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Identification of prognostic and immunotherapy-related eRNA *ID2-AS1* in bladder cancer

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Abstract

Enhancer RNAs (eRNAs) can participate in enhancer regulation and target gene transcription, thus affecting the occurrence and development of tumors. In this study, we identified eRNAs closely related to bladder cancer (BLCA).

Gene expression profiles and clinical information from The Cancer Genome Atlas (TCGA) database were used in this study. The Atlas of Noncoding RNAs in Cancer (TANRIC) co-expression data was also studied to evaluate correlations between the inferred levels of eRNA and its predicted target genes. Moreover, we evaluated differences in tumor microenvironment between high and low *ID2-AS1* expression groups, and predicted the response of high- and low-expression groups to immune checkpoint inhibitor (ICI) treatment. Finally, we analyzed the prognostic value of *ID2-AS1* in different tumors.

ID2-AS1 and *ID2* were identified as eRNAs and target genes related to the prognosis of BLCA. Low *ID2-AS1* levels were associated with advanced age, low overall survival, high histological grade, and late BLCA staging. *ID2-AS1* appeared to regulate epithelial mesenchymal transition, mitotic spindle assembly, and angiogenesis, thereby affecting BLCA progression. The *ID2-AS1* high-expression group had better ICI treatment response. In addition, *ID2-AS1* also had prognostic value in other cancers.

ID2-AS1 helps predict prognostic and immunotherapeutic effects in BLCA.

Abbreviations: ACC = adrenocortical carcinoma, AJCC = American Joint Committee on Cancer, BRCA = invasive breast carcinoma, BLCA = bladder cancer, CESC = cervical squamous cell carcinoma and endocervical adenocarcinoma, COAD = colon adenocarcinoma, DEEs = differentially expressed eRNAs, eRNAs = enhancer RNAs, GBM = glioblastoma multiforme, GSVA = gene set variation analysis, ICI = immune checkpoint inhibitor, KICH = kidney chromophobe, LUAD = lung adenocarcinoma, LUSC = lung squamous cell carcinoma, LGG = brain lower grade glioma, MIBC = muscle-invasive bladder cancer, OS = overall survival, PAAD = pancreatic adenocarcinoma, PRAD = prostate adenocarcinoma, READ = rectal adenocarcinoma, TCGA = The Cancer Genome Atlas, TIDE = tumor immune dysfunction and rejection, TME = tumor microenvironment, UCEC = uterine corpus endometrial carcinoma, UCS = uterine carcinosarcoma.

Keywords: bladder cancer, enhancer RNAs, ICI therapy, immunotherapeutic, prognosis, tumor microenvironment

1. Introduction

Bladder carcinoma (BLCA) is the second-most common urological malignancy worldwide, with an estimated 83,730 new cases and 17,200 deaths in 2021.[1] Ninety percent of BLCAs are caused by malignant transformation of urothelial cells. Approximately 25% of patients with BLCA have muscle-invasive bladder cancer (MIBC) or metastasis, and 75% have non-muscle invasive bladder cancer (NMIBC).[2] If not diagnosed and treated early, NMIBC cells are likely to exhibit muscle invasion and metastasis. As a result, approximately 2/3 of advanced BLCA patients present with MIBC at their first clinical visit.[3] Half of these patients are reported to develop distant metastases within 2 years.[4] Once a tumor has developed to the metastatic stage, surgery combined with general chemotherapy are inadequate for treatment, resulting in a median survival of 14 months.[3,5] According to research, the response rate for novel immunotherapy and immune checkpoint inhibitors (ICIs) is 30% or less.[6] Therefore, there remains an urgent clinical demand for the discovery of novel molecular biomarkers by molecular profiling of BLCA cases.

With the development of high-throughput gene detection technology and the establishment of large-scale gene expression data sets, greater attention is being paid to long noncoding RNAs linked to BLCA.[7–9] The expression of lncRNAs in tissues and cell types has distinct specificity, making them potential cancer biomarkers.[7] Among these lncRNAs, enhancer RNAs (eRNAs) have attracted considerable attention because of their functions in mediating enhancer and gene transcription, and frequent overlap with noncoding risk gene loci associated with disease.[10–12] It has been shown in BLCA that downregulation of P2RY2e diminishes cancer promotion, which thus represents a potential therapeutic target.[13] Estrogen might promote the development of BLCA by inducing SMAD7e production.[14]

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The datasets generated during and/or analyzed during the current study are publicly available.

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Furthermore, high expression of *MARC1* eRNA is associated with malignant cell behavior.[15] To the best of our knowledge, only 3 studies have been conducted regarding BLCA-associated eRNAs.

In this study, we screened differentially expressed eRNAs (DEEs) in normal and tumor tissues, and explored prognostic eRNAs. *ID2-AS1* was found to be significantly associated with survival of patients with BLCA. High expression of *ID2-AS1* resulted in elevated purity of tumor cells in the tumor microenvironment (TME), which suggested that *ID2-AS1* affects the cellular composition of the TME and thus affects the progression and treatment of BLCA.[16,17]

2. Materials and methods

2.1. TCGA data analysis

We obtained RNA expression data for various cancers from The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/). At the same time, we also obtained clinical data (age, gender, pathological stage, etc.) and case survival information from TCGA database. To explore possible target genes regulated by eRNAs, we identified 1585 eRNAs and 2303 predicted target genes using the PreSTIGE algorithm. We also studied TANRIC co-expression data (https://bioinformatics.mdanderson.org/public-software/tanric) to assess correlations between the predicted levels of eRNA and predicted target gene expression. Among these eRNAs, only those that were significantly correlated with the overall survival (OS) rate ($P < .05$) and target gene expression levels ($R > .4$, $P < .001$) were considered as candidate key eRNAs in BLCA. Subsequently, we analyzed the relationship between the expression of eRNA and clinicopathological features of BLCA. In addition, we validated the prognostic value of the selected eRNA at the pan-cancer level in all 32 types of malignancies. All datasets in the present study were downloaded from public databases, thus ethical approval was not required.

