ORIGINAL RESEARCH

Identification of FADS1 Through Common Gene Expression Profiles for Predicting Survival in Patients with Bladder Cancer

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Purpose: Aim of this study was to identify biomarkers between different grades of bladder cancer (BLCA) and its prognostic value.

Methods: mRNA expression data from GSE32549 and GSE71576 were extracted for further analysis. Differentially expressed genes (DEGs) were identified using GEO2R web tool. Gene ontology (GO) analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis and protein–protein interaction (PPI) network were conducted to explore the function and relationship of DEGs. The Cancer Genome Atlas (TCGA) database was used for external validation and Gene set enrichment analysis (GSEA) analysis was used to further identify FADS1 pathways. Bladder cancer cells and patient specimens were used to further demonstrate the function of FADS1.

Results: Datasets from GEO identified a panel of DEGs. Functional enrichment analysis highlighted that DEGs were associated with nuclear division, spindle, cell cycle and p53 signaling pathway. External validation from TCGA demonstrated that FADS1 was an independent prognostic marker in BLCA patients. In cell lines and tumor specimen analysis, FADS1 was overexpressed in the tumor specimen, compared with adjacent tissues, and positively correlated with tumor grade of BLCA. Moreover, FADS1 could enhance the proliferation ability and influence cell cycle of bladder cancer cells.

Conclusion: FADS1 was an independent prognostic biomarker for BLCA and could confer the bladder cancer cells increased proliferation ability.

Keywords: FADS1, bioinformatics, bladder cancer, TCGA, proliferation

Introduction

Bladder cancer (BLCA) is the 10th most common form malignancy, with an estimated about 549,393 new cases and 199,922 deaths per year worldwide.[1] BLCA is more common in men than in women, the respective incidence and mortality among men is reported to be approximately fourfold that among women. Among the urogenital system cancers, BLCA ranked second both in incidence and mortality.[1] Approximately 75% patients with BLCA present with non-muscle-invasive bladder cancer (NMIBC) which include Ta, T1 and Cis tumor stage and 25% as muscle-invasive bladder cancer (MIBC) which include T2-T4 tumor stage.[2] NIMBC is rarely lethal compare to MIBC, but up to 70% patients with NIMBC will recur, 10–15% will progress to MIBC and 5-year survival rate is about 90%.[2,3] As for MIBC, the 5-year survival rate is less than 50%.[3]

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Current European Association of Urology (EAU) recommendations that evaluation of grading in BLCA could use both the 1973 World Health Organization (WHO) and the 2004/2006 WHO classifications.[4] With the improvement in 2004 system, the grading of tumor could be classified into three kinds: papillary urothelial neoplasms with low malignant potential (PUNLMP), low grade (LG) and high grade (HG). This change could standardize the uniform terminology for both pathologists and urologists.[5,6] It has been demonstrated that tumor grade is an important predictor of BLCA prognosis.[7] Precise prediction of prognosis is important for patients to determine appropriate therapy and individual follow-up plan. Despite comprehensive gene expression screening and landscape of molecular alternations have been explored for decades, there are still no reliable biomarkers for early detection or prognosis of BLCA.[8]

Fatty acid desaturase 1 (FADS1) is a member of the fatty acid desaturase gene family.[9] Recent studies have demonstrated that FADS1 plays an essential role in fatty acid metabolism by regulating hepatic fatty acid deposition by altering gene transcription and lipid composition.[10] More evidence had revealed that fatty acid metabolism has been associated with tumorigenesis and progression.[11,12] Wang et al reported decreased FADS1 is correlated to the larger tumor size, poorer tumor differentiation and shorter overall survival (OS) in non-small-cell lung cancer.[13] And reduced FADS1 expression could predict a poor prognosis in esophageal squamous cell carcinoma.[14] However, the correlation between the expression level of FADS1 and BLCA prognosis has not been investigated.

