Research article

A prognostic exosome-related long non-coding RNAs risk model related to the immune microenvironment and therapeutic responses for patients with liver hepatocellular carcinoma

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ABSTRACT

Background: Liver hepatocellular carcinoma (LIHC) is the third largest cause of cancer mortality. Exosomes are vital regulators in the development of cancer. However, the mechanisms regarding the association of exosome-related long non-coding RNAs (lncRNAs) in LIHC are not clear.
Methods: LIHC RNA sequences and exosome-associated genes were collected according to The Cancer Genome Atlas (TCGA), Hepatocellular Carcinoma Cell DataBase (HCCDB) and ExoBCD databases, and exosome-related lncRNAs with prognostic differential expression were screened as candidate lncRNAs using Spearman's method and univariate Cox regression analysis. Candidate lncRNAs were then used to construct a prognostic model and mRNA-lncRNA co-expression network. Differentially expressed genes (DEGs) in low- and high-risk groups were identified and enrichment analysis was performed for up- and down-regulated DEGs, respectively. The expression of immune checkpoint-related genes, immune escape potential and microsatellite instability among different risk groups were further analyzed. Quantitative real-time polymerase chain reaction (qRT-PCR) and transwell assay were applied for detecting gene expression levels and invasion and migration ability.
Results: Based on 17 prognostical exosome-associated lncRNAs, four hub lncRNAs (*BACE1_AS, DSTNP2, PLGLA,* and *SNHG3*) were selected for constructing a prognostic model, which was demonstrated to be an independent prognostic variable for LIHC. High risk score was indicative of poorer overall survival, lower anti-tumor immune cells, higher genomic instability, higher immune escape potential, and less benefit for immunotherapy. The qRT-PCR test verified the expression level of the lncRNAs in LIHC cells, and the inhibitory effect of *BACE1_AS* on immune checkpoint genes levels. *BACE1_AS* silence also depressed the ability of migration and invasion of LIHC cells.
Conclusion: The Risk model constructed by exosome-associated lncRNAs could well predict immunotherapy response and prognostic outcomes for LIHC patients. We comprehensively reveal the clinical features of prognostical exosome-related lncRNAs and their potential ability to predict immunotherapeutic response of patients with LIHC and their prognosis.

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1. Introduction

Liver hepatocellular carcinoma (LIHC) is the third major cause leading to cancer deaths, with the incidence rate of over 30/100 thousand [1,2]. LIHC is characterized by high malignancy, poor prognosis and high mortality, and the high metastatic and invasive nature of LIHC can threaten the prognosis and survival of patients [3]. Despite current advances in cancer-related diagnosis and treatment, many patients with LIHC still have a low 5-year survival rate of only 12.1 %. This is because these patients are already at an advanced stage of cancer when diagnosed [4]. Hence, the diagnosis and treatment of LIHC is urgent by screening effective therapeutic targets and treatments.

LncRNAs are a class of transcription RNA with over 200 length nucleotides [5]. As an important immune-related regulator, lncRNA has an important role in multiple stages of tumor immunity, including immune cell infiltration, immune activation and antigen presentation [6–8]. In addition, lncRNAs have been shown to act as novel regulators in LIHC, and some lncRNAs are capable of inducing ferroptosis and necroptosis in hepatocellular carcinoma cells [9–11]. Although a number of lncRNAs have been studied in LIHC, there are still multiple types of lncRNAs with unknown role in LIHC.

Exosomes are a type of microcapsules of 30–100 nm released by a variety of cells [12], and carry many small molecules, including lncRNAs and miRNAs [13,14]. Cancer cells have been found to produce more exosomes, which play an essential role in tumor progression, metastasis, immune response as well as drug resistance [15,16]. Studies concerning the influence of exosomes on LIHC progression were also conducted. For instance, Peng et al. demonstrated that lncRNA LINC00511 was able to promote LIHC development by regulating exosome secretion and invadopodia biogenesis [17]. In addition, Sun et al. showed that exosome-related lncRNAs can facilitate the diagnosis and prognosis evaluation of cancer patients [18]. However, there is not a comprehensive study of exosome-associated lncRNAs at LIHC.

This study screened differentially expressed exosome-related lncRNAs based on public databases and exosome-related regulatory genes. Prognostic modeling was performed based on candidate lncRNAs and mRNA-lncRNA co-expression networks for LIHC. Next, we used risk models to explore the regulatory role of exosome-associated lncRNAs in immune microenvironment of LIHC patients and their prognosis. For a supplement, the expression levels of hub lncRNAs and the effect of the lncRNAs on LIHC cell biological functions were assessed in vitro experiments, including qRT-PCR and transwell assays. The study revealed that exosome-associated lncRNAs could be considered as promising markers for predicting survival outcomes and immunotherapy responses of LIHC patients.

2. Methods

2.1. Data sources and pre-processing

The LIHC dataset containing RNA sequences and information on clinical characteristics was derived from TCGA (http://cancergenome.nih.gov/) [19]. Samples were processed as follows: (1) Excised samples of primary tumors; (2) Samples with overall survival (OS) greater than 30 days; (3) Only samples with complete data of transcriptome expression and clinical prognostic information were kept. In addition, RNA-sequencing expression data and clinical characteristics were obtained from HCCDB (http://lifeome.net/database/hccdb/home.html) [20] and used as an independent validation set. Finally, we screened a total of 393 TCGA-LIHC samples (343 tumor tissue samples and 50 normal tissue samples) and 380 HCCDB samples (203 tumor tissue samples and 177 normal tissue samples). 121 genes related to exosome were obtained from the ExoBCD (https://exobcd.liumwei.org/) database.

Identification of prognostically differentially expressed lncRNAs related to exosome First, differentially expressed lncRNAs were identified between LIHC and normal liver tissue samples applying the limma package in R [21]. Differentially expressed lncRNAs were selected with p-value <0.05 and | log2fold (FC) | > 1. Next, Pearson correlation coefficients were calculated to determine the correlation of differentially expressed lncRNAs with exosome-related genes, and differentially expressed lncRNAs associated with exosomes were screened under p < 0.01 and |R| > 0.4. Subsequently, prognostic lncRNAs were screened by univariate Cox regression analysis. Finally, we used the overlap between the two lncRNAs as candidate lncRNAs to establish a prognosis model.

2.2. Prognosis model construction and verification of exosome-associated lncRNA

We performed LASSO regression analysis via the glmnet package in R to reduce redundant lncRNAs to avoid risk model overfitting and to identify all independent prognostic lncRNAs [22]. In this regard, we finally selected the four best exosome-related lncRNAs to establish a risk model and calculated the risk score for LIHC patients with the formula:

$$RiskScore = \sum_{i=1}^{n} Coei * Expi$$

(n = 4, *Coei* represented the corresponding Cox regression coefficient, and *Expi* represented the expression level of each exosome-related lncRNA with prognostic differential expression).

LIHC patients were divided into two risk groups (low and high risks) based on median value. The survival curve was generated using the timeROC package in R and the Kaplan-Meier (KM) analysis for validation [23]. Moreover, risk score was also calculated for each LIHC patient in the validation cohort. Finally, the independence of the exosome-associated lncRNA risk model for LIHC prognosis evaluation was determined through univariate and multivariate Cox regression analyses.

2.3. Development of mRNA-LncRNA co-expression network

Correlation of exosome-associated genes with exosome-associated lncRNAs was calculated by using Spearman method. Furthermore, the mRNA-LncRNA co-expression network was generated applying Cytoscape software [24]. $|R| > 0.4$ and $p < 0.01$ were the criteria for screening correlated mRNAs.

