A novel defined basement membrane-related genes signature for predicting the prognosis of Hepatocellular carcinoma

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Research Article

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Abstract

**Background.** Hepatocellular carcinoma (HCC) is a highly heterogeneous disease with poor prognosis, making the prediction of the prognosis much challenges. Basement membrane-related genes (BMRGs) play an important role in the progression of cancer. Thus, they are often used as targets to inhibit tumor progression. However, the value of BMRGs in predicting prognosis of HCC still remains to be further elucidated. This study aimed to find the relationship between BMRGs and HCC and the value of BMRGs in predicting the prognosis of HCC.

**Methods.** We acquired transcriptome and clinical data of HCC from The Cancer Genome Atlas (TCGA) and randomly divided the data into training and test sets in order to develop a reliable prognostic signature of BMRGs for HCC. The BMRGs model was built using multivariate Cox regression, least absolute shrinkage and selection operator (LASSO), and univariate Cox regression. The risk signature was further validated and assessed using the principal component analysis (PCA), Kaplan-Meier analysis, and time-dependent receiver operating characteristics (ROC). To forecast the overall survival, a nomogram and calibration curves were created (OS). Functional enrichment analysis was used to evaluate the potential biological pathways. We also conducted immunological research and a pharmacological comparison between the high- and low-risk groups in this study.

**Results.** We identified 16 differentially expressed genes and constructed a risk model of four BMRGs, including COL2A1, CTSA, LAMB1,P3H1. The PCA analysis showed that the signature could distinguish the high- and low-risk groups well. Patients in the low-risk group showed significantly better outcome compared with patients in the high-risk group. Receiver operating characteristic (ROC) curve analysis show predictive capacity. Moreover, the nomogram showed good predictability. Univariate and multivariate Cox regression analysis validated that the model results supported the hypothesis that BMRGs were independent risk factors for HCC. Furthermore, analysis of clinical characteristics and tumor microenvironment (TME) between risk groups showed significant difference. Functional analysis revealed different immune-related pathways were enriched, and immune status were different between two risk groups. Mediation analysis with IC50 revealed that the two risk group were significantly different, which could be a guidance of systemic treatment. Finally, we further verified in clinical samples that the mRNA and protein expression levels of the four genes in this model are significantly higher in liver cancer tissues than in adjacent tissues.

**Conclusion.** A novel BMRGs signature can be used for prognostic prediction in HCC. This provide us with a potential progression trajectory as well as predictions of therapeutic response.

Introduction

Liver cancer is the sixth most common cancer in terms of incidence among malignancies and is the third leading cause of tumor-originated death worldwide¹. Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer, which is related to diversity risk factors, such as chronic HBV
or HCV infection, alcoholic behavior, nonalcoholic fatty liver disease and so on. As is known to all, HCC is a highly heterogeneous disease that has been demonstrated at the inter-tumoral and intra-tumoral levels. Owing to liver cancers are asymptomatic in the early stage, the 5-year survival rate is still unsatisfactory. Systemic treatment is of great importance in those who cannot undergo surgical operation. Chemotherapeutics and target therapeutics are regular systemic treatments which were reported with failure and side-effect. Immunotherapy has had improved greatly in treatment of malignancies during recent years. But the percentage of those reactivated to checkpoint inhibitors was just 30%, which are mostly attribute to immunotherapy resistance.

Considering the limitations of meditation, new therapeutic targets are urgent to improve the prognosis of HCC patients. Thus, reliable prognostic models are urgently needed in target therapies and immunotherapies. Basement membranes (BMs), which are extensively dispersed parts of the extracellular matrix that surrounds and supports most other tissues as well as the epithelia and endothelia, are composed of self-assembled laminins, type IV collagens, nidogens, and proteoglycans. Additionally, BMs have the ability to control cell polarity, differentiation, migration, and survival. BM proteins are targets of autoantibodies in immune disorders. A significant pathogenic component of cancer, diabetes, and fibrosis is abnormalities in BM protein expression and turnover. According to Reuten et al., the amount of the BM protein netrin-4 was strongly correlated with the prognosis of breast cancer, kidney cancer, and melanoma, and that the stiffness of the BM had a significant role in the development of metastases. Previous research has shown that the destruction or alteration of BM components is strongly connected with a bad prognosis for malignancies. Given the vital part BMs play in the development of cancer, they should be taken into consideration as a potential target for preventing the disease. However, basement membrane-related genes (BMRGs) do not yet have a predictive model. Therefore, we sought to identify the predictive hallmark of BMRGs in order to evaluate and facilitate the prognosis of HCC. We conducted additional analyses based on the signature using the relevant public data, such as ESTIMATE scores, functional enrichment analysis, immunological analysis, and drug sensitivity.

