A seven-Lnc RNA signature for prognosis prediction of patients with lung squamous cell carcinoma through tumor immune escape

zhong lin
Taizhou Hospital of Zhejiang Province

yan hu (✉️ happyhuyan072914@163.com)
Taizhou Hospital of Zhejiang Province

Article

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Abstract

Lung squamous cell carcinoma (LUSC) is a malignant disease with poor therapeutic response and a poor prognosis. Some basic researches had confirmed that dysregulation of long non-coding RNAs (LncRNAs) was associated with cancer development and prognosis. However, related studies in LUSC are rare. In this paper, we purposed to develop a group LncRNAs signature to improve prognosis prediction of LUSC and describe the underlying mechanism. The LncRNAs expression and related clinical information of 471 patients with LUSC from TCGA randomly divided into a training set (n = 236) and a testing set (n = 235). A seven-LncRNAs prognostic signature model were constructed with the data of training group by multivariate Cox regression. All patients were divided into high risk group (n = 101) and low risk group (n = 370) according to the risk-score level calculated by the constructed model at the cutoff value of -0.12 (log2-transformed). The high risk group had a significantly worse overall survival (OS) compared to the low risk group (p < 0.0001). The risk-score also displayed an excellent prognostic predictive ability for LUAC patients by the results of ROC curve (AUC:0.66, 0.67 and 0.67) and nomogram (C-index, Calibration analysis, and Decision Curve Analysis) in years 1, 3, and 5. The risk group [HR = 0.3, 95%CI (0.22–0.4)], stage [HR = 1.78, 95%CI (1.28–2.48)] and age [HR = 1.02, 95%CI (1.00-1.04)] were as an independent predictor among LUAC patients. KEGG enrichment revealed that the mRNA influenced by the hunted seven LncRNAs which involved in immune escape function may be mainly related to the pathway of Chemical carcinogenesis, Th17 cell differentiation, NF-κB and Proteoglycans in cancer, etc. And the CIBERSORT score calculated from the expression level of the immune cell rerated gene between the normal cell and LUSC cell were showed that the immune system in the LUSC patients was activated. To sum up, our study demonstrated the potential clinical significance of 7-LncRNA characteristics in the prediction of survival in patients with LUSC.

INTRODUCTION

In recent years, the incidence of lung cancer is gradually increasing year by year which is the leading source of cancer-associated mortality[1] and its 5-year survival is less than 15%[2]. However, the pathogenesis of lung cancer is not clear and surgery combine with adjuvant therapy is still the main treatment for lung cancer patients. The main pathological type of lung cancer is non-small cell lung cancer, which mainly includes lung adenocarcinoma and lung squamous cell carcinoma (LUSC). Patients with LUSC are usually diagnosed at an advanced stage when currently available treatments cannot be given in time; LUSC patients are also not as sensitive to chemotherapy or radiation as small-cell cancer patients. As far as we know, the well-recognized stratification system of tumor, node, and metastasis (TNM) staging is widely used in clinical practice to guide therapy decision and predict cancer patient’ prognosis including lung cancer[3,4]. However, in the process of clinical application, TNM staging shows some shortcomings including the inability to accurately predict survival outcomes in many patients after surgical resection and even existing conflict among patients with same stage category[5,6]. So the search for new independent biomarkers for diagnosis and prognosis in LUSC is urgent.
Thanks to huge breakthroughs in high-throughput techniques over the past decade, thousands of molecular expression levels are now widely evaluated simultaneously by microarrays, sequencing and mass spectrometry\[7\]. In addition, the accumulation of basic research had shown that certain molecules are closely related to tumor phenotype and clinical behavior, especially LncRNAs, which have been proved holding on a great prospect in clinical practice to predict the long-term outcome of cancer patients\[8–10\]. Abnormal LncRNAs are also frequently detected in many cancers which has been proved associated with tumorigenesis and tumor progression. For example, some well-described LncRNAs, such as HOTAIR, MALAT1 and NEAT1, their expressions were significantly up-regulated in breast cancer, gastric cancer and hepatocellular carcinoma\[11–13\]. Moreover, in many lung cancer studies, multiple differentially expressed LncRNAs also have been identified and some of them play a role in clinical diagnosis and treatment\[14\]. In this paper, we obtained a set of seven prognostic LncRNA biomarkers associated with overall survival (OS) in LUSC patients. Combined with these LncRNAs, a 7-LncRNA risk score model was constructed, which could effectively predict patients’ OS. These results were further confirmed in the testing set and the whole set.