2.2. Gene bioinformatics enrichment analysis

Gene set variation analysis (GSVA) uses standardized RNA-seq data from TCGA database. As an unsupervised gene set enrichment method, GSVA quantifies the pathway level through gene level quantification to analyze the difference in pathway levels between the *ID2-AS1* low- expression group and *ID2-AS1* high-expression cohort. We downloaded the "Hallmark" gene sets from the Molecular Signatures Database (http://software.broadinstitute.org/gsea/index.jsp) for GSVA through the package "GSVA" in *R*. The "MCPcounter" package in *R* was used for analysis of microenvironment cell populations (MCPs) and quantification of immune cells from transcriptomic data. The results were delivered by the package "pheatmap" in *R*. Subsequently, the package "limma" in *R* was used to identify pathways with the most significant differences between patients in the signature group.

2.3. Evaluation of immune cell infiltration in TME and immune checkpoints Inhibitor responses

We evaluated the enrichment of immune and stromal cell gene markers. We used the ESTIMATE algorithm to calculate immunity and matrix scores. In addition, we chose a new TME scoring algorithm to explore differences between high- and low-risk groups based on Immune-related gene pairs. The CIBERSORT algorithm was used to infer the relative proportion of 22 infiltrating immune cells in each sample. In addition, the tumor immune dysfunction and rejection (TIDE) algorithm was used to evaluate ICI treatment responses.

2.4. Statistical analysis

All analyses in this study were performed using *R* software (v.3.6.3); $P < .05$ was considered statistically significant.

3. Results

3.1. Identification of DEEs and prognostic eRNAs

A total of 1585 eRNAs and 2303 predicted target genes were identified using the PreSTIGE algorithm.[18] We extracted eRNA expression data from TCGA-BLCA dataset and found 379 DEEs. Next, according to TCGA-BLCA patient clinical prognostic information, we filtered out 53 of 379 DEEs that were significantly correlated with overall survival (Table 1, Kaplan–Meier

Table 1

53 eRNAs and their predicted target genes.

eRNA	KM	Target	cor	corPval
LINC01122	0.045473483	FANCL	0.40339467	1.63E-17
AC004920.1	0.007582194	GRB10	0.411626332	3.08E-18
RSRP1	0.002617455	RHD	0.412420081	2.61E-18
AC023632.2	0.005158216	DPY19L4	0.414415151	0
AC023794.2	0.002204621	HOXC4	0.420125572	5.25E-19
LINC01820	0.043439249	EPAS1	0.431815996	4.25E-20
AP004608.1	0.03042265	GLB1L2	0.438955464	8.70E-21
AL035587.1	0.029204762	GNMT	0.446748342	0
AC023790.2	0.014139072	HTR7P1	0.452870863	3.55E-22
AC091849.2	0.046824814	SDHAP3	0.46326598	2.95E-23
AL139246.2	0.006990563	TNFRSF14	0.465403499	1.75E-23
AP000424.2	0.010317937	RNF19A	0.465827163	1.58E-23
LINC01940	0.035013722	TWIST2	0.471246138	4.13E-24
ADCY10P1	0.009323816	NFYA	0.474857705	0
STEAP1B	0.036667699	IL6	0.489882296	3.39E-26
AC023632.2	0.005158216	RAD54B	0.493853437	0
BLCAP	0.005434537	NNAT	0.49918123	0
LINC01357	0.025161781	RHOC	0.509990406	1.36E-28
AL118511.1	0.004991953	C1orf198	0.514862257	3.36E-29
LINC01615	0.026431573	THBS2	0.521304029	5.14E-30
LILRP2	0.020981196	LILRB1	0.522919934	3.19E-30
LILRP2	0.020981196	LILRB4	0.524310011	2.11E-30
AP000424.1	0.014852208	RNF19A	0.527188751	8.93E-31
LINC02615	0.043961018	PGRMC2	0.527810686	0
ADCY10P1	0.009323816	UNC5CL	0.544762173	0
AP004608.1	0.03042265	B3GAT1	0.547522101	1.60E-33
AP000696.1	0.028988601	SIM2	0.556052693	9.92E-35
CROCCP2	0.007230662	NBPF1	0.57597325	0
LRRC3-DT	0.015702475	LRRC3	0.577134181	7.16E-38
AC025539.1	0.043428858	HS3ST1	0.578892178	3.83E-38
PCBP1-AS1	9.10E-05	TIA1	0.584023708	0
AP001628.2	0.036292825	PDE9A	0.592521677	2.59E-40
AC073316.2	0.03870831	SDK1	0.599887858	1.58E-41
BLCAP	0.005434537	SRC	0.601001813	0
LINC01357	0.025161781	PPM1J	0.608609553	5.22E-43
LILRP2	0.020981196	NCR1	0.623955584	9.95E-46
AC019117.2	0.012389065	AHR	0.650786839	7.26E-51
ZNF518A	0.007141589	CCNJ	0.657033866	0
AC087672.2	0.044452206	LY96	0.661537466	4.50E-53
ZFHX4-AS1	0.005136828	ZFHX4	0.67490614	5.99E-56
CDK6-AS1	0.004874166	CDK6	0.686888248	1.17E-58
LINC01357	0.025161781	SLC16A1	0.695766686	9.41E-61
ID2-AS1	0.003320138	ID2	0.710186992	2.53E-64
LEF1-AS1	0.033170247	LEF1	0.710507193	2.10E-64
TP53TG1	0.012233638	CROT	0.729915599	0
LNC-LBCS	0.045061	ID4	0.749029601	3.94E-75
TM4SF1-AS1	0.000265242	TM4SF1	0.763220081	1.37E-79
LINC01833	0.026044281	SIX3	0.771680093	2.11E-82
SLC44A3-AS1	0.023976707	SLC44A3	0.780605858	0
NR2F1-AS1	0.003963551	NR2F1	0.784468094	0
AP001189.3	0.014542547	LRRC32	0.784789791	5.25E-87
AC105942.1	0.016728123	CNN3	0.787381061	0
AL162411.1	0.016024105	GLDC	0.79269484	6.09E-90

log-rank test, $P < .05$). *ID2-AS1* was closely related to the prognosis of bladder cancer ($P = .003$), and positively correlated with *ID2* ($R = .71$). In addition, *ID2* has been shown to play an important role in a variety of tumors. So we chose *ID2-AS1* in this study.

