Predicting the difference in treatment response and survival time of lung adenocarcinoma patients based on a prognostic risk model of glycolysis-related genes

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Abstract

Background

Multiple factors affect the survival time of patients with lung adenocarcinoma (LUAD). Specifically, the therapeutic effect of medicines and the disease recurrence probability differs among patients with the same stage of LUAD. Thus, effective prognostic predictors need to be identified.

Methods

Based on the tumor mutation burden (TMB) data obtained by TCGA, LUAD was divided into high and low groups, and the differentially expressed glycolysis-related genes between the two groups were screened out. Cox regression was used to obtain a prognostic model. A receiver operating characteristic (ROC) curve and calibration curve were generated to evaluate the nomogram that was constructed based on clinicopathological characteristics and the risk score. Two datasets (GSE68465 and GSE11969) from Gene Expression Omnibus (GEO) were used to verify the prognostic performance of the gene. Furthermore, differences in immune cell distribution, immune-related molecules and drug susceptibility were assessed for their relationship with the risk score.

Results

We confirmed a 5-gene signature (FKBP4, HMMR, B4GALT1, ERO1L, ENO1) capable of dividing patients into two risk groups. There was a significant difference in overall survival (OS) times between the high-risk group and the low-risk group ($P = 1.085e-4$), with the low-risk group having a better survival outcome. Through multivariate Cox analysis, the risk score was confirmed to be an independent prognostic factor (HR = 1.289, 95% CI = 1.202-1.383, $P < 0.001$), and the ROC curve and nomogram exhibited accurate prediction performance. Validation of the data obtained in the GEO database yielded similar results. Additionally, there were significant differences in cisplatin, paclitaxel, gemcitabine, docetaxel, gefitinib and erlotinib sensitivity between the low-risk and high-risk groups.

Conclusions

Our results reveal that glycolysis-related gene are feasible predictors of LUAD patient survival and response to therapeutics.

Introduction

Lung cancer accounts for a large proportion of cancer-related human deaths worldwide ^{1,2}. As lung adenocarcinoma (LUAD) is a common pathological type of lung cancer ³, individualized therapy for LUAD

has received increasing attention from clinicians. Because the occurrence and development of tumours are based on genetic changes ⁴⁻⁶, the response to therapies and the overall survival times are not necessarily the same in patients of the same sex, performance status score, age and TNM stage when the influence of social, family and economic factors is removed. Therefore, exploring effective microscopic molecular biomarkers to predict the treatment response and prognosis of patients with LUAD is urgently required.

Understanding the differences in metabolism and proliferation between tumour cells and normal cells is essential for predicting the prognosis and clinical treatment response of cancer patients. Cells mainly obtain energy to fulfil their biological activities through glycometabolism, and LUAD cells are not an exception to this rule. Previous studies have revealed that the most significant metabolic change in cancer cells is the occurrence of the Warburg effect, which is manifested by increased aerobic glycolysis of tumour cells and dependence on glycolytic pathways to produce adenosine triphosphate (ATP) ⁷⁻¹⁰. In view of this unique metabolic alteration in tumour cells, many targeted treatments have been tried and improved over time ¹¹⁻¹⁵. Moreover, an increasing number of researchers have used glycolysis-related genes to establish tumour prognosis evaluation models ¹⁶⁻²⁰. TMB which is defined as the number of somatic mutations per megabase (Mb) of the interrogated genomic sequence of a tumor, and it could be used as a predictor of the efficacy of Immune checkpoint inhibitors (ICIs) in multiple tumors²¹⁻²³. Extensive study of TMB as prognostic molecular markers²⁴⁻²⁶ has been carried out in recent years. Since glycolysis-related genes can be used to establish an effective prognosis prediction model for LUAD, integrating TMB into the prediction model is a new and promising research approach with inherent advantages.

Here, we conducted in-depth research on gene expression data from LUAD patients in the TCGA database and used the difference of TMB to screen glycolysis-related genes. Subsequently, we found that the use of statistical tools to obtain a prognostic model and nomogram composed of glycolysis-related gene has good clinical practicality and produces a method for reliable prognosis prediction. Furthermore, the risk score was associated with the tumour immune microenvironment and could be used to estimate the sensitivity of the LUAD patients included in this study to therapeutics.