**MATERIALS AND METHODS**

**Clinical information and RNA expression data**

The raw RNA-Seq counts data and the corresponding related clinical information data of LUAC patients were acquired from TCGA-LUAC database (https://xenabrowser.net/datapages/). The patients missing the crucial analytical data such as RNA expression profiles data of the lung cancer solid tissues, follow-up survival data, age, gender, neoplasm stage (TNM) were excluded. Finally, a total of 471 patients were enrolled and randomly partitioned into a training cohort (n = 236) and a testing cohort (n = 235) for building a LncRNAs risk score model and validation the prognostic precision of LncRS model, respectively. The raw RNA-seq data were annotated by gencode v33 and then normalized by FPKM.

**Establishment of a LncRS model and verification**

The most significant survival-related LncRNAs were screened out by least absolute shrinkage and selection operator (lasso) regression model for training group based on the common prognostic related LncRNAs filter previously by univariate Cox regression (P < 0.05). Stepwise multivariate Cox regression analysis was used to establish the LncRNAs risk signature model (LncRS) after collinearity tested in LUAC based on the Akaike Information Criterion (AIC) that could establish a prognostic signature with the best predictive ability but the least number of LncRNAs. The calculation formula of the LncRS was as follows: LncRS = \[\sum_{i=1}^{k} (Exp_i \ast \beta_i)\], where \(k\) and \(i\) represents the total number and the sequence number of the significant prognostic LncRNAs, \(Exp_i\) the normalized expression values of corresponding LncRNA of each sample and \(\beta_i\) the regression coefficient of the corresponding LncRNA from multivariate Cox regression analysis.
The established formula model (LncRS) was performed to calculate the risk score of each patient from training cohort. Then, the patients from this cohort were separated into high- and low-risk groups based on cutoff point of risk score which was analyzed by the function of survival cutoff point calculation in the “survminer” R package. The Kaplan-Meier (KM) method and the log-rank test was performed to compare the survival difference between high- and low-risk groups. The scatter plot was used to display the survival status and the survival time of the patients sorted by ascending risk score as well as the heat map, which show the LncRS related LncRNAs expression level. The time-dependent (1 year, 3 years, 5 years, 7 years and 10 years) receiver operating characteristic (ROC) analysis was used to evaluated the prognostic diagnosis property of LncRS.

Independent survival prognostic effect of risk group in TCGA cohort

The risk group, and the corresponding clinical factors including age, gender, neoplasm location, tumor stage, pathologic TMN stage were screened by univariate (P < 0.2) and multivariate Cox regression to detect whether the LncRS could be the independent survival prognostic index after crucial clinical factors adjusted in TCGA cohort (P < 0.05). We also tested the correlation between the expression level of the LncRS related LncRNAs and the survival level in the same way. The forest plot was subjected to present the important analysis result (Hazard Ratio and P Value) of univariate and multivariate Cox regression.

Nomogram construction and verification

Nomogram was constructed to visualize the LUAC patient's survival probability of 1 year, 3 years and 5 years, based on the risk group and the crucial clinical parameters. The index of concordance (C-index) and the decision curve analysis (DCA) of the corresponding nomogram were fit to verify the prognostic diagnosis accuracy of the constructed Nomogram.

The prognostic diagnosis accuracy of LncRS verification in TCGA subgroups

The TCGA-LUAC patients were divided into different subgroups according to the crucial clinical parameters including age, gender, neoplasm location, tumor stage, pathologic TMN stage, respectively. Then, KM method and sub-forest plot was performed to compare the survival difference between high- and low-score groups in these sub-cohorts.

Gene Co-expression Network and Gene Functional Enrichment Analysis

The co-expression relationships between the LncRS related LncRNAs and mRNAs were evaluated using Pearson correlation analysis in the TCGA entire set [correlation coefficient (r) > 0.25, P < 0.05]. The tumor immune related genes list were obtained from GeneCards by the keywords “tumor immune” (https://www.genecards.org/). The intersection genes from the above two gene sets were used for GO and KEGG enrichment analysis. Finally, we hunted 14 major regulatory genes related the tumor immune
function and pathways obtained from GO and KEGG enrichment analysis \([r > 0.25; \text{abs (log}_2 \text{Fold change)} > 1.3]\).

