Identifying novel cell glycolysis related gene signature predictive of overall survival in bladder urothelial carcinoma

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Research

Keywords: Bladder urothelial carcinoma, Glycolysis, Overall survival, mRNAs

Posted Date: May 28th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-30639/v1

Abstract

Background: Bladder urothelial carcinoma (BLCA) is the most common pathological type of bladder cancer and featured by a high risk for relapse and metastasis. Although many biomarkers have been developed by data mining and experimental studies to predict the prognosis of BLCA, a single-gene biomarker usually has poor specificity and sensitivity, leading to unsatisfactory prediction. Therefore, novel gene signatures are needed to more accurately predict the prognosis of BLCA.

Methods: Data mining was performed for expression profile analysis of 433 mRNA expression data from the TCGA BLCA patients (n=412). Gene Set Enrichment Analysis (GSEA) was used to interpret the glycolysis-related gene sets. Gene signature related to the prognosis of BLCA was identified by univariate and multivariate Cox regression. A risk score was computed based on three genes by linear regression model and its relation with overall survival was investigated by Kaplan-Meier analysis.

Results: Three genes (CHPF, AK3, NUP188) were found to be significantly correlated to the overall survival of BLCA patients. Based on the signature composed of these three genes, 412 BLCA patients were divided into high-risk and low-risk groups. The survival time of the high-risk group was significantly shorter than that of the low-risk group, indicating a worse prognosis.

Conclusion: A signature composed of three glycolysis-related genes was developed as biomarkers to predict the prognosis of BLCA and to provide a meaningful reference for the clinical treatment of BLCA.

Introduction

Bladder cancer is one of the most common cancers throughout the world. In 2012, there were 429,800 new cases of bladder cancer and 165,100 deaths related to bladder cancer worldwide [1]. Bladder urothelial carcinoma (BLCA) is the most common subtype of bladder cancer, accounting for over 90% of bladder cancer cases [2]. BLCA is more likely to relapse and develop metastasis, which brings enormous pain and economic burden to the patients [3]. Treatments for BLCA include surgical resection, radiotherapy, chemotherapy, and targeted therapy [3–4]. Although the treatments of BLCA show good efficacy, the prognosis of BLCA patients may vary under the same treatment due to individual differences. Therefore, finding proven biomarkers to effectively predict the prognosis of BLCA is of particular importance.

The disorder of the metabolic pathways is one of the hallmarks of cancer. The proliferation of cancer cells involves metabolic processes. Even if there is sufficient oxygen, cancer cells preferably undergo glycolysis to generate materials and energy needed for life activities. This unique phenomenon of energy metabolism is known as the Warburg effect [5]. At present, some glycolysis related biomarkers have already been used to predict the prognosis of BLCA patients. An upregulation of the abnormal spindle-like microcephaly (ASPM) expression level and a downregulation of thyrotroph embryonic factor (TEF) expression level are significantly correlated with a decreased overall survival of the BLCA patients [6]. Forkhead box A1 (FoxA1) depletion can lead to increased proliferation and invasion of the BLCA cells and is considered an independent adverse prognostic factor [7]. Besides, slingshot homolog-1 (SSH-1) expression is associated with the poor prognosis of BLCA patients [8].

With the rapid development of high-throughput sequencing technology, several cancer genomic databases have been established, offering an excellent channel to understand cancer-causing genomic changes [9–10]. Data mining can be performed to discover candidate biomarkers related to the prognosis of cancer patients. However, there are few reports on the metabolic status of cancer patients and its prognostic value. Nearly all of the existing studies use a single-gene biomarker to predict its role in the prognosis of BLCA patients, and the prediction effect is usually far from satisfaction. Therefore, building an expression-based genetic signature is of major clinical significance for predicting the survival of BLCA patients.

In the present study, genome-wide expression profile datasets were analyzed by using GSEA and Cox multivariate regression model to identify novel biomarkers predicting the prognosis of BLCA patients. All mRNA expression data of 412 BLCA patients were downloaded from the TCGA database, and comprehensive analysis was conducted for gene sets. The glycolysis-related mRNAs were identified, and the risk features of three genes were established. The signature composed of these three genes can effectively predict the prognosis of BLCA patients. Much to our surprise and excitement, the risk factor related to glycolysis can be independently used to evaluate high-risk patients with poor prognosis. We developed and verified a novel glycolysis- related gene signature.

Methods And Materials

Data acquisition

MRNA expression profiles and clinical datasets of BLCA patients were downloaded from TCGA(https://cancergenome.nih.gov/). The clinical data of 412 patients were used, including the survival time, survival status, gender, age, the tumor, node, metastasis (TNM) staging, T stage, N stage, and M stage. Mutations of the selected genes were obtained from the cbioportal database (http://www.cbioportal.org/).

GSEA analysis

GSEA was performed to determine whether there was a significant difference in the glycolysis-related gene sets between the normal group and BLCA group. The expression data of 433 samples in TCGA data set were analyzed, including 19 paracancerous samples and 414 BLCA samples. A normalized p-value was used to establish the functions used for subsequent analysis.

Statistical analysis

Log2 transformation was carried out to normalize the expression of each gene in the expression profile. Univariate Cox regression was used to screen for genes associated with the overall survival. After that, multivariate Cox regression was performed to screen out the prediction model with the best explanatory and informational effect. The selected genes were divided into risky type (hazard ratio [HR]>1), and protective type (0<HR<1). The expressions of the genes were subject to linear combinations according to the coefficient, and the risk parameter formula was constructed: risk parameter=$\sum$ (βn×gene expression n). The patients were divided into low- and high-risk groups based on the median of the risk values. The prognostic significance of the risk score was estimated using the Kaplan-Meier survival curve and log-rank test. Student's t-test was used to compare the expressions difference of the optimized genes between paracancerous tissues and BLCA

tissues. All statistical analyses were performed by using R3.6.2 (https://www.r-Project.org/) and Graph Pad Prism 7. P< 0.05 was considered statistically significant.