Materials And Methods

Data acquisition

The gene expression datasets of HCC samples, normal samples and the corresponding clinical information were downloaded from the Cancer Genome Atlas (TCGA) (https://portal.gdc.cancer.gov/). The gene expression data and clinical information of external validation data set was downloaded from International Cancer Genome Consortium (ICGC, LIRI_JP). Datasets were processed with R software (4.1.1) and strawberry perl (v5.30.1). In order to reduce possible statistical bias in this analysis, HCC patients without overall survival (OS) values or short OS values (<30days) were dismissed. Consequently, 350 patients with relevant clinical information were retrieved which were divided into the train group and test group randomly using R caret package with ratio 1:1. The training set was utilized to
construct a basement membrane model, and the entire set and testing set were used to validate the model. The data from TCGA and ICGC was publicly available. The study was out of the approval of local ethics committees.

**Obtaining of basement membrane-related genes.**

We extracted 224 BM-related genes, including genes with confirmed evidence of protein localization to the BM zone (from protein immunolocalization studies), components with confirmed evidence of protein localization to the human BM zone, genes predicted to be in the BM zone based on protein interaction data or BM protein-cleaving protease activity\(^\text{16}\) (Supplementary data 1). Then the ‘limma’ R package was applied to identify differentially expressed BMRGs, with \(|\log_2 (\text{fold change})| > 2\) and \(p < 0.05\) as filtering criteria\(^\text{17}\).

**Construction and Validation of the Risk Model**

Using limma package of R software, 98 BMRGs differentially expressed genes (DEGs) were discovered (FDR\(<0.05,|\log_2\text{FC}|>1\)). With the overall survival (OS) clinical information and DEG expression matrix of HCC cases in the TCGA, we got matrix with OS information. The patients were randomly and equally divided into training (175 cases) and test (175 cases) datasets using R “caret” package. Then we performed univariate Cox proportional hazard regression to screen 16 BMRGs significantly linked with OS. Furthermore, these genes were performed least absolute shrinkage and selection operator (LASSO) Cox regression analysis. Then 4 BM-related genes were further performed multivariate Cox regression analysis and a score model (BMRGs model) of each patient was obtained by applying the gene expression of each BM-related gene and its coefficient. (Lasso regression was run with 10-fold cross-validation, \(\text{maxit} = 1000\), \(p < 0.05\)). The risk score \(= (0.165117630233135 \times \text{COL2A1 exp.}) + (0.283642055740619 \times \text{CTSA exp.}) + (0.36470761635581 \times \text{LAMB1 exp.}) + (0.428660392642715 \times \text{P3H1 exp.}).\) With the median risk score of the training set, the patients were separated into high and low risk groups. ICGC dataset worked as external validation.

PCA and t-SNE analysis based on built-in R prcomp function and "Rtsne" R packages showed that the two risk groups were well separated. Time-dependent ROC curves (1,3,5years) were used to validate the accuracy of the model. Univariate Cox and multivariate Cox regression analyses were used to select independent factors. Nomogram and Calibration analyses were also conducted. They were applied to evaluate the ability of model in prognostic values. Nomogram and Calibration (for 1,3,5years) were plotted applying “regplot”, “rms” and R packages.

The Human Protein Atlas (HPA,https://www.proteinatlas.org/) provided the qualitative data of protein in HCC. We obtained the Immunohistochemical pictures of BMGs of risk model between normal and tumor samples.

**Gene Set Enrichment Analyses**
We discovered immune associated function pathway enriched in both high and low risk groups using GSEA and GO enrichment analysis of pathway in risk samples \((p < 0.05;FDR<0.05)\). The full table was presented in Supplementary data 2. The top 5 enriched pathways in the two risk groups with curated gene sets were displayed using the GSEA software version 4.23 and Strawberry Perl software. (kegg.v7.4.symbols.gmt) \((p<0.05,FDR<0.05)\). The full table was presented in the Supplementary data 2. The “ggplot2” package was used to present the result. R pkgs (clusterProfiler, enrichplot, GOplot) were used to analyze the pathways enriched in two risk groups for GO enrichment analysis.