3.2. LncRNA ID2-AS1 is a key eRNA in BLCA

In tumors, the expression level of lncRNA *ID2-AS1* was lower than that in normal tissues (Fig. 1A). In addition, in patients with BLCA, the *ID2-AS1* high-expression group showed better overall survival than the low-expression group (Fig. 1B). There is a strong correlation between ID2 level and predicted target gene ID2-AS1 expression (Fig. 1C). Moreover, the summary of clinical characteristics of TCGA-BLCA was shown in Table 2. ID2-AS1 expression analysis was performed for different clinical feature subclasses (age, gender, WHO grade, American Joint Committee on Cancer (AJCC) stage, and TNM stage). We observed that patients with advanced BLCA (high WHO grade, high AJCC stage, and MIBC) had lower expression levels (Fig. 2A-G). Subsequently, OS curves using combinations of different clinical features and *ID2-AS1* expression levels showed that lower expression levels were associated with poorer OS in patients aged > 60 years, AJCC-Stage I-II, T0-2 stage, and N0 stage subgroups (Figure 3A-D).

3.3. Multiple aggressive, immunosuppressive, and angiogenic hallmarks related to low ID2-AS1 expression

To investigate differentially expressed features between *ID2-AS1* high- and low- expression groups, we performed GSVA of the TCGA-BLCA cohort. Notably, gene sets related to tumor necrosis factor-α signaling via nuclear factor-κB, Myc targets, mTOR complex 1 signaling, epithelial mesenchymal transition, mitotic spindle, and angiogenesis signaling pathways were upregulated in the *ID2-AS1* low-expression group (Fig. 4A-B).

3.4. Differences in TME between ID2-AS1 high- and low-expression groups, and prediction of whether ID2-AS1 expression is correlated with ICI therapeutic responses

The ESTIMATE algorithm was used to evaluate tumor purity for each patient. There was a significant difference in tumor purity between the high- and low-expression groups ($P < .001$, Figure 5A). Furthermore, the TIDE algorithm, which was established to predict immunotherapy responses through transcriptomic data, was used to explore whether the expression level of *ID2-AS1* could predict immunotherapeutic benefit in the TCGA-BLCA cohort. The results for each patient are shown in Figure 5B. These results revealed that the percentage of ICI therapy responders was significantly higher for the high-expression group (44%) compared to the low *ID2-AS1* expression group (30%) (Chi-squared test, $P = .00367$; Figure 5B). *ID2-AS1* expression was robustly positively correlated with ICI therapeutic response in BLCA patients.

To further characterize the levels of different immune cell subsets in the TME and chemotactic signals involved, we employed the microenvironment cell population-counter (MCP - counter) method, which allows for robust quantification of the absolute abundance of 8 immune and 2 stromal cell populations in heterogeneous tissues from transcriptomic data[19] (Figure 5C). As shown in Figure 5D, compared to the *ID2-AS1* low-expression group, cytotoxic T-lymphocytes and T cells were upregulated in the high-expression group ($P < .001$ and $P < .05$). Strikingly, fibroblasts, a type of stromal cell, were downregulated in the high-expression group ($P < .001$).

3.5. Pan-cancer perspective of ID2-AS1 expression profiles and diagnostic/prognostic potential

Differences in expression of *ID2-AS1* were analyzed in 24 different cancer types in TCGA (Fig. 6). We found that the most prevalent gene expression changes involved downregulation, as opposed to upregulation, in tumor tissue, such as in BLCA ($P < .001$), invasive breast carcinoma (BRCA, $P < .0001$), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC, $P < .05$), colon adenocarcinoma (COAD, $P < .0001$), glioblastoma multiforme (GBM, $P < .05$), kidney chromophobe (KICH, $P < .0001$), lung adenocarcinoma (LUAD, $P < .0001$), lung squamous cell carcinoma (LUSC, $P < .0001$), prostate adenocarcinoma (PRAD, $P < .0001$), rectal adenocarcinoma (READ, $P < .0001$), thymoma (THYM, $P < .0001$), and uterine corpus endometrial carcinoma (UCEC, $P < .01$) compared to normal tissue. In addition, the expression of *ID2-AS1* was independently analyzed for its ability to predict OS in 32 different cancer types using tumor sample data from TCGA. Each cancer type was considered to be independent when examining ability of ID2-AS1 expression to predict OS. Hazard ratios and *P*-values for the 32 cancer types are shown in Figure 7A. Of these, significant survival differences were detected for adrenocortical carcinoma (ACC, $P = .008$), BLCA ($P = .003$), mesothelioma (MESO, $P = .023$), brain lower grade glioma (LGG, $P < .001$), uterine carcinosarcoma (UCS, $P = .007$), and pancreatic adenocarcinoma (PAAD, $P = .013$). Better OS was associated with high *ID2-AS1* expression in BLCA, MESO, LGG, UCS, and PAAD (Figure 7B).

