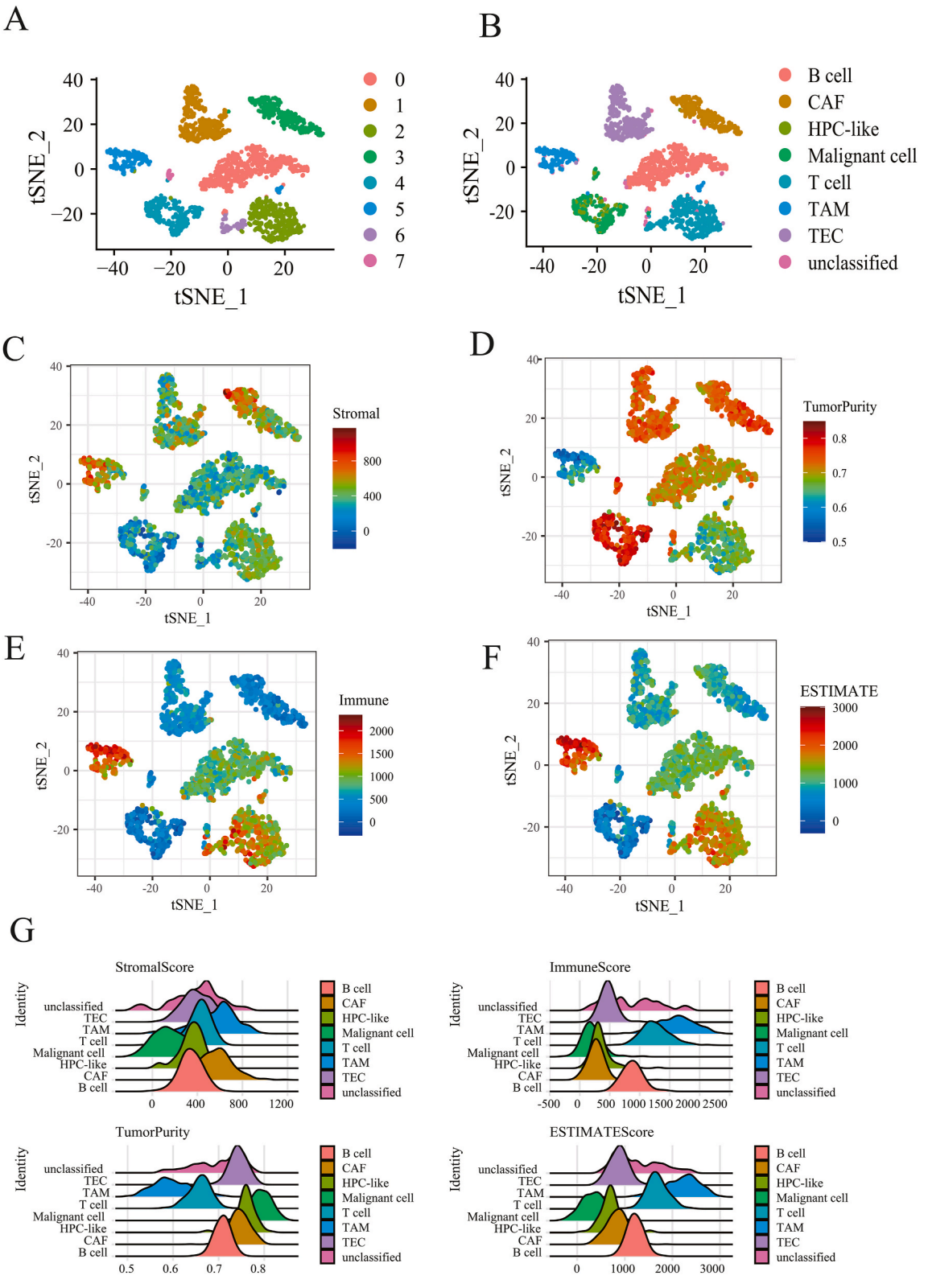

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Fig. 2. ESTIMATE score of hepatocellular carcinomas. (A). Clustering of single-cell sequencing data from nine cases of hepatocellular carcinomas. (B). Dimensionality reduction-based classification and cell annotation of hepatocellular carcinomas from nine cases. (C). Stromal score of hepatocellular carcinomas. (D). Tumor Purity score of hepatocellular carcinomas. (E). Immune score of hepatocellular carcinomas. (F). ESTIMATE score of hepatocellular carcinomas. (G). Mountain map of the distribution of Stromal score, Tumor Purity score, Immune score, and ESTIMATE score in various cells.

screening classifier contained 11 hub genes, while the cholesterol metabolism screening classifier contained 17 hub genes (Fig. 4A). Interestingly, Fig. 4B shows that patients with high tumor immune microenvironment and lower cholesterol metabolism group have higher survival rates, while patients with low tumor immune microenvironment and high cholesterol metabolism group have lower survival rates. Undoubtedly, higher immunity can inhibit the growth of HCC and significantly increase the survival time of HCC patients, as confirmed by our research results (Fig. 4C). The lower survival rate of HCC patients in the high cholesterol metabolism group (Fig. 4D) may be due to the use of cholesterol metabolism to provide energy and substances required for tumor growth. In addition, the tumor immune microenvironment and cholesterol metabolism screening showed excellent accuracy, with AUC values ranging from 0.75 to 0.85 at one, three, and five years (Fig. 4E and F).