**TME and Immune Checkpoints evaluation**

Immune-related pathways were enriched as a result of GSEA and GO. Thus, we first conducted immune cell infiltration analysis. On TIMER2.0 (http://timer.cistrome.org/), we obtained “infiltration_estimation_for_tcga.csv” to perform the analysis to evaluate the degree of immune cell between risk score groups for further TME analysis. With cor.test function (spearman method), limma, scales, ggpubar, ggplot2, reshape2 and ggtext R packages, a correlation bubble chart between immune infiltration cell with risk scores was plotted by the way of 7 softwares including XCELL, TIMER, QUANTISEQ, CIBERSORT-ABS, EPIC, MCPCOUNTER and CIBERSORT. In addition, using the Wilcoxon approach, TME was analyzed in two risk groups using “limma” and “ggpubr” R pakages. The difference in immune infiltration between risk groups was compared using the R packages “GSVA,” “limma,” and “GSEABase.” Significant differences in gene expression of immune checkpoints in risk groups were identified with the R package “limma” (Wilcoxon signed-rank test).

**Evaluation of medical treatment**

In order to evaluate the therapy sensitivities in risk groups, we compared the significant difference in the half-maximal inhibitory concentration (IC50) of potential drugs by pRRophetic R package on Genomics of Drug Sensitivity in Cancer (GDSC) (https://www.cancerrxgene.org/)\(^8\).

**Human Protein Atlas (HPA) database**

The protein expressions of these BMRGs in HCC tissues were analyzed in HPA online database (https://www.proteinatlas.org/), which aims to create a human proteome-wide map through integrated omics technologies\(^9\).

**Detection of the mRNA levels of key genes in clinical samples**

Fifteen pairs of HCC and adjacent non-tumor tissues were obtained from Central South University Xiangya Hospital. Total RNA was extracted from the tissue samples, using RNAiso Plus reagent (Takara). Complementary DNA was synthesized from the extracted RNA using a cDNA reverse transcription kit (PrimeScript™ RT Master Mix, Takara). The mRNA expression level was quantitated by qRT-PCR using SYBR qPCR Master Mix (Vazyme). The relative expression of the target gene was calculated using the
2\(^{-\Delta Ct}\) method (ΔCt = Ct(target gene) - Ct(in vitro control)). The primer sequences are shown in Supplementary Table 2.

**Statistical analysis**

For statistical analysis and relevant visualization graphics, the R version 4.1.2 software and its resource packages were employed. To determine if differences between different risk groups were significant, the Student's t-test was utilized, with p<0.05 as the threshold for statistical significance.

**Result**

3.1 Identification of differentially expressed basement membrane-related genes

The flow map of our study was presented in figure 1. In the TCGA LIHC research, we received a total of 365 tumor samples and 50 normal samples (9 samples was omitted). We discovered 98 BMRGs that were significantly differentially expressed between tumor and normal samples based on various gene expression and analyses (|logFC| > 1, FDR<0.05; correlation coefficient >0.5, p<0.001). The heat map and volcano plot were presented in figure 2 (Figure 2A&B).

3.2 Construction and Verification of the Risk model.

98 BMs differentially expressed genes (DEGs) were found (FDR<0.05,|logFC| > 1) using the Limma package of R software. We obtained a matrix of OS data using the clinical data on overall survival (OS) and the DEG expression matrix of HCC cases in the TCGA. Using the R "caret" package, the patients were randomly and equally split into training (175 cases) and test (175 cases) datasets. Then, to identify 16 BMRGs substantially associated with OS, we used univariate Cox proportional hazard regression (figure 2C). After that, we used LASSO Cox regression and multivariate Cox regression to cut down on the prognostic signature's excessive fitting. Finally, four BMRGs were unmistakably linked to prognosis. Also, a Cox regression analysis using the least absolute shrinkage and selection operator (LASSO) was conducted on these genes. The gene expression of each BMRG and its coefficient were then applied to the results of the multivariate Cox regression analysis done on the 4 BMRGs, and a scoring model (BMRGs model) for each patient was created. (Fig. 2D&E) (Lasso regression was performed with 10-fold cross-validation; maximum iteration was 1000; p <0.05). The risk model was built using the following steps: risk score = (0.165117630233135 * COL2A1 exp.) + (0.283642055740619 * CTSA exp.) + (0.364707681635581 * LAMB1 exp.) + (0.428660392642715 * P3H1 exp.). The patients in the training set, testing set, and full set were divided into high- and low-risk groups using the median risk score of the training set as the demarcation, and PCA and tSNE analyses were then carried out. Dataset from ICGC served as an external validation. The outcomes demonstrated a clear separation between the two risk categories (figure 2F). When the survival periods, risk score distribution, survival status, and levels of four genes' expression were compared between the two sets (Figures 3A–I), it was clear that the high-risk group had a worse outlook (Figure 4A–D). Similar patterns were seen for the clinical parameters of age, grade, gender, and stage (Figure 4E–P).
3.3 Construction and evaluation of the prognostic nomogram