Results

Preliminary gene screening based on GSEA

Detailed information regarding the clinical data of 412 BLCA patients has been presented in Table 1. Based on the clinical data of 412 BLCA patients from TCGA and mRNA expression datasets of 433 non-cancerous and BLCA samples, we have found all glycolysis-related gene sets on the GESA gene database (https://www.gsea-msigdb.org/gsea/msigdb/search.jsp). There were five different datasets, namely, BIOCARTA_GLYCOLYSIS_PATHWAY, GO_GLYCOLYTIC_PROCESS, HALLMARK_GLYCOLYSIS, KEGG_GLYCOLYSIS_GLUCONEOGENESIS and REACTOME_GLYCOLYSIS. Next, GSEA was performed to determine whether there was a significant difference in these five glycolysis-related gene sets between the paracancrous samples and BLCA samples. The datasets GO_GLYCOLYTIC_PROCESS and REACTOME_GLYCOLYSIS were significantly enriched between paracancerous samples and tumor samples(P<0.01). However, the other three glycolysis-related gene sets HALLMARK_GLYCOLYSIS, BIOCARTA_GLYCOLYSIS_PATHWAY, and KEGG_GLYCOLYSIS_GLUCONEOGENESIS were not significantly enriched between paracancerous samples and tumor samples (P>0.05, Table 2, Fig.1).

Identification of glycolysis-related genes predictive of the survival of BLCA patients

In order to determine the novel genetic biomarkers predicting the prognosis of BLCA patients, we selected the core genes from the glycolysis-related datasets. These genes were further analyzed by univariate Cox regression to screen for mRNAs related to overall survival. Five genes were found to be significantly correlated with the overall survival (P<0.05). Multivariate Cox regression confirmed that three independent genes (NUP188, CHPF, and AK3) were significantly associated with overall survival (Table3). Then, the cBioPortal database was used to analyze the genomics alternations and the expression profiles of the three genes from 412 BLCA samples. The results showed that NUP188, CHPF, and AK3 had mutations in 6%, 2.2% and 3% patients (Fig.2a). We further analyzed the differential expression of the NUP188, CHPF, and AK3 genes between BLCA tissues and para-cancerous tissues. As compared to non-cancerous tissues, NUP188,CHPF and AK3 were significantly upregulated in the BLCA tissues (Fig.2b).

Relationship between the risk score and prognosis of BLCA patients

The gene-based prognostic model was built to estimate the survival risk of each BLCA patient: Risk score= 0.058×expression of NUP188 +0.005×expression of CHPF+(-0.031) expression of AK3. The risk value of each BLCA patient was calculated, and ranked in increasing order (Fig.3a). According to the median of the risk value, patients were divided into high-risk (n=203) and low-risk (n=204) groups. Figure 3b shows the risk scores, overall survival (in the unit of year), and survival status of 412 patients in the dataset. The patients with higher risk scores had a lower mortality rate than those with lower risk scores. The heat map (Fig.4) shows the expression profiles of the three mRNAs. As the risk score of the BLCA patients increased, the expressions of the risky-type mRNAs (CHPF and NUP188) were significantly upregulated. In contrast, that of the protective-type mRNA (AK3) was significantly downregulated.

The risk score is an independent prognostic indicator.

In order to compare risk scores with conventional clinical factors, univariate and multivariate Cox regression analyses were performed to evaluate the significance of different indicators in the patient cohort, including risk score, age, gender, grading, and TNM staging. According to the univariate Cox regression, the risk score (HR:1.969; 95%CI:1.547~2.506; P<0.001), age (HR: 1.034; 95% CI: 1.018~1.050; P<0.001), and TNM staging (HR: 1.772; 95%CI: 1.459~2.153; P<0.001) were significantly correlated to overall survival of patients. However, gender and grading didn't display significant correlation with patients' overall survival. The multivariate Cox regression validated risk score, age, and TNM staging also had a significant impact on the prognosis (P<0.01, Table 4), indicating that the risk score was highly valuable for survival prediction.

Verification of the efficacy of the risk score in survival prediction by using the Kaplan-Meier curve

Kaplan-Meier curve analysis showed that age, TNM stage, T stage, N stage, M stage and high risk score were correlated to the adverse overall survival of BLCA patients (P <0.001 for all cases, Fig.5a,b). In order to evaluate whether the proposed prediction model is applicable to different populations, the model built by clinical grouping was verified. As shown in Figure 6, for BLCA patients classified as TNM stage, T stage, N stage (N0), M stage (M0), age (<65) and gender (male), risk score was a reliable prognostic marker. The high-risk group was associated with a poor prognosis. However, when the patients were divided into different subgroups by TNM stage, age, and gender respectively, the predictive effect of the risk score varied. In the subgroup of patients aged above 65, the overall survival of the high-risk patients was significantly lower than that of the low-risk patients (P=0.001,Fig.6b). However, there was no statistical significance in the subgroup aged below 65 (P=0.064,Fig.6b). Similar results were observed in the subgroup divided by gender (male, P<0.001; female, P=0.096;Fig.6a). As shown above, the risk parameter could no longer be used as a sole prognostic marker for the subgroup of patients aged below 65 or the subgroup of females (Fig.6a,b). Moreover,as shown in Fig 6e and 6f, the prognostic predictive value of risk score in patients of N0 stage (P<0.001) and M0 stage (P<0.001) was considerably higher than in patients of N1-3 stage (P=0.086) and M1 stage (P=0.566). These results suggest that, in the early stages of BLCA, risk score might be a more useful prognostic marker.