**Statistical analysis**

All statistics were analyzed by R software (version 4.1.1). Nomogram plot was drawn by the “rms” R package. The KM survival analysis was visualized through “survival” package and the p-value was calculated via log-rank tests. Independent survival prognostic factors were screened by univariate and multivariate Cox regression using “survival” package.

**RESULTS**

Identification of a prognostic LncRNAs signature in the training set

The 471 LUSC patients were randomly divided into a training dataset (\(n = 236\)) and testing dataset (\(n = 235\)). Firstly, we identified the prognostic LncRNAs from the training set by multivariate Cox regression. Finally, seven LncRNAs (RP11.279O17.1, DKFZP434A062, RP11.534L20.5, CTA.292E10.6, CDIPT.AS1, RP6.24A23.7, LINC00628) were identified to be significantly associated with overall survival of LUSC patients. By linear combination of the expression levels of the 7 LncRNAs weighted by their Cox regression coefficients, a 7-LncRNAs was constructed. The relative expression levels of seven prognostic genes sorted by risk score are shown in the heatmap (Fig. 1B). Patients were then divided into low and high risk groups according to the optimal separation threshold of risk score, and the distribution of risk score for each patient was also shown in Fig. 1B. There was a significant difference in survival between the two groups (\(P < 0.0001\)). The mean survival time of high-risk patients was significantly shorter than that of low-risk patients, and the proportion of life state death was higher (Fig. 1B). Kaplan-Meier survival curve analysis showed that the prognosis of patients in the high-risk group was significantly worse than that in the low-risk group (Fig. 1E). In addition, the AUCs for 1-, 3-, 5-, 7-, and 10 year OS in the training group were 0.65, 0.73, 0.69, 0.71, 0.8, respectively (Fig. 2H).

Validation of the seven-LncRNAs signature in the testing set and all set

Secondly, to assess the robustness of the 7-LncRNA signature in OS prediction in LUSC patients, we further examined it in the testing set and in the whole set. As shown in Fig. 1C, in the testing dataset, patients also can be divided into a high-risk group and a low-risk group by using the same risk score formula, and the cutoff point \(-0.12\) (log\(_2\)-transformed) is consistent with which derived from the training dataset; The survival time between the high-risk group and the low-risk group was significantly different. Kaplan-Meier survival curve analysis showed that the prognosis of patients in the high-risk group was significantly worse than that in the low-risk group in the testing dataset (\(P < 0.001\); Fig. 1F). In the same way, the entire TCGA dataset which consisting with 471 patients was performed the same analysis and the same results were found (Fig. 1A, 1D). Moreover, The AUCs for 1-, 3-, 5-, 7-, and 10-year OS in the
testing group were 0.71, 0.68, 0.65, 0.7 and 0.84, respectively (Fig. 2L). The AUCs for 1-, 3-, 5-, 7-, and 10-year OS in the overall sample were 0.66, 0.67, 0.67, 0.68, and 0.73 (Fig. 2J) and the AUCs for 1-, 3-, 5-, 7-, and 10-year OS in the training group were 0.65, 0.73, 0.71, 0.64, 0.69 (Fig. 2K), respectively. Besides, we further investigate the relevant clinical factors (age, stage, multigene) in these groups. The AUCs of age for 1-, 3-, 5-, 7- and 10-year OS were 0.51, 0.51, 0.49, 0.57, 0.6 in overall samples (Fig. 2A), 0.56, 0.6, 0.59, 0.54, 0.6 in training group (Fig. 2B) and 0.59, 0.6, 0.55, 0.67, 0.77 in testing group (Fig. 2C). The AUCs of stage and multigene for 1-, 3-, 5-, 7-, and 10-year OS in all patients were shown in Fig. 2D and 2G, the corresponding results in training group were shown in Fig. 2B, 2E and in testing group (Fig. 2C, 2F). Without a doubt, these results are completely consistent with previous studies which suggesting this seven-LncRNAs signature is a good prognostic indicator among LUSC patients.

**Building and validating a predictive nomogram**

In order to better predict the prognosis of LUSC patients, a prognostic nomogram was constructed based on 471 LUSC patients with complete clinical information about risk score combing clinical factors (age, multigene, stage) (Fig. 3A). The calibration plots showed that the nomogram was best for predicting 1-, 3-, and 5-year OS in patients with LUSC (Fig. 3B-3D). The decision curve analysis showed the nomogram performed better at the threshold probability in Fig. 3E-3G. These results suggested that the prognostic nomogram was superior in predicting the 1-, 3-, and 5-year survival outcomes of patients with LUSC.

