



The role of tumor-infiltrating B cells in the tumor microenvironment of hepatocellular carcinoma and its prognostic value: a bioinformatics analysis

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Background: Accumulating evidence indicates that tumor heterogeneity is characterized by distinct immunosubtypes. However, prior studies have mainly focused on the functions of T cells. The role of tumor-infiltrating B cells in the microenvironment of hepatocellular carcinoma (HCC) requires further investigation.

Methods: We conducted an integrative analysis of single cell RNA sequencing (scRNA-seq) datasets in HCC tumor samples from Gene Expression Omnibus database. We analyzed the features of B cells in normal liver tissue and HCC. Additionally, we conducted a deconvolution analysis using the matrix of scRNA-seq datasets and the RNA-seq datasets in The Cancer Genome Atlas-Liver Hepatocellular Carcinoma (TCGA-LIHC) database. The survival analyses of the TCGA-LIHC cohort with different B cell infiltration rates and was further validated. Finally, we performed immunohistochemistry analysis of primary tumor tissue of HCC patients using antibodies against CD79A and validated the impact of tumor-infiltrating B cells in the prognosis of LIHC.

Results: We identified several subtypes of B cells in the microenvironment of HCC, including the plasma cells and naïve B cells. The relative ratio of B cells, but not the plasma cells, was significantly decreased in HCC as compared to the normal liver tissue ($P < 0.05$). In addition, genes related to antigen presentation and cell proliferation were decreased in tumor-infiltrating B cells ($P < 0.05$). The observation of B cell infiltration was further validated with the TCGA-LIHC cohort. The overall survival and disease-free survival in HCC patients with higher B-cell infiltration rate were significantly longer than those in the lower infiltration group ($P < 0.05$) in the TCGA-LIHC cohort. Moreover, we demonstrated higher infiltration rates of B cells were significantly associated with a better prognosis of HCC in our cohort.

Conclusions: Tumor-infiltrating B cells potentially exert a tumor-suppressive function in the microenvironment of HCC and the higher levels of B cell infiltration are associated with a favorable outcome of HCC. Targeted activation of B cells may improve the tumor immune-targeted therapy.

Keywords: Hepatocellular carcinoma (HCC); tumor microenvironment (TME); B cells; prognosis

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Introduction

According to the latest global cancer statistics, hepatocellular carcinoma (HCC) is the third leading cause of cancer death, with an increasing incidence (1,2). The major risk factors of HCC including infection of hepatitis B virus or hepatitis C virus, heavy alcohol intake, and nonalcoholic fatty liver disease (3). Among them, inflammation plays an important role in the pathogenesis and progression of HCC (3,4). Vaccination, early screening, as well as advances in surgery and targeted medicine have considerably reduced the mortality rate of HCC in recent years (5). Nonetheless, there are still discrepancies among the clinical and pathological stages, drug sensitivity, and prognosis due to tumor heterogeneity (6,7).

Accumulating evidence indicates that tumor heterogeneity is characterized by distinct immunosubtypes, in which T cells, B cells, natural killer (NK) cells, and infiltrating myeloid cells comprised the majority of the tumor immune microenvironment (8-10). In recent years, analysis of single cell transcriptomes has revealed a novel way to access intra-tumor heterogeneity and to predict microenvironment interactions (11). However, studies about the HCC immune microenvironment have mainly focused on the functions of T cells; B cells, as the second most common immune cells, have received less attention in previous studies (12,13). Therefore, clarify the roles of B cells in the microenvironment of HCC is necessary for understanding the crosstalk of distinct immunosubtypes and new therapeutic strategies may be potentially discovered.

This study systematically integrated several single-cell transcriptome sequencing data, and focused on the single-cell analysis of the B cells. We identified the characteristics of the B-cell transcriptome and predicted the interaction network between B cells and other immune cells in the tumor microenvironment (TME). Finally, the association between B cell infiltration and HCC prognosis was further analyzed by immunohistochemical experiments. We present the following article in accordance with the REMARK reporting checklist (available at <https://jgo.amegroups.com/article/view/10.21037/jgo-22-717/rc>).

