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Prediction of immune and targeted drug efficacy in pain-related risk subtypes for bladder cancer patients

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ABSTRACT

Bladder cancer is a complex disease with high morbidity and mortality rates. At least 430,000 cases are diagnosed annually worldwide. Cancer pain is the most common and distressing symptom in cancer patients. Studies have reported depression, anxiety, and decreased quality of life in survivors of various cancers. The study of pain-related genes in cancer patients may provide a basis for developing targeted drugs for cancer therapy, which could reduce pain and improve quality of life of cancer patients. In this study, the mRNA expression and clinical data of bladder cancer patients were downloaded from public databases. A total of 103 pain-related genes were also downloaded from the public databases. Univariate Cox regression analysis identified 17 pain-related genes that were significantly associated with overall survival. We calculated a pain-related risk score for each patient, constructed a bladder cancer pain risk model, and categorized bladder cancer patients into two risk subtypes. Differences in prognosis, differential gene expression, immune cell signatures, hallmarks, metabolic pathways, and somatic mutations between the different risk subtypes were systematically investigated. Eight drugs associated with bladder cancer risk subtypes were identified. Their differences in the high- and low-risk subtypes of bladder cancer were examined. In addition, the response to immunotherapy was analyzed in patients with different pain-related subtypes. Results revealed significant differences in these characteristics. Finally, a predictive model for pain-related risk subtypes in patients with bladder cancer was established. The study findings provide a reference for prognostication and personalized medical treatment of bladder cancer patients.

1. Introduction

Bladder cancer is the most common genitourinary malignancy and urothelial carcinoma is the most common pathological type. According to the GLOBOCAN 2018 statistics, approximately 549,000 new cases and 200,000 deaths from bladder cancer occurred in 2018, ranking bladder cancer as tenth globally in terms of the number of new cases, with approximately six people per 100,000 diagnosed with bladder cancer per year. The risk of developing bladder cancer is approximately four times higher in men than in women [1]. For early stage, non-muscle-invading bladder cancer, the 5-year rate of overall survival (OS) is as high as 95% after trans urethral resection of bladder tumor and subsequent bladder chemotherapy and/or Bacillus Calmette-Guerin therapy [2]. For

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muscle-infiltrating bladder cancer treated with radical bladder cancer surgery in combination with radiation therapy, the 5-year OS rate is approximately 69%. For advanced bladder cancer treated with platinum-containing chemotherapy regimens, the 5-year OS rate is approximately 15% [3].

In recent years, the emergence of immune checkpoint inhibitors and novel targeted drugs has increased the expectation for long-term survival of bladder cancer patients. Although immunotherapy, targeted therapy, and other treatment modalities have emerged in the last few years, cisplatin-based chemotherapy continues to hold an unassailable position in treating advanced bladder cancer. However, the efficacy of the current chemotherapy for bladder cancer patients is only approximately 50%, and several patients continue to progress and die despite extensive treatment [4]. Therefore, improving the therapeutic efficacy in patients with intermediate and advanced bladder cancer is an urgent goal. A thorough study of the onset, development, and regression of this disease, with the goal of reducing the risk of death from bladder cancer, has significant clinical and scientific implications for its prevention and treatment.

Pain is one of the most common complications of cancer. More than one-third of the treated oncology patients experience pain [5]. Pain suppresses the immune response and promotes tumor growth [6]. The association among pain, tumors, and immunity is complex and not completely understood [7]. Pain can trigger inflammation, activation of the hypothalamic-pituitary axis, and an overreaction of the sympathetic nervous system. These factors can interfere with the immune system of tumor patients, leading to immunosuppression. Melanoma cells activate nociceptive neurons by releasing secretory leukocyte proteinase inhibitor, which triggers the release of neuropeptides, such as calcitonin gene-related peptide, resulting in the depletion of cytotoxic RAMP + $CD8^+$ T cells. This limits the ability of these cells to eradicate melanoma. Moreover, inhibition may prevent immune evasion [8]. Cancer pain is a warning sign during the early stages of tumor development. However, as the tumor progresses, the pain becomes increasingly intense and difficult to control. Refractory cancer pain severely impairs the quality of life of tumor patients and is also strongly associated with the worsening of OS [9,10]. These clinical manifestations suggest that cancer pain may have a role in tumor development and that pain control should be a non-negligible part of tumor treatment. However, the biological mechanisms of pain are not yet clear.

The objective of this study was to investigate novel biomarkers that have clinical relevance in the prognosis and treatment of bladder cancer patients and may contribute to managing cancer pain effectively. Utilizing bioinformatics data mining techniques, this study classified patients based on their pain-related risk scores. Furthermore, the study assessed the immunogenomic landscape and prognosis of different pain-related risk subtypes. The results indicated that patients with different risk subtypes exhibited marked variations in their sensitivity to chemotherapy drugs and clinical response to immunotherapy. These findings could facilitate future studies on the advancements in bladder cancer treatment.

2. Materials and methods

2.1. Data collection

We downloaded RNA sequencing data from 408 patients with breast cancer, count data, somatic mutation data, and the corresponding clinical data from The Cancer Genome Atlas (TCGA; https://portal.gdc.cancer.gov/repository) [11]. The pain-related gene set GOBP_SENSORY_PERCEPTION_OF_PAIN (GO:0019,233) was downloaded from the Molecular Signatures Database (MSigDB) by searching for the keyword “Pain”. This entry contains 103 pain-related genes. To study immune infiltration in bladder cancer patients, this study also downloaded data from the ImmPort database, which contains 2449 immune genes and 17 immune cell types [12,13], 28 immune cell signatures [14], and the MSigDB database of 50 Hallmarks [15].

In addition, we calculated the risk scores for IMvigor210 to further assess the relationship between pain-related risk subtypes and immunotherapy response in cancer patients. Expression data and clinical information of patients with advanced uroepithelial cancer treated with anti-programmed death-ligand 1 (PD-L1) agents were downloaded from the R package IMvigor210CoreBiologies (version 1.0.0) [16]. Moreover, the mRNA expression profiles and clinical information of GSE13507 and GSE32894 were obtained from the GEO database and used as validated cohorts.

2.2. Construction of the risk score model

Using TCGA cohort, we performed univariate Cox regression analysis to assess the association between pain-related genes expression and OS in bladder cancer patients. A total of 17 pain-related genes were significantly associated with OS. The following risk score was constructed for the 17 identified pain-related genes:

$$\sum_i (logHRi) * expi$$

where HR_i is the hazard ratio (HR) of the ith pain-related gene and exp_i is the expression of the ith pain-related gene in each sample, where $i = 1, 2, \ldots, 17$. The "surv_cutpoint" function in the survminer package (version 0.4.6) of the Bioconductor platform was used to determine the optimal cutoff value for the pain risk score. Finally, according to the optimal threshold, bladder cancer patients were divided into high- and low-risk subtypes. In this study, the prognosis of bladder cancer patients with high- and low-risk subtypes was compared using the R platform’s survival package. The significance of the difference in survival time was calculated using the Log-rank test and further demonstrated using the Kaplan-Meier survival curve.

