Identification of Novel Immune-infiltration Related miRNAs that can Predict the Prognosis of Patients with Hepatocellular Carcinoma

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Research Article

Keywords: miRNAs, Tregs, hepatocellular carcinoma, immune checkpoint inhibitor therapy, prognosis

Posted Date: February 17th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1263474/v1>

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Abstract

Background: Although immune checkpoint inhibitors (ICIs) have achieved important breakthroughs in the treatment of liver cancer, most hepatocellular carcinoma (HCC) patients do not respond to ICIs, and their clinical outcome remains unsatisfactory. The tumor microenvironment (TME), which contains immune cells and other molecules, probably influences both the prognosis and the response to immunotherapy in HCC. Here, using integrated bioinformatics analyses, we identified key molecules (immune cells and immune-related microRNAs (miRNAs)) as potential prognostic markers for HCC.

Method: mRNA and miRNA expression profiles and clinicopathological data were extracted from the Cancer Genome Atlas Liver Hepatocellular Carcinoma (TCGA-LIHC) data collection. A total of 22 immune cell types from HCC patients were assessed using a deconvolution algorithm (known as CIBERSORT). Both Kaplan-Meier analysis and Cox proportional hazards regression were used to evaluate the prognostic value of the immunocytes. The correlations between differentially expressed miRNAs and tumor-infiltrating immune cells were investigated using the Pearson correlation coefficient. Machine learning approaches were employed to identify prognostic miRNAs. The miRTarBase database was used to construct a DEmiRNA-target gene network. Function enrichment analyses were performed using Metascape software. The relationships between two specific miRNAs and the expression of immune markers (antigen presenting machinery (APM) and tumor-infiltrating lymphocytes (TILs)) as well as cell checkpoint markers were also analyzed.

Results: A total of 19 T regulatory cell (Treg)-related miRNAs were identified by machine learning approaches ($p < 0.001$, $|\text{cor}| > 0.2$). Metascape revealed that Treg-related miRNA-target networks were significantly enriched in biological processes including cellular responses to stress, mitotic cell cycle and myeloid cell differentiation. Using univariate Cox regression analysis on an independent dataset, GSE31384, 19 miRNAs were evaluated for their importance in overall survival (OS). Two hub miRNAs (hsa-miR-877 and hsa-miR-137) were found to significantly impact on OS. Therefore, we assessed the association between these two miRNAs and immune markers (APM, TILs) and cell checkpoint markers (programmed cell death-1 (PD-1), programmed death-ligand 1 (PD-L1), cytotoxic T-lymphocyte antigen-4 (CTLA-4), B- and T-lymphocyte attenuator (BTLA)), and found statistically significant associations in both the TCGA and GSE31384 datasets.

Conclusion: Our study showed that hsa-miR-877 and hsa-miR-137 were significantly associated with poor prognosis in HCC through their effects on Tregs and immune checkpoint markers in the TME. These results suggest that hsa-miR-877 and hsa-miR-137 might serve as novel therapeutic targets for HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is an aggressive and rapidly fatal malignancy, especially in Asians, with increasing incidence and mortality in recent years^[1]. Aside from conventional therapies, there have been several significant advances in HCC treatment options, such as immunotherapy and targeted

therapy [2-4]. ICIs including PD-1/PD-L1 and CTLA-4, have heralded crucial clinical breakthroughs in monoclonal antibodies treatment, targeting immune checkpoint molecules and reactivating the host immune response against cancer cells [5]. Although these agents offer selective and efficient therapeutic strategies, the treatments are not equally effective in all HCC patients [6-8]. Most HCC patients do not respond to ICIs, and the clinical outcome of most HCC remains unsatisfactory.

Several studies have shown that tumor-infiltrating immune cells (TICs) are part of the complex TME and can interfere with primary and secondary resistance to immunotherapy [9]. For example, Kumar et al. reported that tumor-infiltrating myeloid cells induce immune suppression and resistance to ICIs, promoting tumor progression through the production of anti-inflammatory cytokines [10]. On the other hand, Keung et al. found that tumors enriched for activated CD8+ T cells and PD-L1+ macrophages are more responsive to PD-1 blockade [11]. However, the precise mechanisms by which TICs regulate the efficacy of ICIs remain largely unknown. Therefore, investigating the potential regulatory pathways will lead to a better understanding of the benefits of ICI therapy for patients.