Methods

HCC single-cell transcriptome dataset

Raw datasets from the Gene Expression Omnibus database (GEO; www.ncbi.nlm.nih.gov/geo/) were download and analyzed, which comprised 18 HCC samples and 8 normal liver samples. Dataset integration was performed by Seurat software (14) in R, using the Harmony method (15) for integrating different batches of samples. Then, data were visualized using the Yeskit tool (16). The transcriptome data on The Cancer Genome Atlas-Liver Hepatocellular Carcinoma (TCGA-LIHC) were processed using the Cell-type Identification by Estimating Relative Subsets of RNA Transcripts (CIBERSORT) tool (17) for calculating each proportion of immune cell subtype. Cluster analysis of differentially expressed genes (DEGs) was conducted using the topGO package (18). The survival analyses of the TCGA-LIHC cohort with different B cell infiltration rates and was further validated.

HCC specimen collection

A total of 72 HCC specimens were obtained from HCC patients who underwent surgery in Zhongshan hospital affiliated to Fudan University from 2016 to 2018. None of the patients received any anti-cancer therapy before surgery. The follow up data and its correlation to the infiltration level of B cells in HCC samples of patients were retrospectively analyzed. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Clinical Research Ethics Committee of Zhongshan Hospital, Fudan University (No. B2021-020R) and informed consent was taken from all the patients.

Immunohistochemistry

First, formalin-fixed paraffin-embedded (FFPE) tissue sections were dried for one hour at 60 °C. They were then deparaffinized in xylene and rehydrated in decreasing

ethanol concentrations. For antigen retrieval, tissue sections were placed in citrate buffer at pH 6 for 30 min at 95 °C. The sections were incubated in 3% hydrogen peroxide and in serum-free protein blocking solution. Then, the primary antibody (CD20, ab78237, Abcam, Cambridge, MA, USA; CD79A, ab199001, Abcam) were added for 1 hour at room temperature. Biotinylated-secondary antibody and streptavidin-horseradish peroxidase were used for signal amplification. Finally, hematoxylin was counterstained on slides, glycerol was mounted, and digital images were acquired.

Statistical analysis

The statistical significance between different groups was estimated by unpaired two-tailed Student's t-tests. Survival analysis of the TCGA-LIHC cohort with different B cell infiltration rates and HCC specimens of our cohort were assessed by Log-rank tests. A P value <0.05 was considered as statistically significant. All data analyses were performed using SPSS software (v25.0; SPSS Inc., Chicago, IL, USA).

Results

Single cell transcriptomic profiles showed infiltration of B cells in HCC

To systematically investigate the TME of HCC, we merged the single cell transcriptomic profiles from the GEO data, including tumor samples from 24 HCC patients and 8 adjacent normal tissues. We identified 143,104 clusters of cells in the TME of HCC (Figure 1A), including cancer cells, T cells, macrophages, and B cells. In the microenvironment of HCC, we identified several subtypes of B cells, including naïve B cells (MS4A1 positive) and plasma cells (IGHG4 positive) (Figure 1B).

We calculated the infiltration rates of B cells and plasma cells in normal liver tissues and HCC tissues. The results showed that the infiltration rate of B cells was significantly decreased in HCC tissues compared to the normal liver tissues (Figure 1C). However, the relative ratio of plasma cells was not statistically significant in HCC tissues compared to adjacent normal tissues (Figure 1D). This suggests that B cells, but not plasma cells, may be suppressed in HCC; B cells were potentially associated with the progression of HCC.

Genes related to antigen presentation and cell proliferation were decreased in tumor-infiltrating B cells

To clarify the characterization of the distribution and role of the B cell subtypes in the immune microenvironment of HCC, we merged the single cell transcriptomic profiles collected from the TCGA cohorts, and then analyzed the transcriptomic signatures of B cells in HCC tissues and that in normal liver tissues. We found that the genes downregulated in HCC tissues were significantly enriched in the signaling pathways related to antigen presentation and cell proliferation (Figure 2), suggesting that these pathways were potentially inhibited in HCC tissues.

Higher infiltration of B cells was associated with a favorable outcome in the TCGA-LIHC cohort

To clarify the correlation of tumor-infiltrated B cells with the clinical features of HCC, we further analyzed the TCGA-LIHC data. Here, by integrating the single-cell transcriptomic data with the RNA-seq datasets in the TCGA-LIHC, we further confirmed the distribution of immune cell subtypes in the TCGA-LIHC samples.

Through statistical analysis, we found that the infiltration rate of B cells in tumor cells was significantly lower than that in adjacent normal tissues (Figure 3A). In addition, B cells were significantly less infiltrated in advanced HCC [China liver cancer staging (CNLC), stage III and stage IV] than in early HCC (CNLC, stage I and stage II) (Figure 3B).