2.4. Identification and enrichment analysis of DEGs in high and low risk groups with LIHC

Differential expression analysis was conducted using the limma package in R between LIHC high and low risk groups, and genes were considered as DEGs if they satisfied the criteria of p-value <0.05 and $| \log FC | > 1$. Then, the signaling pathways and biological functions correlated with exosome-related risk scores were identified using Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis based on the DEGs. A p-value <0.05 was statistically significant in enrichment analysis.

2.5. Analysis of tumor immune microenvironment and tumor immune infiltration

We used the Tumor Immune Estimation Resource (TIMER) algorithm to count infiltration abundance scores of six immune cells by constrained least-squares method [25]. Subsequently, the degree of infiltration of immune cells and stromal cells was assessed using the xCell algorithm [26]. These two algorithms are applied respectively in the training cohort and in the validating cohort.

2.6. The relationship between risk modeling and immunotherapy

Immunotherapy has been a powerful method for cancer treatment [27]. Therefore, the potential clinical effects of immunotherapy on the two risk groups of patients were determined by The Tumor Immune Dysfunction and Exclusion (TIDE) algorithm (http://tide.dfci.harvard.edu/query/) [28]. Wherein, the higher TIDE score, the more possibility for immune escape. In the opposite, the lower TIDE score, the more benefit from immunotherapy. And we further analyzed the differences in immune checkpoint blockade (ICB) key molecules (KRAS and EGFR) [29,30] between the different risk subgroups. Moreover, the relationship between different risk groups and the expressions of 4 major mismatch repair genes (MLH1, PMS2, MSH6, and MSH2) was analyzed.

Human liver cancer cell lines HepG2 and normal human liver cell lines LO2 were commercially purchased from COBIOER (Nanjing, China). Cells was maintained in RPMI medium containing 10 % FBS, 1 % penicillin/streptomycin (Invitrogen, Grand Island, NY). The negative control (si NC) and *BACE1_AS* siRNA (Sagon, China) were transfected into the cells by Lipofectamine 2000 (Invitrogen, USA) for 48 h.

2.7. RNA extraction and qRT-PCR

Total RNA from each sample was extracted applying TRIzol reagent (Thermo Fisher, USA). For qRT-PCR, a LightCycler 480 PCR System (Roche, USA) with FastStart Universal SYBR Green Master (Roche, Switzerland) was used. The PCR conditions were: 95 °C pre-denaturation for 30 s, followed by 39 cycles. Each cycle contained 95 °C denaturation for 5 s, 55 °C annealing for 30 s, and 72 °C extension for 30 s. Data were analyzed with the $2^{-\Delta\Delta Ct}$ method with GAPDH as an internal reference. Supplementary Table 1 listed the primer sequences.

2.8. Transwell assay

Migration and invasion experiments were performed three times in accordance to Zhang et al.'s study [31]. Briefly, cell migration was examined applying matrigel-uncoated transwells (8.0-μm pore size; Falcon). After incubation at 37 °C for 24 h, the non-invading cells were removed. Invaded cells (on the bottom of the filters) were counted in five random fields under a light microscope (Olympus), after being with fixed 4 % paraformaldehyde for 30 min stained with 0.1 % crystal violet for 20 min. The procedure for cell invasion is similar to that of migration, except for that transwells were coated with matrigel.

2.9. Statistical analysis

Statistical analyses were all conducted using R software (version 3.6.0). The *t*-test or Wilcox test was used to compare and analyze the conventional statistical differences between groups. KM survival curves were plotted for survival analysis accompanied with log-rank test. Statistical significance of p-value <0.05 was determined.

3. Results

3.1. Screening prognostically differentially expressed exosome-related lncRNAs

First, we screened 37 differentially expressed lncRNAs from the TCGA-LIHC database between normal tissue and LIHC samples (Fig. 1A), and obtained 34 differentially expressed lncRNAs associated with exosomes using the Spearman algorithm (Supplementary

Table 2). Univariate Cox regression analysis showed that 18 prognosis-associated differentially expressed lncRNAs were related with OS in LIHC patients (Fig. 1B). Through Venn diagrams, we finally selected 17 overlapped lncRNAs for subsequent analysis (Fig. 1C).

3.2. Development of an exosome-related lncRNA-based risk model and verification

We reduced the number of lncRNAs in this model by performing LASSO regression analysis on 17 exosome-associated lncRNAs predicted to be differentially expressed. Moreover, we provided a distribution of LASSO coefficients for 17 lncRNAs and determined the optimal penalty parameter λ value ($\lambda = 0.03$) by 10-fold cross-validation (Fig. 2A–B). Multivariate Cox regression analysis further determined four predicted differentially expressed exosome-associated lncRNAs (Fig. 2C). Accordingly, a risk model was developed using the risk score as follow:

$$\text{Risk score} = (0.188 \times \text{BACE1\_AS}) + (0.334 \times \text{DSTNP2}) + (-0.121 \times \text{PLGLA}) + (0.18 \times \text{SNHG3})$$

LIHC patients were divided into two risk groups (high and low) based on the median risk score (Fig. 2D–E). Survival analysis showed that patients with high risk score had worse survival than those with low risk score in both the training and validation cohorts (Fig. 2H–I). As shown in Fig. 2F–G, the ROC curves indicated that the area under the curve (AUC) values were 0.76, 0.7, and 0.65 for the training cohort at 1, 3, and 5 years, respectively, and the AUC values were 0.72, 0.69, and 0.72 for the validation cohort at 1,3, and 5 years, respectively.

The risk score was demonstrated to be an independent prognostic variable for LIHC patients in both the training and validation cohorts, as supported by the results of the univariate and multivariate Cox regression analysis (Fig. 3).

3.3. Establishment and analysis of mRNA-LncRNA co-expression network

We obtained 39 exosome-related genes significantly associated with model lncRNAs and constructed co-expression networks to explore the connections of the four lncRNAs with exosome-related genes (Fig. 4A). In addition, the correlation between the target
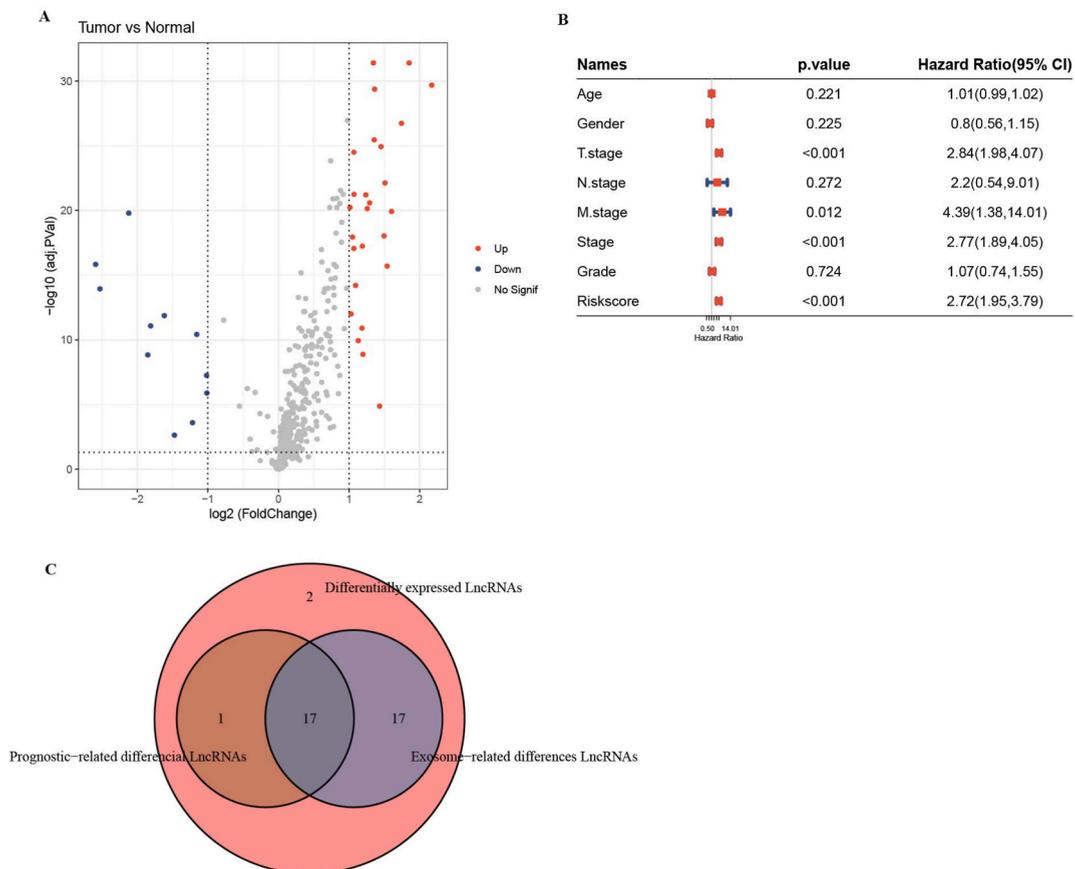

