Development and validation of a recurrence risk assessment model for high-grade bladder cancer based on TCGA and GEO

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Background: Bladder cancer is one of the most commonly diagnosed urinary cancers worldwide. Although muscle-invasive bladder cancer (MIBC) accounts for only 25% of bladder cancer cases, it has a high recurrence rate and poor prognosis, especially among high-grade cases. Despite the existence of some molecular markers, there is a clear clinical need for a robust recurrence prediction model that can assist in patient management and therapeutic decision-making. Therefore, we aimed to use public databases to develop such an effective assessment model.

Methods: We developed a recurrence risk assessment model for high-grade bladder cancer based on the clinical information of 217 cases from The Cancer Genome Atlas (TCGA) and profiles of 87 samples from GSE31684 in the Gene Expression Omnibus (GEO) database. Edge R was used to analyze differences between RNAs of bladder cancer in the TCGA database, with thresholds of $P<0.05$ and $|\log_2(\text{fold change})| >1$; least absolute shrinkage and selection operator (LASSO) Cox regression models were used to screen the RNAs significantly related to recurrence with minimum λ. Survival receiver operating characteristic (ROC) and area under the curve (AUC) was used to assess the predictive accuracy of the model in the training and validation sets of GSE31684.

Results: There were 2,876 differential RNAs obtained from TCGA data. Among a total of 284 RNAs identified as significantly related to recurrence of bladder cancer, 49 were obtained by LASSO regression, and 30 were finally obtained by multifactor risk regression to construct a risk assessment model. The model was found to predict the prognosis of bladder cancer recurrence well, with an AUC of 0.911 in the TCGA training set and an adjusted AUC value of 0.839 in the GEO validation set.

Conclusions: The recurrence assessment model is a relatively accurate recurrence prediction tool for high-grade bladder cancer and could provide a guidance for the treatment of bladder cancer.

Keywords: High-grade bladder cancer; recurrence; risk assessment model; The Cancer Genome Atlas (TCGA); Gene Expression Omnibus (GEO)

Introduction

Bladder cancer is one of the most commonly diagnosed urinary cancers worldwide. It was reported that there were an estimated minimum of 82,290 new cases of and 16,710 deaths from bladder cancer in America in 2023 (1). Muscle-invasive bladder cancer (MIBC) accounts for only 25% of patients with bladder cancer, but it has high rate of invasion and distant metastasis, and results in a poor prognosis, especially among those with high-grade disease (2). The overall incidence of bladder cancer is increasing year by year, which might be associated with the potential influence of tobacco abuse, industrial carcinogens, and population aging (3). However, very few studies have addressed valuable molecular prognostic markers for recurrence in clinical practice; there is an urgent need to discover recurrence assessment markers for high-grade bladder cancer.

Recently, gene microarray and RNA sequencing have been applied to identify novel diagnostic and prognostic signatures for multiple diseases. Schuettfort *et al.* created a model using a panel of systemic inflammatory response biomarkers to predict tumor-specific survival and recurrence-free survival (RFS) in patients with urothelial carcinoma treated with radical cystectomy, but the effectiveness of selected inflammatory response biomarkers in improving the model's discrimination ability was limited (4). Another study revealed that extracellular matrix

genes could predict survival and recurrence of bladder cancer; the combination of follistatin-like 1 (*FSTL1*), stage, age, and gender achieved an area under the curve (AUC) value of 0.76 in predicting bladder cancer recurrence (5). Lucas *et al.* (6) trained a network of digital histopathology slides and clinical data to predict RFS for non-MIBC patients using deep learning. Their results showed that AUC was 0.76 for 5-year recurrence predictions. The abovementioned assessment models focus on clinical data or specific functional genes; they are limited in gene types, sample sizes, and efficiency. A recurrence assessment model with a high prediction efficiency is conducive to early clinical treatment decision-making, and vital for optimizing the prognosis of bladder cancer. Besides, as gene expression detection is convenient and stable, a predictive model constructed by multiple genes is more reliable. Thus, a new model including various gene types and large screening data and presenting a high efficiency in recurrence prediction is required for high-grade bladder cancer. We present this article in accordance with the TRIPOD reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-256/rc).

Methods

Data download and preprocessing

We downloaded The Cancer Genome Atlas (TCGA) RNA expression profile data of bladder cancer in the form of count value from Xena (https://xena.ucsc.edu/). The corresponding clinical information files including gender, age, tumor differentiation, and tumor stage were also downloaded. Only files containing recurrence-free time and recurrence status were included in further data analysis. The information of 413 bladder cancer patients including 77 recurrences was obtained from TCGA. The selected TCGA data were standardized in the form of transcripts per million (TPM), then, according to the distance between different samples in a cluster, Pearson correlation matrix was used to evaluate the microarray quality. We also downloaded the raw gene microarray expression profiles in the form of original files and clinical information from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/). We set the criteria that sample sizes were larger than 80 and clinical information was in the same form as TCGA data. Finally, GSE5479, GSE57933, and GSE120736 were excluded, and only GSE31684 was

suitable for analysis. GSE31684 included 39 recurrences out of 94 bladder cancer patients. All the data were updated as of December 2023. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Screening differentially expressed RNAs and preparation of single factor risk regression input RNAs

