Immune Cell Composition of Liver Hepatocellular Carcinoma and its clinical relevance: A research based on TCGA database

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Research article

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Abstract

Background Liver hepatocellular carcinoma (LIHC), as the main type of liver cancer, has become a main health issue as the third-most common cause of mortality in cancer patients. However, conventional chemo- or radio- therapies shows little improvement in survival, which calls for novel therapies. Because of the immunotolerance mechanism existing naturally in liver, immunotherapy provides significant effect in treatment of LIHC patients. Up to now, various immunotherapies have been proposed, but due to the complex pathways from which LIHC cancerous cell escape immunosurveillance, combined therapies are often needed, which are still under development. **Methods** In the current study, with data downloaded from TCGA database, CIBERSORT was performed for identifying the composition of infiltrating immune cells and further statistical analyses using R 3.5.3 were carried out, aiming at connecting specific immune cells with clinical survival. **Results** With data of immune and stromal scores downloaded from the website of MD Anderson Cancer Centre, both showed significance in survival time. Further analyses based on the result of CIBERSORT demonstrated that the number of macrophages M0 and T cells CD8 infiltration between para-carcinoma and tumour tissues are markedly different. With combination of clinical data, we were able to identify that a higher amount of activated NK cells ($p=0.008$) and a lower amount of resting NK cells ($p=0.047$) presented a longer survival time. **Conclusion** With the help of the TCGA database and multiple techniques, statistical analyses of transcriptome profiling data and clinical data were successfully carried out. The results in this study may pave the way for a new therapeutic strategy which could be combined with current treatments to further improve the clinical outcome of LIHC patients. Further and deeper investigation of other available data, however, were needed in order to verify the results of current study.

Background

Liver cancer has become a main health issue throughout the world. According to the statistics available in GLOBOCAN database(1), despite relatively low incidence of liver cancer overall compared with breast or lung cancer in 2018, its mortality rate was at the fourth place. In males, especially, the second-most cancer-related death were from liver cancer (Figure 1).

Among all types of liver cancer, liver hepatocellular carcinoma (LIHC) is the most common one (2), whereas most patients fail to be detected early enough and are reduced to palliative therapy (3). Because of the immunotolerance mechanism existed naturally in liver, cancer cells can easily escape from the immunosurveillance system, and such tolerance is further enhanced in patients with hepatocellular carcinoma according to previous studies (4,5). As a result, immunotherapy provides significant effect in treatment of LIHC patients, while traditional therapies such as chemotherapy show no benefit for survival rate (6).

Furthermore, increasing researches (7) have proven that tumour microenvironment in LIHC, which mainly includes fibroblasts, immune cells and endothelial cells, plays an important role in pathogenesis and prognosis. The algorithm developed by Yoshihara et al. (8) helps calculate immune and stromal scores

which indicate immune and stromal cells infiltration respectively, which then helps interpret the association between tumour microenvironment and cancer outcomes.

In recent years, novel immunotherapies like anti-CTLA-4 and anti-PD-1 have been proposed (9), and at the same time have longer durability of response and lower cytotoxicity (10). Due to the complex pathways LIHC cancerous cell escape from immunosurveillance, combined therapies are often needed. Thus, this article presents results of immune cells infiltration in LIHC patients and relationship between specific immune cells and clinical survival. Hopefully, the identification of related immune cells would pave the way for a novel combined strategy of various immunotherapies against LIHC.

Methods

Patients and samples

All transcriptome profiling files and clinical data of 376 patients with LIHC were downloaded from TCGA database (11). The results of immune cells in each sample were performed on the website of CIBERSORT developed by Newman et al (12), and those with a p-value less than 0.05 were selected for further study. The results of Stromal and Immune scores were downloaded from the website of MD Anderson Cancer Centre (13) using the ESTIMATE algorithm (8).

Statistical analysis

All analyses were performed with R 3.5.3. Wilcoxon test was used to define the correlations between clinical status and immune data. Survival analysis was performed using Kaplan-Meier curve and log-rank test. Other necessary tests are performed with the help of R packages (Figure 2).

Results

Stromal and Immune scores show correlation with patients' survival

Within all the patients with complete clinical data, 121(32.6%) were female, and 250(67.4%) were male. Clinical staging revealed 171(46.1%) Stage I, 86(23.2%) Stage II, 85(22.9%) Stage III, 5(1.3%) Stage IV and 24 (6.5%) unknown. With data downloaded from the website of MD Anderson Cancer Centre, it showed that stromal score ranged from -1741.56 to 1195.07, while, immune scores ranged from -1209.16 to 2934.36.