Fig. 1. Identification of prognosis-related exosomal lncRNAs in patients with LIHC. (A) Differential expression of lncRNA between normal tissue samples and LIHC samples in TCGA database; (B) Forest plot showing HR (95 % CI) and p-value of prognosis-related differentially expressed lncRNAs; (C) Venn diagram distinguishes between differentially expressed lncRNAs, exosome-related differentially expressed lncRNAs, and prognosis-related differentially expressed lncRNAs of overlapping lncRNAs.
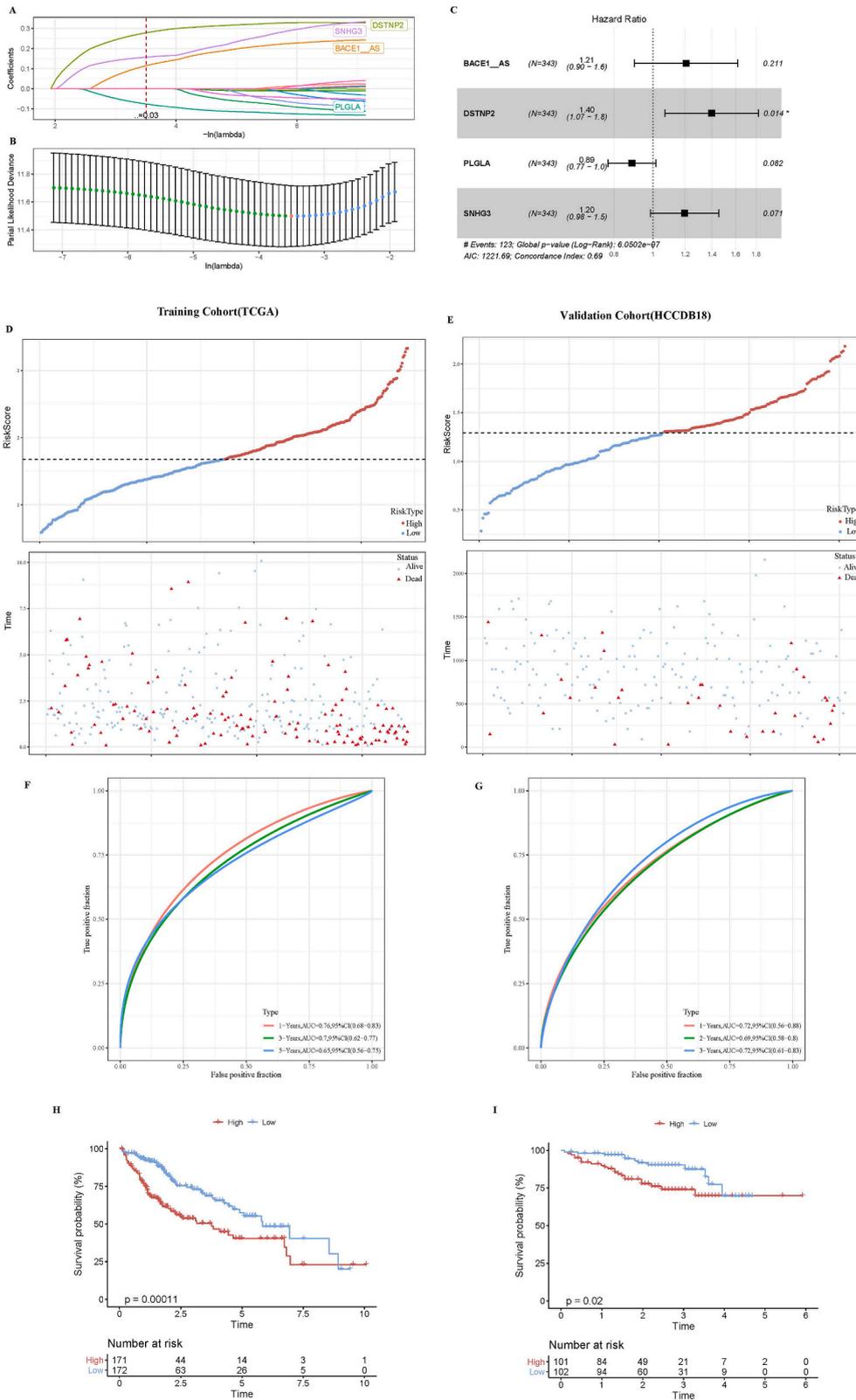
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Fig. 2. Evaluation of a prognostically differentially expressed exosome-related lncRNA risk model. (A) LASSO coefficient profiles of 17 exosome-associated lncRNAs; (B) The optimal value of the penalty parameter λ (λ = 0.03) for 10-fold cross-validation results; (C) Multivariate Cox regression of 4 prognostic exosome-related lncRNAs; (D, E) Expression of exosome-related prognostic lncRNAs in the training and validation cohorts for risk score, survival time and survival status; (F, G) ROC curve analysis model predicts 1-, 3-, and 5-year prognosis of LIHC patients in the training and validation sets; (H, I) K-M curves of LIHC patients in the training cohort and validation cohort.

mRNAs and four exosome-related lncRNAs was demonstrated using Sankey plots (Fig. 4B). All these data further described a close relationship of the four candidate lncRNAs with exosome.

3.4. Identification and enrichment analysis of the DEGs between two LIHC risk groups

In the training cohort, we obtained 691 DEGs in different risk groups of LIHC, including 258 down-regulated DEGs and 433 up-regulated DEGs (Supplementary Table 3). In the validation cohort, we obtained a total of 291 DEGs, including 92 up-regulated DEGs and 199 down-regulated DEGs (Supplementary Table 4).

Secondly, we performed KEGG and GO analysis of DEGs co-expressed up- and down-regulated in the different risk groups in LIHC. Upregulated DEGs were largely related to cell cycle, DNA repair and chromosome function (Fig. 5A). The results suggested that high expression of upregulated DEGs based on exosome-related risk scores promoted cancer cell proliferation. While, the down-regulated DEGs were largely enriched in chemical carcinogenesis, retinol metabolism, and organic acid catabolism (Fig. 5B). These analysis together proposed that DEGs between the two LIHC risk groups may participate in LIHC development through facilitating cell proliferation and metabolic disorder, which are worth further investigation. Finally, we used the same method to analyze the validation cohort as well. The results show a certain degree of consistency with the results of the two cohorts (Fig. 5C–D).