2.3. Predicting response to immunotherapy in patients with bladder cancer

T cell dysfunction and rejection are two important mechanisms of tumor immune escape. The Tumor Immune Dysfunction and Exclusion (TIDE) algorithm is a reliable algorithm that accurately predicts the effectiveness of immunotherapy based on the functional status of T cells [17]. The current study aimed to evaluate T cell dysfunction and rejection scores in bladder cancer patients from the TCGA dataset available on the TIDE website (http://tide.dfci.harvard.edu/). We statistically compared the scores between high- and low-risk subtypes of bladder cancer to predict the immunotherapeutic prognosis in patients with these subtypes.

2.4. Potential drug sensitivity analysis

We used the WGCNA package (version 1.71) [18] in R software to study the patterns associated with 269 drugs in the Genomics of Drug Sensitivity in Cancer (GDSC) database to obtain drug modules related to clinical information. The Pearson correlation coefficient of half maximal inhibitory concentration (IC_{50}) between each drug was calculated. Then, the weighted adjacency matrix amn = | Cmn | β was constructed, where amn represents the adjacency coefficient between drug m and drug n; and C_{mn} represents the Pearson correlation coefficient between drug m and drug n. The construction of a scale-free network was ensured by choosing a soft threshold β = 6, setting the Deepsplit value to 2, and the minimum module size to 30 drugs. Module identification was performed to generate a tree diagram. Highly similar modules were identified using clustering and similar modules were merged with a high shear threshold value of 0.25. The module eigengenes (ME) were then calculated, indicating the first principal component of each module. In addition, the correlation coefficients and *P*-values of the characteristic drugs of the drug modules with each external clinical information were calculated using Pearson correlation analysis and visualized in heat maps using the labeled heatmap function. The module with the highest correlation was studied with the relevant clinical features to search for biologically significant modules. Screening conditions for hub drugs with special modules were Gene Significance (GS, correlation of each gene with clinical traits) > 0.4, Module Membership (MM, correlation of each gene with the module) > 0.5, and a scatterplot between MM and GS. Using the WGCNA package of R software, eight hub drugs associated with pain-related risk subtypes were obtained based on IC_{50} from the GDSC database. Univariate Cox regression analysis was performed to assess the relationship between these eight drugs and the OS. The results were visualized using forest plots. Finally, the Wilcoxon test was used to compare statistical differences in IC_{50} between different pain-related risk subtypes.

2.5. Bioinformatics and statistical analysis

We explored differentially expressed genes (DEGs) in high- and low-risk subtypes in the TCGA cohort. The edgeR package (version 3.36.0) [19] of the Bioconductor platform was used to analyze DEGs based on the count data of gene expression in TCGA bladder cancer patients. The clusterProfiler (version 4.0.5) package [20] of the Bioconductor platform was used for Gene Ontology (GO) enrichment analysis. Kyoto Encyclopedia of genes and Genomes (KEGG) enrichment pathway analysis was performed using the same approach to identify KEGG pathways that were significantly enriched for DEGs. In this study, we used gene set enrichment analysis (GSEA) [21] to discern enrichment differences between different pain-related risk subtypes. In addition, single sample gene set enrichment analysis (ssGSEA) [15] was performed using the R package GSVA (version 1.40) [22]. To explore the differences in immune-related characteristics between the high- and low-risk subtypes, the ESTIMATE scores, tumor purity, stromal scores, and immune scores were evaluated using the ESTIMATE algorithm [23]. The cytolytic activity index (CYT) was determined using the geometric mean expression of granzyme A and perforin-1 secreted by effector T and natural killer cells [24].

Kaplan-Meier survival curves were used to compare the OS of different risk subtypes. A forest plot was generated using the R package forestplot (version 1.7). Somatic mutation data of different subtypes were analyzed using the R package maftools (version 2.14.0) [25]. All analyses in this study were performed in R. All tests were two-tailed, and *P*-value<0.05 was considered statistically significant.

3. Results

3.1. Differential analysis of OS and clinical characteristics of patients with different bladder cancer pain-related risk subtypes

In the TCGA cohort, the expression levels of 103 pain-related genes were subjected to univariate Cox regression analysis, which revealed significant associations between 17 pain genes and overall survival (OS) (*P*-value<0.05; Log-rank test). Due to the substantial HR value of OPRD1, it was excluded from the plot. The remaining 16 pain-related genes were depicted using a forest plot to visualize their risk ratios (Fig. 1A). The 17 pain-related genes were used to construct a risk score model to classify bladder cancer patients into high- and low-risk subtypes based on the best cutoff value determined using the "surv_cutpoint" function of the survminer R package (version 0.4.6). Kaplan-Meier curves were used to compare the differences in OS between the high- and low-risk subtypes. The survival curves showed that OS was significantly higher in patients with low-risk subtype than in those with high-risk subtype (*P*-value<0.05, Log-rank test; Fig. 1B). To further verify the universality of the risk score constructed in this study, the risk model was employed to validate the samples in the GSE13507 and GSE32894 cohorts. The results in the GSE13507 and GSE32894 cohorts demonstrated that the OS of the low-risk subtype was significantly higher than that of the high-risk subtype (*P*-value<0.05, Log-rank test; Supplementary Fig. 1).

In addition, a heatmap revealed the expression of the 17 pain-related genes in high- and low-risk subtypes. As shown in Fig. 1C,