With the development of high-throughput sequencing technology and established. The Cancer Genome Atlas (TCGA) program to map the genome expression profiles. In this study, we identified differentially expressed genes (DEGs) between different grade BLCA groups, and evaluated the prognosis value. Thus, we identified FADS1 is an independent prognostic factor for predicting OS in patients with BLCA.

Materials and Methods
Microarray Data

The gene expression profiles of GSE32549 and GSE71576 were obtained from the National Center of Biotechnology Information (NCBI) GEO database (GEO, http://www.ncbi.nlm.nih.gov/geo/). The GSE32549, which was based on GPL6947 Illumina HumanHT-12 V3.0 expression

beadchip was uploaded by Lindgren D et al. The GSE71576, which was based on GPL6244 Affymetrix Human Gene 1.0 ST Array was uploaded by Pineda S et al. Totally 56 G1 and G2 bladder cancer samples and 75 G3 bladder cancer samples were enrolled in GSE32549, while 25 G1 and G2 bladder tumor samples and 17 G3 bladder tumor samples were enrolled in GSE71576.

Data Processing and Identification of DEGs

GEO2R was performed to analyze the raw data of microarrays and identify DEGs by comparing two groups (G3 vs G1/G2) of samples (https://www.ncbi.nlm.nih.gov/geo/geo2r/). The fold change (FC) >2 ($|\log2FC| \geq 1$) and adj. P.Values <0.05 were used as the threshold to identify DEGs. Volcano plots and heat maps were generated using the R software package ggplot2 and pheatmap, respectively. The Funrich software was used to analyze the overlapping components.

Functional and Pathway Enrichment Analysis

As previously described,[15] The clusterProfiler package was used to perform Gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. An FDR of < 0.05 was used as the cut-off value.

Protein–Protein Interaction (PPI) Network Construction

PPI network analysis is essential to identify the interaction among DEGs. The online Search Tool the Retrieval of Interacting Genes (STRING) database (http://www.string-db.org)[16] and Cytoscape software[17] (Version 3.4.0, http://www.cytoscape.org/) were used to reconstruct a PPI network. The network view summarizes the network of predicted associations for DEGs. The cut-off criterion of interaction confidence score was set as >0.4. The important nodes and hub genes in the network were predicted and explored by CytoHubba, a plugin of Cytoscape, and the top 10 genes were selected from the results of each method.

Validation of the Hub Genes in TCGA Database

The gene expression profiles and clinical data of patients with BLCA using the Illumina RNA Sequencing method

were extracted from the TCGA data portal (https://tcga data.nci.nih.gov/tcga/). The Kaplan–Meier method was used to evaluate the survival rates in different groups, and Kaplan-Meier curves for different genes in high and low tumor grade groups were plotted using the "survfit" function from the R package "survival". Calculated using the "survdiff" function, the equivalences of the survival curves were tested by Log rank tests, and a p-value of <0.05 was considered statistically significant.

Gene Set Enrichment Analysis (GSEA) Analysis

GSEA was used to further understand FADS1-related pathways. The expression level of FADS1 was used as the phenotype annotation and BLCA patients in the TCGA cohort were dichotomized into low and high categories based on the median expression value of FADS1. GSEA version 3.0 software (www.broadinstitute.org/gsea) was used to analyze data. The Molecular Signatures Database (MSigDB) of c2 (c2.cp.kegg. v6.2.symbols.gmt) was used to assess the functional differences between the low and high FADS1 expression groups.[18,19] FDR < 0.01 was used as the cut-off criteria.

Cell Culture

All cells were obtained from ATCC. T24, 5637 and 253J cells were cultured in RPMI-1640 medium (Gibco) containing 10% FBS (Gibco). RT4 cells were cultured in McCoy 5A (Gibco) with 10% FBS, and SV-HUC-1 cells were cultured in F12K medium (Gibco) with 10% FBS.

Plasmid DNA Transfection and RNA Interference

Cells at 70% confluency were transfected with over-expressed FADS1 vector. Cell at 30% confluency were transfected with siRNA. siFADS1-1: 5′-CCGACATCA TCCACTCACTAA-3′,siFADS1-2:5′-GTCCGCTTCTTC CTCACTTAT-3′. The transfection assay was conducted with Lipofectamine 2000 (Invitrogen) reagent according to the manufacturer's protocol.