Stage and risk score were both substantially correlated with OS, according to the results of the univariate Cox regression (HR = 1.682 and HR = 1.327, respectively, p< 0.001). (Figure 5A). Stage (HR = 1.635, p 0.001) and risk score (HR = 1.278, p<0.001) were found to be independent risk factors linked to OS by the multivariate Cox regression analysis (Figure 5B). Combining all parameters, we created 1-, 3-, and 5-year calibration plots and a nomogram that accorded well with the OS prediction (Figures 5C,D). When taking into account the results of the two investigations, the model showed strong prognostic prediction power.

3.4 Risk Model evaluation

We used time-dependent ROC to assess the reliability of the risk model. It revealed that the ROC curve's area under one, three, and five years was, respectively, 0.721, 0.647, and 0.621. (Figure 5E). In all HCC samples, it was seen at the 1-years ROC of the risk model that the risk score had a superior ability to predict than other clinical parameters (AUC=0.721, Figure 5F), which suggested a trustworthy result.

3.5 Functional Enrichment Analysis

We divided all of the patients into two risk groups in accordance with the aforementioned risk signature, and we then screened 1348 genes with differential expression in two sets using the criteria |log2 FC| > 1 and p<0.05. According to GO analysis, BMRGs were substantially associated with These genes were closely associated with the positive control of cell activation, the extracellular matrix that contains collagen, the immunoglobulin complex, and a structural component of the extracellular matrix (Figure 6A&B).

The biological pathways that were enriched in the high-risk and low-risk groups were investigated using GSEA software. 27 significant pathways enriched in the low-risk group and 37 significant pathways enriched in the high-risk group were shown, respectively, using the criterion of p<0.05 and FDR<0.05. Figure 6E showed all major enriched routes in their entirety. Figure 6C&D displays the top five enriched routes in the low- and high-risk groups.

3.6 Estimation of the tumor immune microenvironment and cancer immunotherapy response of the model

Figures 6A and 6B demonstrate a close link between GO enrichment pathways and immune functions. We then compared the immune functions between the two risk groups in light of this. the high-risk group had higher estimation scores, immunological scores, and stromal scores, according to the TME study (figure 6 F-H). Also, we analyzed the enrichment scores of 16 different immune cell types and the 13 immune-related pathway activities and discovered that the high-risk group had higher numbers of most immunocytes (Figure 7A). Moreover, the high-risk group displayed significantly increased immune system activity not related to the type-2 IFN response pathway (Figure 7B). The majority of immunological checkpoints were also more activated in the high-risk group (Figure 7C). we also discovered the majority
of therapeutic medications, including tipifarnib, bleomycin, mitomycin, and doxorubicin, which were given to the high-risk group, had lower IC50 values (Figure 7D).

3.7 Study of the expression patterns of BMRGs at mRNA and protein levels.

In regards to the levels of protein expression in HCC tissues, the immunohistochemistry findings from the HPA data-base showed that COL2A1, CTSA, LAMB1, and P3H1 protein expression was higher in HCC tissues (Figure 8A). To investigate the clinical implications of the signature, the expression levels of the 4 genes in the HCC and paired neighboring normal tissues were examined. According to the results of qRT-PCR, COL2A1, CTSA, LAMB1, and P3H1 have higher levels of mRNA expression in tumor samples than in normal tissue samples (Figure 8B).