3.5. Analysis of tumor immune infiltration

The abundance of immune cell infiltration in each LIHC patient was counted using the TIMER algorithm. In both the validation cohort and training cohort, compared with low risk groups, LIHC high-risk patients displayed higher immune scores, especially in dendritic cells (Fig. 6A–B).

As for xCell analysis, the distribution of multiple immune cells and stromal cells between the two risk groups of LIHC patients was significantly different. In particular, the abundance of cell infiltration such as HSC and hepatocytes was significantly lower in the high-risk group. However, immune infiltration scores were lower in the low-risk group in terms of smooth muscle cells (Fig. 6C–D).

3.6. Exosome-related risk models for immunotherapy evaluation

We further analyzed ICB key molecules (KRAS and EGFR) and microsatellite instability (MSI) in LIHC to explore the potential of the risk profiles of lncRNAs in predicting immunotherapeutic response. In the two cohorts, KRAS in high-risk LIHC patients was significantly overexpressed (Fig. 7A–B). High-risk patients with LIHC were more likely to experience immune escape, because of higher TIDE
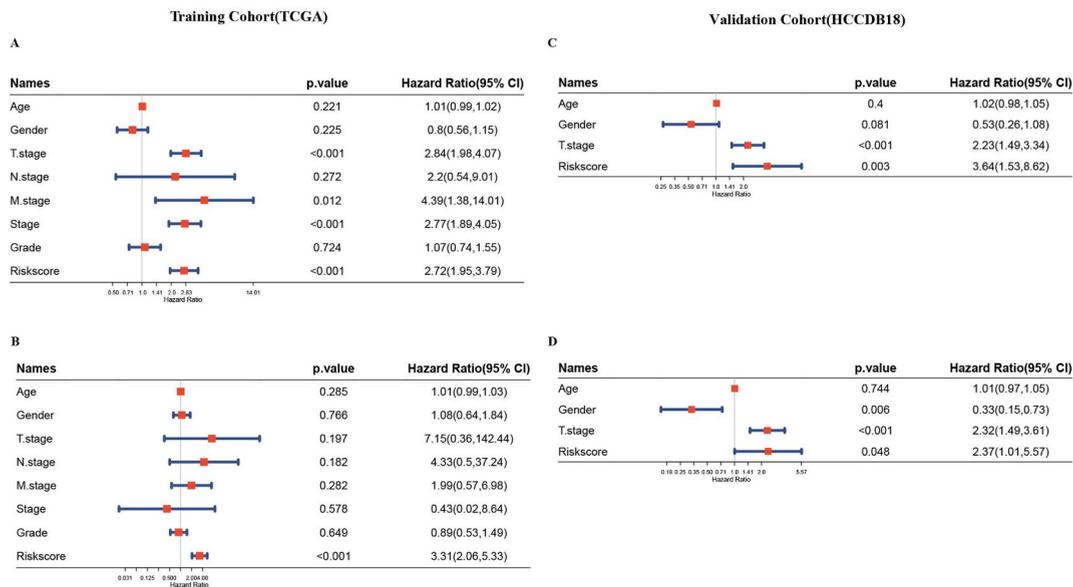
Training Cohort(TCGA)

A

Names	p.value	Hazard Ratio(95% CI)
Age	0.221	1.01(0.99,1.02)
Gender	0.225	0.8(0.56,1.15)
T.stage	<0.001	2.84(1.98,4.07)
N.stage	0.272	2.2(0.54,9.01)
M.stage	0.012	4.39(1.38,14.01)
Stage	<0.001	2.77(1.89,4.05)
Grade	0.724	1.07(0.74,1.55)
Riskscore	<0.001	2.72(1.95,3.79)

B

Names	p.value	Hazard Ratio(95% CI)
Age	0.285	1.01(0.99,1.03)
Gender	0.766	1.08(0.64,1.84)
T.stage	0.197	7.15(0.36,142.44)
N.stage	0.182	4.33(0.5,37.24)
M.stage	0.282	1.99(0.57,6.98)
Stage	0.578	0.43(0.02,8.64)
Grade	0.649	0.89(0.53,1.49)
Riskscore	<0.001	3.31(2.06,5.33)

Validation Cohort(HCCDB18)

C

Names	p.value	Hazard Ratio(95% CI)
Age	0.4	1.02(0.98,1.05)
Gender	0.081	0.53(0.26,1.08)
T.stage	<0.001	2.23(1.49,3.34)
Riskscore	0.003	3.64(1.53,8.62)

D

Names	p.value	Hazard Ratio(95% CI)
Age	0.744	1.01(0.97,1.05)
Gender	0.006	0.33(0.15,0.73)
T.stage	<0.001	2.32(1.49,3.61)
Riskscore	0.048	2.37(1.01,5.57)

Fig. 3. Exosome prognosis-related lncRNA risk score was an independent prognostic factor for LIHC. (A, B) Univariate and multivariate Cox regression analysis for training cohort risk models; (C, D) Cox regression analysis for validation cohort risk models.
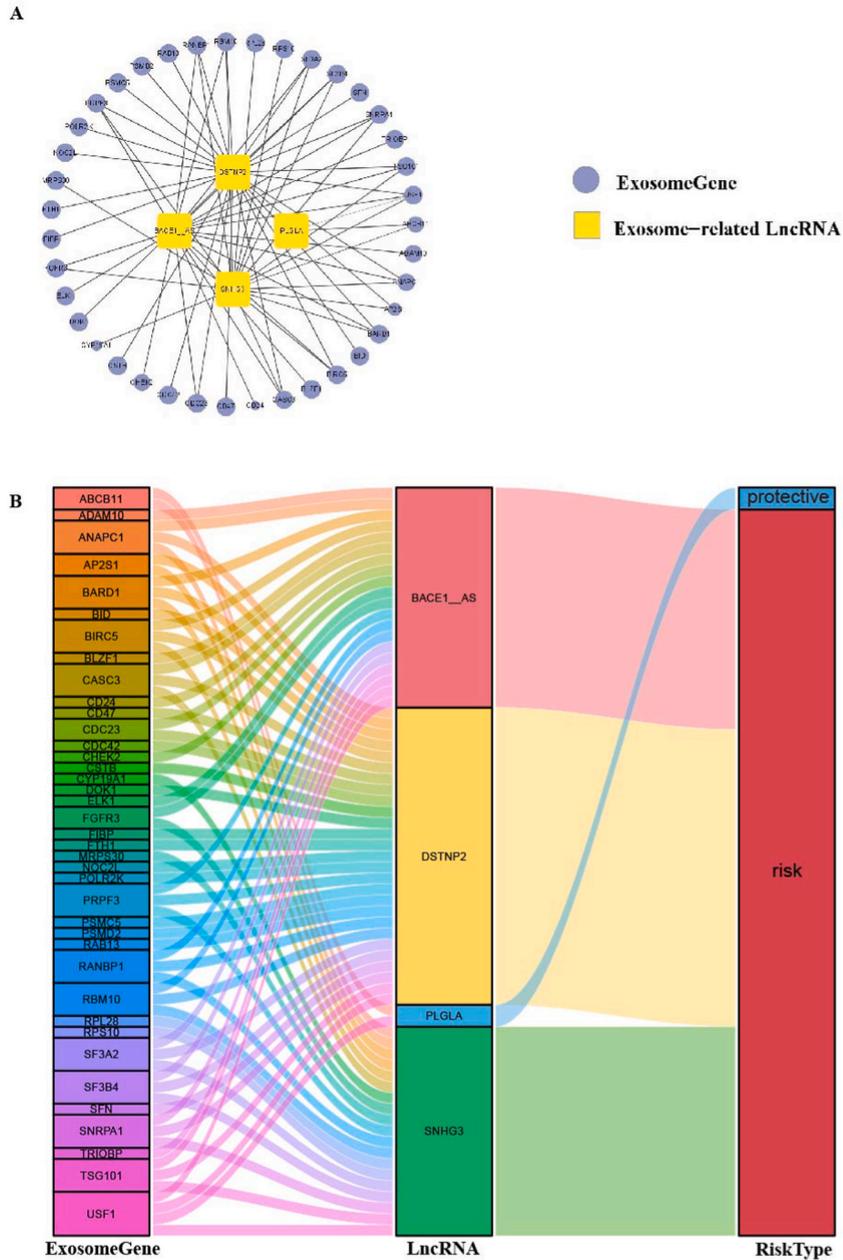
Fig. 4. Construction of mRNA-LncRNA co-expression network. (A) Co-expression network of candidate lncRNAs and exosome-associated genes; (B) Sankey diagram showing the relationship between candidate lncRNAs, exosome-associated genes and risk types.