3.4. Immune and cholesterol metabolism-related gene characteristics can evaluate patient survival

Based on the previous results, we combined the advantages of tumor immune microenvironment and cholesterol metabolism classifiers to construct a liver cancer risk assessment model. We define the group with high cholesterol metabolism and low immune microenvironment as the high-risk group, and the group with low cholesterol metabolism and high immune microenvironment as the low-risk group. The liver cancer risk assessment model exhibited even better performance (Fig. 5A and B). The risk model includes 20 hub genes, of which eleven genes (HSPA8, S100A16, DNAJB4, CALM1, SPINK1, PGF, IGFBP3, CYB5R3, SPP1, FKBP1A, APOLD1) have a relatively high risk and are associated with poor patient prognosis, while nine genes such as CD69, GJA4, RPL18A, MTRNR2L12, IL1B, TM4SF1, TINAGL1, SPARCL1, ADAMTS9 have a lower risk (Fig. 5C). We found that HSPA8, S100A16, DNAJB4, CALM1, SPINK1, PGF, IGFBP3, CYB5R3, SPP1, FKBP1A and APOLD1 were expressed at higher levels in the high-risk group, while CD69, GJA4, RPL18A, MTRNR2L12, IL1B, TM4SF1, TINAGL1, SPARCL1 and ADAMTS9 were expressed at higher levels in the low-risk group (Fig. 5D). Meanwhile, when overexpressed in cancer, genes with higher risk ratios have poorer prognosis, while genes with lower risk ratios have better prognosis when overexpressed, which is the opposite of the former (Fig. 5E). To ensure the accuracy of the model, we validated it separately in independent datasets GSE116174 and GSE14520. The 1-year, 3-year, and 5-year roc curves for GSE116174 were between 0.65 and 0.75, while the roc curves for GSE14520 ranged from 0.5 to 0.6, indicating that our liver cancer risk model has good performance in liver cancer screening and classification (Fig. 5F and G).

3.5. HCC patients with high cholesterol metabolism have lower immune levels

In order to detect the abundance of all immune cells in all HCC patients, we scored and categorized them based on the optimal model constructed earlier correlating cholesterol with the tumor immune microenvironment based on clinical data (Fig. 6A). The tumor immune microenvironment and cholesterol metabolism model we previously constructed showed good performance. We found that the content of T cells and B cells was significantly lower in the high cholesterol metabolism group (low immune microenvironment group) compared to the low cholesterol metabolism group (high immune microenvironment group). At the same time, we also found a significant increase in M2 type macrophages in the high cholesterol metabolism group (low immune microenvironment group), while there were more M1 type macrophages in the low cholesterol metabolism group (high immune microenvironment group) (Fig. 6B). In addition, we found that there were more macroscopic and mast cells in the high cholesterol metabolism group (low immune microenvironment group), and more lymphocytes in the low cholesterol metabolism group (high immune microenvironment group) (Fig. 6C). These results strongly indicated that the risk score could classify immune cells and the abundance of immune cells in different risk groups. It also indicates that M2-macrophages and T cells are important factors for the poor survival rate in high-risk group patients.

3.6. HCC patients in the high cholesterol group are more prone to mutations

To further evaluate the efficacy of tumor immunotherapy, we conducted TMB analysis on cancer patients in the high cholesterol metabolism group (low tumor immune microenvironment group) and the low cholesterol metabolism group (high tumor immune microenvironment group), respectively. Interestingly, we found that 42 % of patients in the high cholesterol metabolism group had a P53 mutation, while only 20 % in the low cholesterol metabolism group had this mutation. In addition, we also found that many genes in the high cholesterol metabolism group (low tumor immune microenvironment group) and low cholesterol metabolism group (high tumor immune microenvironment group) had a higher frequency of mutations and the main type of mutation that occurs is Missense Mutation (Fig. 7A, E and H). The frequency of variant alleles refers to the proportion of individuals with one or more variant genotypes at a certain gene locus in the total population. It reflects the prevalence of a genetic mutation in a population. Generally, the higher the frequency of variant alleles, the more common the mutation is in the population. The frequency of variant alleles is one of the important indicators in genetic epidemiology research, which can help us understand the transmission and role of genetic variations in human populations. By analyzing the frequency of variant alleles, we found that the high cholesterol metabolism group (low tumor