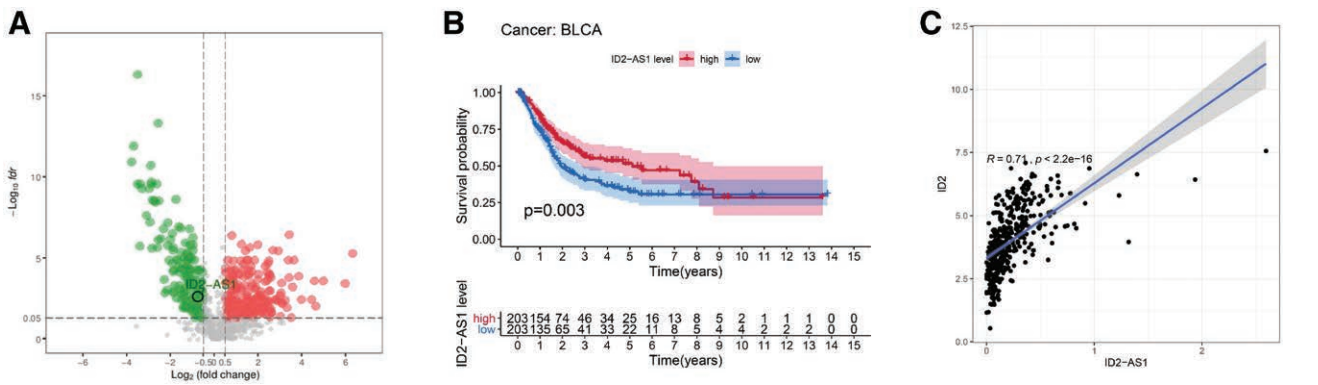

Figure 1. Expression of ID2-AS1 in different tissues. (A) Expression of ID2-AS1 in normal and tumor tissues, (B) Kaplan–Meier curves of overall survival according to IRL-signature groups in the TCGA cohort. (C) Correlation analysis between ID2-AS1 and its prediction target ID2.

Table 2

Summary of clinical characteristics of TCGA-BLCA patient data sets in the study.

Characteristic	TCGA-BLCA data set (n = 406)
Vital status, n (%)	
Alive	226 (55.7)
Dead	180 (44.3)
Age, n (%)	
<60	86 (21.2)
≥60	320 (78.8)
Grade, n (%)	
Low grade	21 (5.2)
High grade	382 (94.1)
Unknown	3 (0.7)
Gender, n (%)	
Female	106 (26.1)
Male	300 (73.9)
WHO-Stage, n (%)	
I	2 (0.5)
II	129 (31.8)
III	140 (34.5)
VI	133 (32.7)
Unknown	2 (0.5)
AJCC-T stage, n (%)	
T0	1 (0.2)
T1	3 (0.7)
T2	118 (29.1)
T3	193 (47.5)
T4	58 (14.3)
Unknown	33 (8.1)
AJCC-N stage, n (%)	
N0	236 (58.1)
N1	45 (11.1)
N2	75 (18.5)
N3	8 (2.0)
Unknown	42 (10.3)

4. Discussion

In recent years, studies have found that eRNAs regulate gene expression by promoting the enhancer promoter chain, and knockout of eRNAs is usually accompanied by a decrease in target gene expression. A large body of evidence shows that abnormal expression of eRNAs in various cancers is closely related to tumorigenesis. In various types of cancer, eRNAs may modulate the occurrence and progression of tumor cells by regulating certain signaling pathways. However, few researchers have examined BLCA prognosis-related eRNAs. Our study provides insights into the potential role of *ID2-AS1* in tumor immunology and its use as a new BLCA-related molecular biomarker.

In our study, we searched for eRNAs related to BLCA prognosis. Through a series of comprehensive analyses, we found that *ID2-AS1* and its corresponding target gene, *ID2*, had a significant impact on survival of BLCA patients. Therefore, we regarded *ID2-AS1* as a primary eRNA in BLCA. The results showed that low expression of *ID2-AS1* represented an independent prognostic factor in patients with BLCA. We further evaluated the clinical characteristics and relationships between *ID2-AS1* expression and BLCA. Interestingly, low expression of *ID2-AS1* predicted advanced tumor staging better, and the same results were also found in different clinical subgroup analyses. As an important member of the DNA-binding inhibitor family, *ID2* has been shown in previous studies to play key roles in the growth and invasion of various tumor cells.[20,21] Upregulation of *ID2* indicates poor prognosis of BRCA patients after breast-conserving surgery.[21] In head and neck squamous cell carcinoma, *ID2* is a new molecule involved in the regulation of tumor cell invasion and lymph node metastasis.[20] These findings coincide with the results of our pan-cancer analysis.

Subsequently, the results of GSVA revealed that there were significant differences in biological behavior between the *ID2-AS1* high-expression group and the *ID2-AS1* low-expression group. Compared to the high-expression group, the low-expression cohort scored higher in epithelial mesenchymal transformation, mitotic spindle formation, and angiogenesis signaling pathways. There is evidence that these signals are closely related to the occurrence and development of tumors. In a study by Zhou et