Patients included in this study were divided into two groups according to tumor status. RNA count value in the different groups were extracted with clinical data, and Edge R (version 3.3.0; https://www.r-project.org/) was used to analyze the difference between the two groups; $P<0.05$ and $|\log_2(\text{fold change})| >1$ were selected as the thresholds. Gene Ontology (GO) (http://www.geneontology.org) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed to predict the function and interactions among differentially expressed RNAs. For GSE31684 data, Affy package was used to merge the matrix, robust multiarray average (RMA) method was used to standardize the data, and the probe names were transformed to gene names (7).

Evaluation of the model and risk analysis of recurrence

The expression matrix of differently expressed RNAs and the clinical recurrence status were used for single factor Cox analysis. R survival package was used for Cox analysis. RNAs related to bladder cancer recurrence with $P<0.05$ were obtained for further analysis. The glmnet and survival packages in R were used for least absolute shrinkage and selection operator (LASSO) analysis (8). After removing redundant RNAs, the remaining RNAs were used for multi-factor risk analysis. Finally, the recurrence risk-associated RNAs were used to construct a disease-free or recurrence evaluation model. A risk score was calculated, and according to the maximum Youden value, patients with high-grade bladder cancer in TCGA were divided into high- and low-risk groups. Then, the difference between the two groups in disease-free recurrence and survival was calculated to evaluate the effect of the model. The survival receiver operating characteristic (ROC) package of R was used to evaluate the prediction ability of the model based on the expression of screened RNAs. Finally, the model was verified in GSE31684. The tumor-node-metastasis (TNM) stages of the patients were horizontally compared with those of the model to evaluate the prediction ability in recurrence of bladder cancer. An AUC larger than 0.7 was

identified as having a good prediction.

Immune assessment and drug sensitivity prediction

KEGG pathway analysis of selected genes was performed with R package. The enrichment factor was the value ratio between genes and all annotated genes enriched in the pathway. Oncopredict package of R was used to predict sensitivity to chemotherapy drugs (9). ImmuneCellAI (https://guolab.wchscu.cn/ImmuCellAI/#!/) was used to estimate the proportion of 18 types of T cells and 6 other types of immune cells [B cells, natural killer (NK) cells, monocytes, macrophages, neutrophils, and dendritic cells (DCs)], and predict the patient's response to immune checkpoint inhibitor therapy.

Statistical analysis

Statistical analysis of different RNAs was performed with the analysis of variance (ANOVA) and $P<0.05$ was considered statistically significant. In comparison, $P<0.05$ between the two sample groups signaled the existence of differences.

Results

Characteristics of selected data

As shown in *Table 1*, 217 cases of clinical information of high-grade bladder cancer were selected in TCGA expression spectrum, which included 79 cases of recurrence or progression and 138 cases of complete remission (CR). In *Table 2*, 87 samples including 38 cases of bladder cancer recurrence and 49 cases of CR were screened from GSE31684 profiles.

Characteristic and function prediction of included differently expressed RNAs

The screening process and results are presented in *Figure 1*. Clustering analysis showed the characteristics of the selectively included data of TCGA. A total of 2,876 differentially expressed RNAs including 905 up-regulated RNAs and 1,971 down-regulated RNAs were obtained between tumor and normal tissues (table available at https://cdn.amegroups.cn/static/public/tcr-24-256-1.xlsx). The Volcano map well distinguished tumor and normal tissues (*Figure 2A*). The heatmap analysis demonstrated no

Table 1 Clinical pathological characteristic of patients with high grade bladder cancer in the TCGA database

Parameters	Patients, n (%)
Recurrence	
Recurrent/progressive	79 (36.41)
Disease-free	138 (63.59)
Age (years)	
>67	112 (51.61)
≤67	105 (48.39)
Gender	
Male	159 (73.27)
Female	58 (26.73)
Pathologic stage	
Stage I	1 (0.46)
Stage II	68 (31.34)
Stage III	73 (33.64)
Stage IV	75 (34.56)
Pathologic M	
M0	95 (43.78)
M1	8 (3.69)
Mx	113 (52.07)
Pathologic N	
N0	120 (55.30)
N1	24 (11.06)
N2	42 (19.35)
N3	4 (1.84)
Nx	27 (12.44)
Pathologic T	
T1	7 (3.23)
T2	105 (48.39)
T3	76 (35.02)
T4	24 (11.06)
Tx	5 (2.30)

Mx, Nx, Tx: data that had not been thoroughly evaluated. TCGA, The Cancer Genome Atlas; M, metastasis; N, node; T, tumor.