Discussion

Studies on the mechanism of glycolysis affecting tumor occurrence, development, proliferation and invasion are emerging. However, very few of them are devoted to glycolysis- based prediction of survival of cancer patients. For example, FOXJ1 can promote aerobic glycolysis and proliferation of bladder cancer cells. FOXJ1 upregulation is associated with a poor clinical prognosis of the patients [11]. MiR-4999-5p can regulate the glycolysis of the colorectal cancer cells and promote the clone formation, proliferation and invasion of the colorectal cancer cells. The overall survival of the colorectal cancer patients with MiR-4999-5p upregulation is generally low, and MiR-4999-5p upregulation can be used as an independent predictor [12]. However, currently no single glycolysis-related gene signature has been developed to predict the survival of BLCA patients. In the present study, bioinformatics methods were used to screen and analyze glycolysis-related mRNAs, which were significantly correlated to the prognosis of BLCA patients. Three marker genes (CHPF, AK3, NUP188) were identified. Among them, chondroitin polymerizing factor (CHPF) is a type II transmembrane protein composed of many amino acids. Studies have shown that CHPF is abnormally upregulated in cancer tissues, such as

glioma, lung cancer, and colorectal cancer [13-14]. CHPF upregulation can promote the proliferation of glioma cells while inhibit the apoptosis, thereby predicting a poor prognosis[14]. Adenylate kinase 3 (AK3) is localized to the mitochondrial matrix and participates in maintaining the dynamic balance of nucleotides in cells. Human chromosome 9 carries the AK1, AK2 and AK3 genes[15]. It has been reported that chromosome 9p deletion occurs in the bladder cancer and has close connections with pathological staging and prognosis of bladder cancer [15-16]. AK3 compounds are blockers of mitosis, which block the proliferation and apoptosis of the cells in colonic and breast cancers. AK3 upregulation positively correlates with the overall survival of patients with colonic cancer and breast cancer [17-18], AK3 downregulation can lead to severe inhibition of the growth of the cancer cells in chronic lymphocytic leukemia [19], which is in line with our finding that AK3 is a protective type mRNA. NUP188 is a nucleoporin regulating chromosome segregation and promotes tumor cell division and growth by participating in mitotic process. In mitotic cells, Nup188 depletion can lead to mitotic arrest and cell division arrest [20]. There are only a limited number of reports on the relationship between the three genes and BLCA and the molecular mechanism of these genes. Our research in detail reported the involvement of the three genes in the prognosis of BLCA.

GSEA is a method to integrate data of different levels and sources [21]. In the present study, expression data of 19 paracancerous samples and 414 BLCA samples were used to perform GSEA, and two gene sets with significant functional difference were identified. Then, GSEA was used to select genes predicting the survival of patients, and these genes were further investigated by univariate and multivariate Cox regression of the selected genes from the BLCA patients. The signature composed of three genes was finally determined to be of potential prognostic predictive value for BLCA patients. As compared with the confirmed prognostic biomarkers, this gene signature might be more targeted and convincing in terms of prognostic predictive value, and can be used as an effective tool for classification of BLCA patients. BLCA gene sets were downloaded from TCGA and the glycolysis-related genes were analyzed. Comparison was done between tumor tissues and the adjacent normal tissues. The Kaplan-Meier survival curve showed that a high risk score was closely associated with a low survival rate. It is thus implied that estimating the risk score for BLCA patients can help predict the prognosis.

Although a prognostic prediction model was built based on this gene signature, our study had some limitations. Firstly, TCGA database was the only data source in the present study, and no clinical data of relapse and metastasis of the BLCA patients were included. Therefore, we could only perform prognostic analysis using overall survival data. In the stratified analysis, the risk parameter was a reliable prognostic predictor except for the subgroups where the patients were aged below 65, were females, of N1-3 stage, or of M1 stage. The reasons for these findings remain unknown and need to be further discussed. Besides, the signature composed of the three genes was not verified for the molecular biological function in BLCA, which might need further studies in the future.

Conclusion

The three glycolysis-related genes associated with the overall survival of the BLCA patients were verified by GSEA and Cox regression. The signature composed of these three genes could predict the prognosis of the BLCA patients. The higher the risk score, the worse the prognosis. The use of this gene signature may improve the success rate of individualized cancer therapy. Our findings may provide potential glycolysis-related

biomarkers for predicting the prognosis of BLCA patients, and may also provide a basis for further understanding of the occurrence and development mechanism of glycolysis in BLCA.

Abbreviations

BLCA
Bladder urothelial carcinoma;
TCGA
The Cancer Genome Atlas;
GSEA
Gene Set Enrichment Analysis;

Declarations

Acknowledgements

None.

Authors' contributions

Ruijiang and Xin Zhao designed and guided the study. Jia Li and Wenju Xiong downloaded clinical information of patients from TCGA. Xin Zhao and Jiafeng Li wrote the manuscript. All authors read and approved the final manuscript.

Funding

None.

Availability of data and materials

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

References

1 Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65(2):87–108.

2 Akhtar M, Al-Bozom IA, Ben Gashir M, Taha NM. Intrinsic Molecular Subclassification of Urothelial Carcinoma of the Bladder: Are We Finally there?. Adv Anat Pathol. 2019;26(4):251–256.

3 Bladder cancer: diagnosis and management of bladder cancer: © NICE (2015) Bladder cancer: diagnosis and management of bladder cancer. BJU Int. 2017;120(6):755–765.

4 Steffensen EH, Cary C, Jensen JB, Larsson H, Weiner M, Nørgaard M. Preadmission antidepressant use and bladder cancer: a population-based cohort study of stage at diagnosis, time to surgery, and surgical outcomes. BMC Cancer. 2018;18(1):1035.

5 Bose S, Le A. Glucose Metabolism in Cancer. Adv Exp Med Biol. 2018;1063:3-12.

6.Saleh AA, Gohar SF, Hemida AS, Elgharbawy M, Soliman SE. Evaluation of ASPM and TEF Gene Expressions as Potential Biomarkers for Bladder Cancer [published online ahead of print, 2020 Apr 9]. Biochem Genet. 2020;10.1007/s10528-020-09962-1.

7 Reddy OL, Cates JM, Gellert LL, et al. Loss of FOXA1 Drives Sexually Dimorphic Changes in Urothelial Differentiation and Is an Independent Predictor of Poor Prognosis in Bladder Cancer. Am J Pathol. 2015;185(5):1385-1395.

8 Luo Q, Liu Y, Zhao H, et al. Slingshot homolog-1 expression is a poor prognostic factor of pT1 bladder urothelial carcinoma after transurethral resection [published online ahead of print, 2020 Jan 21]. World J Urol.