score in the high-risk group (Fig. 7C–D). Furthermore, among the cell types that TAM M2 (tumor-associated M2 subtype macrophages) and MDSC (myeloid-derived suppressor cells) were at higher levels in the high-risk group and were more prone to immune exclusion. Furthermore, the high-risk group showed significantly higher expression of all four MSI-related DNA repair protein genes (including MLH1, PMS2, MSH2, and MSH6) (Fig. 7E–F), which indicated more stable microsatellites in high-risk LIHC patients.

3.7. Expression level of 4 lncRNAs in cell lines

The four key lncRNAs screened in this study were further validated in hepatocellular carcinoma cell line (HepG2) and normal liver cell line (LO2) using qRT-PCR. As shown in Fig. 8, compared to normal controls, we found that BACE1_AS, DSTNP2 and SNHG3 were all highly expressed in hepatocellular carcinoma cell lines. In particular, PLGLA was downregulated in LIHC (Fig. 8A–D). These results indicated that the four lncRNAs can be used as biomarkers for the prognostic prediction of LIHC patients.
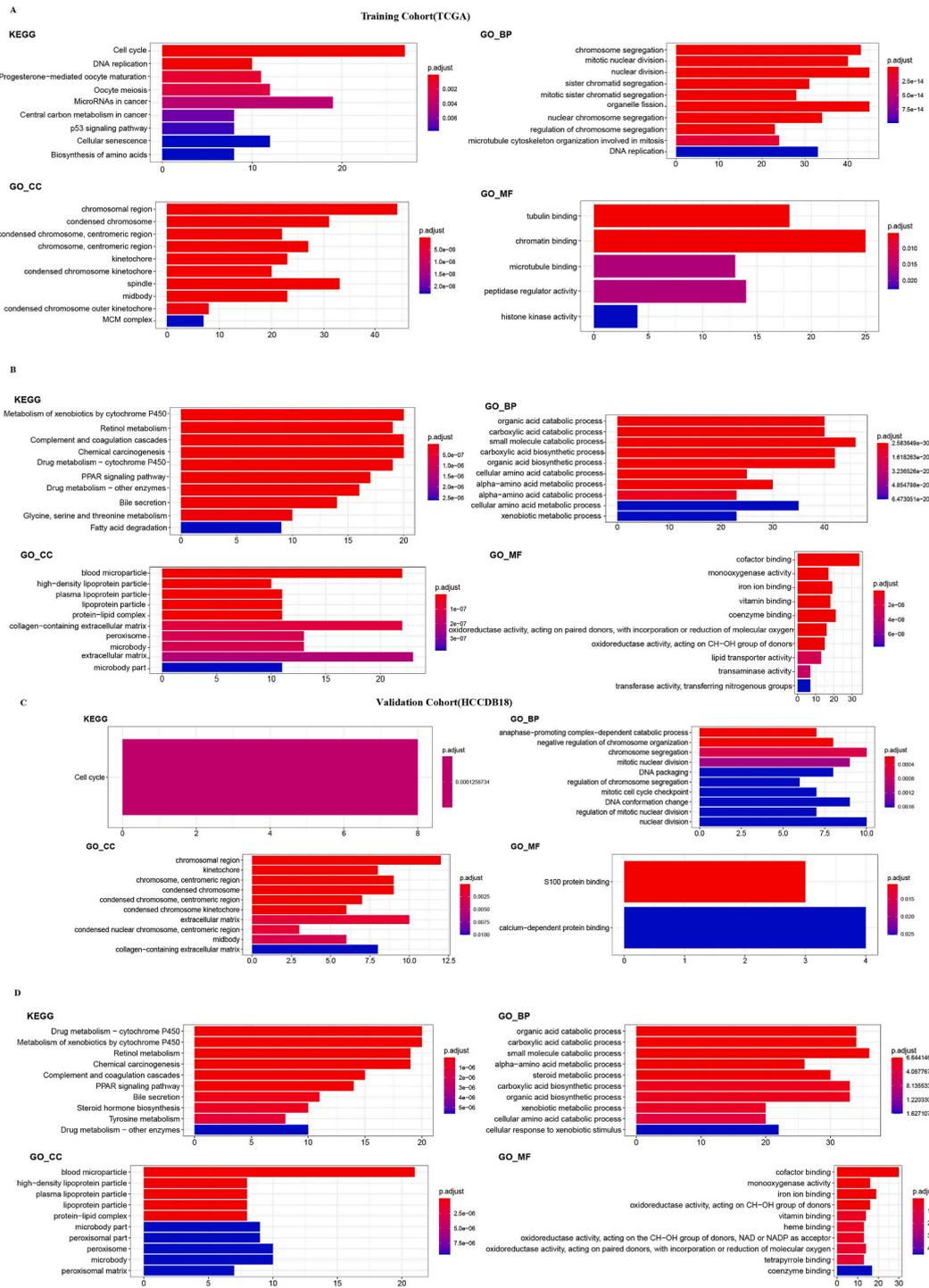
Fig. 5. Results of KEGG and GO enrichment analysis. (A, B) Enrichment results for DEGs in the training cohort; (C, D) Enrichment results for DEGs in the validation cohort.