MiRNAs, a class of non-coding short (approximately 22 nucleotides) single-stranded endogenous RNA molecules, are key post-transcriptional regulators, causing both translational repression and degradation of target genes by binding to the 3'-untranslated region sites of mRNA transcripts [12-14]. Aberrantly expressed miRNAs have frequently been detected in tumor-associated genomic regions in various human cancers and have been observed in both HCC tissues and cells [15]. Accumulating evidence indicate that miRNAs are involved in a wide range of pathological processes during HCC genesis and development, and play important roles in the TME [16-20].

Increasing evidence indicate that partial miRNAs are also involved in immune regulation. These immune-related miRNAs (immuno-miRs) can regulate cell proliferation, cytokine production, immune functions in a range of different immune cells, and contribute to the immune escape of tumors [21]. Pua et al. reported that co-clustered miR-24 and miR-27 mediates an IL4-network-related suppression of Th2 immunity by regulating the protein expression of multiple target genes [22]. Moreover, miR-146a was identified as a link in the chain of a negative feedback loop regulating T-cell activation by suppressing NF- κ B activity. A recent study showed that [miRNA-146a modulated immune-related adverse events caused by ICIs](#) [23-24]. All of these studies strongly suggest that TICs and miRNAs are closely connected and affect the immune microenvironment and immunotherapy of tumors. Therefore, the development and clinical application of immuno-miRs as adjunctive immune modulatory agents in HCC patients may be a promising strategy in the foreseeable future.

In this study, we used systematic analyses to identify and validate a novel biomolecular risk model based on the immuno-miR signature to predict the prognosis of patients with HCC. Moreover, we identified two key regulators, hsa-miR-877 and hsa-miR-137, that played roles in recruiting and facilitating the infiltration of T regulatory cells (Tregs) in the TME. We explored the role of these immuno-miRs in regulating the expression of the immune checkpoint related genes PDCD1, CD274, CTLA-4, CD47 and

BTLA, and the resulting effect on tumor immunity. In brief, this study established an immuno-miRs signature, in terms of miRNAs expression levels, which could predict the response to immunotherapy and the prognosis of HCC patients. These data give promising insights into approaches to predicting prognosis and responsiveness to ICI treatment in HCC patients.

2. Materials And Methods

2.1 mRNA and miRNAs expression datasets

Data from two publicly available datasets were incorporated into our study. The miRNA and mRNA expression data and the corresponding clinical information from samples from patients from TCGA with LIHC were downloaded from the Genomic Data Commons (GDC, <https://portal.gdc.cancer.gov/>): a total of 425 samples, including 50 normal and 374 LIHC tumor samples. miRNA expression levels detected by microarray from 166 LIHC from the GSE31384 data set, with corresponding OS data, were downloaded from the Gene Expression Omnibus (GEO) (<http://www.ncbi.nlm.nih.gov/geo>), and served as the validation set. GSE31384 miRNA expression data were quantile normalized across multiple arrays and log2 transformed. Only the miRNAs with expression in more than 15% of samples were subjected to further statistical analyses.

2.2 Evaluation of prognostic tumor-infiltrating immune cells

The numbers of each type of tumor-infiltrating immune cells were quantified using CIBERSORT ^[25]. CIBERSORT is an analytical tool that provides an estimation of the abundance of specific cell types in a mixed cell population using a gene expression-based approach. In this study, the range of the 22 infiltrating immune cells of the TCGA-LIHC samples was calculated based on the LIHC mRNA expression data. The CIBERSORT score after running 1000 permutations is available in the CIBERSORT website (<https://cibersort.stanford.edu/index.php>). A univariate Cox proportional hazards regression model was used to select immune cell components associated with OS in the TCGA cohort.