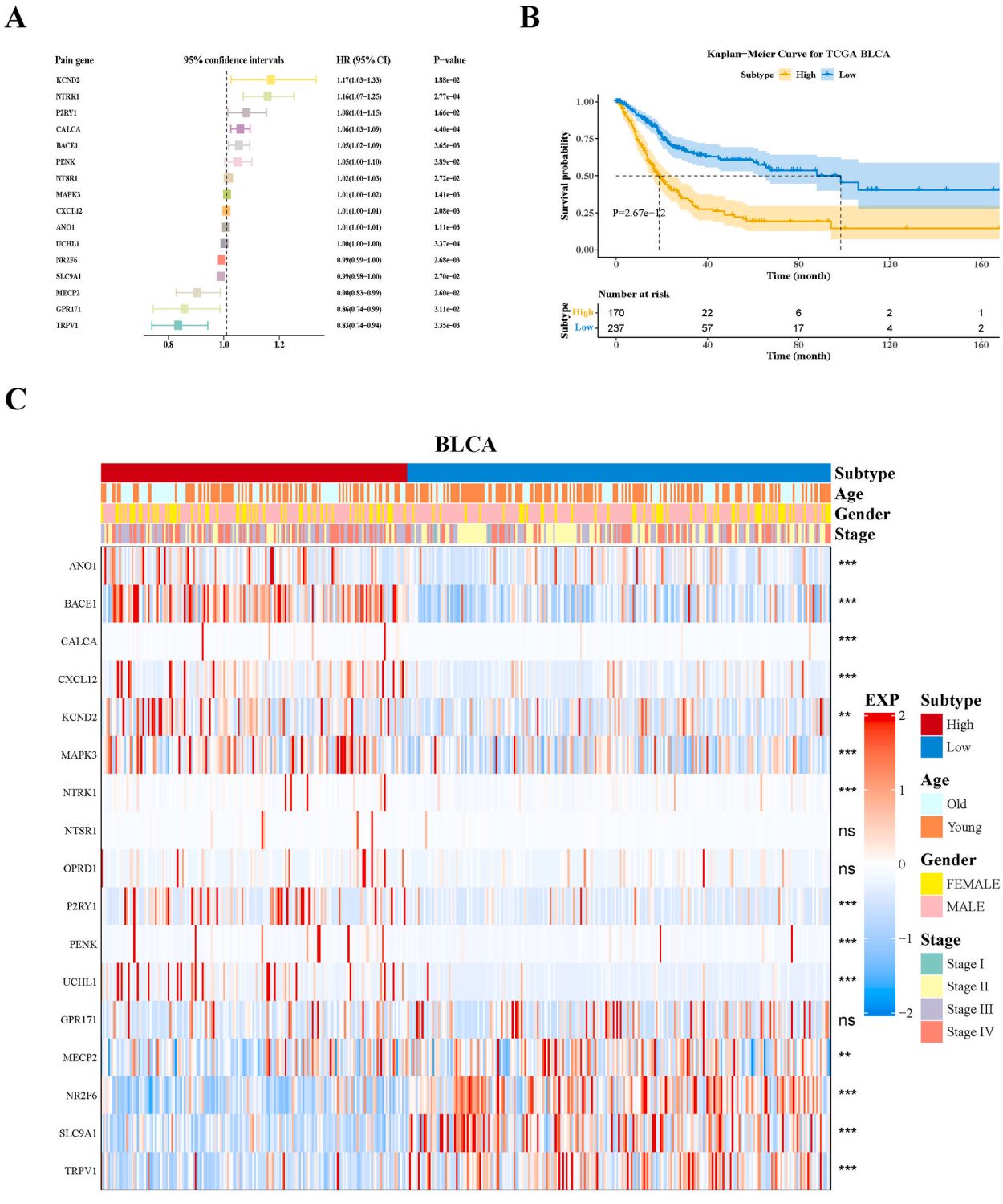


Fig. 1. Construction and analysis of the pain-related risk score model. (A) Forest plot of the associations between the expression levels of 17 pain-related genes and OS in the TCGA cohort. The hazard ratio (HR), 95% confidence interval (CI), and *P*-value were determined using univariate Cox regression analysis. (B) Kaplan–Meier estimate of the OS of TCGA cohort two risk subtypes. (C) Heatmap of the different expression levels of 17 pain-related genes horizontally clustered in TCGA cohort and clinical features ranked by risk scores.

excluding three genes *NTSR1, OPRD1*, and *GPR171*, the expression of the remaining 14 pain-related genes was significantly different in the high- and low-risk subtypes (*P*-value<0.05; Wilcoxon test). Furthermore, 12 of the 17 genes exhibited higher mean expression in the high-risk subtype than in the low-risk subtype. The remaining five genes had higher mean expression in the low-risk subtype than in the high-risk subtype. Overall, the expression levels of 17 pain-related genes in the high- and low-risk subtypes did not correlate with clinical characteristics such as age, sex, and tumor stage.

3.2. Analysis of DEGs between high- and low-risk subtypes of patients with bladder cancer

A total of 1126 upregulated and 2032 downregulated DEGs (|logFC|>1 and *P*-value<0.05) were identified between two pain-related risk subtypes in the TCGA cohort using the edgeR package in glmQLFTest (Fig. 2A). Fig. 2B shows the location on the chromosome of the top 322 significantly downregulated DEGs, top 70 significantly upregulated DEGs, and the correlation between gene expression and pain-related risk scores. GO enrichment analysis revealed (Fig. 2C) that the DEGs were mainly enriched in biological processes, such as collagen-containing extracellular matrix, synaptic membrane, neuronal cell body, and skin and epidermis development. KEGG enrichment analysis showed that the DEGs were mainly enriched in neuroactive ligand-receptor interactions, drug metabolism-cytochrome P450, retinol metabolism, cytochrome metabolism of xenobiotics, chemical carcinogenesis-DNA adducts, and other KEGG pathways (Fig. 2D).

In addition, GSEA analysis of hallmarks, 17 ImmPort immune signatures, 28 immune cell signatures, and metabolic pathways were performed on TCGA cohort. Twenty-two hallmarks were significantly enriched in the high-risk subtype (Fig. 3A). Four ImmPort immune signatures were significantly enriched in the high-risk subtype (Fig. 3B). Two immune cell signatures were significantly enriched in the low-risk subtype, whereas three immune cell signatures were significantly enriched in the high-risk subtype (Supplementary Fig. 2A). Corresponding to the metabolic pathways, we observed that four metabolic pathways were significantly enriched in the high-risk subtype and thirteen metabolic pathways were significantly enriched in the low-risk subtype (Supplementary Fig. 2B). In addition, GSEA results for the eight most significantly enriched hallmarks, ImmPort immune signatures, immune cell signatures, and