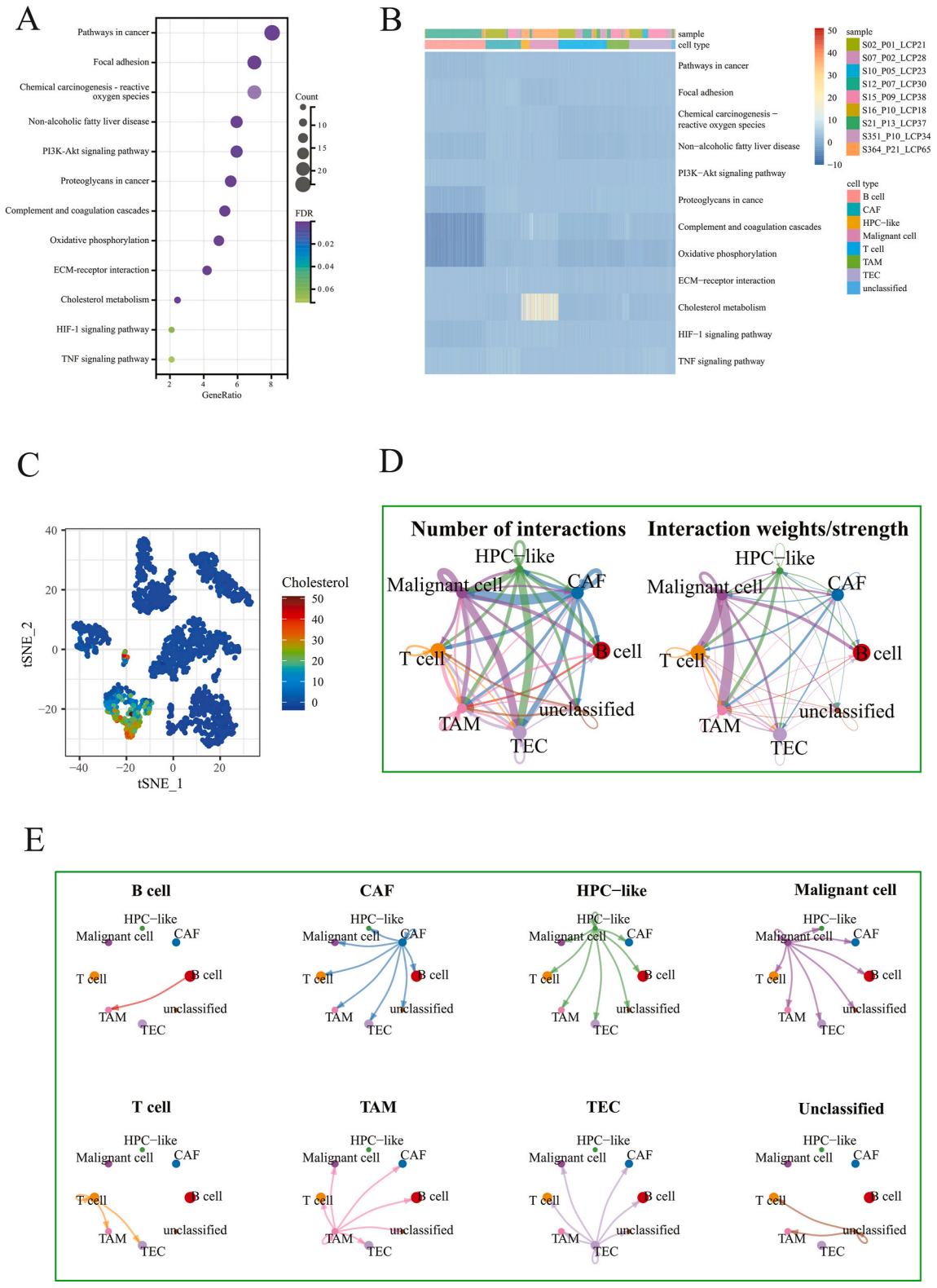

Fig. 3. Characteristic analysis of malignant tumor cells in hepatocellular carcinoma. (A). KEGG enrichment map of differential immune score genes in hepatocellular carcinoma. (B). Heat map of high expression of cholesterol metabolism in malignant hepatocellular carcinoma. (C). Cholesterol metabolism score in hepatocellular carcinoma. (D). The quantity and intensity of communication between eight types of cells. (E). Cellular communication network diagram among different types of cells.