2.3 Identification of immune- and prognosis-related miRNAs

To identify the miRNAs differentially expressed in the LIHC and normal samples, the downloaded miRNA data were standardized, and differential expression analysis was performed using the edgeR software package. A total of 238 differentially expressed miRNAs with a false discovery rate (FDR) < 0.01 were considered for subsequent analysis. Next, using the Tregs selected via CIBERSORT, we performed a Pearson correlation analysis to calculate the correlation between the expression of the 238 differentially expressed miRNAs and the Tregs score. We found 39 differentially expressed miRNAs that were significantly associated with the infiltration of Tregs ($p < 0.001$, $|\text{cor}| > 0.2$). A univariate Cox proportional

hazards regression model was then performed to assess the association of these 39 miRNAs with OS in the TCGA cohort. We found 19 immune- and prognosis-related miRNAs with $p < 0.05$. The results of the differential expression, Pearson correlation and univariate Cox proportional hazards regression analyses are shown in Table S1.

2.4 miRNAs-target interactions and functional analysis

Experimentally validated miRNA-target interactions were obtained from the miRTarBase database (<http://mirtarbase.cuhk.edu.cn/php/index.php>). We extracted 2330 miRNA-target interactions involving 19 miRNAs and 1593 target genes, which were used to construct an interaction network. The degrees of the 19 miRNAs in the interaction network are shown in Table S2. We performed pathway and process enrichment analyses using the following ontology sources: the Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway, the Gene Ontology (GO) Biological Processes, and Reactome Gene Sets by Metascape^[26], a web-based tool (<https://metascape.org/gp/index.html>). We chose Metascape because the database is updated monthly to ensure up-to-date content. The Metascape analysis was performed using the default settings. Terms with a $p < 0.01$, a minimum count of 3, and an enrichment factor > 1.5 were collected and grouped into clusters based on their membership similarities. To further capture the relationships between the terms, a subset of enriched terms was selected and rendered as a network plot, where terms with a similarity > 0.3 are connected by edges.

2.5 miRNAs immune correlation analysis

Pearson correlation analysis was used to explore the association between the expression of miRNAs and immune checkpoint-related genes, including PD-1, PD-L1, CTLA-4, CD47 and BTLA. We divided the LIHC samples into two groups based on the median value of miRNA expression and a one-sided Wilcoxon rank test was used to evaluate the differences in five immune checkpoint-related gene expression between the high and low expression groups. We then collected APM genes from several studies^[27-29], and TIL genes from He et al., Bindea et al., Ali et al. and Garnelo Ali et al.^[30-33]. Single-sample gene set enrichment analysis (ssGSEA) in the Gene Set Variation Analysis (GSVA) package for R was used to calculate the enrichment scores for each sample based on the APM and TILs gene sets.

2.6 Association between miRNAs, survival and clinical characteristics

We downloaded OS data from TCGA and GSE31384. A log-rank test was used to assess the difference in the survival time between patients with high and low expression of miRNAs. The patients were grouped by the median value of their miRNA expression, and Kaplan-Meier plots were used to present the results. Clinical information of the TCGA-LIHC samples were obtained from the GDC data portal (<https://gdc->

portal.nci.nih.gov/legacy-archive/). The LIHC tumor samples were grouped according to stage (S) and grade (G), and a one-sided Wilcoxon rank test was used to evaluate the differences between the groups.

3. Results

3.1 Tumor-infiltrating immune cells in LIHC

To evaluate immune cell infiltration in the LIHC samples, we calculated the abundance of 22 types of immune cells in the TCGA-LIHC samples using CIBERSORT (Figure 1A). Next, we explored the proportions of different subpopulations of tumor-infiltrating immune cells in the TCGA-LIHC samples (Figure 1B). Naïve CD4 T cells, M2 macrophages and M0 macrophages accounted for a large proportion of the LIHC immune cell infiltrate. To assess the prognostic value of the 22 different types of immune cells for patients with LIHC, a univariate Cox proportional hazards regression model was performed to select immune cell components associated with OS in the TCGA cohort (Figure 1C). M0 macrophages ($p = 0.0014$), follicular helper T cells ($p = 0.0023$) and regulatory T cells ($p = 0.039$) were found to be significant risk factors. Resting CD4 memory T cells ($p = 0.018$) and resting mast cells ($p = 0.027$) were found to be significant protective factors.