3.8. Validating the biological functions of BACE1_AS on LIHC cells

Given the highest expression levels of BACE1_AS on HepG2 cells, we selected this gene for further biological functions validation. As seen in Fig. 9A–B, we could see that BACE1_AS silence inhibited the migration and invasion ability of HepG2 cells in comparing with NC vectors (p < 0.01). QRT-PCR revealed that BACE1_AS silence effectively decreased the levels of BACE1_AS accompanied with
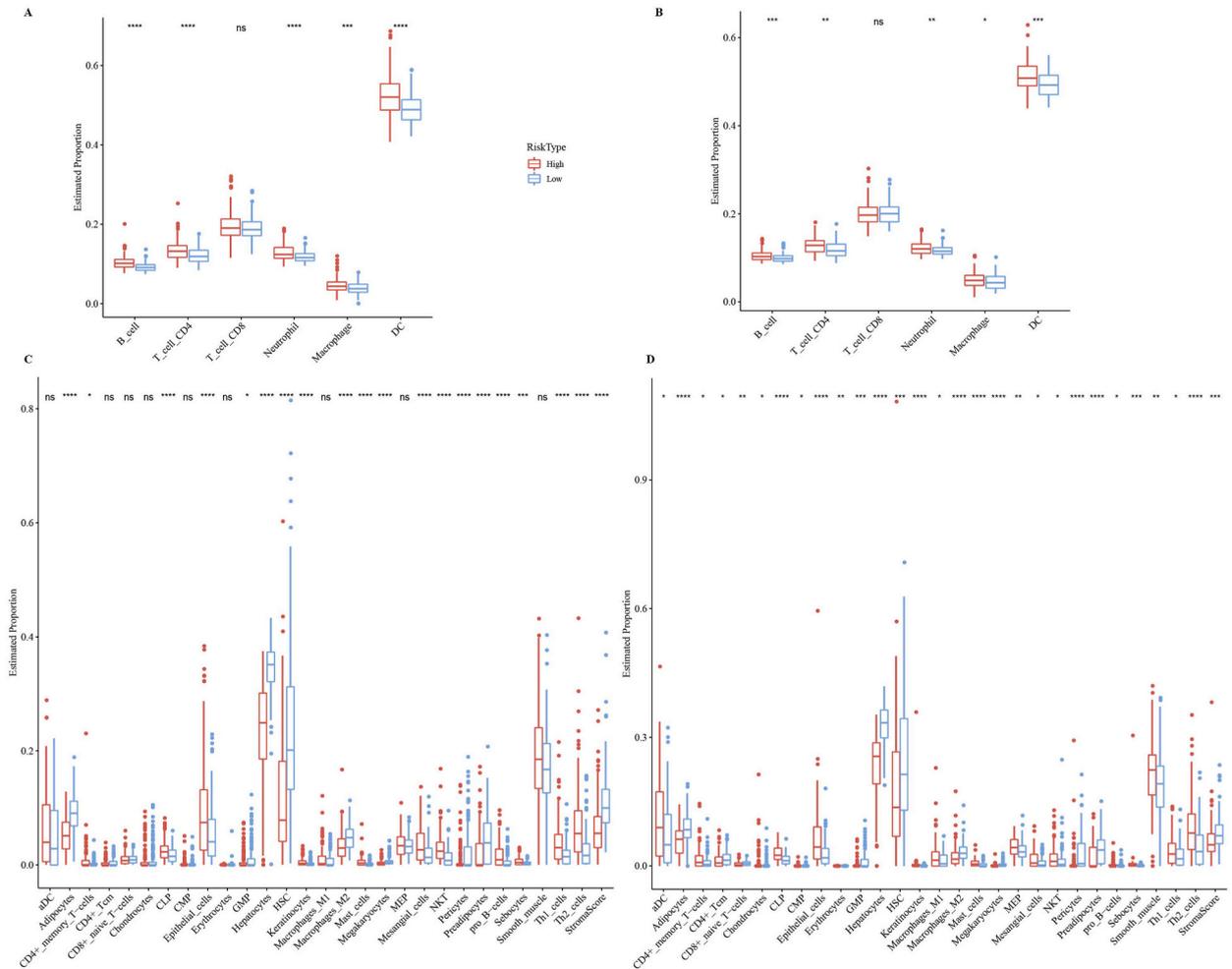
Fig. 6. Differential analysis of the abundance of immune cell infiltration in different LIHC risk score groups. (A, B) Assessment of the infiltration abundance of six immune cell types in the training cohort (A) and validation cohort (B) in high and low risk groups using the TIMER algorithm; (C, D) Evaluation of the abundance of immune and stromal cell infiltrates in the training cohort (C) and validation cohort (D) for different risk groups using the xCell method. * indicated p < 0.05, ** indicated p < 0.01, *** indicated p < 0.001, and **** indicated p < 0.0001.

reduced PD-L1 and PD-L2 (Fig. 9C–E), which are commonly expressed in cancer cells [32]. Collectively, these tests suggested that BACE1_AS may facilitate the progression of LIHC through enhancing migration and invasion ability and the expressions of immune checkpoint genes on cancer cells for immune escape.

4. Discussion

LIHC is the most common primary live cancer [33]. Conventional treatments for LIHC (including surgery, radiation therapy, and radiofrequency ablation) provide only modest benefits in terms of overall survival, but patients with advanced LIHC do not respond satisfactorily to them [34,35]. In recent years, immunotherapy has provided opportunities for effective treatment of malignancies, and research related to the tumor immunology has become the fast-growing area for a variety of tumors, including LIHC [36]. As a multicellular organ, the liver requires intercellular communication to perform its vital functions [37]. Exosomes, as small vesicles only nanoscale in size, which transport a variety of biologically active analyses between cells via multiple biomolecules (such as DNA, RNA, and proteins), thereby regulating the intercellular immune system and microenvironment [38–40]. Exosomal lncRNAs have been demonstrated to be crucial for treatment resistance, cancer cell growth, angiogenesis, and invasion [41,42]. An example on of the role of lncRNA in exosome-mediated communication in LIHC was provided by Yang et al. who discovered that the lncRNA HOTAIR stimulates exosome release by causing the translocation of multivesicular bodies to the plasma membrane [43]. In addition, exosome H19 is considered as an oncogene and a promising biomarker for bladder cancer due to its detection in the plasma and serum of bladder cancer patients [44]. Nonetheless, the comprehensive study of exosome-associated lncRNAs in LIHC is incomplete.

In this study, through a series of analysis, we finally identified four hub exosome-associated lncRNAs, including *BACE1_AS*, *DSTNP2*, *PLGLA*, and *SNHG3*. Liu et al. showed that lncRNA *BACE1_AS* was overexpressed in both tissues and cell lines of LIHC and that
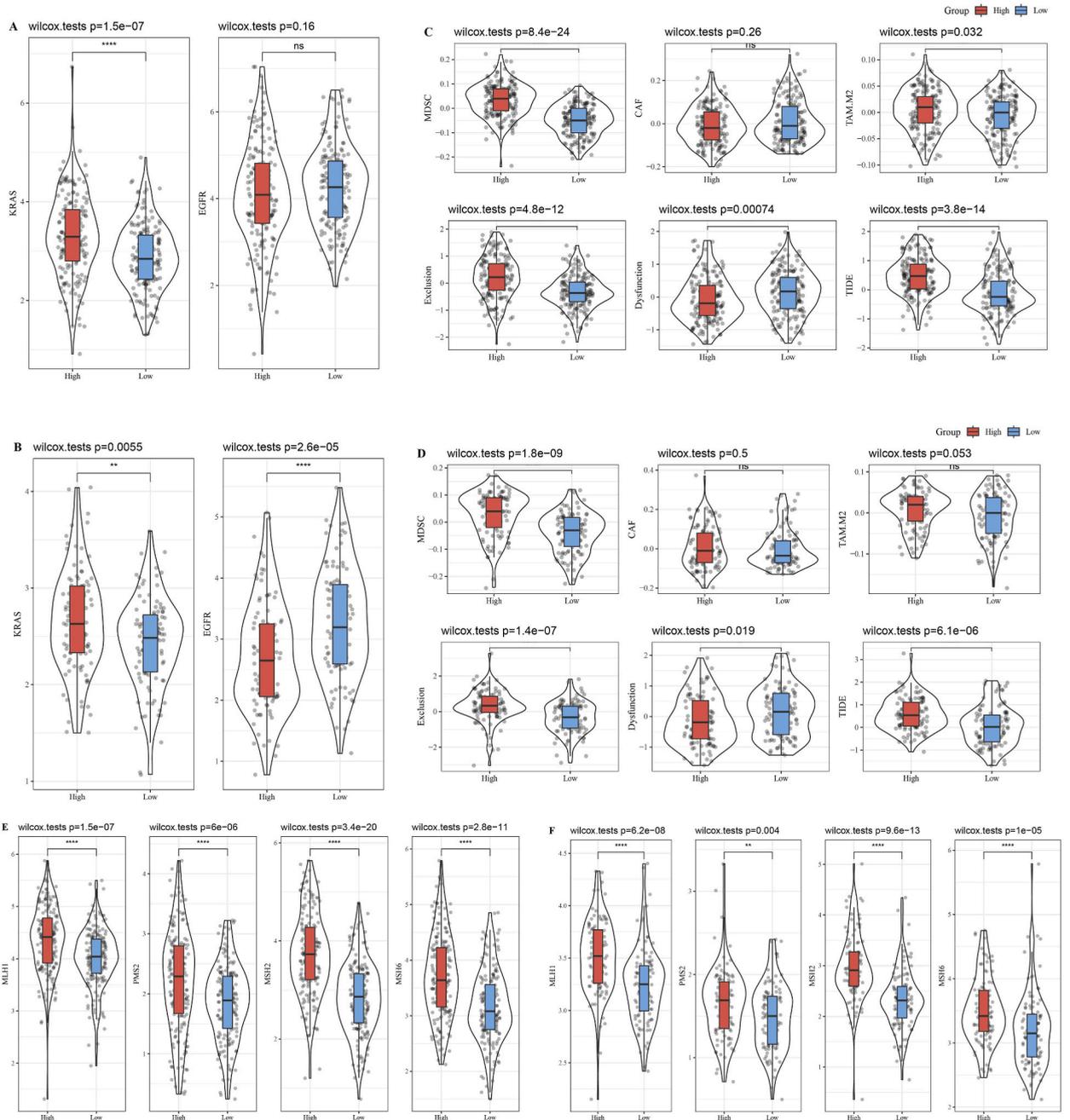
Fig. 7. Exosomal lncRNA-associated risk model for its prediction of immunotherapeutic response. (A, B) Analysis of differences in immune check blocking key molecules in different risk score groups in training cohort (A) and validation cohort (B); (C, D) TIDE predicts immune escape potential in the different risk groups in training cohort (C) and validation cohort (D); (E, F) Expression of four MSI-related DNA mismatch repair genes were significantly elevated in the high-risk group in both the training cohort (E) and validation cohort (F).