Table 2 Clinical pathological characteristic of patients with high grade bladder cancer in GSE31684

Parameters	Patients, n (%)
Recurrence	
Recurrent/progressive	57 (65.52)
Disease-free	30 (34.48)
Age (years)	
≥67	63 (72.41)
<67	24 (27.59)
Gender	
Male	5 (5.75)
Female	17 (19.54)
Pathologic T	
Ta	54 (62.07)
T1	10 (11.49)
T2	1 (1.15)
T3	38 (43.68)
T4	49 (56.32)
Metastasis	
M1	51 (58.62)
M0	36 (41.38)

T, tumor; M, metastasis.
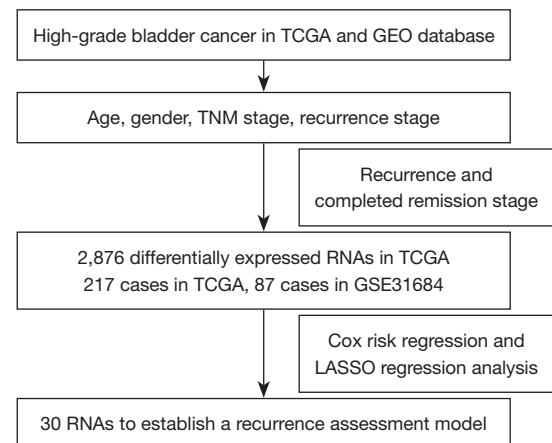
Figure 1 Schematic representation of screening process for recurrence assessment model. TCGA, The Cancer Genome Atlas; GEO, Gene Expression Omnibus; TNM, tumor-node-metastasis; LASSO, least absolute shrinkage and selection operator.
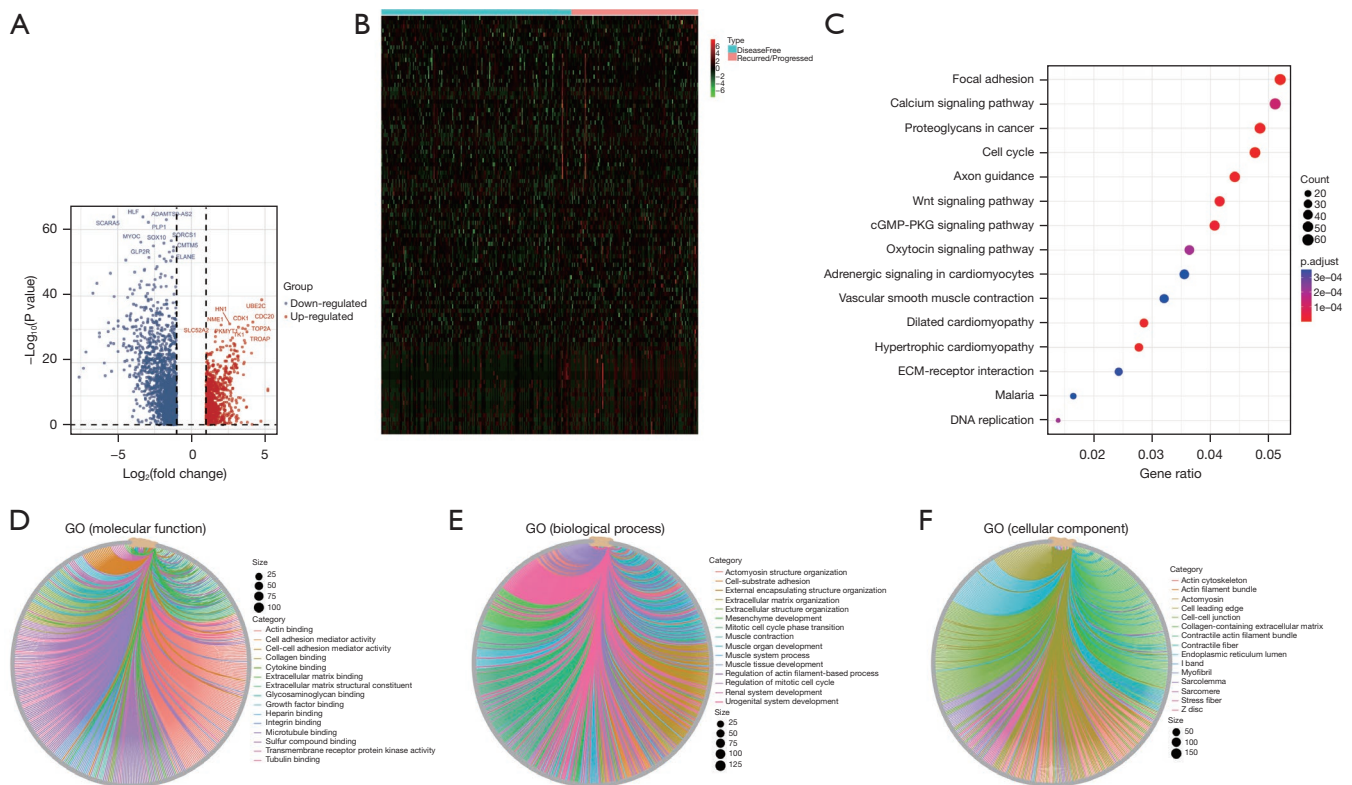
Figure 2 Characteristic and function prediction of differently expressed RNAs. (A) Volcano plots show differently expressed RNAs between tumor group and normal group. Red points, upregulated RNAs; blue points, downregulated RNAs; Red or blue points correspond to RNAs with two-fold changes between the two groups. (B) Clustering analysis of top 100 of significant differentially expressed RNAs between recurrent/progressive group and disease-free group. These dysregulated RNAs showed no difference between recurrent/progressive and disease-free groups. (C) KEGG analysis for differently expressed RNAs. Enrichment factor represents the ratio between the differentially expressed RNAs and all annotated genes enriched in the pathway. Bubble scale represents the number of different RNAs; depth of bubble color represents P value. (D-F) GO analysis for differently expressed RNAs. Different lines refer to different terms and size of bubble represents enriched genes. All enriched terms with P value <0.05. ECM, extracellular matrix; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