3.2 Identification of immune- and prognosis-related miRNAs

To identify miRNAs that play critical roles in tumor progression, we performed differential expression analysis of the LIHC and normal samples. We found that 238 miRNAs were differentially expressed with an FDR < 0.01 (Figure 2A). To further identify miRNAs that correlated with immune infiltration, we performed a Pearson correlation analysis of the Treg infiltration scores and the 238 differentially expressed miRNAs. We found that 39 miRNAs were associated with Treg cell infiltration, with $P < 0.001$ and $|COR| > 0.2$. Of these, 33 were positively correlated, and 6 were negatively correlated, with Treg infiltration (Figure 2B). Next, we used a univariate Cox proportional hazards regression model to analyze the association of the 39 miRNAs with OS in the TCGA cohort. Our results showed 19 immune- and prognosis-related miRNAs with $P < 0.05$, and all 19 miRNAs were risk factors (Figure 2C).

3.3 miRNAs-target network construction and survival analysis

To explore the effect of miRNA on target genes, we constructed a miRNA-target gene network according to the interactions provided in the miRTarBase database. In the network, the 5 miRNAs with the highest degrees were hsa-miR-18a, hsa-miR-877, hsa-miR-760, hsa-miR-137 and hsa-miR-6844 (Figure 3A; Table S2). Survival analysis (log-rank test) showed that TCGA-LIHC patients with high expression of hsa-miR-877 had a poorer prognosis than those with low expression of hsa-miR-877 (Figure 3B; $p = 0.035$).

Similarly, TCGA-LIHC patients with high expression of hsa-miR-137 had a poorer prognosis than those with low expression of hsa-miR-137 (Figure 3C; $p = 0.089$). Next, we assessed the risk score for 166 LIHC samples based on their expression of hsa-miR-877 or hsa-miR-137 using the univariate Cox proportional hazards regression model and the GSE31384 dataset (Figure 3D, F). The number of patients alive in the low-risk group was greater than the number of patients alive in the high-risk group based on both the hsa-miR-877 and the hsa-miR-137 risk score (Figure 3D, F). Survival analysis (log-rank test) of the GSE31384 dataset also showed that patients with high expression of hsa-miR-877 had a poorer prognosis than those with low expression of hsa-miR-877 (Figure 3E; $p = 0.011$), and patients with high expression of hsa-miR-137 had a poorer prognosis than those with low expression of hsa-miR-137 (Figure 3G; $p = 0.019$).

3.4 Functional analysis of target genes

To identify the functional processes regulated by the 19 miRNAs, we performed pathway and process enrichment analyses for their target genes using Metascape, including 10 pathway resources. We found that the biological processes that were significantly enriched included cellular responses to stress, mitotic cell cycle, myeloid cell differentiation and regulation of cellular response to stress (Figure 4A). To further capture the relationships between the terms, a subset of enriched terms were selected and rendered as a network plot, where terms with a similarity > 0.3 were connected by edges (Figure 4A). We then explored the biological functions of the 19 miRNAs target genes using the GO database. The biological processes that were significantly enriched included immune system process, cellular component organization or biogenesis, response to stimulus, and cellular process (Figure 4B).

3.5 Relationship between hub miRNAs and immune and clinical characteristics

To further examine the association between the hub miRNAs and immunity, we assessed the relationship between the two survival-related miRNAs (hsa-miR-877 and hsa-miR-137) and various immune checkpoint related genes. Pearson correlation analysis showed that the expression levels of both hsa-miR-877 and hsa-miR-137 were significantly correlated with five immune checkpoint related genes (Figure 5A). In particular, hsa-miR-877 showed significant correlation with CTLA4 (Figure 5B; $p = 2.8E-11$) and PD-1 (Figure 5C; $p = 9.3E-11$). Next, we evaluated the differences in expression levels of five immune checkpoint related genes in the hsa-miR-877 and hsa-miR-137 low and high expression groups using the one-sided Wilcoxon rank-sum test. We found that the expression of CD47 ($p = 2.0E-02$), CTLA4 ($p = 1.2E-07$), PD-1 ($p = 1.5E-07$) and BTLA ($p = 5.3E-03$) in the hsa-miR-877 high expression group was significantly greater than that in the hsa-miR-877 low expression group (Figure 5D). We also found that the expression of CTLA4 ($p = 1.2E-08$), PD-1 ($p = 7.3E-06$), BTLA ($p = 3.7E-04$) and CD47 ($p = 7.6E-02$) in the hsa-miR-137 high expression group was significantly greater than in the hsa-miR-137 low expression group (Figure 5E).