Materials And Methods

Clinical information and gene expression data from patients

The clinical characteristics, genetic mutations, and mRNA expression data from LUAD patients in the TCGA database (<https://cancergenome.nih.gov/>) were obtained for subsequent analyses by using Perl (version 5.32.1.1). The clinical features of 522 patients with LUAD, including gender, age, T stage (tumour), N stage (lymph node), M stage (metastasis), tumour stage and survival status are detailed in Table 1.

Table 1
LUAD patient characteristics in
the TCGA.

Clinical feature	N	%
Gender		
Male	242	46.4
Female	280	53.6
Age (years)		
≤65	241	46.2
>65	262	50.2
Unknown	19	3.6
Stage		
Stage I	279	53.4
Stage II	124	23.8
Stage III	85	16.3
Stage IV	26	5.0
Unknown	8	1.5
T (tumor)		
T1	172	33.0
T2	281	53.8
T3	47	9.0
T4	19	3.6
TX	3	0.6
N (lymph node)		
N0	335	64.2
N1	98	18.8
N2	75	14.4
N3	2	0.4
NX	11	2.0
Unknown	1	0.2

Clinical feature	N	%
M (metastasis)		
M0	353	67.6
M1	25	4.8
MX	140	26.8
Unknown	4	0.8
Survival status		
Death	188	36.0
Alive	334	64.0

Screening of differentially expressed glycolysis-related genes

By searching glycolysis gene sets in the Gene Set Enrichment Analysis (GSEA) database (<http://www.broadinstitute.org/gsea/index.jsp>), a total of 326 genes were obtained. Use the gene mutation data obtained to calculate the TMB value of each sample, and divide it into high and low groups based on the median value. Subsequently, the analysis of differentially expressed glycolysis-related genes between the high TMB group and low TMB group was executed in R, with the following cut-off criteria: $|\log_2\text{fold change (logFC)}| > 0.2$; $P\text{-value} < 0.05$; $\text{FDR (false discovery rate)} < 0.05$.

Gene prognosis model development

In R, the “survival” package was used to perform the univariate Cox regression analysis of differentially expressed genes, and the filter condition was $P < 0.05$. Then, the subsequent multivariate Cox proportional hazards regression were performed. We used the following risk formula: $\text{riskscore} = \beta_{\text{gene1}} \times \text{Expression}_{\text{gene1}} + \beta_{\text{lncRNA2}} \times \text{Expression}_{\text{gene2}} + \beta_{\text{lncRNA3}} \times \text{Expression}_{\text{gene3}} + \dots + \beta_{\text{genen}} \times \text{Expression}_{\text{genen}}$, where β is defined as the coef and $\text{Expression}_{\text{gene}}$ indicates the expression level in patients with LUAD. The prognostic value of genes in the model was verified by using UALCAN database (<http://ualcan.path.uab.edu/index.html>) and Kaplan-Meier (KM) plotter database (<http://kmplot.com/analysis/>). Finally, integrating GSE68465 and GSE11969, a total of 536 patient data were obtained, and they were divided into high and low risk groups using the risk score formula.

Model evaluation and nomogram construction

The ROC curve was employed to evaluate the validity of the prognostic model by using the “survival ROC” package. The LUAD patients were reclassified into a low-risk group and a high-risk group by using the cut-off value at which the best time-dependent AUC was obtained using the risk score. Then, the difference in survival times between the low-risk and high-risk groups were assessed by using the “survival” package and the “survminer” package, and the Kaplan-Meier curve was plotted to display the results directly in R.

The Wilcoxon signed-rank test was used to obtain the risk score difference among different clinicopathological features. Then, the gene prognostic model was compared with gender, age, T stage (tumour), N stage (lymph node), M stage (metastasis), and tumour stage for independent prognostic analysis. The subsequent operations were performed with the “ggpubr” R packages. Additionally, we constructed a nomogram to improve the practicality of the model and validated it through the “rms” package.