Discussion

The most prevalent histological kind of liver cancer is hepatocellular carcinoma, which has a poor prognosis and a high probability of metastasizing and returning. The control of cell polarity, differentiation, migration, and survival is largely dependent on BMs. Earlier studies have shown that BMs are strongly associated with the emergence of cancer and may serve as potential targets for preventing the emergence of cancer. However, there haven't been any HCC models that involve the genes for the basement membrane. In this investigation, we created a trustworthy prognostic signature with a satisfactory predictive value.

From the TCGA, RNA-seq data and clinical details were gathered. We found four BMRGs that could serve as a risk signature using LASSO and Cox regression analysis, and we saw that patients who were classified as high-risk had considerably worse prognoses. By combining clinical signs and risk factors, we created a nomogram for predicting prognosis. A functional enrichment analysis was then carried out. Using functional analysis, we found that the DEGs associated with the immune response varied amongst the categories. The analysis revealed that certain immune cells and pathways were highly expressed in high-risk groups.

Earlier studies have demonstrated that BMRGs play a crucial role in the development of malignancies. The four BMRGs in the risk model for the current investigation were all risk factors. The alpha-1 chain of type II procollagen is encoded by the COL2A1 gene. Type II collagenopathies, which have a wide range of clinical manifestations, are hereditary illnesses with COL2A1 gene variations as its etiological agents. At least 460 distinct COL2A1 mutations, 663 distinct probands, and 21 illnesses have been identified so far. One of the most often altered genes that contribute to the development of chondrosarcoma is COL2A1. COL2A1 was a risk factor in our model as a result.

One of the RAS molecules, CTSA, is a multifunctional lysosomal enzyme with a clear catalytic and protective role. In both primary and metastatic human melanocytic malignancies, CTSA is expressed in platelets, lymphocytes and primary human antigen-presenting cells. According to earlier research,
the mRNA expression of CTSA differs between breast ductal carcinoma in situ and invasive breast cancer. Michael S. Toss discovered that greater CTSA expression had a poor predictive relevance and was generally associated with invasive recurrence and progression.

LAMB1 has been linked to a number of malignancies, and it has a role in attachment, migration, and organization during development. LAMB1 has been implicated in a number of tumor types, including prostate cancer, hepatocellular carcinoma, breast cancer, and glioblastoma multiforme, according to recent investigations. Prior research demonstrated that the cell growth and motility factor LAMB1 is increased in the tissues of gastric cancer patients. By PDGF/La axis-mediated LAMB1 translation, LAMB1 expression accelerates tumor growth during invasion in hepatocellular carcinoma (HCC). LAMB1 can be utilized as a potential serological biomarker because colorectal cancer patients’ serum contains high levels of LAMB1.

P3H1 is also known as procollagen-proline3-dioxygenase, growth suppressor 1, or leucine-proline-enriched proteoglycan (leprecan). Prior research has revealed P3H1 to have three distinct biological activities. It has the ability to control intracellular pathways like endoplasmic reticulum signaling and interactions with the cell matrix as leprecan. On chromosome 1, P3H1 acts as a growth inhibitor in humans. Last but not least, P3H1, a member of the 2-oxoglutarate dioxygenase family, is essential for the production, folding, and assembly of collagen. P3H1 was recently found to be markedly elevated in osteosarcoma tissues and cell lines (MG63 and Saos2), and reduction of P3H1 prevented osteosarcoma from proliferating, migrating, and invading. P3H1 hypermethylation levels were connected to favorable prognosis in a number of cancers, including LIHC. According to Chunlei Li and colleagues, rising LIHC grades dramatically increased the expression of P3H1. P3H1 expression levels may be used as an independent prognostic factor for patients with LIHC. Moreover, P3H1 knockdown greatly reduced the capacity of liver cancer cells to proliferate, migrate, and invade. P3H1 expression levels may be used as a separate prognostic factor for patients with LIHC. Moreover, P3H1 knockdown greatly reduced the capacity of liver cancer cells to proliferate, migrate, and invade.

According to our risk model, immune cell functions such CCR, cytolytic activities, checkpoint, T cell co-inhibition, T cell co-simulation, and so on were enriched in the high-risk group. In the high-risk group, the majority of immune cells were found to be enriched, as well. Additionally, the high-risk group showed elevated expression of the majority of checkpoint genes. In our study, the HCC patients with minimal risk had a superior response to immunotherapy. We identified 15 candidate compounds for HCC differentiation. Finally, we further confirmed in clinical samples that the four genes in this model have substantially higher levels of mRNA and protein expression in liver cancer tissues compared to healthy tissues.