**Independence of the LncRNAs signature for survival prediction and the subgroup analysis**

Univariate and multivariate Cox regression analyses were performed to examine whether the prognostic power of 7-LncRNAs features was independent of clinical data. We evaluated the prognostic value of risk group and other clinic information characteristics, such as gender, age, AJCC stage and TNM stage, founding that the risk group and the seven LncRNAs served as independent prognostic factors for LUSC patients in univariate analysis (Fig. 4A, 4B) and age, AJCC stage, risk group and the seven LncRNAs served as independent prognostic factors in multivariate analysis after other clinic information characteristics were adjusted (Fig. 4C, 4D).

Further, the prognostic power of the 7-LncRNA signature (Fig. 4E) was consistently observed across LUSC patients by stratifying the whole dataset into 2 stratums subgroup based on different age (Fig. 5A), gender (Fig. 5B), tumor AJCC stage (Fig. 5C) and TNM grade (Fig. 5D-5F).

**Potential biological roles of the LncRNAs signature**

KEGG pathways were analyzed with the protein coding intersection genes, the expression level of which significantly related with model LncRNAs from TCGA-LUSC, using the whole human genome as the background. The results showed that the prognostic LncRNAs mainly enriched in the pathways related tumor immune function, such as Th17 cell differentiation, TNF signaling pathway, NF-kappa B signaling pathway, JAK-STAT signaling pathway, Toll-like receptor signaling pathway, Cytokine-cytokine receptor
interaction, etc (Fig. 6B, P < 0.05) that the expression level of the related enriched genes were obviously suppressed in LUSC patients. In addition, the Gene Ontology (GO) functional annotation enrichment analysis demonstrated that the co-expressed genes were significantly inhibited and enriched in representative GO terms related immune function, such as mast cell activation involved in immune response, negative regulation of tumor necrosis factor production, regulation of leukocyte degranulation, negative regulation of tumor necrosis factor superfamily cytokine production etc (Fig. 6A, P < 0.05). The similar results also showed in the mRNA expression level in the cnetplot analyses results for KEEG and GO (Fig. 6C, 6D). The emapplot analysis results displayed the network interaction among the hunted KEEG pathways and GO terms (Fig. 6E, 6F). We detected significantly positive correlation (Fig. 7A, 7B) among the 14 hunted gene (BMP2, CCL4, CFLAR, CISH, CSF1, CSF2RA, CSF3, CXCL3, ICAM1, IL17D, IL18R1, NFKBIA, PYGM, TNFSF14).

DISCUSSION

Lung cancer is the leading cause of cancer-related death worldwide, but treatment options are limited. Clinical success of treatment is not very promising due to late diagnosis, limited therapeutic tools, and the development of relapse and resistance[15]. LUSC is a subtype of non-small cell lung cancer and accounts for nearly 40% of all lung cancer. Early diagnosis and treatment of LUSC can greatly improve the prognosis of LUSC patients, which will not only reduce the economic burden of patients, but also improve the quality of life[16]. For decades, research into cancer biology focused on the involvement of protein-coding genes[17]. Only recently studies focus on the function, regulatory mechanism and therapeutic potential of the non-coding RNAs(ncRNAs) including microRNA(miRNA), LncRNA, circular RNA(circRNA) and PIWI interacting RNA(piRNA) in different type of cancers, which have proved that these ncRNAs play a crucial role in regulating the occurrence and development of various cancers[18,19]. Chen et al. and Zhou et al. have established a several distinctive LncRNAs panel with considerable diagnostic value for lung adenocarcinoma (LUAD) cancer prognosis prediction[20,21]. Zhang et al. constructed a 9-LncRNAs (AC013457.1, AC124067.2, AP001189.1, AP002360.1, BANCR, LINC00519, LINC01807, MIR3945HG, FAM83A – AS1 and POU6F2 – AS2) related model for prognostic evaluation of LUSC patients[22]. However, this kind of research for LUSC patients were still infrequent.