Immune microenvironment and therapeutic response

To clarify the relationship between the immune microenvironment and the risk score, XCELL, TIMER, QUANTISEQ, MCPcounter, EPIC, CIBERSORT and CIBERSORT-ABS were used to obtain differences in the distribution of immune cells in LUAD patients from the TCGA database. The “ggplot2” R package was used to perform the procedure, the Wilcoxon signed rank test and Spearman’s correlation analysis were used for the statistical analysis, and $P < 0.0001$ was set as the threshold value for significance. The expression levels of immune checkpoint inhibitor-related genes were compared between the low-risk group and the high-risk group. We showed the results and the P value was labelled as follows: $< 0.001 = ***$, $< 0.01 = **$, and $< 0.05 = *$. Subsequently, we added the pRRophetic package to evaluate clinical treatment response differences among LUAD patients grouped by the gene prognostic model by calculating the IC_{50} (half maximal inhibitory concentration) of commonly used drugs in R.

Results

Data processed by GSEA

Using “glycolysis” as the search keyword, the BioCarta, Hallmark, KEGG, REACTOME and WP gene sets were selected from the Molecular Signatures Database (MSigDB) to obtain a glycolysis-related gene signature. After using the above detailed data to perform GSEA, significant differences were detected between the tumour tissue sample group and the normal tissue sample group among the gene sets (Figure 1A-D), with normalized P values < 0.05 .

Establishment of a glycolysis-related gene model

By comparing the high TMB group with low TMB group, 95 differentially expressed genes were obtained (Figure 2 and Supplementary file 1). Univariate Cox analysis was performed, and we obtained 29 prognosis-related genes (Table 2), and multivariate Cox proportional hazards regression analysis was used to calculate the risk score to construct the prognostic model (Table 3). The prognostic was calculated as follows: $(0.00854 * \text{expression level of FKBP4}) + (0.0295 * \text{expression level of HMMR}) + (0.00651 * \text{expression level of B4GALT1}) + (0.00732 * \text{expression level of ERO1L}) + (0.000671 * \text{expression level of ENO1})$.

Table 2
Univariate Cox regression analysis.

ID	HR	95% CI		P-value
		Lower	Upper	
SLC16A3	1.021	1.014	1.028	2.80e-08
KIF20A	1.053	1.026	1.081	1.30e-04
TPI1	1.002	1.001	1.004	1.48e-04
DSC2	1.028	1.004	1.053	2.06e-02
RPE	1.042	1.015	1.069	1.88e-03
PFKP	1.007	1.003	1.011	3.41e-04
ALDOA	1.002	1.001	1.002	1.43e-03
FKBP4	1.011	1.006	1.015	1.29e-05
GAPDH	1.001	1.000	1.001	4.30e-08
HMMR	1.062	1.031	1.092	4.55e-05
P4HA1	1.007	1.002	1.013	8.88e-03
PLOD2	1.010	1.004	1.015	3.52e-04
B4GALT1	1.009	1.006	1.013	5.39e-07
PYGL	1.020	1.008	1.032	8.68e-04
SLC2A1	1.006	1.004	1.009	9.44e-08
GPI	1.009	1.004	1.014	8.90e-04
PGAM1	1.039	1.020	1.059	7.24e-05
PGM2	1.053	1.029	1.078	1.12e-05
STC1	1.009	1.004	1.013	7.94e-05
DEPDC1	1.075	1.021	1.132	5.72e-03
ERO1A	1.013	1.008	1.017	2.61e-08
MIF	1.006	1.001	1.010	8.97e-03
TXN	1.001	1.000	1.002	1.40e-02
CDK1	1.017	1.004	1.031	1.24e-02
AURKA	1.022	1.007	1.036	2.93e-03
FBP1	0.994	0.991	0.998	5.40e-03

ID	HR	95% CI		P-value
		Lower	Upper	
ENO1	1.001	1.001	1.002	2.29e-04
NUP37	1.060	1.017	1.105	6.28e-03
PSMC4	1.007	1.001	1.013	3.36e-02

Table 3
Multivariate Cox proportional hazards regression analysis to construct a risk model.