Additionally, our study has some drawbacks. This was an early investigation into the prognostic utility of BMRGs with the intention of offering some theoretical support for subsequent investigations. We question whether the aforementioned regulatory factors in BM-related pathways in patients with HCC due
to the lack of pertinent studies, and additional research is needed to verify this claim. We believe that future research from our lab will support these findings in the real world, and we intend to perform additional prospective studies to support our findings.

**Conclusion**

In conclusion, our study identified 4 BMRGs with prognostic value and created a prognostic and predictive BMRGs prognostic signature, which is likely to be useful in determining the best course of treatment for patients as well as elucidating the functional and molecular mechanisms underlying the development and progression of HCC. Additionally, there was a strong correlation between the tumor immune microenvironment and the BMRGs risk score, which was important for selecting the best possible course of therapy for HCC patients.

**Declarations**

*Ethics approval and consent to participate*

The studies were approved by the Ethics Committee of Xiangya Hospital of Central South University. Written informed consent was obtained from all patients. We confirmed that all methods were performed in accordance with the relevant guidelines and regulations.

*Consent for publication*

Not applicable

*Availability of data and materials*


*Competing interests*

The authors declare that they have no competing interests.

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*Authors’ contributions*
Haoyang conceived the study and wrote the manuscript; Yijiang Luo and Xueyong Zhang conducted the experiments and contributed to the analysis of data. All authors reviewed the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Not applicable

References


Figures
Figure 1

Flow diagram of the study.
Figure 2

Identification of BMRGs and extraction of BMRGs prognostic signature in patients with HCC. (A) The heatmap of expression profiles of 98 prognostic BMRGs. (B) The volcano plot of 98 differentially expressed BMRGs. (C) The forest map of prognostic BMRGs extracted by univariate Cox regression analysis. (D) The 10-fold cross-validation for variable selection in the LASSO model. (E) The LASSO coefficient profile of 4 BMRGs. (F) The PCA of high-risk risk and low-risk groups.
Figure 3

Prognosis value of the 4 BMRGs model in the train, test and entire set. (A–C) Exhibition of BMRGs model based on risk score of the train, test and entire set, respectively, (D–F) Survival status and time of patients between two groups in the train, test and entire set, respectively, (G–I) The heatmap of 4 BMRGs between two groups in the train, test and entire set, respectively.
Figure 4

survival curves of patients between two groups. (A–D) The survival curve of patients between two groups in the train, test, entire and ICGC set, respectively. (E–P) Survival curves stratified by age, gender, grade, stage, T, N or M between two groups in the entire set.
Figure 5

Nomogram and assessment of the risk model. (A,B) Uni-Cox and multi-Cox analyses of clinical factors and risk score with OS. (C) The nomogram that integrated the risk score and clinical parameters to predict the 1-, 3-, and 5-years OS rate. (D) The calibration curves for 1-, 3-, and 5-years OS. (E) The ROC curves for 1-, 3-, and 5-years OS rate in TCGA cohort. (F) The ROC curves for 1-years OS rate of risk score and clinical parameters.
Figure 6

Functional enrichment for differentially expressed m7G genes between two groups (A) The top 30 significant terms of GO functional enrichment. (B) The circle diagram enriched in the GO analysis. (C&D) GSEA of the top 5 pathways significantly enriched in the two groups. (E) The full significant enriched pathways significantly enriched in the two groups. (F-H) The comparison of ESTIMATE scores, immune scores and stromal scores between two groups.
Figure 7

The investigation of tumor immune factors and immunotherapy. (A) The bar plot for different types of immune cells between two groups. (B) The bar plot for different immune functions between two groups. (C) The difference of 40 checkpoints expression in two groups. (D) Immunotherapy prediction of 15 drugs in high- and low-risk groups.
Figure 8

The expression patterns of BMRGs at protein and mRNA levels. (A) Differences in protein expression of the key genes in HCC tissue and normal tissue from Human Protein Atlas immunohistochemistry. (B) Differences in mRNA expression of the key genes in clinical samples.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementarytable1224BMrelatedgeneslist.docx
- supplementarytable2TheprimerssequenceofkeyBMrelatedgenes.docx