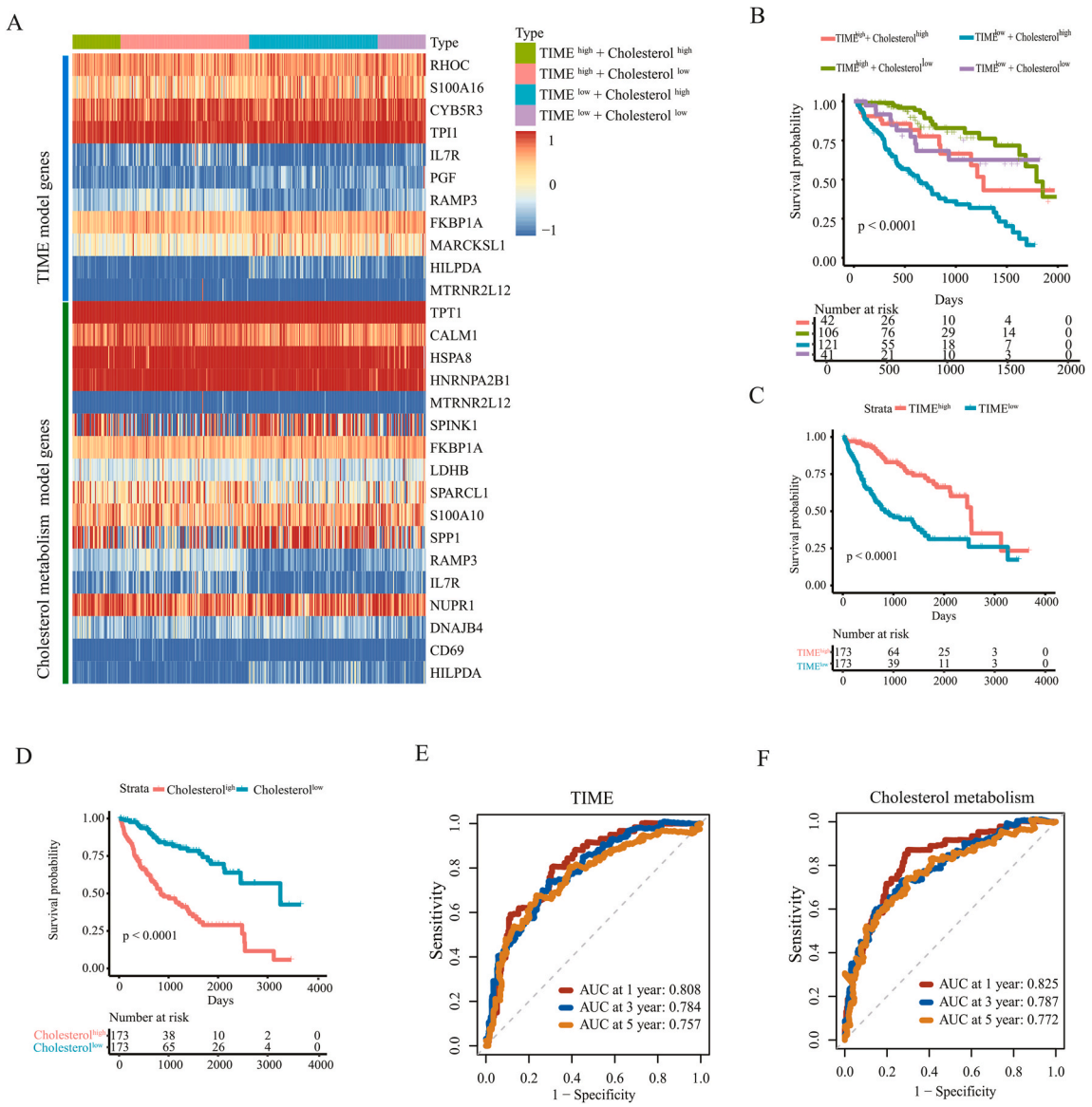

Fig. 4. The prognosis of different subtypes of hepatocellular carcinoma is based on the immune microenvironment and cholesterol metabolism. (A). The heatmap of the hub genes of the immune microenvironment and cholesterol metabolism models in different combinations. (B). Survival curves of cancer patients grouped by immune microenvironment and cholesterol metabolism. (C). Survival curve of liver cancer patients grouped based on the immune microenvironment. (D). Survival curve of liver cancer patients grouped based on cholesterol metabolism. (E). The AUC curve of the immune microenvironment model at 1 year, 3 years, and 5 years. (F). The AUC curve of cholesterol metabolism model at 1 year, 3 years, and 5 years.

immune microenvironment group) does indeed have a higher frequency of allelic mutations (Fig. 7B). CTNNB1 is the gene that encodes beta-catenin. Beta-catenin is a component of the intracellular adhesion structure and also plays an important role in signal transduction. Overactivated beta-catenin is closely related to the occurrence and development of various human cancers. Through Mutual exclusive and Co-occurrence analysis, we found there was a significant Mutual exclusive between TP53 and CTNNB1 in the high cholesterol metabolism group (low tumor immune microenvironment group), while there was a significant co-occurring between TP53 and PCLO as well as OBSCN. Meanwhile, compared to the low cholesterol metabolism group (high tumor immune microenvironment group), the high cholesterol metabolism group (low tumor immune microenvironment group) has many co-occurring cancer-related genes (Fig. 7C). The high cholesterol metabolism group (low tumor immune microenvironment group) mainly affects related signaling pathways such as RTK-RAS, Hippo, TP53, and NRF2, while the low cholesterol metabolism group (high tumor immune microenvironment group) mainly affects signaling pathways such as RTK-RAS, PI3K, and TGF-Beta (Fig. 7D). In addition, we found that the high-risk group has a higher tumor mutation burden (Fig. 7F), and the 20 modeled hub genes also exhibit higher mutations in