ID	Coef	HR	95% CI		P-value
			Lower	Upper	
FKBP4	0.00854	1.009	1.002	1.015	6.33e-03
HMMR	0.0295	1.030	0.993	1.068	1.15e-01
B4GALT1	0.00651	1.007	1.002	1.011	1.86e-03
ERO1L	0.00732	1.007	1.002	1.012	4.80e-03
ENO1	0.000671	1.001	1.000	1.001	6.71e-02

Assessment of model performance

Validation in the UALCAN database suggests that each gene in the model has prognostic value (Figure 3A-E), and similar results are also obtained in the Kaplan-Meier (KM) plotter database (Figure 3F-K). The areas under the ROCs (AUCs) of the 1-, 2-, and 3-year overall survival rate analysis for the 5-gene prognostic model were 0.673, 0.671 and 0.687, respectively (Figure 4A). Similar verification results obtained in the GEO databases, the ROCs (AUCs) of the 1-, 2-, and 3-year overall survival rate analysis for prognostic model were 0.710, 0.701 and 0.650, respectively (Figure 4B). Then, we used risk score cut off point to classify LUAD patients in the TCGA database into a low-risk group and a high-risk group, and the survival time was significantly different between the two groups ($p = 1.085e-4$), and the low-risk group in the GEO database also had a better prognosis ($p = 2.78e-2$) (Figure 4C and D). The risk score and survival status of all patients are plotted in Figure 6A and B. Next, we conducted an independent prognostic analysis of age ($p = 0.312$, HR = 1.008, 95% CI [0.993-1.023]), gender ($p = 0.518$, HR = 1.102, 95% CI [0.821-1.480]), stage ($p < 0.001$, HR = 1.632, 95% CI [1.420-1.876]) and the risk score ($p < 0.001$, HR = 1.348, 95% CI [1.263-1.438]) by univariate Cox regression analysis (Figure 5A) and confirmed that stage ($p < 0.001$, HR = 1.548, 95% CI [1.338-1.791]) and the risk score ($p < 0.001$, HR = 1.289, 95% CI [1.202-1.383]) can be used as predictors of prognosis in LUAD patients by multivariate Cox regression analysis (Figure 5B). We also found that the AUC of the risk score of all patients was similar to the AUC of the tumour stage (Figure 5C).

Clinical evaluation and nomogram construction

We found that T stage, N stage, and clinical stage (Figure 6A - C) were significantly correlated with the risk score, while there were no differences in risk scores between gender group, age group, and M stage, (Figure 6D - F) through the Wilcoxon signed-rank test. Subsequently, to enhance the clinical utility of the gene model, we constructed a nomogram (Figure 7A). Risk score, age, gender and clinical stage were basic elements included in the nomogram. Moreover, the prediction results of the nomogram were generally consistent with the actual survival outcomes based on the prediction calibration curves of 1-, 2-, and 3-year survival rates (Figure 7B - D). The AUCs of the 1-, 2-, and 3-year survival rate predictions for the nomogram were 0.750, 0.719, and 0.740 (Figure 7E - G), respectively.

Estimation of tumour immune-related cells and molecules and patient therapeutic response with the gene assessment model

We revealed that the presence of more tumour immune-related cells, such as myeloid dendritic cell, T cell CD4+, endothelial cell, and macrophage M2, were positively correlated with low risk, while macrophage M0, macrophage M1, and NK cell were positively associated with high risk (Figure 8A). Detailed results of the Wilcoxon signed-rank test are exhibited in Supplementary file 2. Since it is not uncommon to use immune checkpoint inhibitors to treat lung adenocarcinoma in the clinic, we aimed to clarify whether ICI-related biomarkers were related to the risk model and discovered that low risk scores were positively correlated with high expression of CD28 ($p < 0.001$), CD274 ($p < 0.001$), LAG3 ($p < 0.05$) (Figure 8B - D). Next, we explored the relationship between the risk score and the response of TCGA LUAD patients to therapeutics. Our results indicated that a low risk score correlated with a higher IC50 for medications such as cisplatin ($p = 3.1e - 08$), paclitaxel ($p = 2.22e - 16$), docetaxel ($p = 2.22e - 16$), gemcitabine ($p = 1.7e - 15$), erlotinib ($p = 2.5e - 08$), and gefitinib ($p = 5.1e - 09$) (Figure 9A - F).