9 Feng A, Tu Z, Yin B. The effect of HMGB1 on the clinicopathological and prognostic features of non-small cell lung cancer. Oncotarget. 2016;7(15):20507-20519.

10 Wang X, Li G, Luo Q, Xie J, Gan C. Integrated TCGA analysis implicates lncRNA CTB-193M12.5 as a prognostic factor in lung adenocarcinoma. Cancer Cell Int. 2018;18:27.

11 Xian S, Shang D, Kong G, Tian Y. FOXJ1 promotes bladder cancer cell growth and regulates Warburg effect. Biochem Biophys Res Commun. 2018;495(1):988-994.

12 Zhang Q, Hong Z, Zhu J, et al. miR-4999-5p Predicts Colorectal Cancer Survival Outcome and Reprograms Glucose Metabolism by Targeting PRKAA2. Onco Targets Ther. 2020;13:1199-1210.

13 Hou XM, Baloch Z, Zheng ZH, et al. Knockdown of CHPF suppresses cell progression of non-small-cell lung cancer. Cancer Manag Res. 2019;11:3275-3283.

14 Fan YH, Xiao B, Lv SG, Ye MH, Zhu XG, Wu MJ. Lentivirus-mediated knockdown of chondroitin polymerizing factor inhibits glioma cell growth in vitro. Oncol Rep. 2017;38(2):1149-1155.

15 Chang X, Ravi R, Pham V, Bedi A, Chatterjee A, Sidransky D. Adenylate kinase 3 sensitizes cells to cigarette smoke condensate vapor induced cisplatin resistance. PLoS One. 2011;6(6):e20806.

16 Bond MJ, Bleiler M, Harrison LE, et al. Spindle Assembly Disruption and Cancer Cell Apoptosis with a CLTC-Binding Compound. Mol Cancer Res. 2018;16(9):1361-1372.

17 Chopra AS, Kuratnik A, Scocchera EW, Wright DL, Giardina C. Identification of novel compounds that enhance colon cancer cell sensitivity to inflammatory apoptotic ligands. Cancer Biol Ther. 2013;14(5):436-449.

18 Qin WY, Feng SC, Sun YQ, Jiang GQ. MiR-96-5p promotes breast cancer migration by activating MEK/ERK signaling [published online ahead of print, 2020 Mar 20]. J Gene Med. 2020;e3188.

19 Admoni-Elisha L, Nakdimon I, Shteinfer A, et al. Novel Biomarker Proteins in Chronic Lymphocytic Leukemia: Impact on Diagnosis, Prognosis and Treatment. PLoS One. 2016;11(4):e0148500.

20 Labade AS, Karmodiya K, Sengupta K. HOXA repression is mediated by nucleoporin Nup93 assisted by its interactors Nup188 and Nup205. Epigenetics Chromatin. 2016;9:54.

21 Subramanian A, Kuehn H, Gould J, Tamayo P, Mesirov JP. GSEA-P: a desktop application for Gene Set Enrichment Analysis. Bioinformatics. 2007;23(23):3251-3253.

Tables

Table I. Clinical data of BLCA patients (n = 412) obtained from The Cancer Gene Atlas

Variables	Patients,n(%)
Sex	412
Male	304(73.79%)
Female	108(26.21%)
Age, years	
≤65	162 (39.32%)
>65	250(60.68%)
TNM stage	
	2(0.48%)
	131 (31.80%)
	141(34.22%)
	136(33.01%)
Unkonw	2 (0.48%)
T stage	
T1	4(0.97%)
T2	120(29.17%)
T3	196(47.57%)
T4	59(14.32%)
Tx	1(0.24%)
Unknow	32(7.77%)
N stage	
N0	239(58.01%)
N1	47(11.41%)
N2	76(18.45%)
N3	8 (1.94%)
NX	36(8.73%)
Unknow	6(1.46%)
M stage	
M0	196(47.57%)

M1	11(2.67%)
MX	202(49.03%)
Unknow	3(0.73%)

Table 2. Gene sets enriched in BLCA (412 samples)

GS follow link to MSigDB	SIZE	NES	NOM p-val	FDR q-val
GO_GLYCOLYTIC_PROCESS	106	1.91	0.006	0.006
REACTOME_GLYCOLYSIS	72	1.97	0.004	0.004
HALLMARK_GLYCOLYSIS	200	1.36	0.144	0.144
BIOCARTA_GLYCOLYSIS_PATHWAY	3	0.58		0.941
KEGG_GLYCOLYSIS_GLUCONEOGENESIS	62	-1.30		0.182

Table 3. Three prognostic genes were selected via univariable and multivariable Cox regression analysis,

mRNA	Univariate analysis		P	Multivariate analysis		P
	HR	95% CI		HR	95% CI	
NUP188	1.045	1.016-1.075	< 0.05	1.060	1.030-1.090	<0.01
CHPF	1.005	1.001-1.009	< 0.05	1.005	1.001-1.009	<0.01
AK3	0.970	0.951-0.990	<0.05	0.969	0.949-0.989	<0.01

Table 4 Univariable and multivariable analyses for each clinical feature

Clinical feature	Univariate analysis		P	Multivariate analysis		P
	HR	95% CI		HR	95% CI	
riskScore	1.969	1.547-2.506	< 0.001	1.928	1.496-2.485	<0.001
age	1.034	1.018-1.050	< 0.001	1.032	1.015-1.048	<0.001
gender	0.894	0.644-1.241	0.503	0.893	0.642-1.242	0.502
grade	2.882	0.713-11.652	0.137	1.103	0.265-4.580	0.891
stage	1.772	1.459- 2.153	< 0.001	1.681	1.375-2.055	<0.001