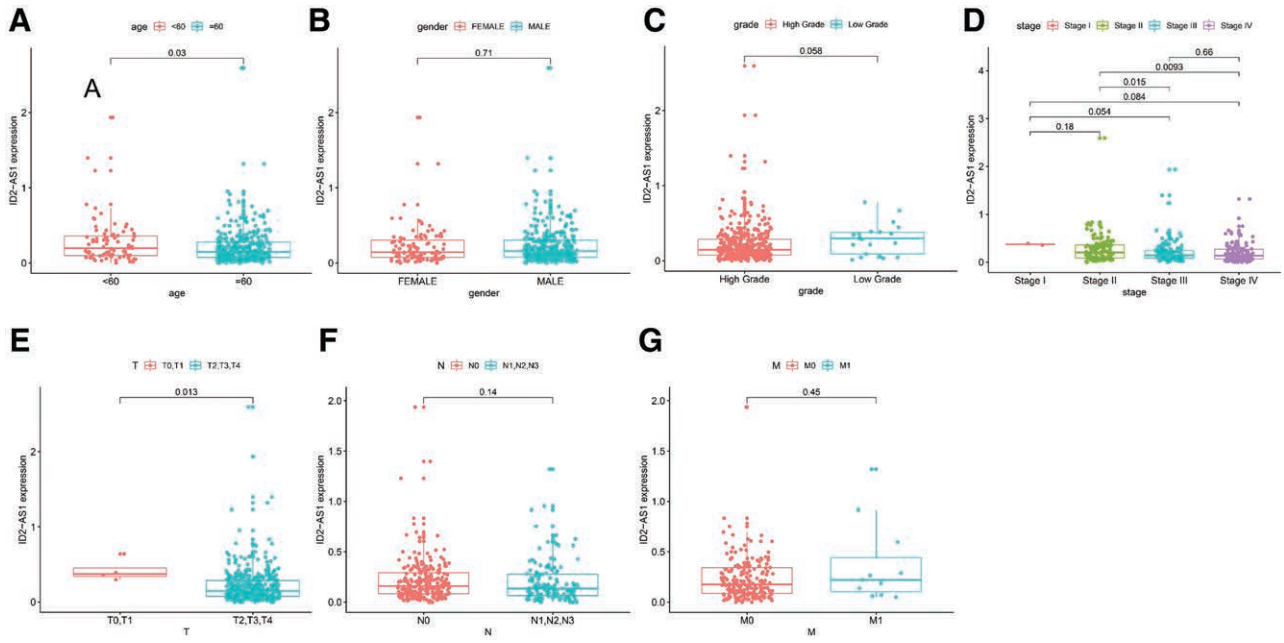

Figure 2. Analyze ID2-AS1 expression in different clinical features. Kaplan–Meier curves analyses of clinical subgroups in TCGA cohort: (A) Age < 60 years, (B) Gender, (C) WHO Grade: high, (D) AJCC Stage: Stage I, II, III, IV, (E) AJCC-T stage: T0-1, T2-4, (F) AJCC-N stage: N0, N1-3, (G) AJCC-M stage: M0, M1. AJCC = American Joint Committee on Cancer, WHO = World Health Organization; .

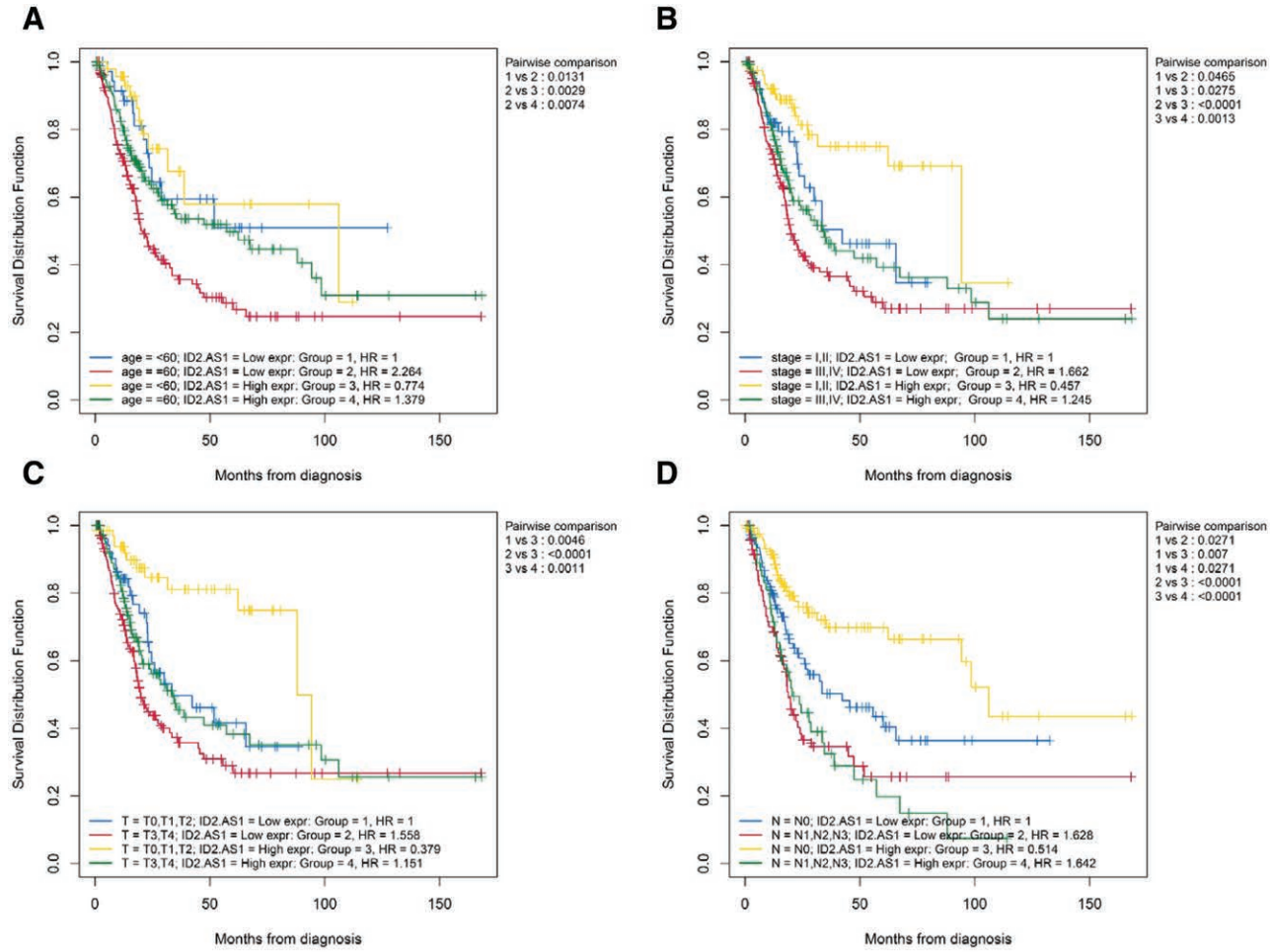

Figure 3. Evaluate whether lncRNA signature is an independent prognostic factor and relationship between risk score and clinical characteristics. Two factors Kaplan–Meier curves of overall survival for patients in TCGA cohort stratified by IRL-signature, age (A), AJCC Stage (B), AJCC-T stage (C), and AJCC-N stage (D).