Discussion

The survival rate and quality of life of LUAD patients has improved with the development of multiple aggressive treatments, such as surgery, chemotherapy, targeted therapy and radiotherapy; however, patient responses to individualized treatment remain inconsistent. Since the occurrence and development of tumours are accompanied by changes in the expression of a variety of genes, only by fully evaluating the risks associated with gene changes can the formulation of appropriate treatment plans be improved. LUAD is a pathological type of lung malignancy and can be further divided into different therapeutic subtypes and prognostic subtypes according to the status of multiple genes. For instance, LUAD patients with EGFR mutations and ALK fusion mutations have better treatment options available and higher survival rates than patients without these mutations²⁷⁻³⁰. Moreover, as immunotherapies have been developed, researchers have found that only some patients with LUAD benefit from these treatments³¹. Therefore, the problem of different patient responses to treatment remains to be solved. To date, many studies have revealed that scoring can be used to assess multiple molecular markers and effectively predict patient prognosis and evaluate the potential patient response to drugs. The breast cancer 21-Gene Expression Assay is one of the most well-developed methods that can provide a prediction of patient

prognosis, disease recurrence and tumour metastasis and can be used to guide treatment plans and assist in the development of individualized patient treatment strategies³². There have been many studies on molecular markers in lung adenocarcinoma; however, the research directions of these studies have been different (such as the development of an immune prognostic model³³, an autophagy-associated gene prognostic model³⁴, a ferroptosis-related gene prognostic model³⁵, and a glycolysis-related gene prognostic model¹⁶ and it is not known whether any one approach is effective for all individuals. Therefore, constantly improving the predictive model methods will provide a variety of options for specific patients. The nomogram is a prognostic evaluation tool that can integrate several prognostic determinants, including molecular and clinicopathological parameters, and can calculate and visualize the numerical probability of clinical events with a relatively simple output and is widely used in clinical oncology³⁶.

To obtain a more reliable prognostic model for LUAD, we used prognostic models constructed from glycolysis-related genes as a reference. First, glycolysis-related genes in LUAD were obtained, and based on the difference of TMB, differentially expressed glycolysis-related genes were selected as the cornerstone for constructing the prognostic model. Then, after performing Cox regression analyses, we found that a prognostic model composed of 5 glycolysis-related genes had better independent prognostic prediction performance, and the nomogram combined with the clinical characteristics of this model had better performance and more practical clinical application value. Meanwhile, we have also used the data in the GEO database for good verification. Since there was a difference in survival times between patients grouped according to the model, we investigated the reason for this difference. The results of our in-depth study revealed that there were differences in tumour pathological characteristics and immune responses among patients grouped according to glycolysis-related genes, as well as differences in sensitivity to therapeutic drugs. Therefore, we have presented sufficient evidence to demonstrate that the gene model obtained by the method in this paper has a better auxiliary effect in the prediction of LUAD patient response to clinical treatment.

More recently, numerous studies have been conducted on the use of gene or lncRNA to construct a prognostic model for LUAD. Sun et al. reported that immune-related genes could be used to construct a prognostic model. However, nomogram was not combined with the model and clinicopathological characteristics, so it was impossible to evaluate the effects of age, gender and stage for a specific clinical patient using this model³⁷. Although Xu et al. obtained prognostic biomarkers by analysing the tumor microenvironment of LUAD, they did not calculate the AUC value of the model³⁸. Most of the risk models are based on the detection of the expression level of the molecule of interest and the calculation of the total risk score to determine the prognosis of the patient. The first requirement is to judge the accuracy of the model before considering whether it can be used in clinical practice. Li et al. found that RNA binding proteins could be prognostic signatures for LUAD, the model obtained had good prediction performance, and a nomogram was also constructed³⁹. However, the differences in the immune microenvironment between the groups based on that model have not been further explored. It is well known that the prognosis of patients is related to a variety of factors, and the aforementioned model is of limited use for

predicting survival time. Additionally, the clinical treatment plan for patients is somewhat volatile. Therefore, a model is more valuable if it also has the ability to predict patient response to medication. Wu et al. validated a LUAD patient prognostic biomarker constructed using autophagy-related long noncoding RNAs⁴⁰, but the risk model did not specify the 1-year, 2-year and 3-year survival rate AUCs in detail, nor did it analyze the relationship with clinicopathological features. Zhang et al. also constructed a prognostic model based on glycolysis-related genes, but it did not specify the AUC value of 1, 2 and 3 years, did not use TMB for differential analysis, and did not verify it⁴¹. And the number of genes in the model is more than that in this study. Our prognostic model based on the metabolic characteristics of tumours has the following characteristics. First, the theoretical basis is sufficient. Glycolysis, as a metabolic characteristic of common tumour changes, has been confirmed to be relevant by many researchers. Second, the data screening was reasonable, and the data processed by the Cox regression analysis were more reliable. Finally, the assessment of the nomogram and its ability to predict patient drug sensitivity provides better clinical applicability.