Figures

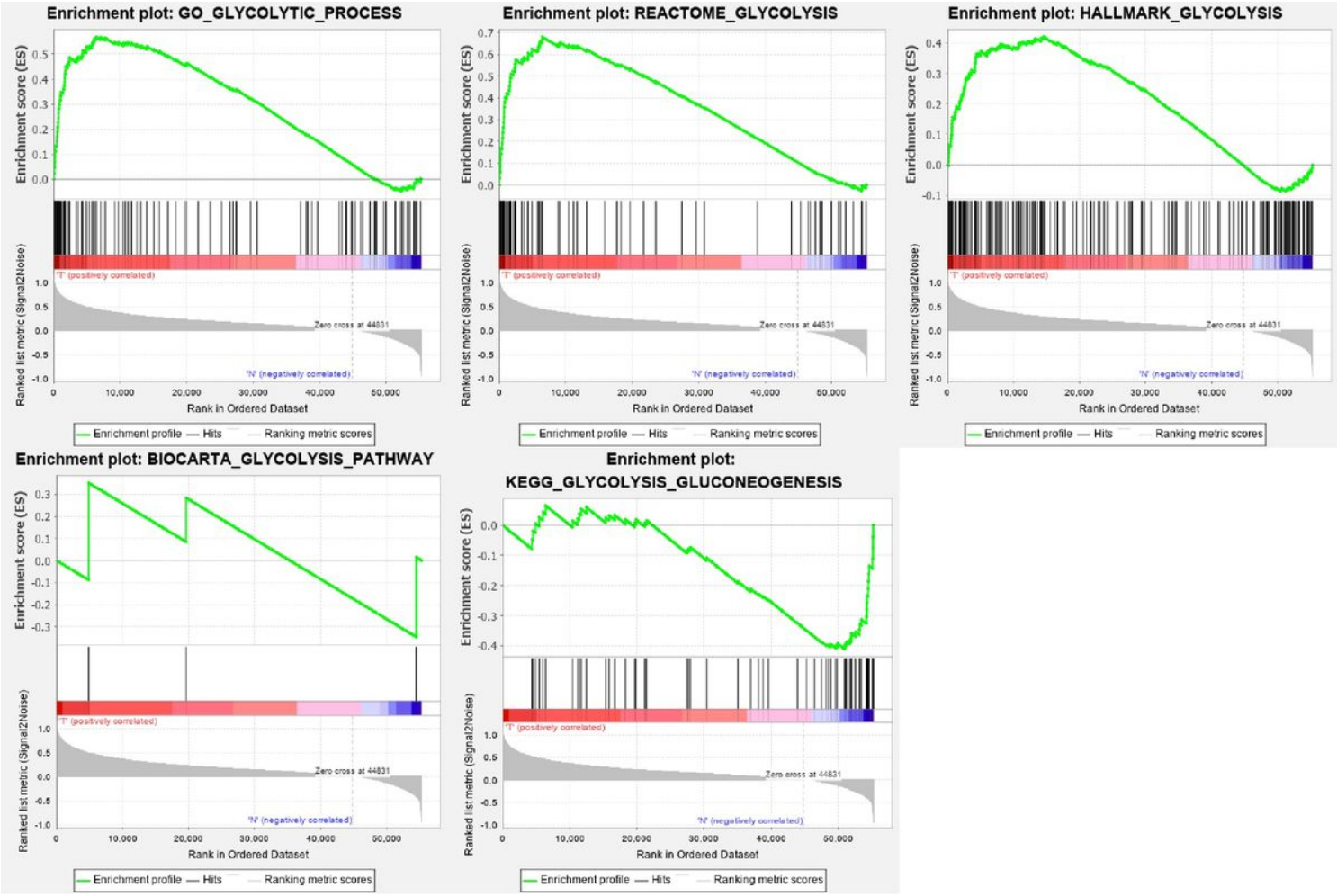

Figure 1

GSEA results of enrichment profiles of five gene sets (GO_GLYCOLYTIC_PROCESS REACTOME_GLYCOLYSIS,HALLMARK_GLYCOLYSIS,BIOCARTA_GLYCOLYSIS_PATHWAY and

KEGG_GLYCOLYSIS_GLUCONEOGENESIS)