significant differences between patients who had achieved CR and those with disease recurrence (*Figure 2B*). KEGG pathway analysis showed that the dysregulated RNAs mainly enriched in focal adhesion, calcium signaling pathway, and cell cycle pathway (*Figure 2C*). GO analysis revealed that regulation of mitotic cell cycle, extracellular matrix organization, and cell-substrate adhesion were mainly enriched in biological process; actin binding, microtubule binding and cell-cell adhesion mediator activity were mainly enriched molecular function; cell-cell junction, endoplasmic reticulum lumen, and actin cytoskeleton were main enriched cellular component (*Figure 2D-2F*).

Cox risk regression and LASSO regression analysis

At last, 284 RNAs related to bladder cancer recurrence were obtained (P<0.05) in Cox risk regression (table available at https://cdn.amegroups.cn/static/public/tcr-24-256-2.xlsx). Some 49 from 284 redundant RNAs were obtained in LASSO regression analysis with minimum λ of 0.061. We performed multifactor risk analysis and obtained 30 recurrence-associated RNAs (*Figure 3A*). We used these 30 RNAs to establish a RFS model for bladder cancer. The risk score in the model for each patient was calculated based on expression levels of these RNAs and weighted by their

4978

Wang et al. Recurrence assessment mode for high-grade bladder cancer
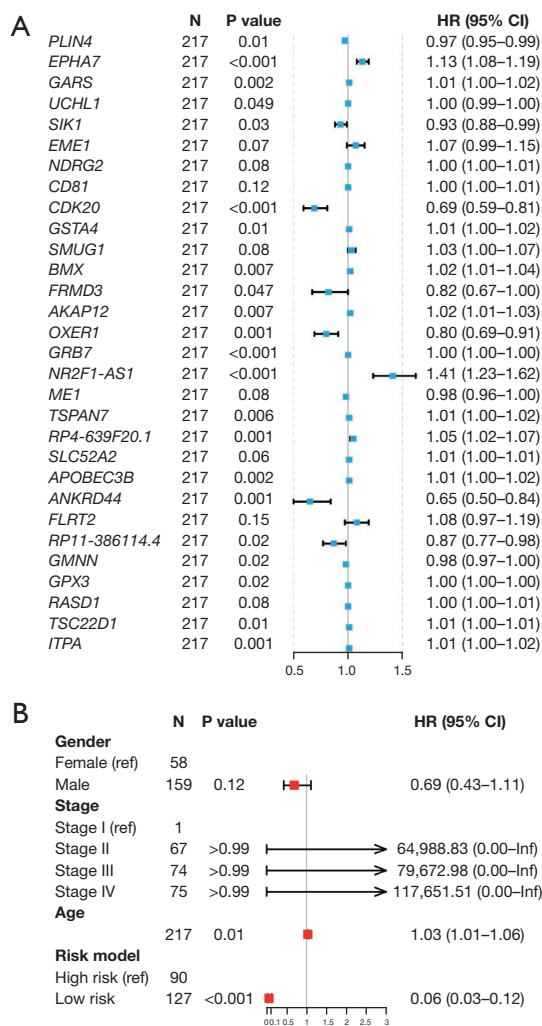
Figure 3 Forest of recurrence risk assessment model. (A) Forest of 30 RNAs included in recurrence risk assessment model, the HR was listed in form of 95% CI. (B) Forest of recurrence risk assessment model and clinical characteristics in predicting recurrence of bladder cancer. The global P value of risk assessment model in log-rank scale was lower than 0.001. HR, hazard ratio; CI, confidence interval; ref, reference.

regression coefficients (Table S1). This risk model presents a higher ability than gender, stage, and age in predicting recurrence of high-grade bladder cancer (*Figure 3B*).