it could regulate the *miR-377-3p/CELF1* axis by participating in the epithelial mesenchymal transition pathway to promote the invasion and metastasis of LIHC cells [45]. Moreover, Nie et al. found that higher *BACE1_AS* expression was related to poorer OS and relapse-free survival outcomes and concluded that *BACE1_AS* was able to predict prognostic biomarkers in patients with LIHC [46]. These results are consistent with our findings. We also revealed that *BACE1_AS* may facilitate LIHC progression through enhancing migration and invasion ability and the expressions of immune checkpoint genes on cancer cells for immune escape. Therefore, we hypothesize that lncRNA *BACE1_AS* is indeed a risk factor for LIHC development. *PLGLA* is a transcriptionally unprocessed pseudogenes whose sequence is thought to be highly homologous to the parental gene, but lacks the ability to encode protein [47]. High expression of pseudogenes in LIHC is associated with poor survival and promotes cancer cell proliferation, invasion and migration [48,
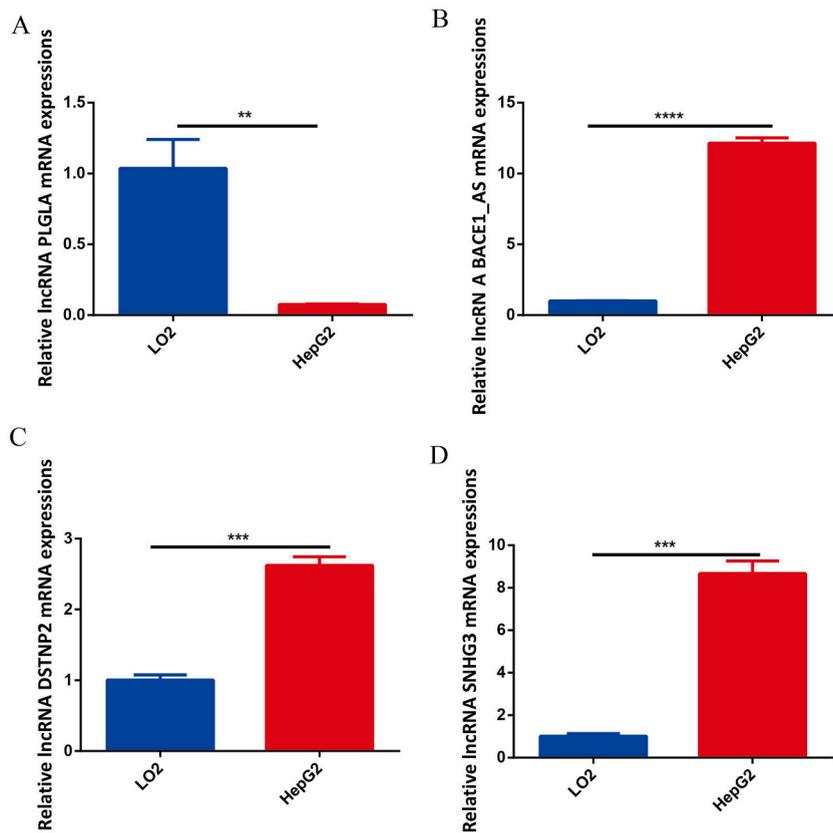
Fig. 8. Expression levels of risk model lncRNAs in hepatocellular carcinoma cell lines and human normal hepatocyte cell lines. (A–D) Relative lncRNA PLGLA, BACE1-AS, DSTNP2, and SNHG3 mRNA expressions.

49]. However, Bao and his colleagues found significantly downregulated expression of *PLGLA* in LIHC tissues, and that low *PLGLA* expression contributed to tumor progression and poor prognosis. *PLGLA* binds miR-324-3p competitively and acts as endogenous RNA to enhance the expression of the protein-coding gene *GLYATL1* [47]. All of this evidence is strengthening the idea that *PLGLA* can act as a protective factor for LIHC in our study. Currently, several studies have demonstrated than lncRNA *SNHG3* plays an oncogenic role in LIHC [50–52]. Zhang et al. explored in depth the molecular regulatory mechanism of lncRNA *SNHG3* on the malignant progression of LIHC. Their study disclosed that reducing SNHG3 expression can reduce tumor size and effectively suppress cancer cell proliferation, and induce apoptosis and G0/G1 phase block [51]. Moreover, epithelial mesenchymal transition in LIHC cells could be induced via *miR-128/CD151* cascade activation by overexpression of *SNHG3* [52]. However, no studies have yet demonstrated the prognostic function of lncRNA *DSTNP2* in tumors. Therefore, it is necessary to further investigate how this lncRNA affects the prognosis of LIHC patients through the tumor microenvironment.