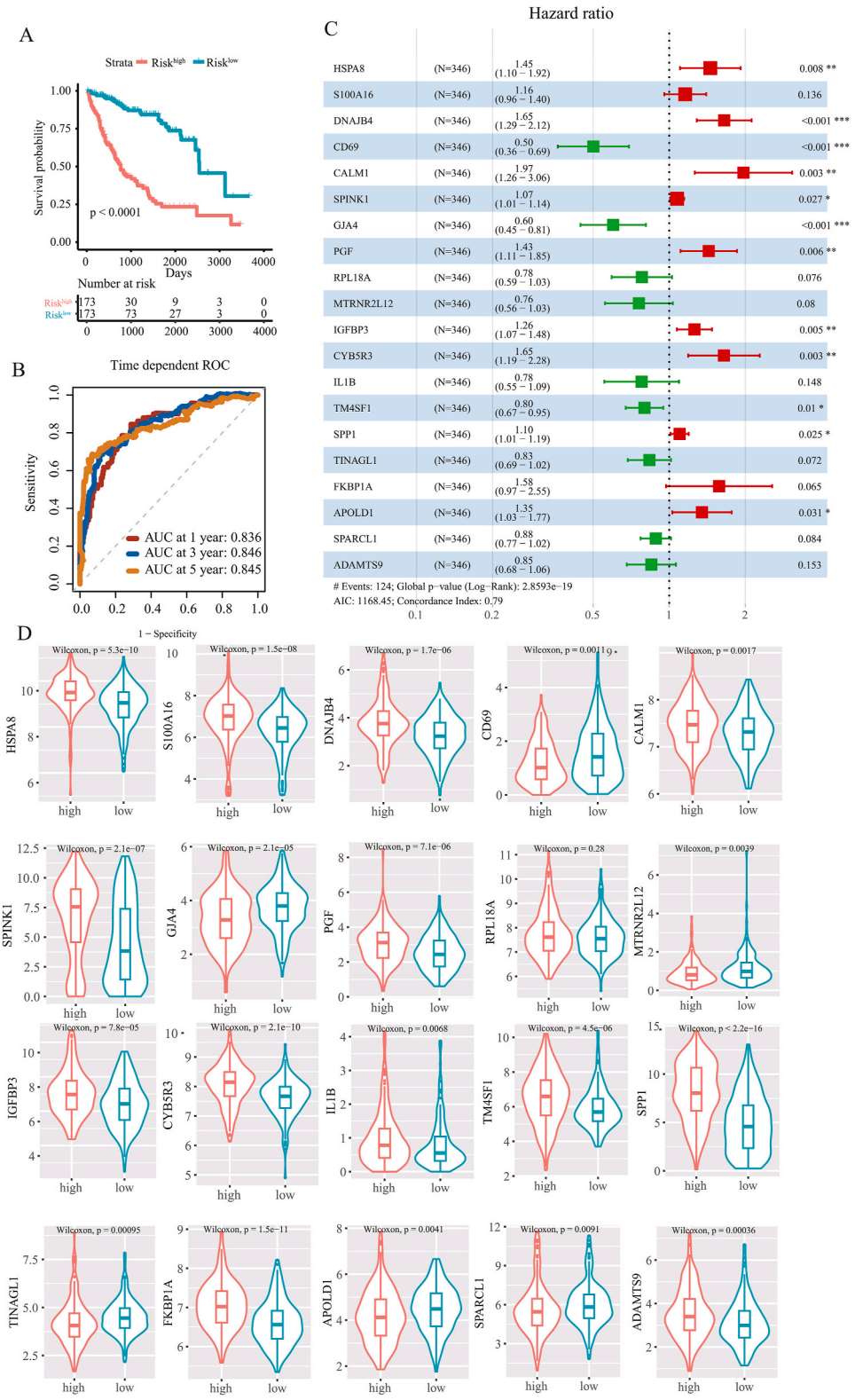

Fig. 5. Risk model based on immune microenvironment and cholesterol metabolism. (A). Survivorship curve of patients with liver cancer in different risk groups. (B). The AUC curve of the risk model at 1 year, 3 years, and 5 years. (C). Multivariate Cox regression analysis of hub genes in risk models. (D). The expression levels of risk model hub genes in different risk groups. (E). The survival curve of the risk model hub gene is grouped based on the risk score. (F). AUC curves for test set 1 at 1 year, 3 years, and 5 years. (G). AUC curves for test set 2 at 1 year, 3 years, and 5 years.

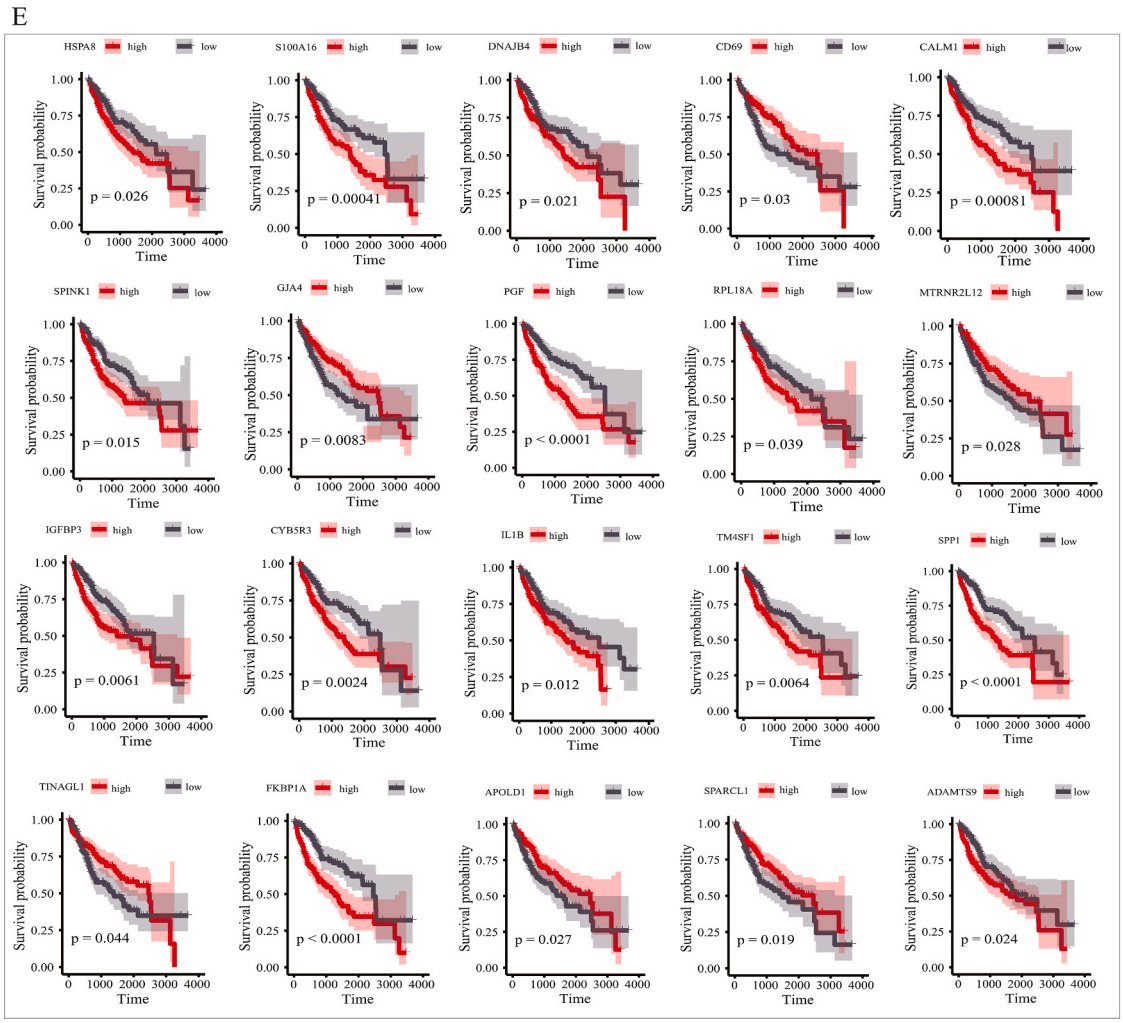

Fig. 5. (*continued*).

the high cholesterol metabolism group (low tumor immune microenvironment group) (Fig. 7G). In summary, the high burden of tumor mutations in high cholesterol metabolism group (low tumor immune microenvironment group) populations is more likely to lead to the occurrence and poor prognosis of cancer.