In order to determine whether there was correlation between survival time and stromal or immune scores, we divided the patients into two groups based on scores: the lowest 25% as 'low' and the rest 75% as 'high'. Both Kaplan-Meier curve (K-M curve) and log-rank test showed stromal and immune scores were significantly co-related with patients' survival. (Figure 3a & 3b)

Defining immune cells composition in acquired TCGA LIHC transcriptome profiles

As shown in figure 3a & 3b, a high percentage of immune cells infiltration in tumour tissues provides long survival time, which is in accordance with evidence previously reported (14). Therefore, we suggested that immune cells play an important role in LIHC progression and prognosis. To find out specific immune cells composition for further analyses, CIBERSORT (Newman et al.) was carried out using R script downloaded from its website. Results were selected if P value was less than 0.05, and were presented with heatmap and correlation heatmap of 22 types of immune cells (Figure 4a & 4b).

Immune cell related survival analyses

We combined clinical data with immune cell profiles and carried out survival analyses, producing K-M curves for each type of immune cells. However, neither macrophages M0 nor T cells CD8 showed significant correlation with survival time ($p > 0.05$). Interestingly, when samples with a high amount of activated NK cells presented a longer survival time ($p = 0.008$), and samples with a low amount of resting NK cells exactly presented a shorter survival time ($p = 0.047$; Figure 5 (a)-(d)). This indicates that the activity of NK cells in tumour tissues may have a great impact on the prognosis of patients, which is not clearly shown previously.

Discussion

In the current study, we identified macrophages M0 and T cells CD8 as the two mostly infiltrated cells in LIHC, and further analyses demonstrated a negative relationship between them, which meant LIHC patients could be divided into either macrophages-M0-dominated or T-cells-CD8-dominated. Thus, survival analysis was carried out, with a surprising result that neither cell showed significant influence on the survival time. Interestingly, the NK cells pairs, activated and resting NK cells, obviously affect prognosis, suggesting a possible role of NK cells in eliminating malignant tumour cells. However, considering the relatively small number of cases involved and the low amount of cell counts as shown in Figure 4 (a), more repeated analyses with disparate samples should be performed in the future.

As the third-most common cause of mortality in cancer patients (15), LIHC responds poorly to conventional chemotherapy, which calls for novel therapeutics. So far, new drug development mainly focused on T cells and B cells, which are the two primary composing cells of immune repertoire, serving as potential biomarkers for the diagnosis and treatment targets for LIHC. (16) Unfortunately, neither of them demonstrated significance in the current study using TCGA database, but the valid results of NK cells corresponded with what Juengpanich et al reviewed (17): therapies based on NK cells can subsequently lead to higher survival rate of HCC patients. Moreover, the completely opposite results that higher activated NK cells are associated with longer survival time, while higher resting NK cells with

shorter time, provides possibility for developing combined treatment which includes transforming NK cells into active forms.

The main limitation of this study lies in the limited number of valid cases (with $p < 0.05$) after applying the CIBERSORT algorithm. Possible reasons include diversity of cell infiltration in LIHC and insufficient number of cases for a more accurate CIBERSORT prediction. Hence, further studies should include other available databases and/or real-time clinical data.

Abbreviations

LIHC: liver hepatocellular carcinoma; NK cell: natural killer cell;

Declarations

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Authors' contributions

Yu-cheng Ji performed data selection, analysis and was a contributor in writing the manuscript. Guang-xiang Gu helped interpret results, advise appropriate statistical approach and proper language use.

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Availability of data and materials

All data used are included in this manuscript.

Ethics approval and consent to participate

Not applicable (using open-access online database)

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

Estimated age-standardized cancer incidence and mortality rates (World) in 2018 in males of all ages

Figure 2

Flow chart of the main analysis procedures in this study

Figure 3

(a) showed the result of Kaplein-Meier test. Patients were divided into two groups, the lowest 25% as 'low' and the rest 75% as 'high'. The curve shows that mean survival time of 'high' group is longer than the 'low' group (587.5 days vs 421 days, $p=0.038$); (b) showed the result of Kaplein-Meier test. Patients were also divided into two groups, the lowest 25% as 'low' and the rest 75% as 'high'. The curve shows that mean survival time of 'high' group is longer than the 'low' group (577 days vs 428 days, $p=0.026$)

Figure 4

. (a) Heatmap of immune cell composition of 58 samples whose p value is less than 0.05, 3 of which are para-carcinoma tissues (indicated as blue blocks in the top bar) and 55 of which are tumour tissues (indicated as pink blocks in the top bar). While most cells present no significant difference, the number of macrophages M0 and T cells CD8 infiltration between para-carcinoma and tumour tissues are markedly different. (b) Correlation heatmap of the 22 types of immune cells. T cells follicular helper and T cells CD8, T cells CD4 memory activated and T cells CD8 were the two most positively correlated pairs, while macrophage M0 and T cells CD8, T cells CD4 memory resting and activated were the two most negatively correlated pairs.

Figure 5

(a)-(d) show K-M curves of four types of immune cells divided by medians into high and low groups. Surprisingly, though macrophage M0 and T cells CD8 both show obvious difference between para-carcinoma tissues and tumour tissues, correlations with survival time were not as significant as predicted. But interestingly, activated and resting NK cells expressed impact on survival status.