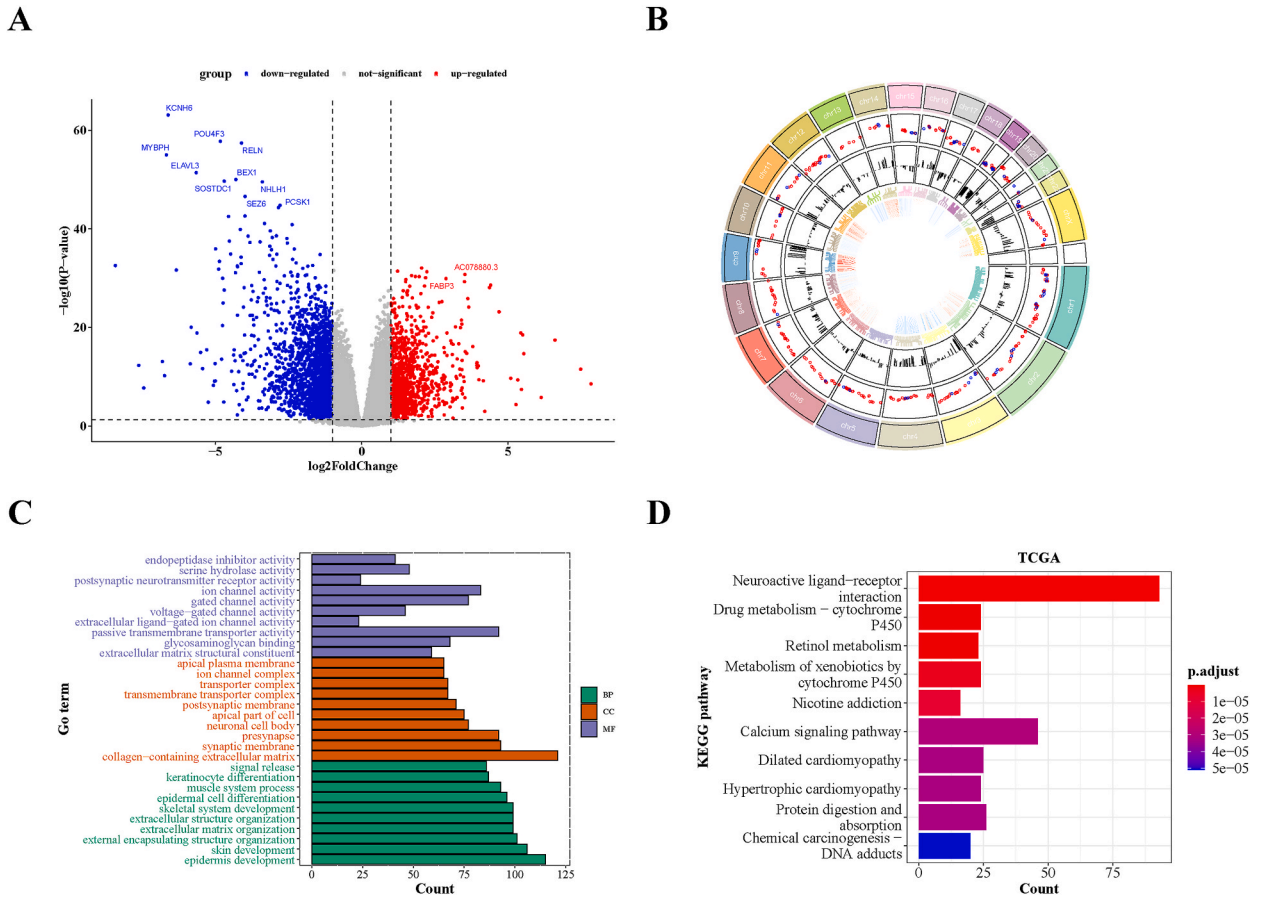
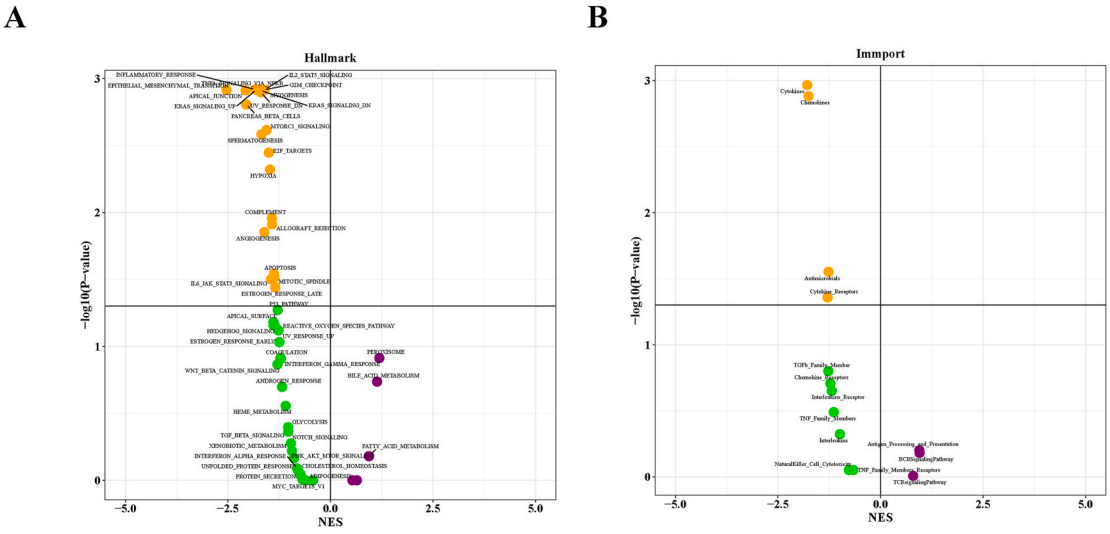
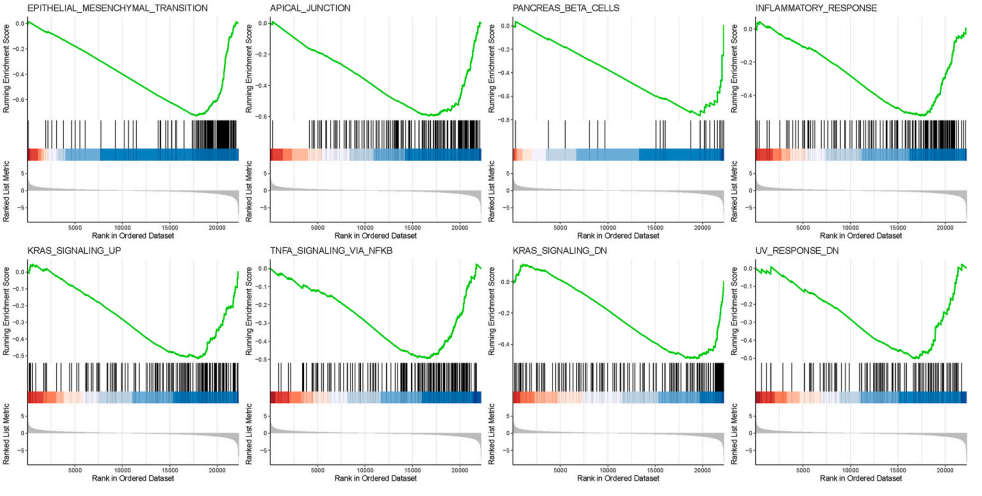


Fig. 2. Differential gene analysis in different risk subtypes. (A) Volcano plot of DEGs related to pain using |logFC|>1 and *P*-value <0.05 as screening criteria. The horizontal line is -log. The two vertical lines are −1 and 1. (B) The chromosome locations of 392 DEGs. (C) Enrichment results of significantly DEGs in GO terms for biological process, cellular component, and molecular function. (D) Enrichment results of significant DEGs in KEGG pathways.





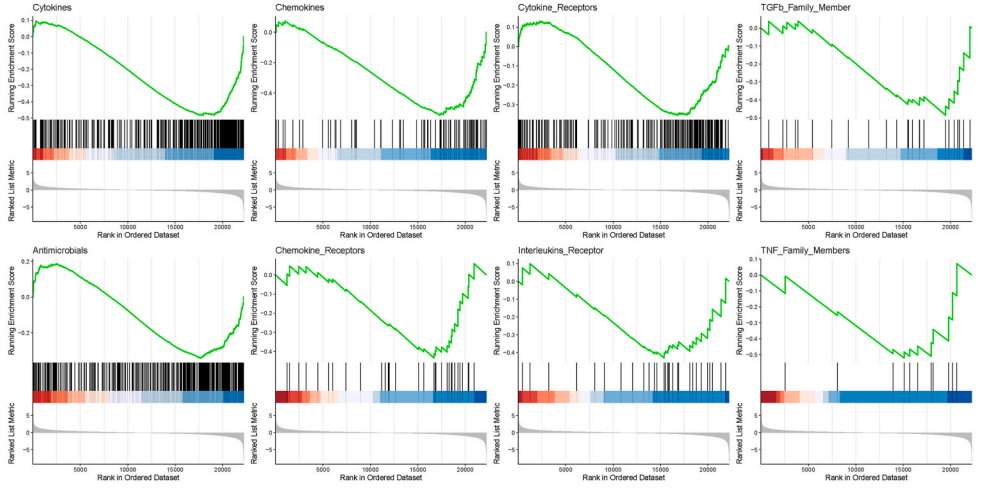


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Fig. 3. GSEA results of different risk subtypes in TCGA cohort. (A) Volcano plot of the hallmark gene enrichment analysis in TCGA cohort. The X-axis is normalized enrichment score (NES), and the Y-axis is the -log of the *P*-value for significance in the enrichment result. (B) Volcano plot of the ImmPort immune signature gene enrichment analysis in TCGA cohort. (C) GSEA results of the top eight enriched hallmarks. (D) GSEA results of the top eight enriched ImmPort immune signatures.