In the present paper, we further investigated the role of LncRNAs in LUSC. Finally, seven LncRNAs which have not been studied previously were identified to be significantly associated with overall survival of LUSC patients. There was a significant different in survival between low risk group and high risk group grouped by the risk score calculated with the construct model in the training set. In addition, the mean survival time, the proportion of life state death and the prognosis were also different between these groups (Fig. 1). Then, we verified the results in the testing dataset and all patients, there were the same conclusions which were completely consistent with previous results and reported. The ROC curve analysis was used for further studied to evaluate prognostic ability of the constructed model. The results of AUCs (Fig. 2I) for 1-, 3-, 5-, 7-, and 10-year OS in the testing group were 0.65, 0.73, 0.69, 0.71 and 0.80, which has more detail information and better accuracy than Zhang et al. reported only one result of AUCs.
= 0.65 for 3-years survival data in all patients group\(^{[22]}\). Which surprised us was that the prognostic ability of the model was much more superior to other variables including TNM stage (AUCs equal to 0.57, 0.55, 0.54, 0.55, 0.4) and age (AUCs equal to 0.56, 0.60, 0.59, 0.54 and 0.60) for all the same time frame. In order to predict the prognosis of LUSC patients, a prognostic nomogram was constructed, and it was excellent for predicting 1-, 3-, and 5-year OS in patients with LUSC which has not been reported in previous study (Fig. 3). The multivariate Cox regression showed that 7-LncRNA signatures were consistently identified as independent predictors of OS in LUSC patients (Fig. 4). Moreover, the subgroup analysis also stated the excellent survival predictive ability of the risk score for the sub population of LUSC patients which grouped by the difference of the age, gender, tumor stage and other clinical features (Fig. 5). Taken together, these findings suggest that the predictive value of 7-LncRNA characteristics for OS of LUSC is independent of other clinical variables.

GO and KEGG enrichment analysis was used for expounding the potential tumor immune inhibited mechanism of the LUSC patients regulated by the model related 7-LncRNAs. Th17 cell associated with its relevant cytokines has been detected in a wide variety of tumor\(^{[23]}\). IFN-\(\gamma\) and IL-17 stimulates Th17 leading CXCL9 and CXCL10 production, the latter can recruit Th1 and NK to the tumor microenvironment for antitumor defense and advanced tumor immune response\(^{[24]}\). Interestingly, as our result shown the expression level of the protein coding mRNAs enriched (Fig. 6D) related the pathway of Th17 cell differentiation (Fig. 6B) was significantly inhibited in LUSC patients. NF-kB stimulates immune cell function and acts in a pro-inflammatory manner by inducing the expression of cytokines, chemokines and their receptors, which lead to tumor inhibited\(^{[25]}\); And it also has been shown an activation in tumorigenesis\(^{[26]}\). TNF-\(\alpha\) and IL-1 activation of their relevant receptors following up stimulate intensely NF-\(\kappa\)B, which contributes to amplify and extend the endurance of the innate immune response leading to apoptosis of tumor cell\(^{[27,28]}\). TNF also display an antineoplastic activity\(^{[29]}\) and the expression of TNF receptors is significantly down-regulation in high stage non-small cell lung cancer (NSCLC). Besides, STATs have displayed as a double-edged sword, being widely detected in multiple tumor. Among the members of the STAT family, STAT3 and STAT5 have been related to tumor origination and advancement\(^{[30]}\). Some papers have proved the expression of TNF-\(\alpha\) is reduced through STAT3 inactivation, which lose the ability of binding to the promotor of TNF-\(\alpha\)\(^{[31,32]}\). As shown in Fig. 6, it exhibited a significant inhibited in the expression of NFKBIA, CSF1, ICAM1 and CXCL3 associated TNF pathway, which was consistent with the result of NF-\(\kappa\)B pathway (CCL4, CFLAR, NFKBIA, TNFSF14, ICAM1 and CXCL3), JAK-STAT signaling pathway (CSF2RA, CISH, CSF3), Toll-like receptor signaling pathway (CCL4 and NFKBIA) and Th17 cell differentiation signaling pathway (NFKBIA).