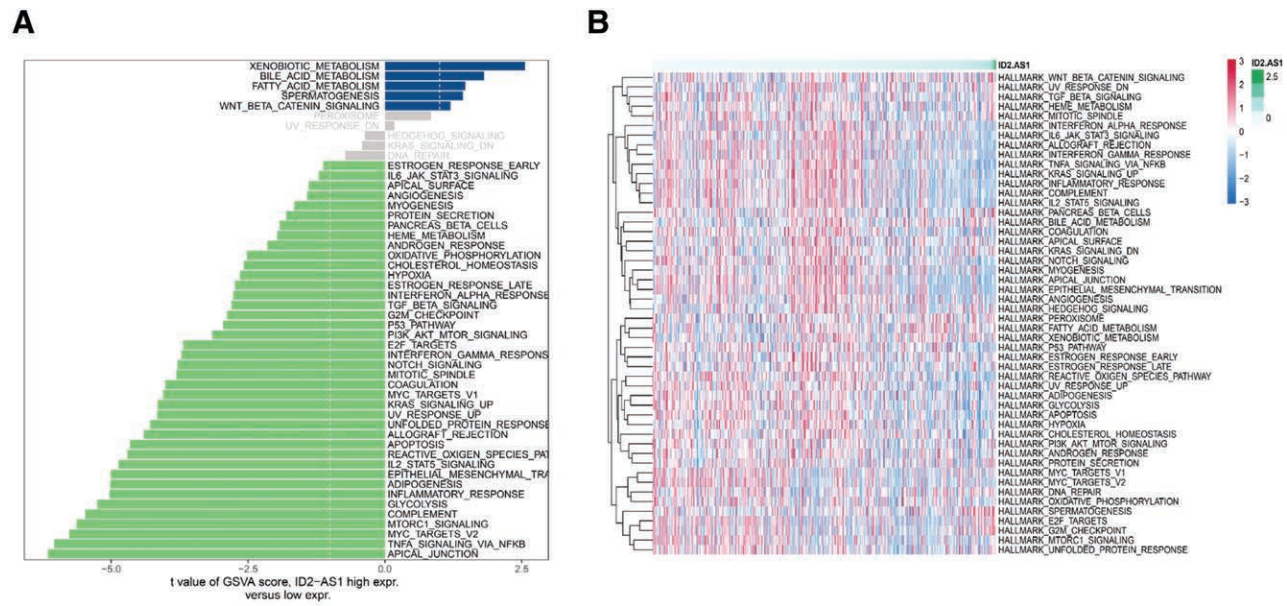

Figure 4. Biological function of 2 groups of patients. (A) Bar plot of "Hallmark" pathway score calculated by GSVA for 2 groups of patients. Heat map of GSVA analysis between 2 risk groups. (B) Heat map of most valuable pathway.