metabolic pathways are shown in Fig. 3C and D, and Supplementary Figs. 2C and D. In this study, ssGSEA was used to calculate the enrichment scores for 50 hallmarks in TCGA patients with high- and low-risk subtypes. The statistical differences between the enrichment scores for the 50 hallmarks in the two subtypes were also calculated. TCGA dataset analysis revealed that with the exception of five hallmarks (DNA_REPAIR, ADIPOGENESIS, MYC_TARGETS_V2, OXIDATIVE_PHOSPHORYLATION, and XENOBIOTIC_METABOLISM), the enrichment of the hallmark scores was significantly different between high- and low-risk subtypes (Fig. 4). In addition, the enrichment scores for majority of hallmark were significantly higher in the high-risk subtype than in the low-risk subtype (*P*-value<0.05; Wilcoxon test; Supplementary Fig. 3).

3.3. Analysis of somatic mutation differences between high- and low-risk subtypes in bladder cancer patients

Genetic mutations play an important role in tumor progression and patient prognosis. Accordingly, further mutational landscape analysis of bladder cancer pain-related risk subtypes was performed. The top five most frequently mutated genes in both subtypes were TP53, TTN, KMT2D, MUC16, ARIDIA, KDM6A, and PIK3CA (Fig. 5). TP53 and RB1 play key roles in regulating cell division. Inactivation, mutation, and deletion of TP53 and RB1 are among the major causes of bladder cancer. The mutation frequency of RB1 was much higher in the high-risk subtype than that in the low-risk subtype. STAG2 was one of the most commonly mutated genes in bladder cancer. The frequency of mutations was significantly higher in the high-risk subtype than in the low-risk subtype.

3.4. Characterization of immune landscape in bladder cancer with high- and low-risk subtypes

This part of the study focused on the differences in the immune system between the high- and low-risk subtypes. Stromal and immune scores are often used to predict the degree of mesenchymal and immune cell infiltration of the tumor and provide the basis for

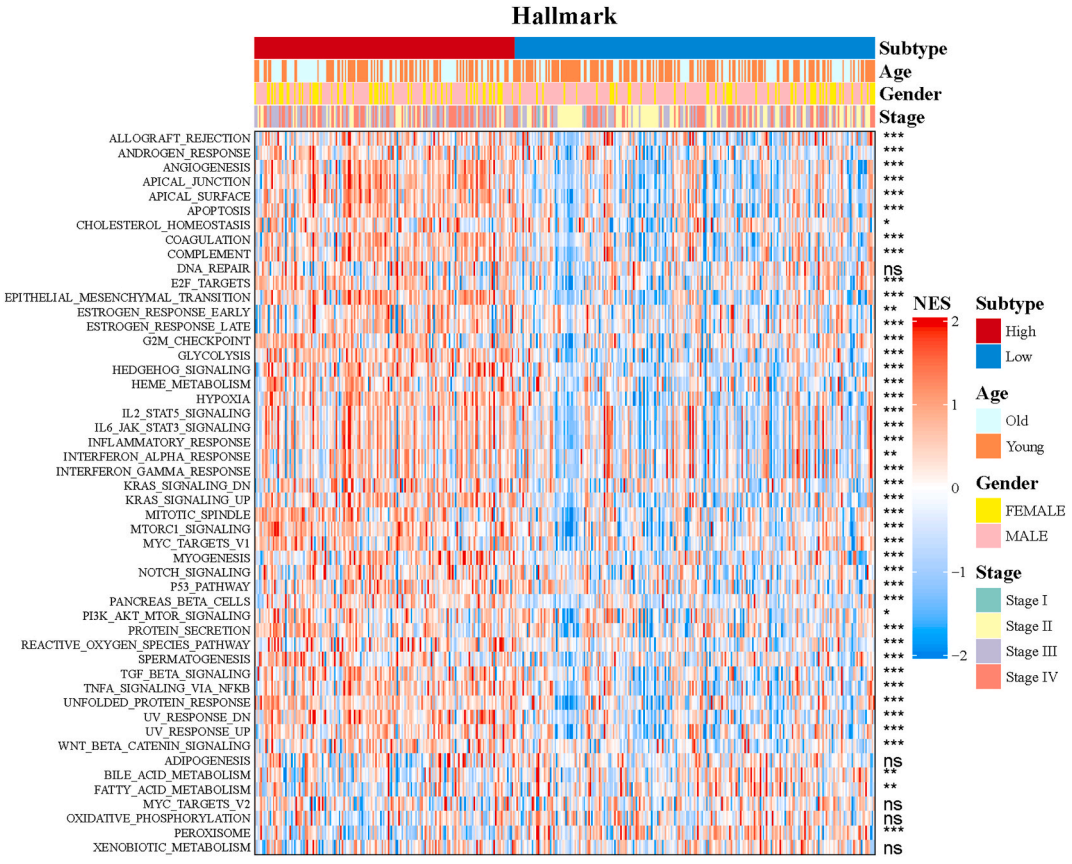

Fig. 4. GSVA analysis of 50 hallmarks with different risk subtypes.
Heatmap of the hallmark enrichment scores of the different risk subtypes in TCGA cohort.

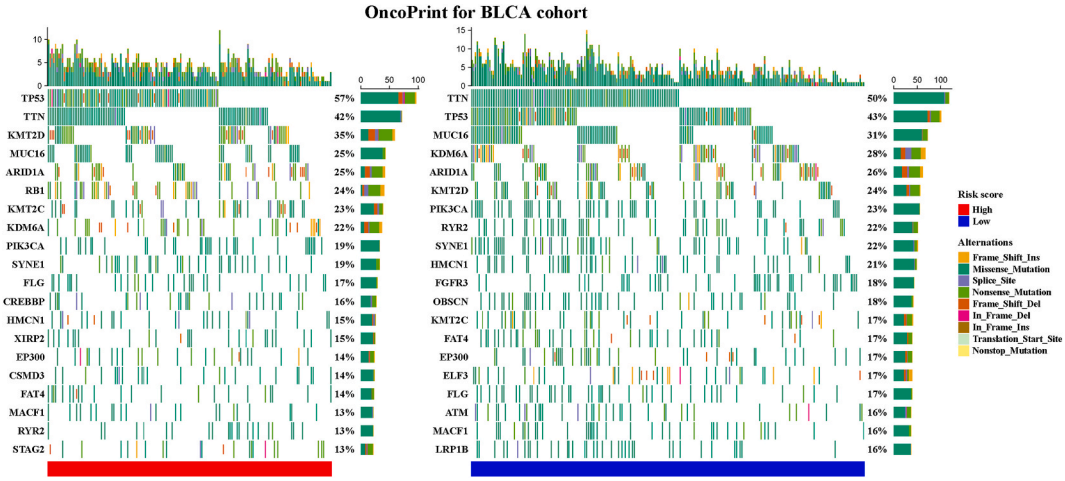

Fig. 5. Somatic mutations in high- **and low-risk subtypes in TCGA cohort**.
Waterfall plot of the top 20 mutant genes in high- and low-risk subtypes in TCGA cohort.