Immune escape is an important mechanism in tumorigenesis. One paper has reported that long intergenic non-protein coding RNA 1140 (LINC01140) overexpression protects c-Myc and PD-L1 mRNA from miRNA-mediated inhibition and contributes to the proliferation, migration, invasion, and immune escape of LC cells\(^{[33]}\). Another paper showed Long intergenic non-coding RNA LINC01088 directly targeted miR-548b-5p and miR-548c-5p, promoting G3BP1 and PD-L1 expression, which facilitated colorectal cancer progression and immune escape\(^{[34]}\). A recent study also revealed SKIL promoted
tumorigenesis and immune escape of NSCLC cells through upregulation of TAZ/autophagy axis and inhibition on downstream STING pathway\cite{35}. And LncRNA small nucleolar RNA host gene 12 (SNHG12) facilitated the immune escape of NSCLC by binding to HuR and increasing PD-L1 and USP8 levels\cite{36}. However, in our study, we also revealed that the 14 hunted genes from several signaling pathways related tumor immune system have been significant inhibited in LUSC patients. However, the immune infiltration reaction including T cells, B cells, NK cells and monocytes were prominent active in LUSC patients as Fig. 8 shown. Multiple papers have shown that immune infiltration is prevalent in tumor cells, such as breast cancer and glioblastoma multiforme\cite{37,38}; however, not all immune infiltrates are capable of anti-tumor effects\cite{39,40}. In our study, how the seven prognostic LncRNAs facilitated the immune escape of LUSC was still unclear.

In this study, there are still having some short comings. First of all, our study just included a total of 236 patients created the 7-LncRNAassignature, the number of cases was relatively small. Second, although we used bioinformatics methods to explore the functional roles of seven prognostic LncRNAs, the exact molecular mechanisms remain unclear and need to be clarified through experimental studies. Third, due to a lack of data, we were unable to assess the impact of confounding factors, such as treatment strategies or medications, on outcomes in patients with LUSC. In conclusion, we identified a total of seven LncRNA biomarkers which cloud effectively predict LUSC patient’s OS and provided novel insights to predict the prognosis of LUSC patients.

Declarations

Data availability statement

The datasets used during the current study available from the corresponding author on reasonable request.

Click on the link: https://pan.baidu.com/s/14J3fQAhx80V9yXKFS7zQsA.

References


Figures
Figure 1

The risk score distribution, duration and survival statuses of LC patients and heatmaps of the seven-gene signature relative expression in the total TCGA cohort (A), training TCGA cohort (B), and testing TCGA cohort (C). Kaplan-Meier analysis of the low- and high-risk group patients in the total TCGA cohort (D), training TCGA cohort (E), and testing TCGA cohort (F).
Figure 2

ROC curve analysis of age, stage, multigene and all union index according to the 1, 3, 5, 7, and 10-year survival of the area under the AUC value in the total TGCA cohort (A, D, G, J), training TGCA cohort (B, E, H, K), and testing TGCA (C, F, I, L).
Figure 3

A prognostic nomogram predicting 1-, 3-, and 5-year OS of LC (A). Calibration plots of the nomogram for predicting the proportion of patients with 1-, 3-, or 5-year OS (B-D). Decision curve analysis of nomogram predicting 1-, 3-, and 5-year OS of LC comparing the age, stage and multigene (E-G).
**Figure 4**

Forrest plot of the univariate Cox regression analysis OS of clinical factors (A) and seven-gene signatures (B). Forrest plot of the multivariate Cox regression analysis OS of clinical factors (C) and seven-gene signatures (D). Forrest plot of the univariate Cox regression analysis OS of risk score group in subgroup of clinical factors (E).
**Figure 5**

Kaplan-Meier survival analysis of risk score group in subgroup of clinical factors. (A) age; (B) gender; (C) TNM stage; (D) N stage; (E) M stage; (F) T stage.
Figure 6

Biological functions (A) and the signaling pathways (B) of co-expressed genes related to the model LncRNAs. The network regulation relationship between the co-expressed genes and biological functions (C)/the signaling pathways (D). The network regulation relationship among the biological functions (E)/the signaling pathways (F).
Figure 7

(A) The ChordDiagram displayed the positive correlation among the 22 hunted genes ($r > 0.25$; abs (log2 Fold change) > 1). (B) The network plot displayed the correlation level among the 14 hunted genes (BMP2, CCL4, CFLAR, CISH, CSF1, CSF2RA, CSF3, CXCL3, ICAM1, IL17D, IL18R1, NFKBIA, PYGM, TNFSF14) which were screened out from co-expressed genes related the model LncRNAs ($r > 0.25$; abs (log2 Fold change) > 1.3).
Figure 8

The immune infiltration reaction including T cells, B cells, NK cells and monocytes were prominent active in LUSC patients.