Next, we divided the HCC samples into a higher infiltration group and lower infiltration group according to the infiltration rates of B cells. The prognoses of HCC patients in both groups were also analyzed. It was found that the overall survival and disease-free survival in HCC patients with higher B-cell infiltration rate were significantly longer than those in the lower infiltration group (Figure 3C,3D).

Immunohistochemistry analysis of HCC tissues and the prognostic value of infiltrated B cells

To further identify the infiltration of B cells in tumor tissues and its correlation to the prognosis of HCC, immunohistochemical staining of 72 HCC samples was performed using antibodies to CD79A to analyze the association of B cell infiltration and HCC prognosis. Based

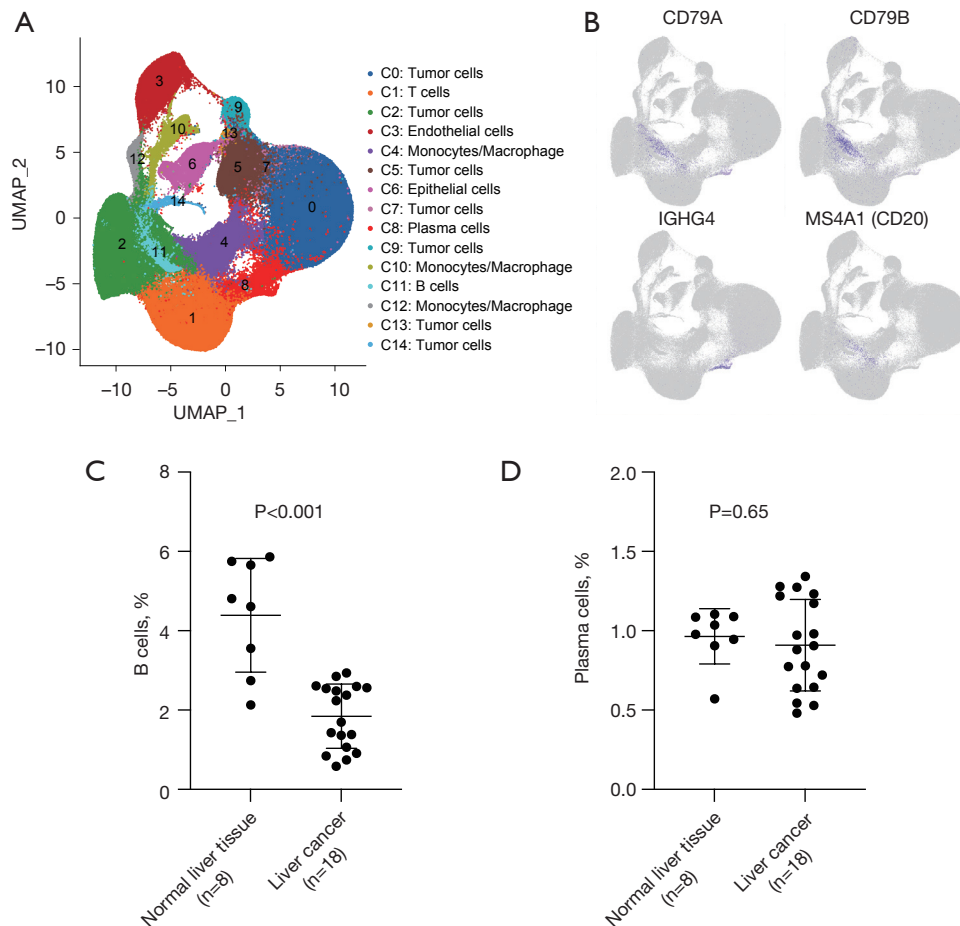


Figure 1 Two subtypes of B cells in the TME of HCC. (A) Visualization of different cell populations in the TME of HCC by Umap. Different colors represent different cell populations. (B) The expression of the specific markers of B cells. Positive expressing cells are shown in blue and negative expressing cells in gray. (C) Proportion of B cells in single-cell sequencing data of HCC tissues and adjacent normal tissues. (D) Proportion of plasma cells in single-cell sequencing data of HCC tissues and adjacent normal tissues. TME, tumor microenvironment; HCC, hepatocellular carcinoma.

on the positive degree and area of CD79A staining, the HCC cases could be divided into a high infiltration B-cell group and low infiltration B-cell group. The prognosis of HCC cases in the high infiltration B-cell group was significantly better than that in the low infiltration B-cell group (*Figure 4*), indicating the important prognostic value of infiltrating B cells in HCC.