Prognostic analysis of the recurrence risk model

A total of 30 RNAs were used to construct an assessment model for recurrence prediction of high-grade bladder cancer. The results showed that the prediction model

obvious distinguish disease-free or recurrence status in TCGA, with an AUC of 0.911, sensitivity of 0.81 and specificity of 0.89, compared to stage with an AUC of 0.641 (*Figure 4A*). The prediction ability to differentiate disease-free or recurrence status in GSE31684 was low, with an AUC of only 0.533. However, these selected RNAs can construct an adjusted prediction model with an AUC of 0.837 (*Figure 4B*), whereas tumor stage in prediction of recurrence can reach an AUC of 0.694 in TCGA and 0.617 in GSE31684.

Evaluation and validation for recurrence risk model

The maximum Youden as a cutoff point was used to divide the included patients into high- and low-risk groups for the training set and the verification set. As shown in *Figure 5A*, the AUC of the prediction model for 3-year recurrence of bladder cancer in TCGA was 0.95. To further evaluate the predictive power of the model risk score, we confirmed its ability in GSE31684 with an AUC of 0.63 for 3-year recurrence (*Figure 5B*). The prognosis model predicted obvious lower disease-free recurrence time in the high-risk group in the training set (*Figure 5C*) and the validation set (*Figure 5D*).

Functional prediction of model genes and efficacy evaluation of adjuvant therapy

We conducted pathway prediction on the included genes of the risk model, and the results showed that these genes were mainly enriched in the glutathione metabolism and peroxisome proliferators-activated receptor (PPAR) signaling pathway (*Figure 6A*). We used the oncoPredict R package to predict drug sensitivity in the two groups of the risk model, and found that the high-risk group had no difference in sensitivity to traditional chemotherapy drugs such as gemcitabine, paclitaxel, and carboplatin compared to the low-risk group. However, the high-risk group may be more sensitive to chemotherapy with ifosfamide (*Figure 6B*). At the same time, we analyzed the immune responses of the two groups and found no difference in the expression of immune genes programmed cell death protein ligand 1 (*PD-L1*) and cytotoxic T-lymphocyte-associated protein 4 (*CTLA-4*), and there was no difference in immune scores, but the high-risk group had a higher immune response rate (*Figure 6C*). In addition, we found that the target genes of the PPAR signaling pathway were dysregulated. Poly ADP-ribose polymerase 2 (*PARP2*) was
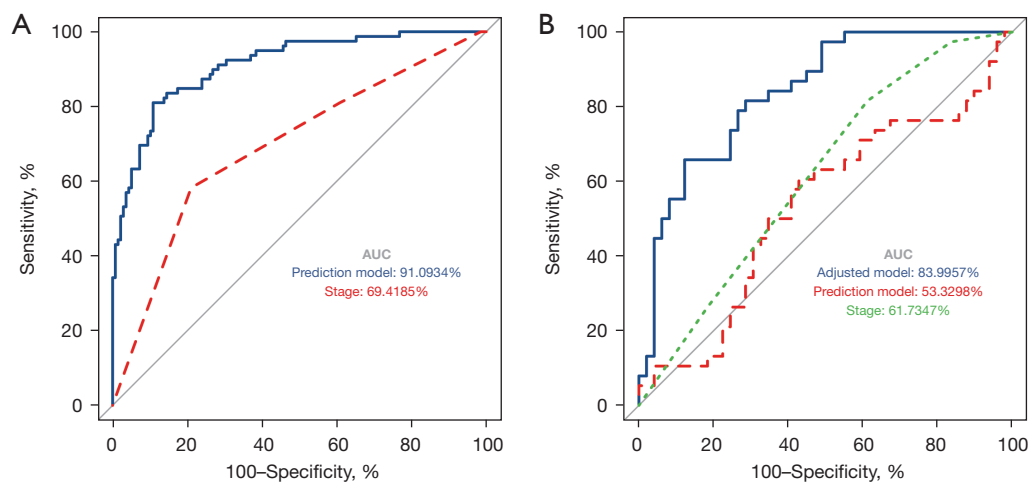
Figure 4 ROC curves of recurrent/progressive group and disease-free survival analysis for 30 RNAs. (A) ROC curves of TCGA showed risk assessment model constructed by 30 RNAs significantly distinguish recurrence status of high-grade bladder cancer. The AUC represents the identification ability of risk assessment model and stage. (B) ROC curves of GSE31684 showed risk assessment model constructed by 30 RNAs had a lower distinguishing ability in prediction of recurrence status of high-grade bladder cancer. The adjusted ROC curve was also constructed by 30 RNAs but with new coefficients, its AUC can reach 0.839. AUC, area under the curve; ROC, receiver operating characteristic; TCGA, The Cancer Genome Atlas.

highly expressed in the high-risk group, whereas the *PARP3* gene was lowly expressed in the high-risk group, and *PARP1* gene was of no significant difference between these two groups, indicating that the high-risk group might benefit from PARP inhibitors. Besides, antibody-drug conjugate (ADC) of human epidermal growth factor receptor 2 (HER2) revealed no significant differences between the two groups, but the overall expression level of HER2 was higher in the high-risk group (*Figure 6D*), suggesting that the high-risk group may benefit from HER2 ADC drugs.