calculating the ESTIMATE score. This score can be used to infer tumor purity, defined as the proportion of tumor cells in solid tumor samples. The ESTIMATE method predicts immune-related scores of the tumor microenvironment, including stromal and immune cell scores, using the expression profiles of tumor patients. Therefore, stromal score, immune score, and ESTIMATE score and tumor purity were calculated based on this algorithm for high- and low-risk subtypes. Statistically significant differences in the scores for these four characteristics were evident between the high- and low-risk subtypes. Analysis of TCGA bladder cancer dataset revealed significantly lower tumor purity scores in the high-risk subtype compared to the low-risk subtype (*P*-value<3.5e-06; Wilcoxon test; Supplementary Fig. 4). In contrast, stromal scores, immune scores, and ESTIMATE scores were significantly higher than the corresponding scores in the low-risk subtype. In addition, the CYT reflected the cell killing function by the geometric mean of granzyme A and perforin-1 gene expression, with CYT expression levels significantly lower in the high-risk subtype than in the low-risk subtype.

3.5. Analysis of drug sensitivity differences between high- and low-risk subtypes in bladder cancer patients

We further performed WGCNA analysis to identify drugs associated with pain-related risk scores in the GDSC database. The optimal soft threshold power β was set to 6 to ensure the construction of a scale-free network (scale-free $R^2 = 0.9$) (Fig. 6A). The constructed dendrogram revealed that drugs with similar drug sensitivity patterns clustered into four modules (Fig. 6B), with the minimum number of drugs per module set at 30. Of the four modules, the blue module was significantly positively correlated with the high-risk subtype (ME = 0.4 *P*-vvalue = 3e-17) and significantly negatively correlated with the low-risk subtype (Fig. 6C). Therefore, we selected the blue module as the hub module, from which eight hub drugs were screened with the screening criteria of MM > 0.5 and GS > 0.4 (Fig. 6D). Univariate Cox regression analysis was performed to assess the relationship between IC_{50} and OS for these eight drugs. Of these, seven were significantly associated with OS (*P*-value<0.05; Log-rank test). Forest plots were constructed to visualize the HRs for the seven drugs. All seven had HR > 1 and were prognostically favorable (Fig. 6E). In addition, the IC_{50} values of these eight drugs were analyzed in the high- and low-risk subtypes. The IC_{50} values of all eight drugs were significantly different in the high- and low-risk subtypes. The IC_{50} values of each drug were significantly higher in the high-risk subtype than in the low-risk subtype, indicating that patients in the low-risk subtype were more sensitive to the drugs than those in the high-risk subtype (Fig. 6F). These results suggest that the pain-related risk score model may be a useful filter for the use of chemotherapeutic agents in the treatment of bladder cancer.

3.6. Prediction of immunotherapy outcome in patients with high- and low-risk subtypes of bladder cancer

To explore the response of the different pain-related risk subtypes to immunotherapy, immune signature scores of TCGA bladder cancer patients were downloaded from the TIDE website. Information included the myeloid-derived suppressor cell (MDSC), cancer-associated fibroblast (CAF), M2 subtype tumor-associated macrophage (M2), T cell rejection, and T cell dysfunction scores. Significant differences between the two subtypes in the CAF, M2, MDSC, and T cell rejection scores were evident (Fig. 7A). Three cell types limited T cell infiltration in tumors: CAF, MDSCs, and tumor-associated macrophages TAMM2. MDSC and CAF scores were higher in the high-risk subtype, whereas the TAMM2 score was higher in the low-risk subtype. The high-risk subtype had higher T cell rejection scores than the low-risk subtype. The findings indicate that high-risk subtype have less T cell infiltration into the tumor, poor immune competence, and poor survival prognosis. Analysis of variance revealed that the efficacy of immunotherapy differed between the high-risk and low-risk subtypes, with the proportion of immunotherapy responders in the low-risk subtype being >2.5 times that observed in the high-risk subtype (Fig. 7B). Data from the urothelial carcinoma dataset (IMvigor210) were used to compare the prognosis of the different pain-related risk subtypes after immunotherapy. The survival curves revealed that patients with low -risk subtype had significantly better survival than those with high-risk subtype (Fig. 7C). In addition, more patients in the low-risk subtype had a