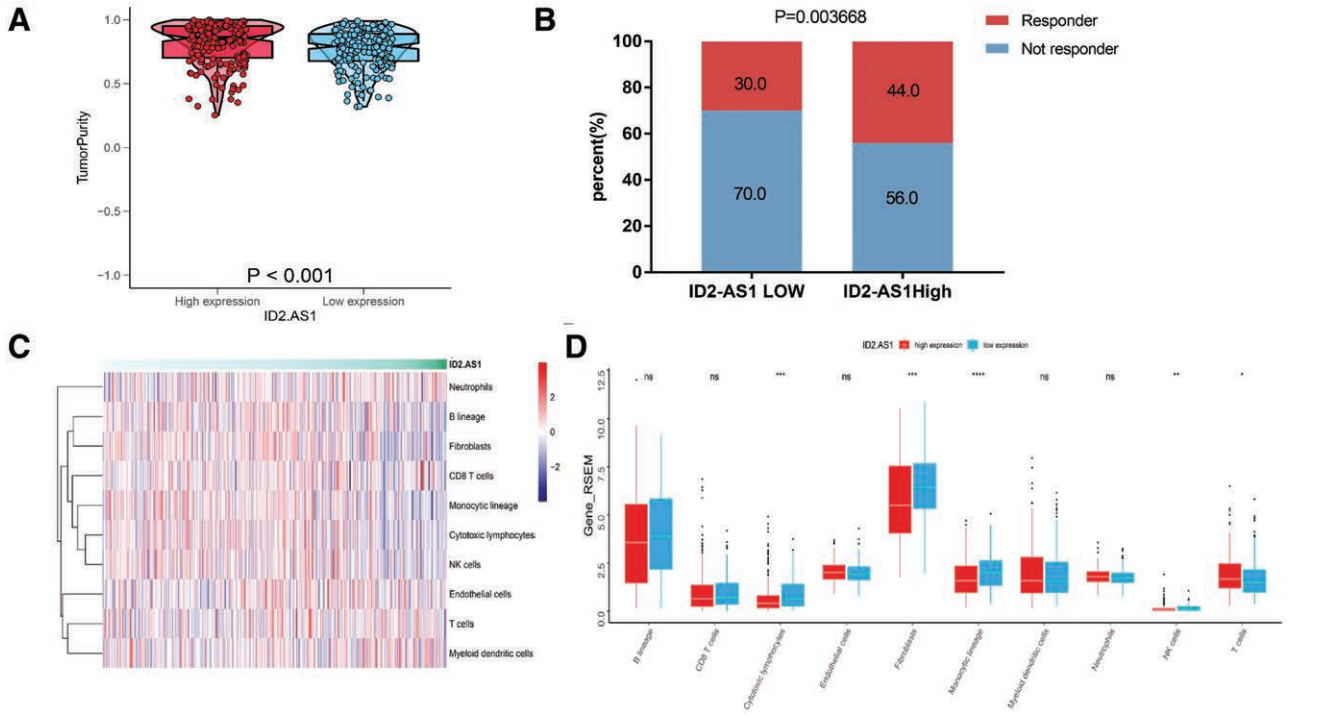

Figure 5. ID2-AS1 may affect the cellular composition of TME and thus affect the immunotherapy of BLCA. (A) ESTIMATE algorithm was used to evaluate tumor purity for each patient. Assess the difference in immune infiltration between the 2 groups. (B) IRL-signature was efficient in prediction the immunotherapeutic benefit in BLCA. (C) Differences of 10 cell abundances calculated by MCP-counter method between 2 groups of patients. (D) Box plot of ten gene sets calculated by MCP-counter. ns: $P \geq .05$, $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$, $^{****}P < .0001$.

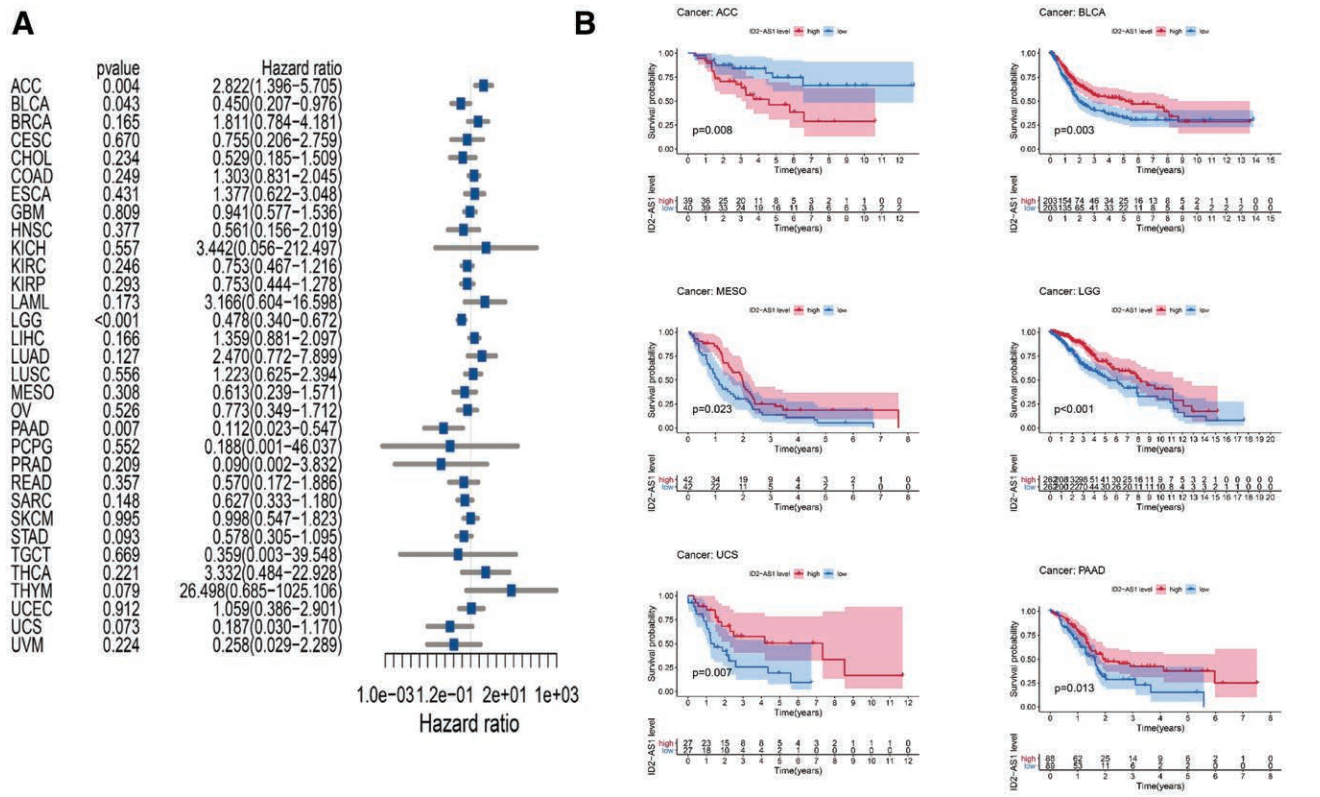

Figure 6. Gene expression differences for ID2-AS1 were analyzed in 24 different cancer types. ns: $P \geq .05$, $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$, $^{****}P < .0001$.