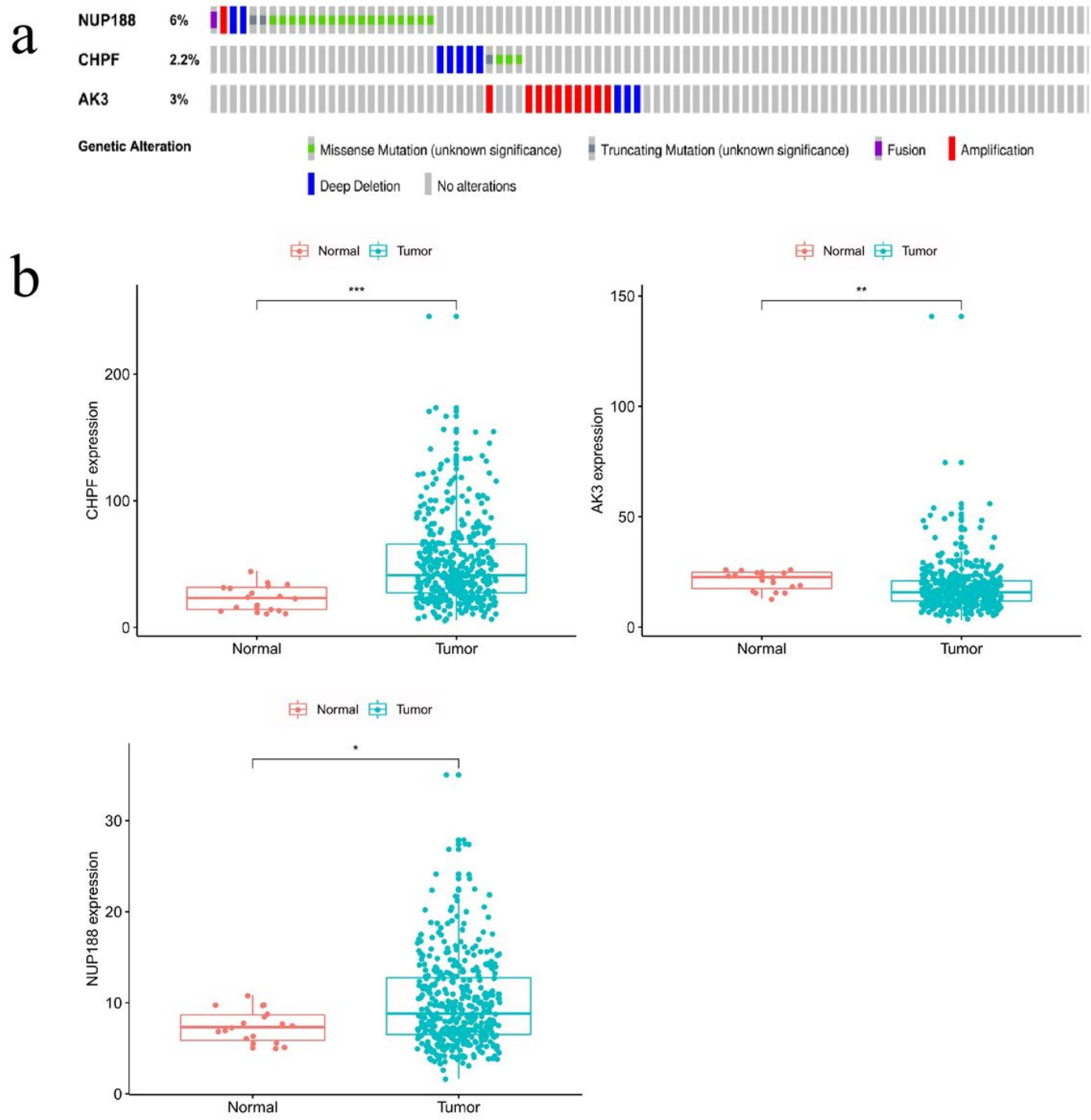

Figure 2

Identification of mRNAs associated to patients' survival. (a) The alteration proportion for the three selected genes in 412 clinical samples of bladder urothelial carcinoma in the cBioPortal database. (b) Different expression of three genes in the normal tissues and tumor tissues obtained from TCGA. (***represent P < 0.001,**represent P < 0.01,*represent P < 0.05).

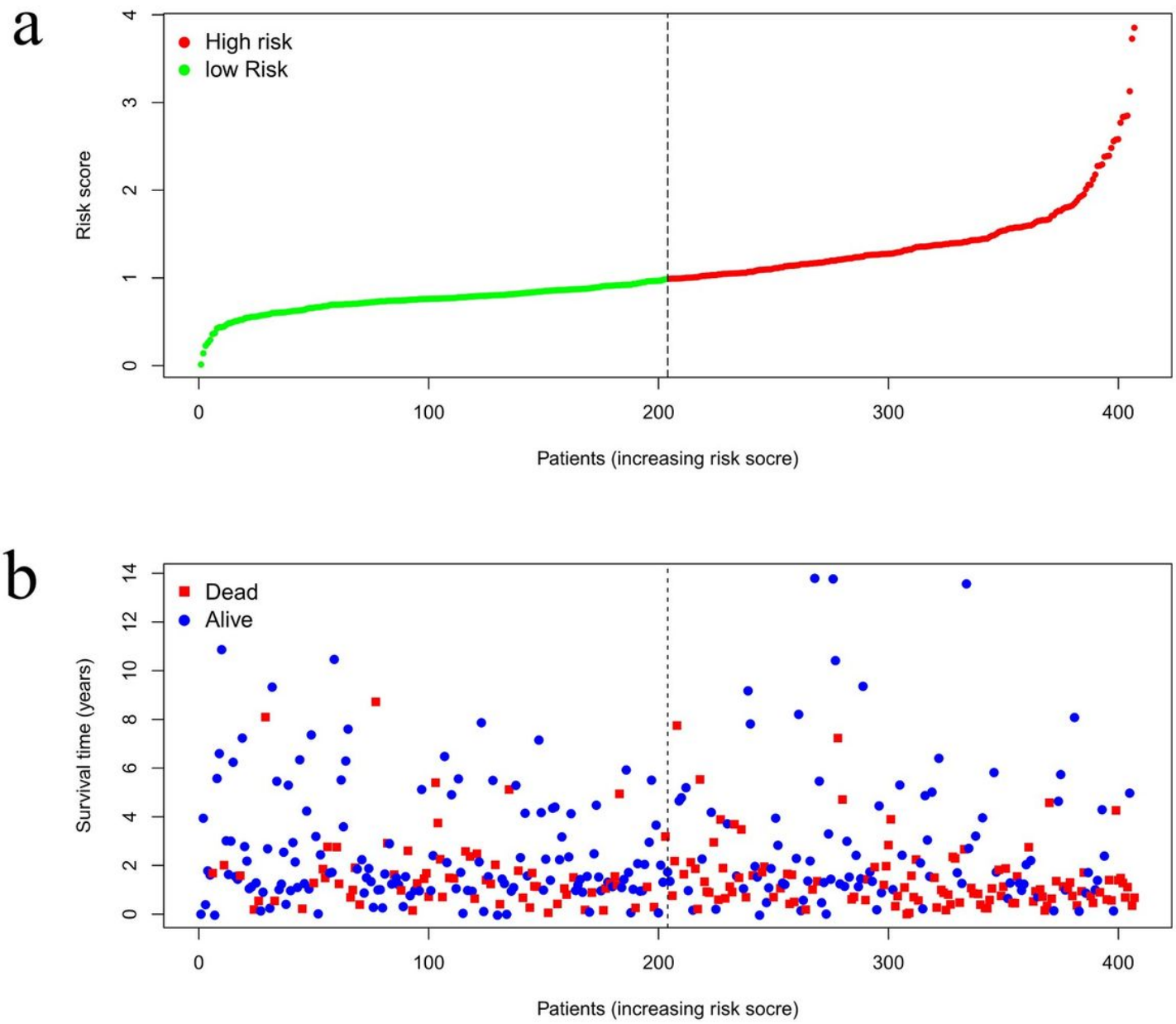

Figure 3

The three-mRNA signature related to risk score predicts overall survival in the patients with bladder urothelial carcinoma. a mRNA risk score distribution. b Survival days of patients