Discussion

In our study, we analyzed the significantly dysregulated RNAs in the TCGA database, and used LASSO Cox regression to construct a risk assessment model to predict the recurrence for bladder cancer. Through the training set in the TCGA database and validation sets in the GEO database, we found that the risk model was accurate in recurrence prediction, and it was more accurate than the gender, age, and stage model. From function prediction of genes in the risk model, we found that the high-risk group of the risk model may benefit from chemotherapy of ifosfamide, immunotherapy, target drug of PARP inhibitor, and HER2-ADC drugs.

Although a statistically significant difference is vital to

obtain more reliable results, a high level of criteria limits the study sample size (10). Thus, we set a moderate level of criteria with P<0.05 and |log₂(fold change)| >1 for difference selection. Differentially expressed genes present significant function in disease progression (11), so we chose differentially expressed RNAs from tumor tissues and normal tissues rather than directly comparing samples from recurrence and disease-free patients. Our study firstly analyzed differently expressed RNAs in the TCGA data rather than GEO data or other previous studies to construct a risk assessment model to predict tumor recurrence; because TCGA included a sufficient number of patients with bladder cancer, we also selected GSE31684 with 93 patients to construct a model with high reliability. We obtained the risk assessment model with 30 RNAs through Cox and LASSO regression, which presented an AUC of 0.911 in TCGA and 0.533 in GSE31684. However, with the same RNAs, the adjusted risk model reached an AUC of 0.839. We speculated that the differences in diagnostic efficacy between databases were mainly related to the differences in data results caused by detection methods. Even though the original AUC of the validation set GSE31684 was low, the diagnostic model with adjusted coefficients still had high diagnostic effectiveness. This indicated that the screening results had a good ability to distinguish disease status.

Tumor prognosis is often related to stage (12,13).