Although the model we constructed has the above advantages, there are also some shortcomings. We are not in a position to conduct in vitro studies to further verify the function of these genes. FKBP4, HMMR and B4GALT1 are associated with the occurrence and development of LUAD or the survival prognosis of patients^{17, 41}. ERO1L was found to be a potential biomarker in LUAD and shapes the immune-suppressive tumor microenvironment. ENO1 was confirmed to be able to promote glycolysis and tumor progression in lung adenocarcinoma through CircRNA-ENO1⁴². When better experimental research resources become available in the future, the predictive ability of these genes can be verified in more LUAD patients.

Conclusion

In summary, a LUAD risk prognostic signature of 5 glycolysis-related genes was identified in this study. For predicting the survival time of patients with LUAD and their potential response to therapeutics, the model obtained in this study has excellent performance. This is the first study to predict the survival and drug response of LUAD patients by combining glycolysis-related genes with TMB. Furthermore, the combination of glycolysis-related genes and immune responses could not only enhance the accuracy of the model but also lead to a new direction in immunotherapy.

Declarations

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Author contributions

Rongchang Zhao wrote the manuscript. Rongchang Zhao, Dan Ding, and Xiujuan Wang performed the statistical analysis. Yan Ding, Rongbo Han, and Chunrong Zhu prepared the figure and wrote the figure legend. All authors approved the final version to be published.

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Figures

Figure 1

GSEA revealed that four gene sets were significantly enriched: (A) BioCarta glycolysis, (B) glycolysis, (C) REACTOME glycolysis and (D) WP glycolysis and gluconeogenesis.

Figure 2

Heatmap diagram of differentially expressed glycolysis-related genes, by comparing the high TMB group with low TMB group

Figure 3

Prognostic validation of genes in the prognostic model. (A) FKBP4, (B) HMMR, (C) B4GALT1, (D) ERO1L, and (E) ENO1 all have the ability to predict survival time, in UALCAN database.

Figure 4

Prognostic validation of genes in the prognostic model. (A) FKBP4, (B) HMMR, (C) B4GALT1, (D) ERO1L, and (E) ENO1 also all have the ability to predict survival time, in Kaplan-Meier (KM) plotter database.

Figure 5

Evaluation and validation of the glycolysis-related gene risk model. (A) AUC values of 1-, 2-, and 3-year survival rates are shown in the ROC curve, by integrating TCGA database data. (B) TAUC values of 1-, 2-, and 3-year survival rates are shown in the ROC curve, by integrating GEO database data. (C) Kaplan-Meier survival curves of patients based on the risk score of 5 glycolysis-related genes, in TCGA database. (D) Kaplan-Meier survival curves of patients based on the risk score of 5 glycolysis-related genes, in GEO database.

Figure 6

Comparison with clinicopathologic features. (A) Univariate cox regression analyses. (B) Multivariate cox regression analyses. (C) AUC of the risk score, age, gender and stage.

Figure 7

Use risk assessment models for clinical evaluation. (A) A strip chart for information overview. The scatter diagram of (A) T stage, (B) N stage, (C) tumour stage, (D) gender, (E) age, and (F) M stage.

Figure 8

A nomogram for survival prediction was constructed and evaluated. (A) The nomogram for predicting the 1-, 2-, and 3-year survival rate of LUAD patients. The consistency of the actual proposal and nomogram-predicted probability of OS at (B) 1, (C) 2 and (D) 3 years. AUC calculated for (E) 1, (F) 2 and (G) 3 years based on the nomogram.

Figure 9

The risk model was used to evaluate tumour-infiltrating cells and immune costimulatory molecules. (A) Correlation between immune cells and the risk score was obtained using different methods. A low risk score was positively related to the upregulation of (B) CD28, (C) CD274, and (D) LAG3 expression.

Figure 10

The risk model was used to evaluate IC50 differences. Analysis of (A) cisplatin, (B) paclitaxel, (C) docetaxel, (D) gemcitabine, (E) erlotinib, and (F) gefitinib showed that there was significant difference between the low- and high-risk groups.

Supplementary Files

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