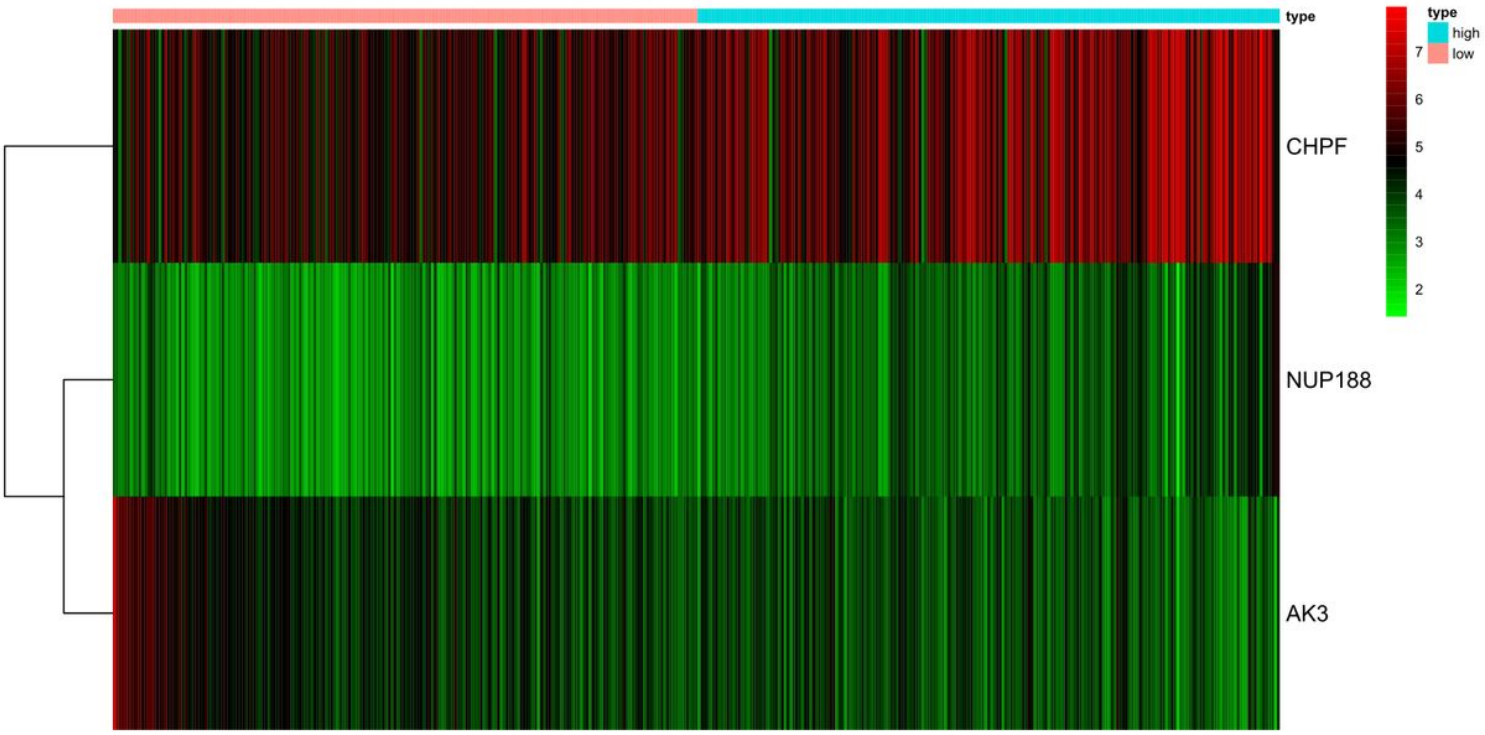

Figure 4

Heatmap of three genes expression profile in TCGA.The figure shows that each gene is differentially expressed in the high-risk score group and the low-risk score group