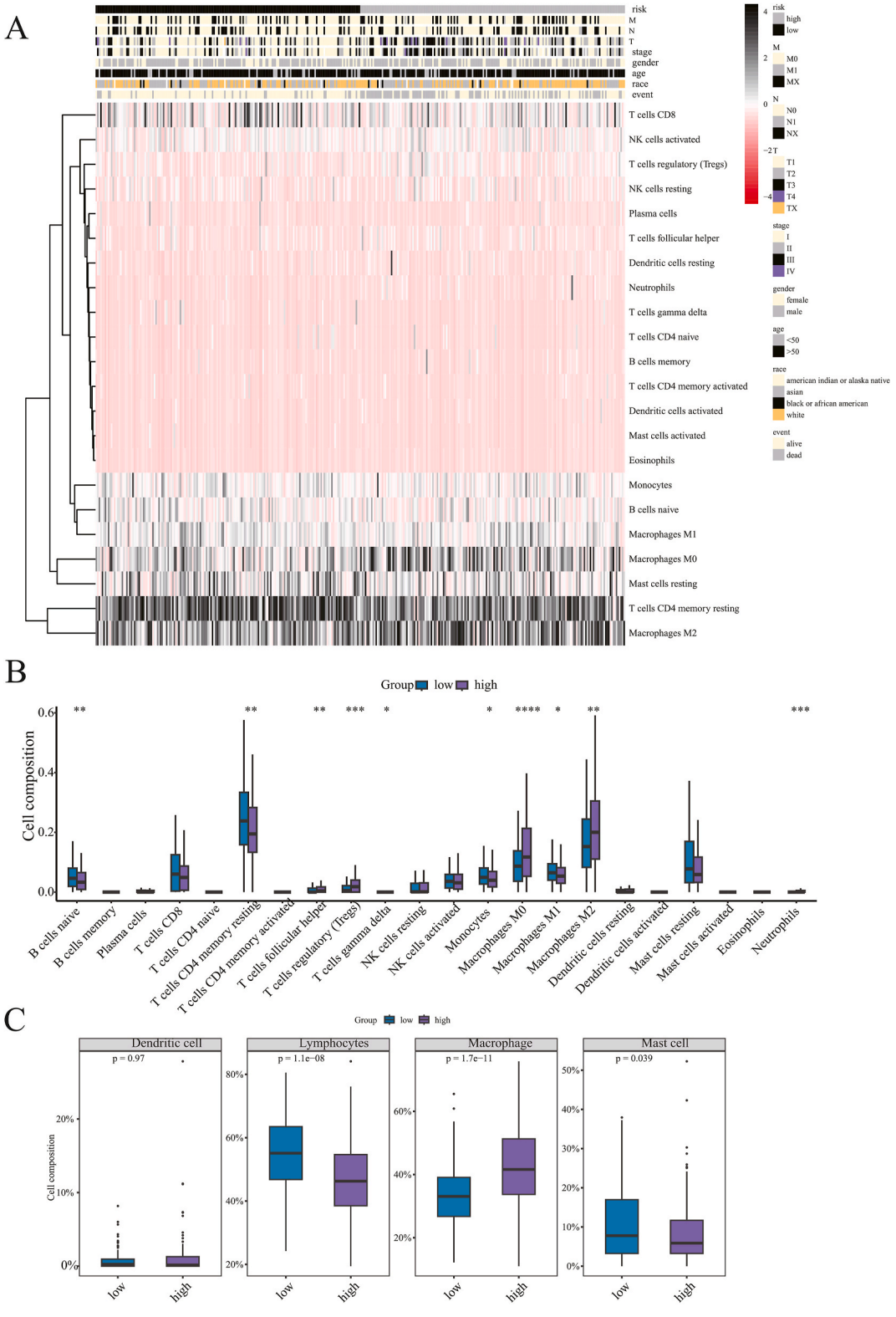

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Fig. 6. Immune infiltration analysis of different risk groups (A). The expression calorimetry of each immune cell was grouped according to clinical data and risk score. (B). Wilcoxon rank-sum test accurately compared the difference and indicated that several immune cells conferred significantly lower infiltrating density in high-risk groups. *P < 0.05; **P < 0.01; ***P < 0.001, statistically significant. (C). The 20 types of immune cells calculated by Cibersort were divided into four major groups and their expression levels were calculated in different risk groups: Total lymphocytes, Total dendritic cells, Total macrophage, and Total mast cells.

3.7. BPD-00008900 and ML323 have high drug sensitivity in the treatment of patients with high cholesterol metabolism

Patients with high drug sensitivity often achieve better treatment outcomes after receiving a certain dosage of medication. Conversely, low drug sensitivity can lead to poor treatment outcomes or may require higher dosages of medication to achieve the same treatment effect. The treatment of liver cancer also faces the challenge of drug resistance. Therefore, it is important to analyze the drug sensitivity of different liver cancer patients. Our results indicate that Doramapimod and AZD2014 have a positive correlation with risk scores (Fig. 8A–D) and show better treatment outcomes in low cholesterol metabolism group (high tumor immune microenvironment group) liver cancer patients. BPD-00008900 and ML323, on the other hand, have a negative correlation with risk scores (Fig. 8E–H) and demonstrate better treatment effects in high cholesterol metabolism group (low tumor immune microenvironment group) liver cancer patients.