3.6 Single-sample gene set enrichment analysis (ssGSEA)

We used ssGSEA to evaluate the enrichment of two immune related gene sets in the TCGA-LIHC cohort. We found that, using the one-sided Wilcoxon rank-sum test, the gene set enrichment score for the expression of both APM ($p = 9.5E-03$) and TIL ($p = 2.4E-02$) in the hsa-miR-877 high expression group was significantly greater than that in the hsa-miR-877 low expression group (Figure 6A). Similarly, the gene set enrichment score for the expression of both APM ($p = 1.1E-03$) and TIL ($p = 7.1E-02$) in hsa-miR-137 high expression group was significantly greater than that in hsa-miR-137 low expression group (Figure 6B). In addition, using the one-sided Wilcoxon rank-sum test, we examined the association between the expression of hsa-miR-877 and hsa-miR-137 and clinicopathological features (Figure 6C-D). We found that there was a significant difference in the expression of hsa-miR-877 between G1 and G3 ($p = 0.0029$), G1 and G4 ($p = 8.8E-03$), G2 and G3 ($p = 1.9E-03$) and G2 and G4 ($p = 1.2E-02$). Similarly, there was also a significant difference in the expression of hsa-miR-877 between Stage I and Stage II ($p = 0.059$) and Stage I and Stage III ($p = 0.054$).

4. Discussion

While the prognostic role of miRNAs in HCC has been reported in several studies^[34], their role in the modulation of the TME remains largely unclear. In this study, we identified Tregs as a prognostic predictor for HCC using the CIBERSORT deconvolution machine learning algorithm, and identified 19 immune-related miRNAs significantly associated with the infiltration of Tregs in the TME. Two hub miRNAs showed significant prognostic performance in both the training and the validation datasets. These two miRNAs (hsa-miR-877 and hsa-miR-137) could, therefore, potentially serve as indicators for the infiltration of Tregs into the TME and predictors for the prognosis and immunotherapy responses of HCC patients under various clinical settings, including microenvironment cells, immune checkpoint markers, and antigen-presenting cells. Our analyses provide novel references for using miRNAs as potential candidate agents in ICI immunotherapy.

Tregs, a subset of CD4⁺ T cells, are central regulators of tolerance and immunity, stabilizing the suppressive phenotype of the TME^[34]. Previous studies reported that the infiltration of Tregs in the TME is indicative of poor OS in various cancer subtypes^[35-37]. Using machine learning techniques, we found that the enrichment of Tregs in the TME is one of the top predictors of poor prognosis in HCC patients. Consistent with our finding, Hsu P et al. reported that Tregs are major components of the immunosuppressive TME^[38]. Moreover, another study showed that high levels of Tregs correlated with low responses in patients treated with ICIs^[39]. This may be one of the main reasons for the correlation between high numbers of tumor-infiltrating Tregs and poor prognosis; in fact, the effect of Tregs on the microenvironment and immune tolerance may be key to poor outcomes.

Our studies highlight the fact that Tregs are regulated by more than Treg-intrinsic pathways, such as TGF- β activity and CCL22^[40], we found that Treg-related miRNAs also played a significant regulatory role.

The targets of the 19 Treg-related miRNAs were significantly enriched in biological processes, including cellular responses to stress, mitotic cell cycle, myeloid cell differentiation and regulation of cellular response to stress, all of which have been implicated in the regulation of the differentiation of Tregs [41-43]. This finding further suggests a critical role for Treg-related miRNAs in Treg-induced pathogenesis. For example, miR15a/16 has been reported to directly target the 3'UTR regions of FOXP3, thus regulating Tregs at the post-transcriptional level [44]. Xia Lin et al. reported that miR-155-deficient mice exhibited a decrease in the numbers of Tregs in both the thymus and the spleen, and that miR-155 specifically targeted suppressor of cytokine signaling 1 (SOCS1), favoring Treg homeostasis [45]. Moreover, miR-146a-deficient Tregs were unable to suppress effector T cell activation [46]. Hence, miRNAs play an important role in regulating the differentiation and function of Tregs. The discovery of Treg-related miRNAs provides new insights into the underlying regulatory mechanisms of Tregs that result in the alteration of the TME and contribute to tumor biology and the therapeutic response to ICI.