Discussion

Evidence indicates that HCC is a highly heterogeneous malignant tumor involving complicated interactions between tumor cells and immune cells (19,20). Previous studies have

tried to clarify the landscape of molecular heterogeneity in HCC (4,5). However, the functions of various factors in liver carcinogenesis and progression remain unclear. With the progress of sequencing technology, single cell transcriptomics can analyze the distribution and roles of cell subsets at the single-cell level (21,22).

With the rapid development of tumor immunotherapy in recent years, tumor immune microenvironment studies have flourished of immune cell subtypes and relevant functions. Although B cells could not directly kill tumors, B cells can not only play an important function as antigen-presenting cells, but also directly act on tumor cells through the secretion of antibodies and cytokines (23,24). Here,

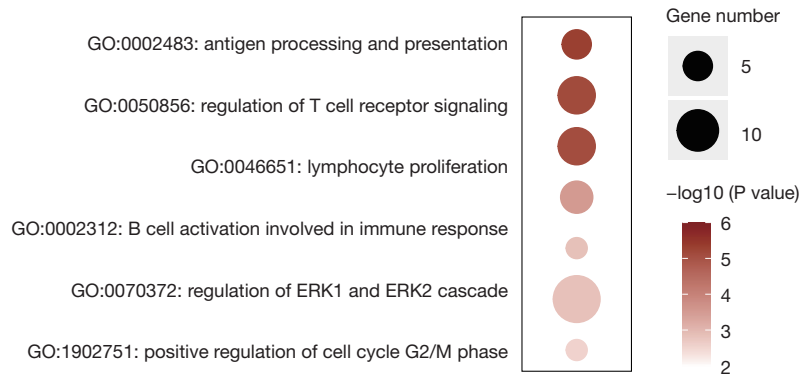


Figure 2 Genes related to antigen presentation and cell proliferation were decreased in tumor-infiltrated B cells. Cell cluster deregulated genes in B cells from HCC tissues and adjacent normal tissues were analyzed and the GO functional annotation was also performed. Cell cluster specifically downregulated genes in HCC-infiltrating B cells were enriched in some pathways. Colors represent P values, and darker colors indicate more significant differences. Circle size represents the number of genes, with larger circles enriched with a larger number of genes. HCC, hepatocellular carcinoma; GO, Gene Ontology.