4. Discussion

HCC is the most malignant subtype of liver cancer, characterized by poor prognosis [50–52] and significant heterogeneity [53,54]. Regardless of the cancer stage, chemotherapy remains the preferred treatment modality [55,56]. However, HCC exhibits high drug resistance and recurrence rates [57–59]. The primary reason for this phenomenon is the high tumor heterogeneity observed in HCC [9, 60,61]. The malignant tumor cells in HCC are the main cause of HCC progression, and it is important to understand the metabolic levels and differences in immune levels between them and immune cells and normal cells. In this study, we aim to identify the location of malignant tumor cell abnormalities and provide insights into cancer treatment strategies. Through data analysis of nine HCC patients, we found significant heterogeneity among the seven cell types in HCC. Among these seven types of cells, malignant tumor cells exhibit lower immune levels and higher tumor purity compared to other cells, while immune cells and other cells exhibit higher immune levels. Tumor heterogeneity can reshape the tumor microenvironment and subsequently influence drug resistance [62]. Therefore, we conducted differential analysis and enrichment analysis on HCC cells with high and low immune scores. Based on the comparison of these seven types of cells, we found that the cholesterol metabolism signaling pathway is significantly activated in malignant tumor cells. Cholesterol is a necessary lipid for maintaining the homeostasis of mammalian cells in normal environments [17,63,64]. It is mainly synthesized in the liver and bound to low-density lipoprotein (LDL), which is transported to various parts of the body through blood [65,66]. After being transferred to the target site, LDL enters the cell through endocytosis, is transported to lysosomes through endocytosis, and hydrolyzes into free cholesterol molecules [67–69]. These molecules shuttle to the cell membrane and other organelles bound to the cell membranes, regulating membrane fluidity and stability [70]. Cells typically rely on de novo synthesis of acetyl-CoA to meet their cholesterol requirements, with even greater importance for this process during the cancer cell proliferation process [71]. Additionally, cholesterol also forms lipid raft structures within cells, playing a role in signal transduction. Abnormal cholesterol metabolism promotes tumor growth. Cholesterol activates the Wnt signaling pathway by regulating Fzd5, thus promoting the growth of pancreatic ductal adenocarcinoma [72]. Cholesterol-lowering interventions can reduce the mTOR complex 2 signaling pathway in prostate cancer, enhancing anti-tumor immunity [73]. In addition, cholesterol homeostasis is disrupted, and excessive accumulation of cholesterol can increase the resistance of cancer cells to iron death, leading to the further development and deterioration of liver cancer [64]. In summary, the heterogeneity of malignant tumor cells in HCC and the changes in the tumor immune microenvironment due to abnormal cholesterol metabolism are crucial for the treatment of liver cancer.

Through our comparative analysis, we found that the main reason for poor prognosis in liver cancer patients is a high cholesterol metabolism score and low tumor immune microenvironment score. Additionally, in the high-risk group of cancer patients (high cholesterol metabolism score and low immune score), M2 macrophages have higher levels and fewer lymphocytes (T cells, B cells) and M1 macrophages. The large accumulation of M2 macrophages in cancer not only does not prevent the progression of the tumor but also promotes the rapid generation of tumor blood vessels, ultimately leading to the proliferation of tumor cells [41], which is also the reason for the poor prognosis of some patients.

Furthermore, we conducted TMB analysis in both the high cholesterol metabolism (low tumor immune microenvironment or high-risk group) and low cholesterol group (high tumor immune microenvironment or low-risk group). We found that multiple tumor suppressor genes, including P53, were mutated in the high-risk group. As a tumor suppressor, the P53 protein plays a crucial regulatory role in various stages of the cell cycle and helps maintain genomic stability [74]. P53, also known as the "guardian of the genome," can inhibit the formation and progression of cancer. It achieves this by promoting the repair of damaged cells through multiple pathways or selectively inducing apoptosis in damaged cells, preventing uncontrolled cell proliferation and mutation [75]. This suggests that the mutations in cancer-related genes in the high-risk group may be associated with the occurrence and progression of HCC.

Finally, based on these results, we proposed treatment strategies. Through drug sensitivity analysis, we found that BPD-00008900 and ML323 had better chemotherapeutic effects in the high-risk group, while Doramapimod and AZD2014 had better chemotherapeutic effects in the low-risk group. Although our research has provided valuable insights, it is important to acknowledge certain limitations. Specifically, further studies with a larger sample size are necessary to enhance our analysis.