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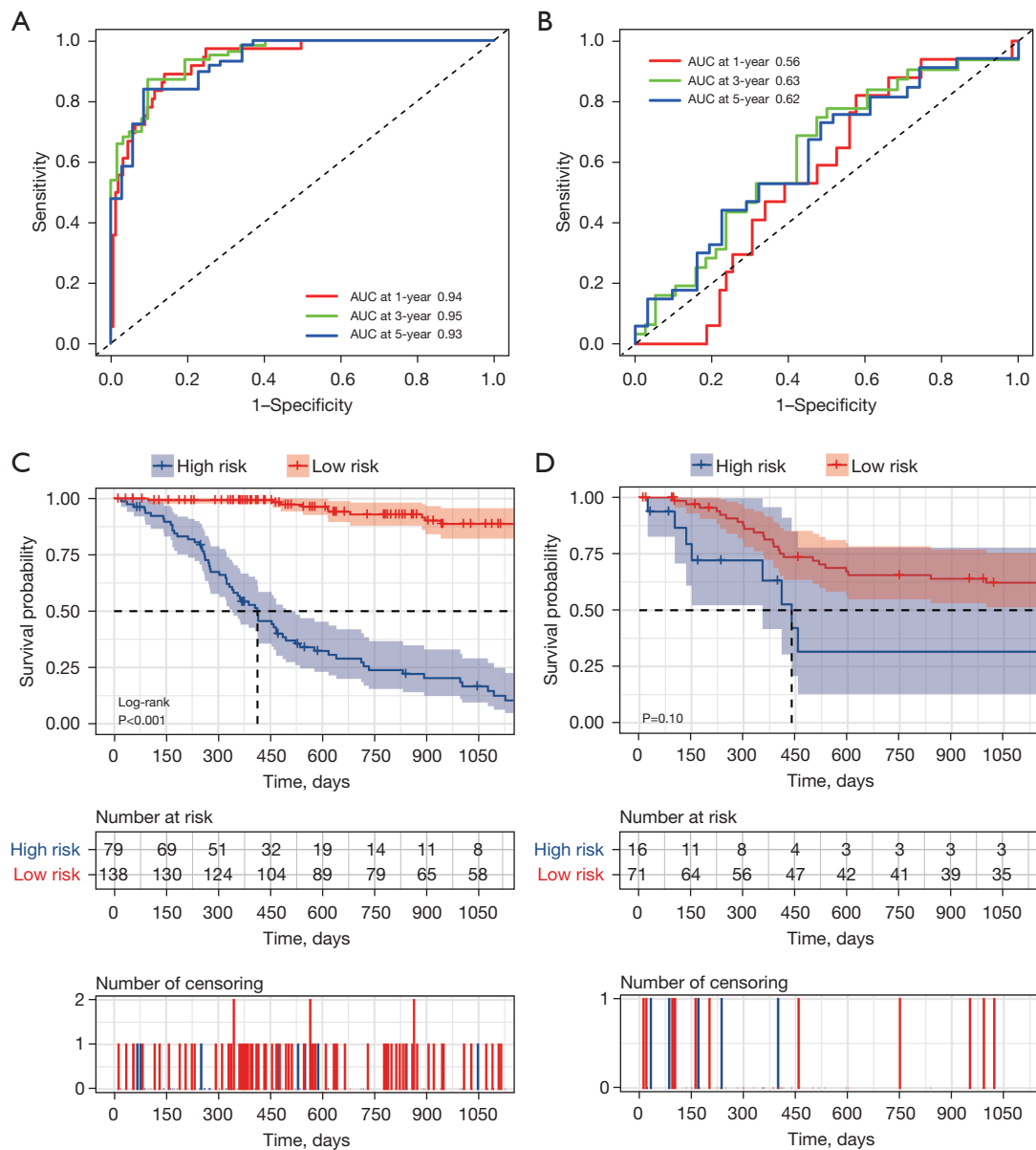
Figure 5 Accuracy of risk assessment model in recurrence prediction of high-grade bladder cancer. (A) Time-ROC curves of TCGA showed high accuracy of risk assessment model in predicting recurrence of bladder cancer, with an AUC of 0.95 in 3-year. (B) Time-ROC curves of risk assessment model in GSE31684 showed a lower accuracy of in predicting recurrence of bladder cancer. (C) Risk assessment model constructed by 30 RNAs significantly distinguish recurrence status of high-grade bladder cancer in TCGA. (D) Risk assessment model has a lower accuracy in distinguishing recurrence status of high-grade bladder cancer in GSE31684. AUC, area under the curve; ROC, receiver operating characteristic; TCGA, The Cancer Genome Atlas.
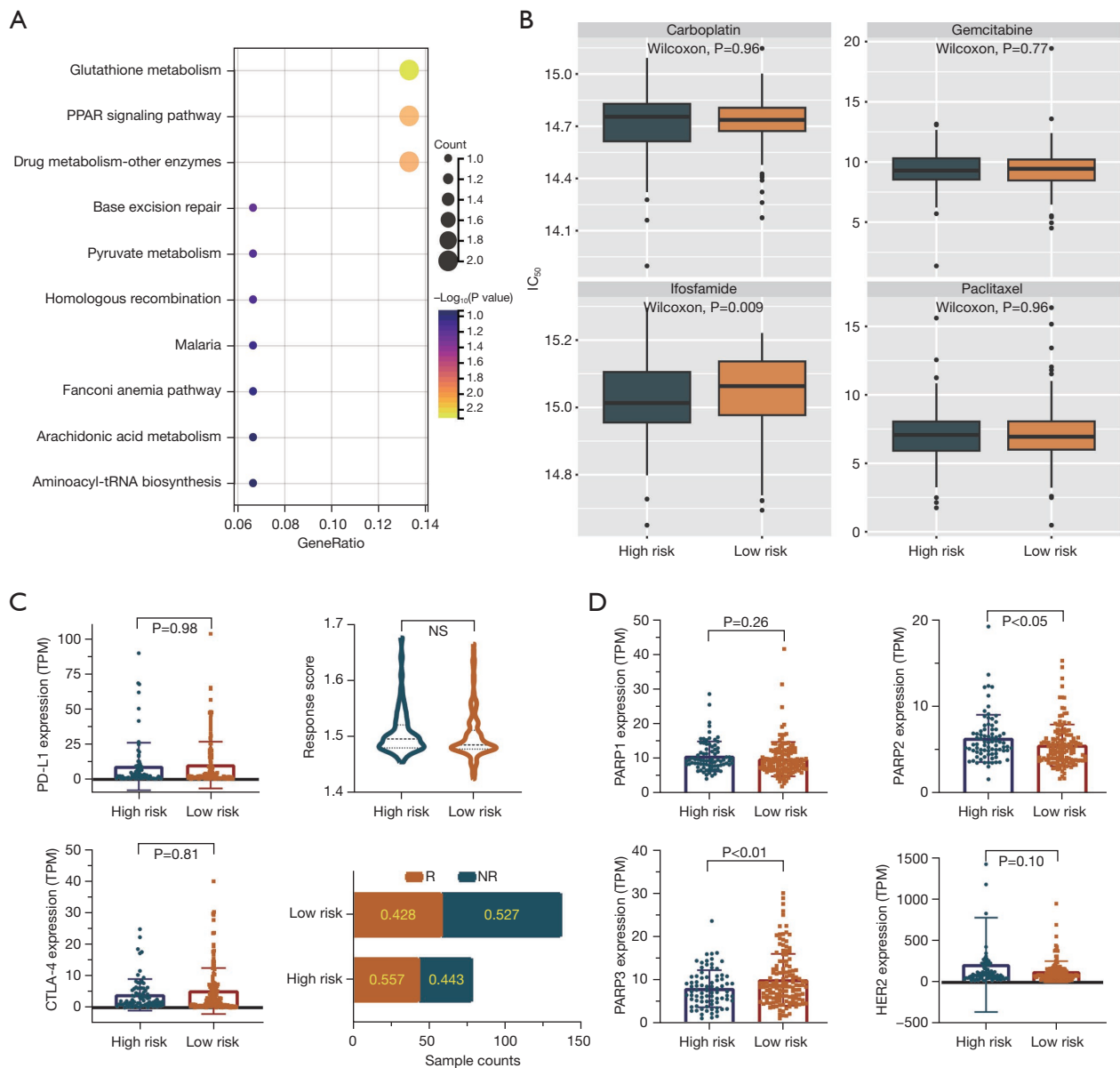
Figure 6 Functional prediction of model genes and efficacy evaluation of adjuvant therapy. (A) KEGG pathway of included genes of risk assessment model. Enrichment factor represents the ratio between the differentially expressed RNAs and all annotated genes enriched in the pathway. Bubble scale represents the number of different RNAs; depth of bubble color represents P value. (B) Predicted drug sensitivity on two groups of risk model. P value <0.05 presents significance between high- and low-risk models. (C) Difference of PD-L1 and CTLA-4 expression, immunity score and in response ratio between high- and low-risk model. (D) Difference of PARP1, PARP2, PARP3, and HER2 expression between high- and low-risk model group. IC_{50}, half maximal inhibitory concentration; PD-L1, programmed cell death protein ligand 1; NS, no significance; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; R, responsive; NR, no responsive; PARP, poly ADP-ribose polymerase; TPM, transcripts per million; HER2, human epidermal growth factor receptor 2; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Bladder cancer with later stage is more likely to recur, but the prognosis prediction accuracy of stage has been limited (5). Besides stage, other clinical information as well as parts of RNA detection have been reported as effective tools for predicting recurrence of bladder cancer (14-16). However, these assessment tools or models have also had their limitations in sample sizes or efficiency. Our research based on TCGA and GEO databases showed that the AUC of stage prediction for high-risk bladder cancer was only 0.69. In contrast, the AUC of tumor model prediction of 3-year no-recurrence could reach 0.95, and the RFS rate of low-risk group was significantly lower than that of high-risk group. We also found that the median RFS time of the high-risk group was less than 1.5 years, so accurate grouping and early intervention are extremely important for prognosis.