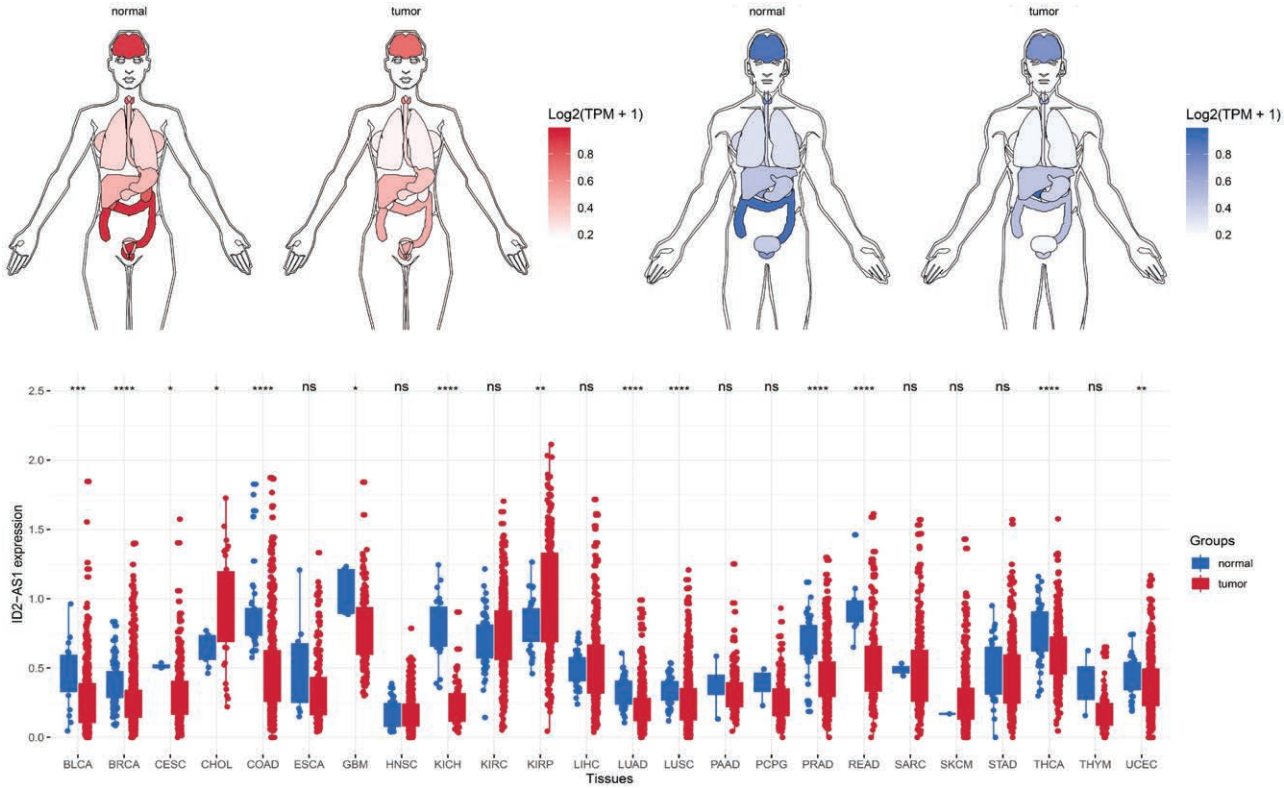

Figure 7. Identification the prognostic effect of ID2-AS1 in pan-cancer. (A) Correlation of ID2-AS1 expression and Hazard ratio in pan-cancer. (B) ID2-AS1 expression downregulation was significantly correlated with poorer overall survival in BLCA, MESO, LGG, UCS and PAAD. However, ID2-AS1 expression downregulation was significantly correlated with better overall survival in ACC.

al, *ID2-AS1* and *ID2* inhibited twist-induced epithelial mesenchymal transformation in hepatocellular carcinoma cells to inhibit tumor metastasis,[22] which is consistent with our results.

Tumor tissues are mainly composed of tumor cells, stromal cells, and immune cells. Tumor purity represents the proportion of tumor cells in the tumor tissue. Interestingly, in studies of glioma, gastric cancer, and colon cancer, patients with low tumor purity tended to have higher malignancy and poorer prognosis.[23–25] In our study, the *ID2-AS1* high expression group had a higher tumor density and better OS. TME immune cell infiltration is considered an important and unpredictable phenomenon to predict the prognosis of various cancers and immunotherapeutic responses. The difference analysis of immune cell infiltration showed that the infiltration abundance of CTLs and T cells in the high expression group was significantly higher, and the infiltration abundance of fibroblasts in the low expression group was higher, which is consistent with the results of GSVA to a certain extent. We used the TIDE algorithm to predict the response of patients with BLCA to immunotherapy. It was encouraging that patients in the high expression group are more likely to benefit from immunotherapy.

The significant advantage of our research is the comprehensive analysis conducted, which revealed that *ID2-AS1* is an independent prognostic factor for BLCA. We also predicted the relationship between high expression of *ID2-AS1* and positive ICI treatment, and found that it is associated with OS in many tumors. Our research has potential value in future tumor prognosis and immunotherapy. However, we did not verify the prognostic ability of *ID2-AS1* in the external database; therefore, the predictive value of *ID2-AS1* in terms of immunotherapeutic responses needs to be verified in future experiments. Other researchers can use this study as a cornerstone to further reveal the cellular mechanisms involving *ID2-AS1* in the occurrence and development of various tumors.

5. Conclusions

In conclusion, *ID2-AS1* is an independent protective factor in BLCA, and may become a potentially powerful tool to improve the diagnosis and prognosis of BLCA patients. Hence, *ID2-AS1* is a potential promising immune-related therapeutic target for the precise treatment of a variety of malignant tumors in the future.

Author contributions

Conceptualization: Jie Liu, Lei Zhang.
Data curation: Jie Liu.
Formal analysis: Jie Liu.
Investigation: Jie Liu.
Methodology: Jie Liu, Lei Zhang.
Resources: Junfeng Liu.
Software: Junfeng Liu.
Supervision: Jianjun Liu, Degang Ding.
Validation: Ning Wang.
Writing - original draft: Lei Zhang.
Junfeng Liu, Ning Wang, Jie Liu.
Writing - review & editing: Lei Zhang, Junfeng Liu, Ning Wang, Jie Liu.

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