There exist complex interactions between LIHC-derived exosomal lncRNA-mediated tumor and tumor microenvironment [53]. Previous studies reported that B cells and T cells infiltrated around the tumor could improve the survival of liver cancer patients [54]. Macrophage infiltration in patients with LIHC is associated with a good prognosis and neutrophils are related to low infiltration of immune cells in LIHC [55]. However, in our study, dendritic cells, B cells, neutrophils, macrophages, T cells CD4 were highly invasive in LIHC high-risk patients. When linking exosomal lncRNA risk models to immune infiltration in LIHC, our study predicted that exosome-associated lncRNAs had the potential to be new targets for ICB therapy. This study illustrated that LIHC patients with high risk scores were highly associated with KRAS, a key molecule for ICB therapy. Dietrich et al. indicated that KARS was prone to dysregulation in LIHC and that its upregulation was correlated with advanced tumor size and poor survival, while its downregulation inhibits cancer cell growth [56]. Although, low levels of EGFR was discovered in low risk groups, we suspected that the downstream KARS of EGFR was more vital for ICB response in LIHC patients. As the mutations of these two genes could be more helpful for immunotherapy prediction [29,30], the mutation frequencies of these two genes will be detected lately to more accurately evaluate their benefits in immunotherapy. Zhu et al. showed that the poor treatment outcome of breast cancer may be due to the influence of MDSC and TAM in M2 [57]. In addition, there was also evidence that MDSC and TAM M2 limited the efficacy of anti-PD-L1 therapy in non-small cell lung cancer [58,59]. This suggested that MDSC and TAM M2 may affect the therapeutic efficacy of ICB therapy in high-risk patients with LIHC. Therefore, our future studies will aim to eliminate the effects of MDSC and TSM M2 and thus improve the therapeutic efficacy of ICB therapy for patients with LIHC.

This is the first study based on four validated exosome-associated lncRNA signatures from a public database. However, there are
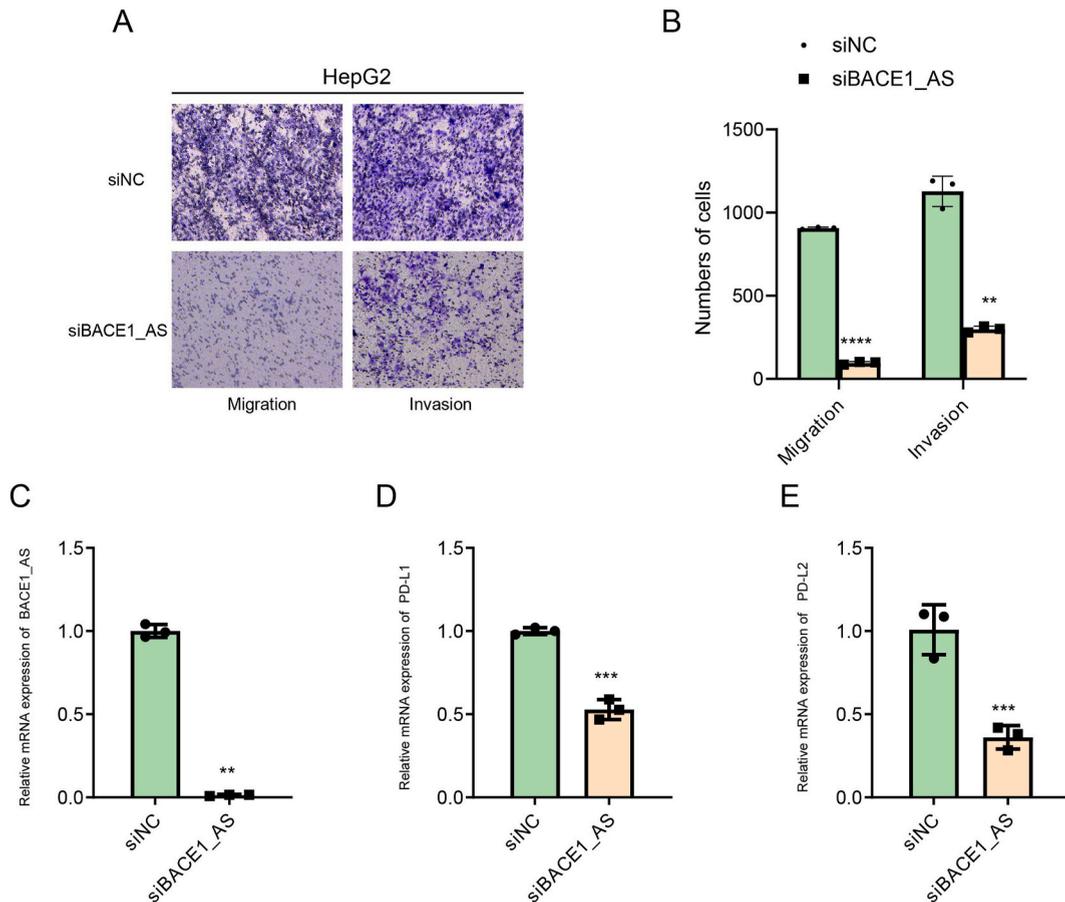
Fig. 9. Validating the biological functions of BACE1-AS on hepatocellular carcinoma cell lines. (A, B) Migration and invasion assays after BACE1-AS silence. (C–E) QRT-PCR detected the expressions of BACE1-AS, PD-L1 and PD-L2 in HepG2 cell lines. N = 3, ** indicated $p < 0.01$, *** indicated $p < 0.001$. **** indicated $p < 0.0001$. The results are presented as mean $\pm$ SEM.

currently some limitations in this study. First, wet experiments did not deeply verify the role of lncRNAs selected in this study. In the future, we plan to collect LIHC patients and separate exosomes in accordance to published papers [60] to detect the levels of selected lncRNAs. At cell levels, carriers with overexpressed or knockdown lncRNAs will constructed to explore their functions on LIHC cell growth, migration, invasion and apoptosis. Second, our study is only a preliminary insight into the correlation of the risk score and immune cell infiltration and immunotherapy response. Therefore, further studies such as gathering LIHC cohorts to calculate their risk scores and giving different immunotheraptic treatment and detecting the expressions of screened lncRNAs are necessary to validate the significance of these lncRNAs in LIHC prognosis.

5. Conclusion

This research developed a new risk model for exosome-correlated lncRNAs through a bioinformatic approach and demonstrated that the model was associated with immune cell infiltration. Our results suggested that exosome-associated lncRNA signatures exhibited superior performance in determining prognosis and immunotherapeutic response in patients with LIHC. Furthermore, exosome-associated lncRNA was an independent prognostic factor, providing new directions for immunotherapy in LIHC.

Funding

Data availability statement

The dataset in our study includes The Cancer Genome Atlas (TCGA), Hepatocellular Carcinoma Cell DataBase (HCCDB) and ExoBCD databases. The raw data generated and/or analyzed during the current study are available in the https://github.com/1LeShi/Experimental-Data.git

Ethical statement

Informed consent was not required for this study because it is not involved any human experiments.

CRediT authorship contribution statement

Yuan Yue: Writing – review & editing, Writing – original draft, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Jie Tao:** Writing – original draft, Validation, Supervision, Resources. **Dan An:** Writing – review & editing, Validation, Investigation, Data curation. **Lei Shi:** Writing – review & editing, Visualization, Software, Resources, Project administration, Formal analysis.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24462.

Abbreviations

LIHC   Liver hepatocellular carcinoma
LncRNA   long non-coding RNA
DEGs   Differentially expressed genes
TIMER   The Tumor Immune Estimation Resource
FC   fold change
AUC   area under ROC curve
HR   hazard ratio
LASSO   least absolute shrinkage and selection operator
OS   overall survival
ROC   receiver operating characteristic analysis
ssGSEA   single-sample gene set enrichment analysis
TCGA   The Cancer Genome Atlas
TIDE   Tumor Immune Dysfunction and Exclusion
TME   tumor microenvironment
ICB   immune checkpoint blockade
MDSC   Myeloid-derived suppressor cells
TAM M2   Tumor-associated M2 subtype macrophages
BACE1_AS   β-site APP-cleaving enzyme 1 antisense
PLGLA   Plasminogen like A
SNHG3   Small nucleolar RNA host gene 3

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