The first-line treatment for advanced bladder cancer is chemotherapy (17), the main drugs of which are platinum combined with gemcitabine or paclitaxel (18). However, through chemotherapy sensitivity prediction, we found there was no difference in the chemosensitivity of the high- and low-risk groups to platinum, paclitaxel, and gemcitabine, whereas the high-risk group was more sensitive to ifosfamide, a second-line chemotherapy drug for advanced bladder cancer (19), suggesting that ifosfamide may benefit high-risk group. According to the pathway analysis of the included risk-related genes, we found that the PARP pathway was the main enrichment pathway, and PARP inhibitors were also targeted drugs for bladder cancer. Research has shown that combination treatment with PARP inhibitors to MIBC reached 50% of pathological CR rate (20). We found that *PARP2*, a main target of PARP, was significantly overexpressed in the high-risk group, indicating that the high-risk group may be more limited in the targeted therapeutic effect of PARP inhibitors. Immunotherapy has also represented a main treatment for advanced bladder cancer (21). We analyzed the immune score, immune response, and the expression of commonly used immunosuppressive targets in different groups. The results showed that although there was no difference in immune scores between the two groups, and there was no difference in the expression levels of *PD-L1* and *CTLA-4*, but the high-risk group had a higher proportion of immune responses, which also suggested that the high-risk group would benefit more from immunotherapy. A previous study showed that ADC drugs were an important means of treatment for advanced bladder cancer. The objective response rate of an ADC drug of HER2 for advanced bladder cancer was

51.2%, the disease control rate was 90.7%, and the adverse reactions were mild (22). We found that the expression level of HER2 was higher in the high-risk group, although there was no statistically significant difference between the two groups, which suggested that the high-risk group might benefit more from HER2-ADC drugs.

There were some limitations in our studies. Although datasets GSE5479, GSE57933, and GSE120736 were also associated with recurrence of bladder cancer, they had no clinical information about recurrence or had small sample sizes, thus they were excluded for validation. The included GSE31684 dataset contained incompletely inconsistent clinical information with TCGA, lymph metastasis or distal metastasis were not detailed in this dataset, thus more detailed analysis was unable to proceed. Unlike an accuracy serum prediction model, our model, constructed from solid tumor data, was obviously high in the TCGA database but slightly less effective in the GEO database, but with promotion and popularization of accurate genetic sequencing, the recurrence risk assessment model would present a significant value in clinical practice.

Conclusions

The recurrence risk assessment model is accurate in predicting recurrence of high-grade bladder cancer and can provide guidance for treatment of bladder cancer.

Acknowledgments

Footnote

to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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