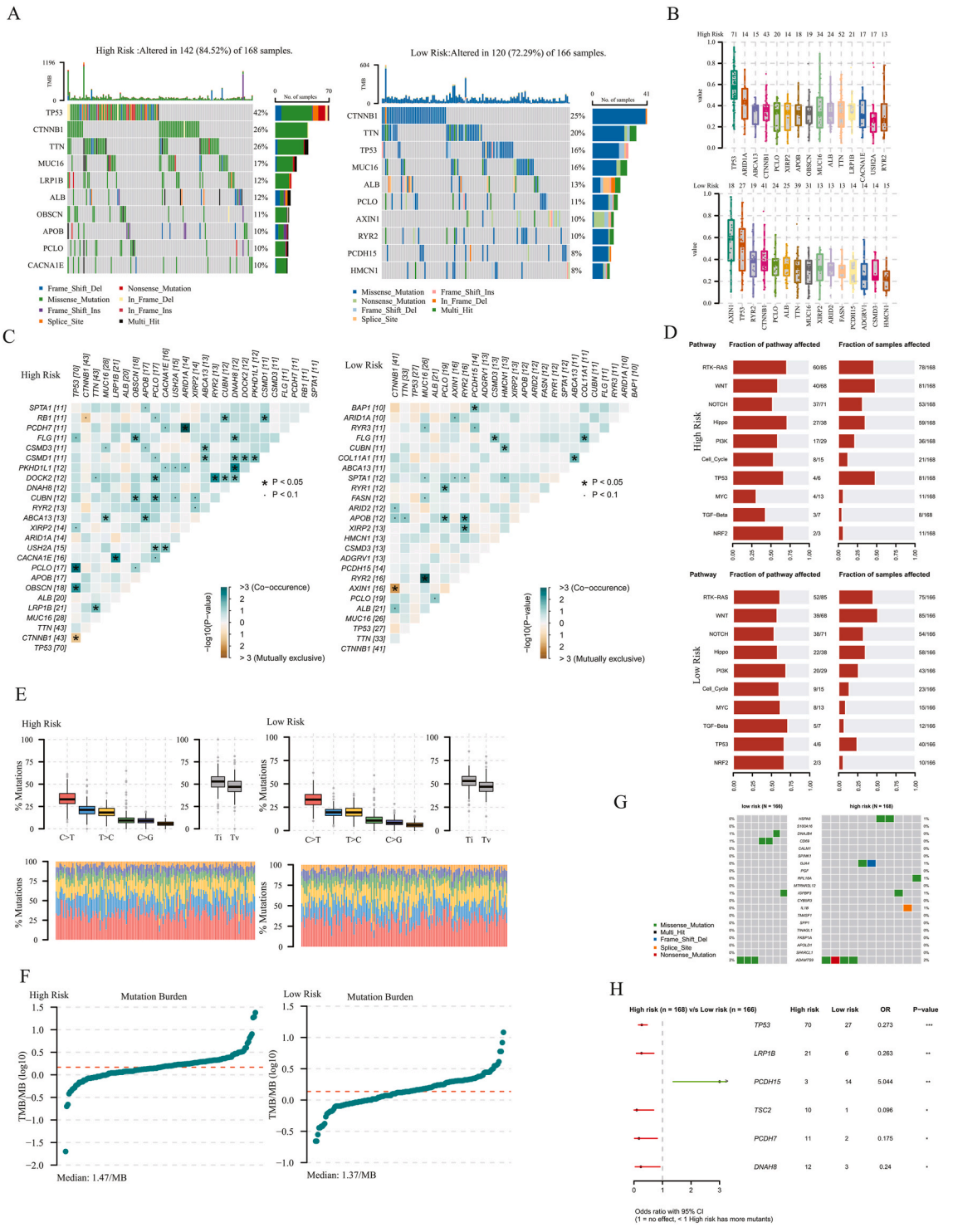

Fig. 7. Somatic cell Mutation Analysis in Different Risk Groups. (A). Waterfall maps of the somatic mutations in different groups. (B). Mutation frequency of variant alleles in different risk groups. (C). Exclusive and co-occurrence analysis of different risk groups. (D). Analysis of carcinogenic signal pathways in different risk groups. (E). Transition/Ti and transition/TV statistics for different risk groups. (F). Tumor mutation burden in different risk groups. (G). Mutation frequency of model genes in different risk groups. (H). Mutant genes with significant differences in different risk groups.

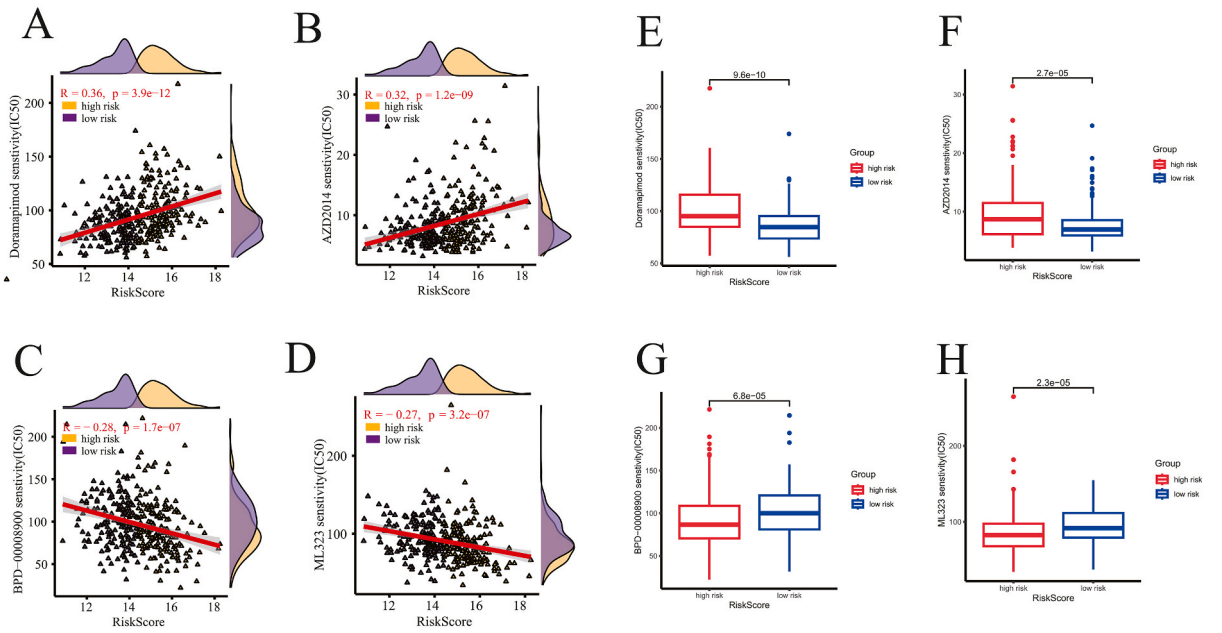

Fig. 8. Drug sensitivity analysis of different risk groups. (A–D). The two groups compared the Doramapimod, AZD2014, BPD−00008900, and ML323 sensitivity (IC50). (E–F) Correlation between Risk Score and Doramapimod, AZD2014, BPD−00008900, ML323.

Funding

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Data availability statement

The data of nine HCC cases and the validation of cholesterol metabolism and tumor immune microenvironment models can be obtained from the GEO database (GSE125449, GSE14520, GSE125449), and the model construction data can be obtained from The Cancer Genome Atlas.

CRediT authorship contribution statement

Weiyu Bai: Writing - review & editing, Writing - original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

HCC	hepatocellular carcinoma
TIME	tumor immune microenvironment
TME	tumor microenvironment
TAMs	tumor-associated macrophages
GEO	gene expression omnibus
KEGG	Kyoto Encyclopedia of Genes and Genomes
ROC	receiver operating characteristic
AUC	area under the curve

TCGA	the cancer genome atlas
LIHC	liver hepatocellular carcinoma
CAF	cancer-associated fibroblasts
HPC	like hematopoietic progenitor-like cell
TEC	tumor endothelial cell
TMB	tumor mutational burden
IC50	half-maximal inhibitory concentration
LDL	low-density lipoprotein

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.heliyon.2023.e22885.

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