We further examined the main hub Treg-related miRNAs (hsa-miR-877 and hsa-miR-137), which we showed were independent indicators of poor outcome. Hsa-miR-877 was found to be overexpressed in gastric cancer, and also antagonized the promoting effect of Substance P in the occurrence and progression of gastric cancer, suggesting that miR-877-5p could be a potential drug target [47]. Further, miR-877-5 has been reported to participate in gene regulation of competing endogenous RNA (ceRNA) mechanisms in multiple tumors, including cervical cancer, malignant gliomas, and non-small-cell lung cancer [48-50]. Yijun Yang et al. showed that Hsa-miR-137 effectively induced apoptosis and diminished tumorigenicity in multiple myeloma [51]. Chonglin Luo et al. also reported miR-137 could inhibit the invasion of melanoma cells [52]. Another report also suggested the prognostic relevance of miR-137 in patients with hepatocellular carcinoma [53]. We found that patients with high expression of either hsa-miR-877 or hsa-miR-137 had poorer prognosis than those with low expression of either hsa-miR-877 or hsa-miR-137. This shows that these hub Treg-related miRNAs (hsa-miR-877 and hsa-miR-137) are important prognostic markers for HCC patients.

It is widely accepted that the immune system homeostasis plays a key role in controlling tumor growth and metastasis. Multiple factors have been reported to affect escape from host's immune system and resistance to anti-PD-1 monotherapy. These factors include lack of PD-L1 expression (TIL+/PD-L1-) [54], lack of cytotoxic T lymphocyte infiltration [55] and defects in antigen presenting cells [56]. To investigate the role of hsa-miR-877 and hsa-miR-137 in the host immune system, we evaluated the expression levels of TILs, HLA class I APM component and checkpoint markers, and found significant correlations between them. The results suggest that the regulation of tumor immunity by hsa-miR-877 and hsa-miR-137 may occur not only through Tregs, but also through the dysregulated expression of the APM component and by affecting the enrichment of TILs. Moreover, miRNAs have been found to be expressed in specific cells and tissues at special developmental stages and conditions. These facts suggest that the specific expression of hsa-miR-877 and hsa-miR-137 plays a key role in the fight against tumor cells by the host adaptive immune system. Further, we identified potential effects of hsa-miR-877 and hsa-miR-137 on the tumor immune microenvironment (TIM) of HCC, which suggests that hsa-miR-877 and hsa-miR-137 may

be potential predictive biomarkers for the cancer immunotherapy response. As the immunosuppressive microenvironment is a major obstacle for successful tumor immunotherapy, the implementation of “combination immunotherapy” is a new hope for liver cancer treatment.

This study had some limitations. First, although the association between miRNA and Tregs could successfully predict HCC patients’ prognosis, there was only limited information on the clinical characteristics of the patient cohorts that we investigated. In addition, although a favorable performance in the external validation suggested that hsa-miR-877 and hsa-miR-137 could be potential candidates as regulators of the TIM, it is too early to conclude that immune-related miRNAs could act as novel immunotherapy reagents to reverse resistance in ICI non-responders in HCC. Therefore, further studies are required to confirm our findings.

This study aimed to provide new insights into the potential mechanisms involved in the role of miRNAs in immune escape and immune microenvironment regulation in HCC. Our data showed that hsa-miR-877 and hsa-miR-137 are key immune-related miRNAs that could affect the infiltration of Tregs in the TME. These data provide vital information that could help to guide further studies exploring potential combination immunotherapeutic approaches for HCC.

Declaration

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from TCGA-LIHC database and GEO database with accession number GSE31384.

Competing interests

The authors declare that they have no conflict of interest.

Funding

This work was supported by Beijing CSCO Clinical Oncology Research Foundation (Y-2019AZMS-0055); the Natural Science Foundation of Liaoning Province (No. 20180550781), and Liaoning Cancer Hospital & Institute–Dalian University of Technology Medical Engineering Cross Research Fund (LD202031); the Provincial doctoral research start-up fund project (2021-BS-040).