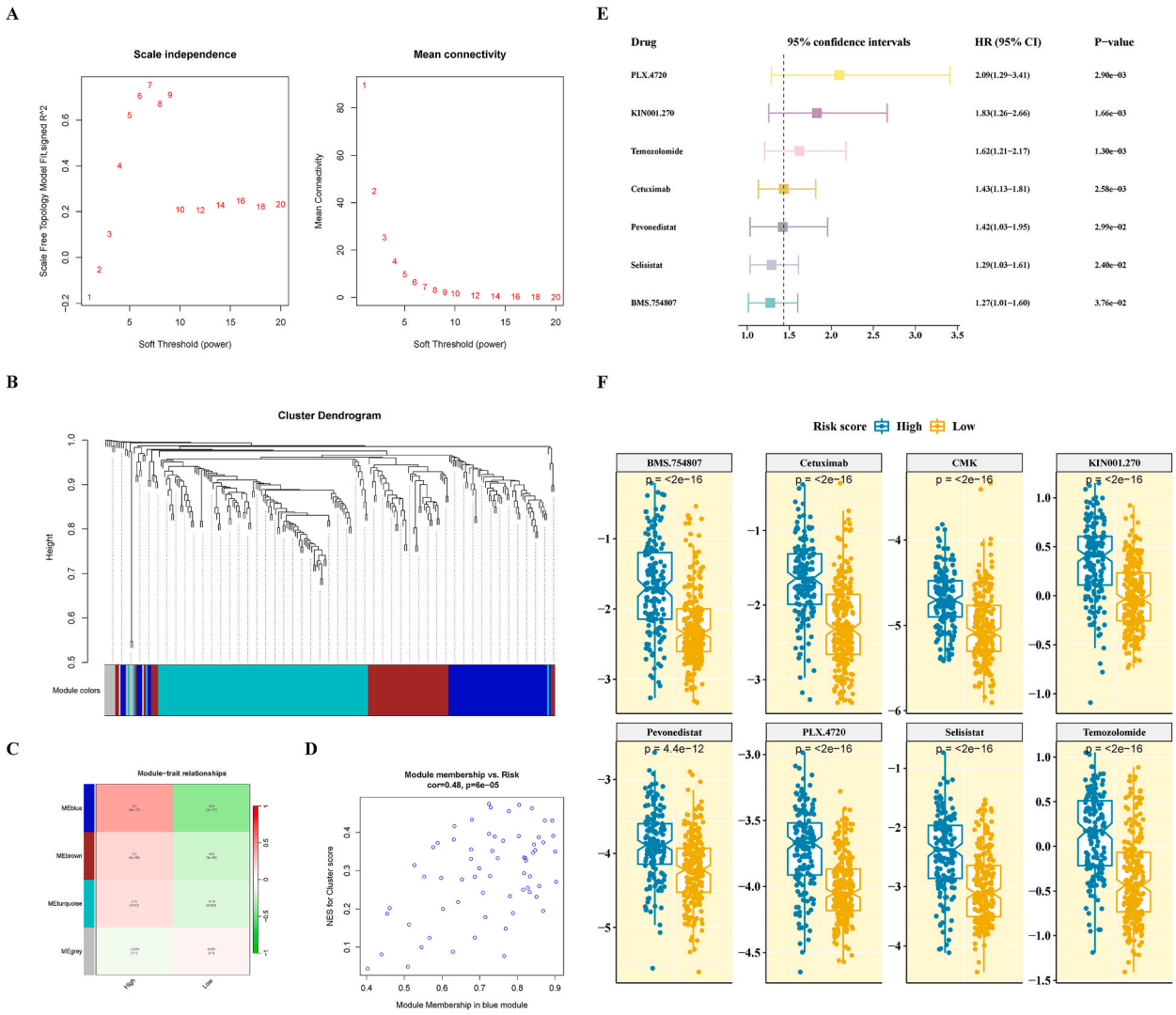

Fig. 6. Network construction, module detection in the GDSC database, and evaluation of chemotherapeutic reaction of different risk subtypes in TCGA cohort. (A) Analyses of network topologies for various soft threshold powers through scale-free fit index and mean connectivity. (B) Clustering dendrogram of genes based on topological overlapping. Different colors were assigned to corresponding modules. (C) Heat map of the correlation between drug modules and risk subtypes. (D) Scatter diagram on the coefficient of correlation between the MM and GS of the blue module. (E) Forest plot of the associations between the IC_{50} of seven hub drugs and OS in TCGA cohort. HR, 95% CI, and *P*-values were determined using univariate Cox regression analysis. (F) Comparison of the IC_{50} of the eight hub drugs between the two risk subtypes. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

complete response (CR) and a partial response (PR) after immunotherapy (Fig. 7E). In addition, patients in CR and PR with anti-PD-L1 therapy had lower risk scores than patients with stable disease (SD) and progressive disease (PD) (*P*-value = 0.021; Wilcoxon test) (Fig. 7D, F). These results suggest that bladder cancer patients with low-risk scores may be more sensitive to immunotherapy and have a better prognosis. This may be because the high-risk subtype had fewer T cells infiltrating into the tumor tissue, resulting in poor survival.

4. Discussion

Bladder cancer remains the most common malignancy of the genitourinary system. In 2018, 549,393 patients were diagnosed with bladder cancer worldwide and 199,922 patients died from this disease [1]. The incidence of bladder cancer is expected to continue to increase over the next decade [26]. There is no widely accepted screening protocol for bladder cancer [27], probably because of the low prevalence of invasive disease and the lack of optimal screening tools. As a consequence, majority of patients have already advanced to the infiltrative stage of bladder cancer at the time of diagnosis. Clinical management of bladder cancer has changed little. For patients with muscle invasion, surgical resection remains the mainstay of treatment. Pain is a common complaint in cancer

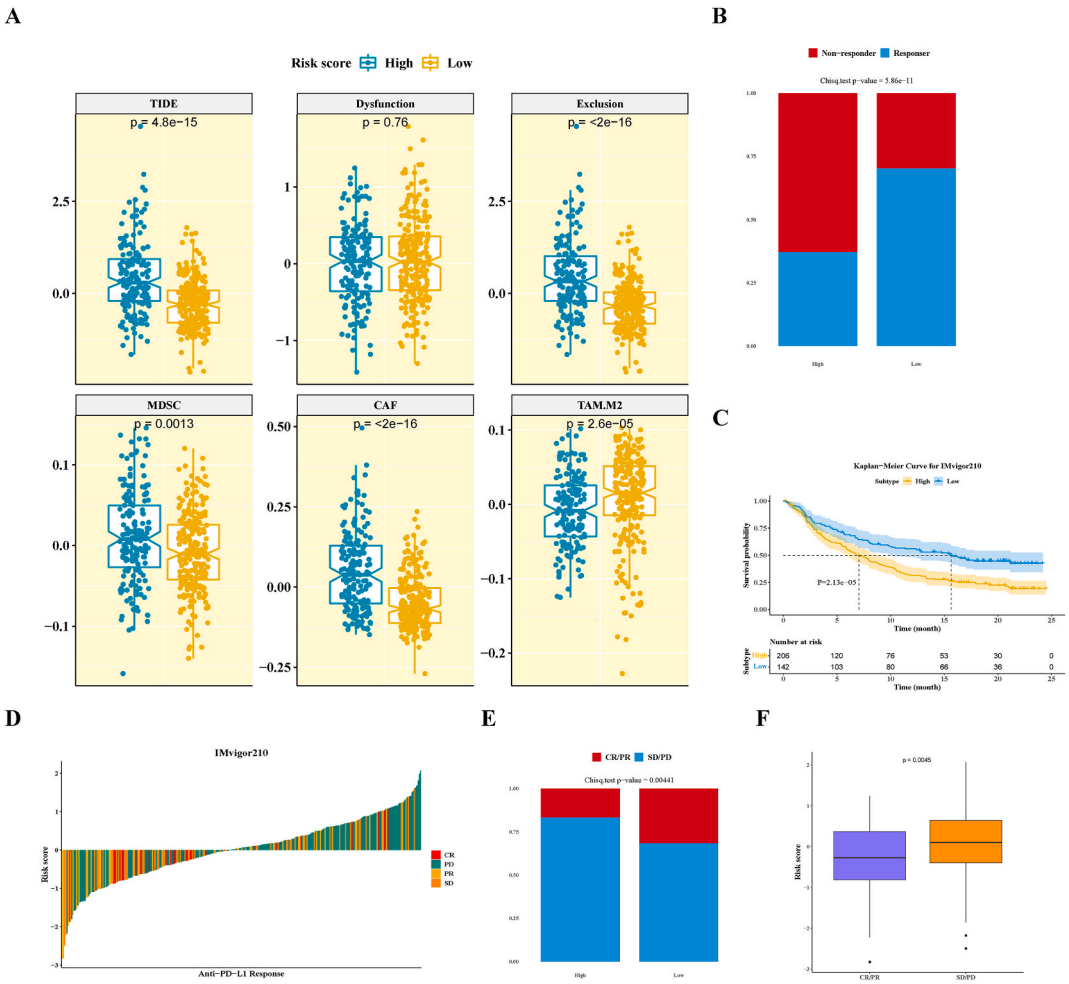

Fig. 7. Differences in response of different risk subtypes to immunotherapy. (A) Comparison of TIDE scores between the different risk subtypes. (B) Distributions of responder and non-responder to immunotherapy in the distinct risk subtypes estimated by TIDE algorithm. (C) Kaplan-Meier curves of OS according to risk subtypes in the IMvigor210 cohort. (D) Bar chart of patients' risk score in IMvigor210 dataset. The color of the column indicates the response to immunotherapy. Each bar represents a sample. CR/PR refers to complete response or partial response to the drug. SD/PD refers to stable disease or progressive disease. (E) Distribution ratio of anti-PD-L1 immunotherapy response of patients with high- and low-risk score subtypes in IMvigor210 cohort. The horizontal axis represents the different risk subtypes and the vertical axis represents the proportion of the different treatment responses. (F) Boxplot illustrating the distribution of risk score for patients with different immunotherapy responses in the IMvigor210 cohort. Significance was determined using the Wilcoxon test. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