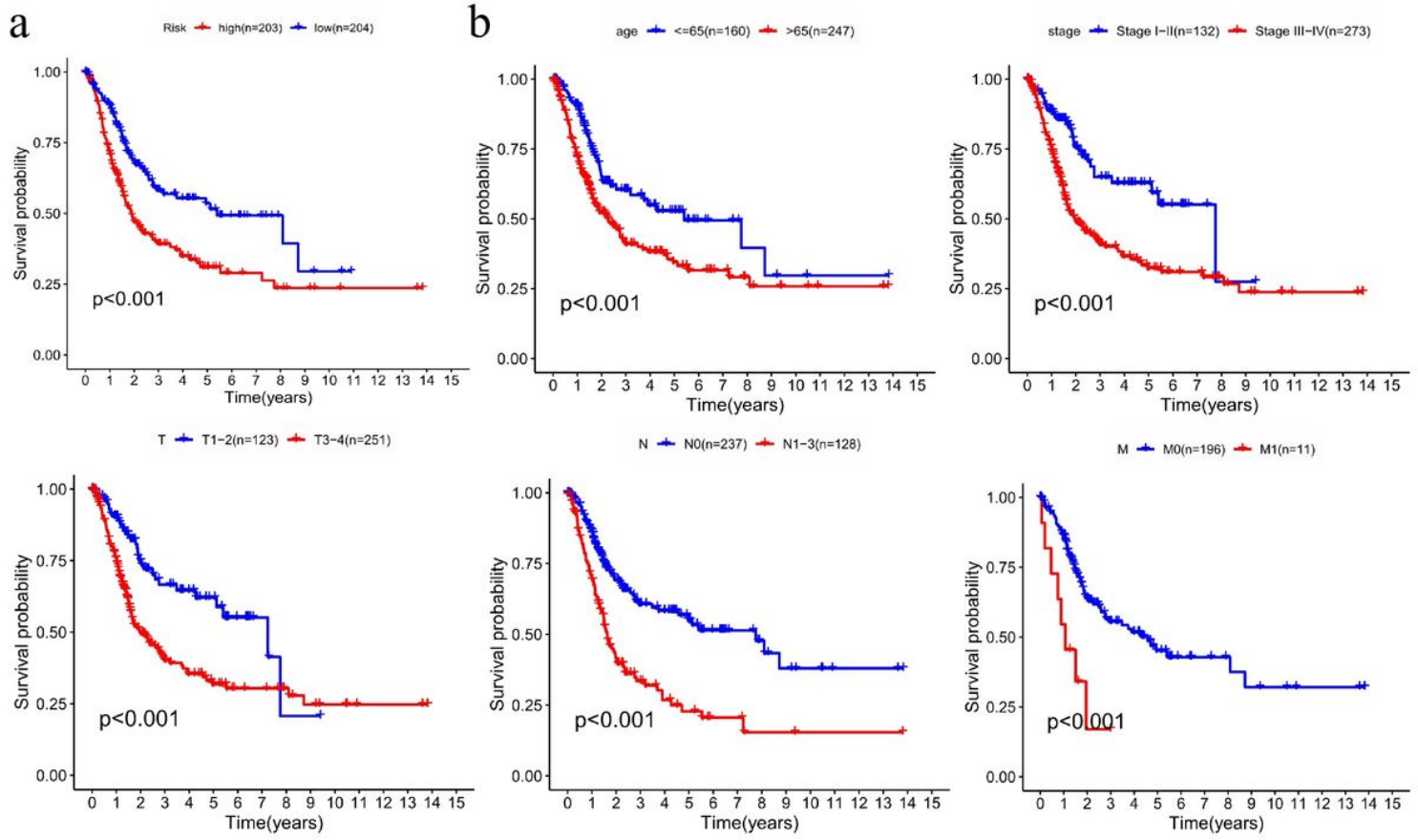

Figure 5

Kaplan-Meier survival analysis for patients with bladder urothelial carcinoma in TCGA data set. (a) Kaplan-Meier curve of patients in high risk group and low risk group (b) The clinical features including age, TNM stage, T stage, N stage and M stage predict patients survival.

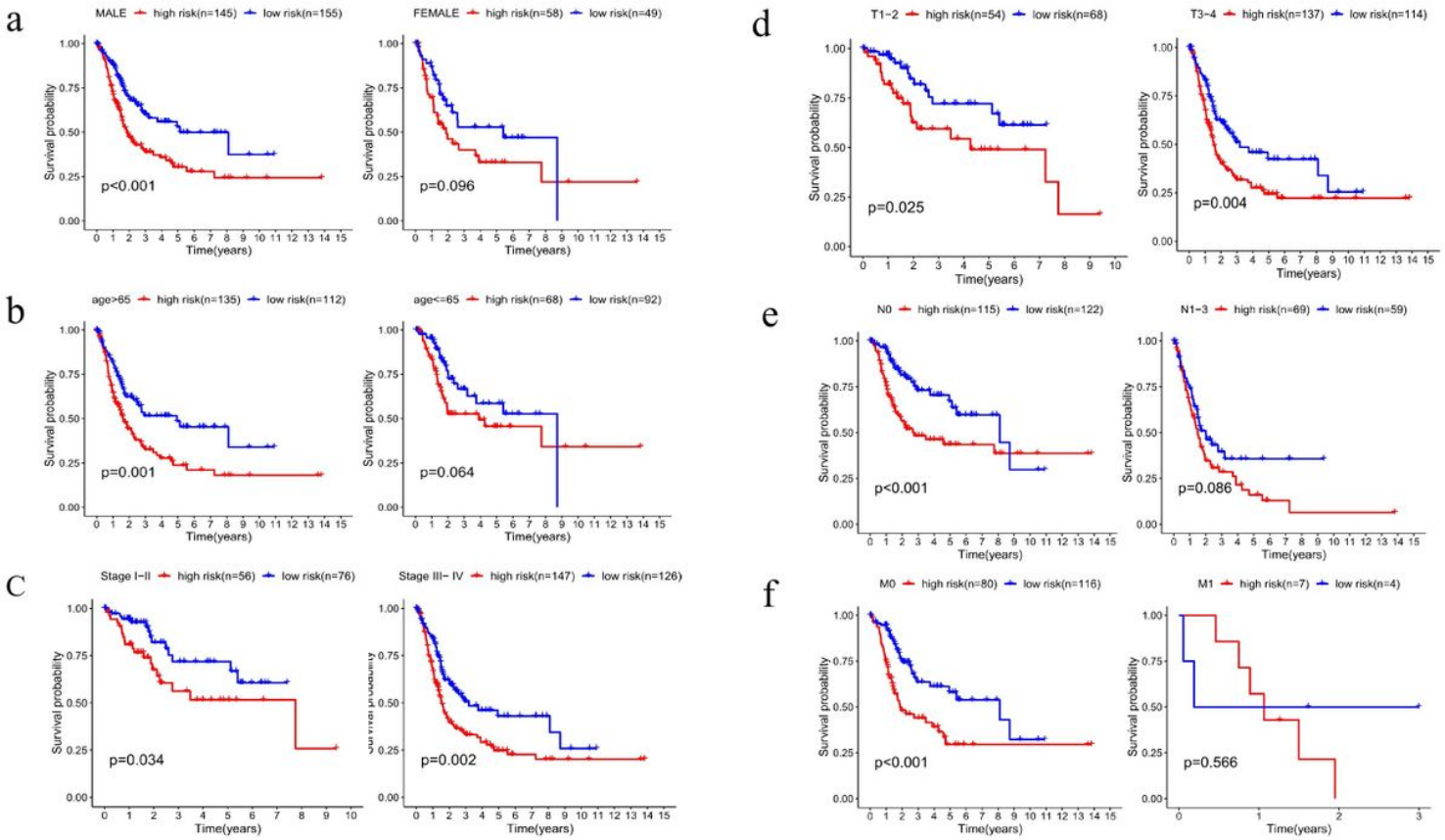

Figure 6

Prognostic value of the Kaplan-Meier curve for the signature of the patient's risk score by each clinical feature.(a gender,b age, c TNM stage,d T stage,e N stage,f M stage)