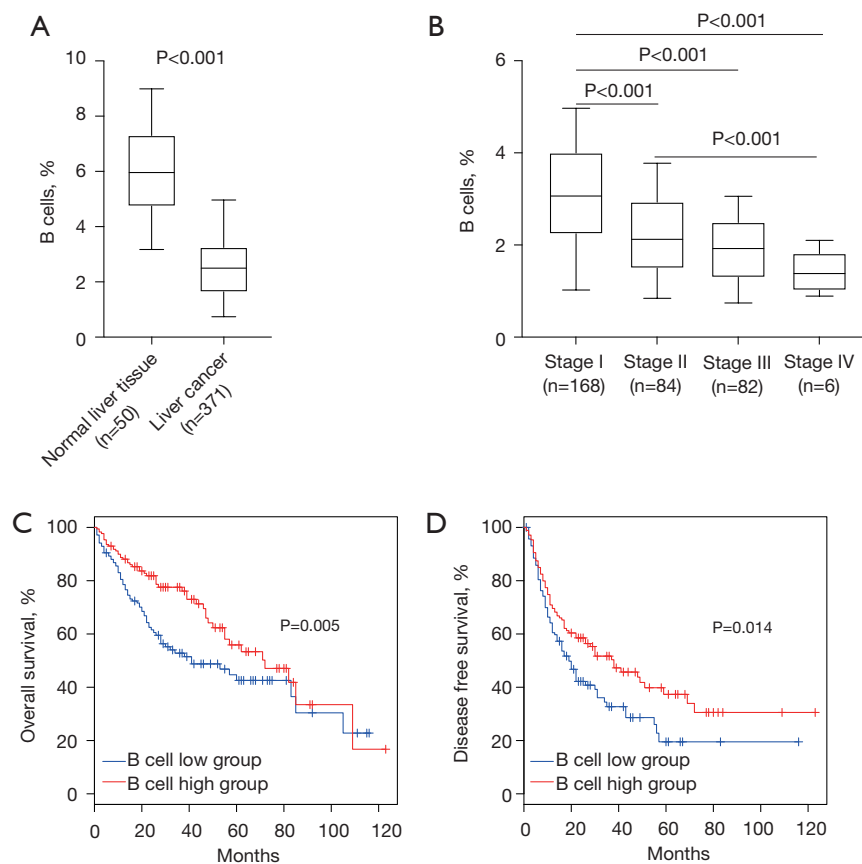


Figure 3 The B cell infiltration and its prognostic value in the TCGA-LIHC cohort. (A) B cell infiltration rates in HCC samples and adjacent normal samples; (B) B cell infiltration rates in different stages of HCC samples; (C) overall survival of the B cell high infiltration and B cell low infiltration groups; (D) disease-free survival of the B cell high infiltration and B cell low infiltration groups. TCGA-LIHC, The Cancer Genome Atlas-Liver Hepatocellular Carcinoma; HCC, hepatocellular carcinoma.

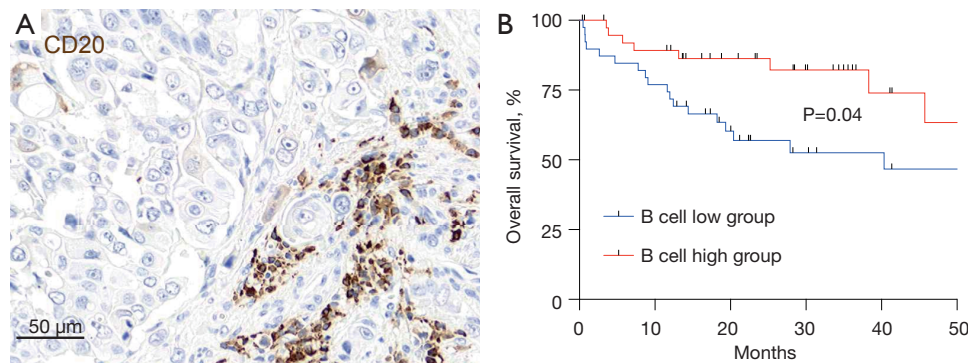


Figure 4 Correlation between B cell infiltration and HCC prognosis. (A) Immunohistochemistry analysis of primary tumor tissue of HCC patients using antibodies against CD79A; (B) the impact of tumor infiltrated B cells in the prognosis of HCC. HCC, hepatocellular carcinoma.

we identified B cells and plasma cells that had infiltrated in HCC via single cell transcriptomic profiles. With the increase of the tumor malignant invasion, the facilitation rate of B cells decreased. Higher B-cell infiltration closely correlated to a favorable outcome of HCC patients. This revealed that B cells presented a tumor suppressive activity, and the B cells were constantly exhausted along with the tumor progression.

Tumor-infiltrating immune cells play a significant role in the promotion or inhibition of tumor growth (25). The B cells are one major class of immune cells in the adaptive immune system and have several functions in the pathogenesis and progression of cancers (26,27). However, the functions of tumor-infiltrating B cells remain incompletely characterized. We found that B cell antigen presentation as well as cell proliferation-related pathways were significantly suppressed in the tumor immune microenvironment. This revealed that the antigen-presenting function of B cells was impaired in the TME. The proliferation capability of B cells may not be reversed by antigen stimuli due to the decrease of cell proliferative potential. This study indicated a lasting malfunction of B cells in the TME. Targeted activation of B cells may be effective on reactivation and may assist other immune cells, such as T cells, to perform an anti-tumor function, which could improve the tumor immune-targeted therapy. However, functional analyses of B cells in the TME of HCC and the crosstalk between different cell types still need further researches in the future.

In summary, our studies identified several subtypes of B cells in the microenvironment of HCC, including the plasma cells and naïve B cells. The relative ratio of B cells,

but not the plasma cells, was significantly decreased in HCC as compared to the normal liver tissue. In addition, genes related to antigen presentation and cell proliferation were decreased in tumor-infiltrating B cells. The observation of B cell infiltration was further validated with the TCGA-LIHC cohort. Moreover, we found that a higher infiltration of B cells was associated with a favorable outcome of LIHC in both the TCGA-LIHC cohort and our cohort. Tumor-infiltrating B cells potentially exert a tumor-suppressive function in the microenvironment of HCC.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <https://jgo.amegroups.com/article/view/10.21037/jgo-22-717/coif>). The authors have no conflicts of interest to declare.

Ethical Statement: The 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. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Clinical Research Ethics Committee of Zhongshan Hospital, Fudan University (No. B2021-020R) and informed consent was taken from all the patients.

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