patients. In a systematic review of 19 studies conducted in 2014, the pooled prevalence of cancer-related pain was 59.2%, ranging from 39.9% in outpatient settings to 80.5% in hospice care settings [28]. The presence and severity of pain are clinically important because pain, as a variable in health-related quality of life factors, provides prognostic information for survival [29,30]. Two decades ago, an effect of pain on the body's immune function was proposed [31]. Solid tumors are innervated by nerve fibers from the autonomic and sensory peripheral nervous systems. Whether the reinnervation of tumors by pain-triggered sensory neurons affects cancer immunosurveillance remains unclear. To date, no studies have classified bladder cancer based on pain gene sets. Therefore, we used specific pain gene sets to identify and validate our new classification of bladder cancer.

The series of events required for an organism to receive a painful stimulus, convert it to a molecular signal, and recognize and characterize the signal. Pain is medically defined as the physical sensation of discomfort or distress caused by injury or illness, so can hence be described as a harmful stimulus which signals current (or impending) tissue damage. Pain may come from extremes of temperature, mechanical damage, electricity or from noxious chemical substances. This is a neurological process. Cancer pain is an unpleasant feeling and emotional experience caused by the information that tissue damage needs to be repaired or adjusted transmitted to the nerve center. Therefore, we selected 103 pain-related genes as the background gene for analysis.

Using TCGA data related to bladder cancer patients, 17 pain-related genes significantly associated with OS were identified using univariate Cox regression analysis. Recent research has discovered that the genetic composition and corresponding polymorphisms are

the fundamental determinants of an individual's pain sensitivity. The bladder cancer prognosis model has implicated 17 pain-related genes that are intricately involved in pain perception via the mechanisms of ion channels, neurotransmitters, action receptors, and drug metabolism enzymes. Remarkably, the polymorphisms of these aforementioned genes at various levels are capable of affecting pain perception and the resultant individual performance. Bladder cancer patients were classified into high- and low-risk subtypes using the optimal pain risk score cutoff determined using the surv_cutpoint function in the survminer package of the Bioconductor platform (version 0.4.6). In recent years, considerable scientific investigation has been devoted to evaluating the correlation between pain and tumor progression. Patients experiencing pain symptoms are subject to a substantial decrease in survival rates, thereby underscoring the crucial necessity of effective pain management for optimal patient outcomes. Stressful conditions have the ability to activate both the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS), culminating in an elevated discharge of catecholamines and adrenal ketones from the adrenal glands. Consequently, this may potentially result in immunosuppression due to reduced immune function of macrophages and lymphocytes, as well as the hindered development, transportation and activation of both central and peripheral immune cells. Moreover, prolonged cancer-related pain may lead to damage of the immune system and hastened cellular aging. Furthermore, the tumor-stimulating effects of stress could potentially be attributed to endocrine dysfunction. Studies have revealed that adrenalin signals may regulate tumor cells and vascular endothelial cells, thereby inducing tumor neovascularization and promoting metastasis. The immune system and endocrine system are both regulated by the nervous system. Thus, the aberrant nervous excitation associated with pain-induced stress can disrupt normal cellular homeostasis, leading to functional impairment of different cellular mechanisms. Intriguingly, some signaling pathways in tumor cells and nerve cells are jointly implicated, imparting a biological underpinning for pain-induced promotion of tumor progression. Our results imply that discrepant expression profiles of genes related to pain perception in the high- and low-risk subtypes may contribute to differences in their prognosis and survival durations. The correlation between pain-induced disruption of the endocrine and immune systems, as well as the direct influence of the nervous system on tumors, may explain these observations.

Subsequently, we systematically investigated DEGs, immune cell signature enrichment, hallmarks enrichment, metabolic pathway enrichment, and somatic mutations among the different risk subtypes. These characteristics differed significantly. In addition, differences in immunotherapy response and drug sensitivity between the two risk subtypes were evaluated based on TIDE scores and drug response data from the GDSC database. Bladder cancer patients with lower risk scores were more sensitive to immunotherapy and drugs. Tumorigenesis is associated with mutations in oncogenes and defective immune surveillance [32]. Tumors have immunogenic properties similar to those of other pathogens while retaining several specific biological responses. Multiple immune cells are involved in antitumor immune processes. The survival of malignant cells in tissues and organs is usually determined by the state of the tumor microenvironment and the infiltration of immune cells [33–37]. In addition, individual or cellular heterogeneity, such as tumor mutational load, metabolic status, microbiome, and other specific characteristics, also have a critical impact on the outcomes of the tumor microenvironment and immunotherapy. In this study, we also analyzed the immune characteristic scores between high- and low-risk subtypes of bladder cancer and observed that these immune-related characteristics differed significantly between the two subtypes. Therapeutic prospects for patients with advanced disease are expanding as studies have attempted to link molecular signatures to response. Based on our analytical studies, we hypothesize that pain-related risk subtypes in bladder cancer patients could be used as patient screening indicators for immunotherapy and that patients in the low-risk subtype group for bladder cancer would be more suitable for immunotherapy.

This study has some limitations. The limited literature on the direct relationship between the two and the role of pain-related genes in tumorigenesis and progression is unclear. Furthermore, there is a lack of detailed clinical and experimental data to accurately assess whether pain-related genes have the potential to alleviate bladder cancer-related pain and predict prognosis as biomarkers. The risk score model needs to be further validated in multicenter clinical trials and prospective studies. In addition, our study did not include all human pain-related genes for analysis, and the number of genes was relatively small. The regulatory mechanisms of pain-related genes in bladder cancer remain ambiguous, and which is exactly what future work arising from this study should continue to explore.

Despite the above limitations, our study reveals associations between pain-related risk subtypes and prognosis, clinical information, and immune-related characteristics of bladder cancer patients. Staging of these patients based on pain-related genes may be an important prognostic marker. As an important part of subsequent research, we will continue to collect bladder cancer data and establish more accurate staging systems to provide the necessary basis for targeted treatment of bladder cancer.

By identifying bladder cancer subtypes through pain-related genes, we systematically analyzed the relationship between these subtypes and immune cells, immunotherapy/chemotherapy responses, and the corresponding pathways in the tumor microenvironment. These results provide a basis and reference for the clinical diagnosis and treatment of bladder cancer.

Author contribution statement

Yan Wang: Performed the experiments; Wrote the paper. Qingling Kong; Mingming Li: Analyzed and interpreted the data; Wrote the paper. Jing Gu; Jing Chen: Contributed reagents, materials, analysis tools or data. Lei Yang: Conceived and designed the experiments. Meng Chi: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Data availability statement

Data associated with this study has been deposited at https://github.com/wy678/BLCA.

Additional information

Supplementary content related to this article has been published online at [URL].

Declaration of competing interest

The authors declare no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e17690.

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