Authors' contributions

Wenxin Li performed data collection, data analysis and manuscript writing. Yefu Liu executed the study design. All the authors read and approved the final manuscript.

Acknowledgments

The authors would like to thank TopEdit (www.topeditsci.com) for English language editing of this manuscript.

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Figures

Figure 1

Infiltration of 22 types of immune cells and their relevance in the prognosis of TCGA-LIHC

(A) The proportion of 22 different immune cells was evaluated by CIBERSORT among LIHC tumor samples. (B) The distribution of 22 immune cells in TCGA-LIHC cohort. (C) A univariate Cox proportional hazards regression analysis to assess the relationship between the immune cell components and prognosis in the TCGA cohort. Horizontal axis is $-\log_2$ (HR).

Figure 2

Identification of immune- and prognosis-related miRNAs. (A) Volcano plot showing differential expression of miRNAs in TCGA-LIHC and normal samples. Blue points show upregulation, orange points show

downregulation. (B). Pearson correlation analysis identified significant differences in differentially expressed miRNAs and Tregs ($p < 0.001$, $|cor| > 0.2$). Purple bars depict positive correlation, yellow bars depict negative correlation. (C) A univariate Cox proportional hazards regression analysis showing the relationship between immune cell components and prognosis in the TCGA cohort.

Figure 3

miRNAs-target network construction and analysis of prognosis. (A) Network of 19 miRNAs and their target genes. Purple nodes depict miRNAs, pink nodes depict target genes. (B)-(C) Log-rank test was used to assess the differences in OS between patients from the TCGA-LIHC cohort with high and low expression of hsa-miR-877 and hsa-miR-137. (D) Distribution of survival time and risk score based on the expression of hsa-miR-877 in the GSE31384 patient cohort. (E) Log-rank test was used to assess the difference in OS between GSE31384 patients with high and low expression of hsa-miR-877. (F) Distribution of survival time and risk score based on the expression of hsa-miR-137 in the GSE31384 patient cohort. (G) Log-rank test was used to assess the difference in OS between GSE31384 patients with high and low expression of hsa-miR-137.

Figure 4

Functional analysis of target genes. (A) For the 19 miRNA target genes, pathway and process enrichment analysis was carried out using the following ontology sources: KEGG Pathway, GO Biological Processes, Reactome Gene Sets, Canonical Pathways, CORUM, TRRUST, DisGeNET, PaGenBase, Transcription Factor Targets and COVID. The graph shows the top 20 enrichments with $p < 0.01$. P value was multi-test adjusted in log base 10. Terms with a similarity > 0.3 are connected by edges. (B) GO enrichment biological processes terms for 19 miRNA target genes.

Figure 5

Relationship between expression of immune checkpoint related genes and hsa-miR-877 and hsa-miR-137. (A) Pearson correlation analysis to assess the association between the expression of hsa-miR-877 and hsa-miR-137 and immune checkpoint related genes, including PDCD1 (PD-1), CD274 (PD-L1), CTLA-4, CD47 and BTLA in the TCGA-LIHC cohort. The color of heatmap depicts $-\log_{10}(p)$ values. (B)-(C) Scatter diagram showing the distribution of hsa-miR-877 and hsa-miR-137 with immune checkpoint related genes expression. (D)-(E) The expression differences of five immune checkpoint related genes in hsa-miR-877 or hsa-miR-137 low or high expression groups in the TCGA-LIHC cohort. P values were calculated using a one-sided Wilcoxon rank-sum test.

Figure 6

ssGSEA and clinical characteristics correlation analysis. (A) Distribution of APM and TIL gene sets ssGSEA score in hsa-miR-877 low and high expression group in TCGA-LIHC patient cohort. (B) Distribution of APM and TIL gene sets ssGSEA score in hsa-miR-137 low and high expression group in the TCGA-LIHC patient cohort. (C) Differences in hsa-miR-877 expression among TCGA-LIHC patients based on their clinical pathological grade. (D) Differences in hsa-miR-877 expression among TCGA-LIHC patients based on their clinical pathological stage. P values were calculated using a one-sided Wilcoxon rank-sum test.

Supplementary Files

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- [TableS119miRNAsresults.xlsx](#)
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