Cell Proliferation and Colony Formation Assays

Cell proliferation assay was carried out by using the MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide) assay. In brief, cells were seeded in 96-well cell culture plate overnight for attachment. And MTT regent solution was added into each well and incubated for 4 hours at 37°C. The cells were re-suspended in 100μL DMSO and the absorbance was determined at wavelength 570nm using microplate reader.

For colony formation assay, 1×10^3 cells were planted into each well of six-well plate at 37°C for 10–14 days. Colonies were fixed with methanol and stained by Giemsa. Colonies which were containing more than 50 cells were counted as one.

RNA Isolation and Quantitative RT-PCR

RNA isolation and quantitative RT-PCR (qRT-PCR) were performed according to the manufacturer's instruction. The cDNA was reverted by using RevertAid Reverse Transcriptase (Thermo) according to the manufacturer's instructions. qRT-PCR was performed by FastStart Essential DNA Green Master (Roche). GAPDH was used as an internal reference.

Western Blot and Antibodies

Cells were lysed in RIPA buffer (Beyotime). The samples were boiled at 95°C for 5 minutes. The proteins were separated by 10% SDS-PAGE gel, and transferred onto PVDF membranes by electroblotting for 60 minutes. The blots were blocked with containing 5% non-fat dry milk for 1 h at room temperature and then incubated at 4°C overnight with primary antibodies. After incubation secondary antibody at room temperature for at least 1 hour, the blots were detected by ECL and visualized by hyperfilm ECL film. The primary antibodies were against FADS1 (1:1000, Proteintech, 10627-1-AP), and Actin (1:2000, Proteintech, 20536-1-AP).

Patients and Tumor Samples

Five bladder cancer and paired adjacent tissue samples were obtained from surgically removed tissues. And the pathology was confirmed as urothelial bladder cancer. All clinical experiments in our study were conducted in accordance with the Declaration of Helsinki. This study was approved by the Institutional Ethics Committee of Shandong University Qilu Hospital (Qingdao) (KYLL-2019010), and informed consent was obtained from each patient.

Immunohistochemistry (IHC) Analysis

Slides were incubated in xylene and different concentrations of ethanol, and antigen retrieval was performed. The

slides were then blocked with hydrogen peroxide for 30 min and 5% goat serum for 1 hour. The tissues were incubated with primary antibody against FADS1 at 4°C overnight, and then a biotinylated secondary antibody and ABC Peroxidase Staining Kit (ZSGB-Bio) were used to examine FADS1, then the tissues were stained with haematoxylin.

EdU Incorporation Assay

EdU (5-ethynyl-2′-deoxyuridine) incorporation assay was performed using the Cell-Light EdU DNA Cell Proliferation Kit (RIB-Bio) according to the manufacturer's instructions.

Flow Cytometry Analysis

For cell cycle assay, the cells were collected by trypsin and fixed with 75% cold ethanol overnight at −20°C, then the samples were washed with PBS once and stained with propidium iodide and RNase for 15 min at room temperature. The cell cycle distribution was analyzed by flow cytometry.

Statistical Analysis

The Statistical Package for Social Science (SPSS version 23.0) program was performed in this study. And the Cox proportional hazards regression model was applied in univariate and multivariate analyses. Correlation analysis was assessed by using chi-square test and Pearson's correlation coefficient test. The two-tailed p-value was used in this study, and a p-value of <0.05 considered statistically significant (* p< 0.05, ** p< 0.01, *** p<0.001 and **** p<0.0001).

Results

Identification of DEGs and Heat Map Clustering Analysis

Gene expression profiles from GSE32549 and GSE71576 were conducted to analyze DEGs by using GEO2R tool. Genes were considered as DEGs according to their log2FC and adj.P.Values. The results showed that 251 genes (152 up-regulated and 99 down-regulated genes) were identified to be differentially expressed in GSE32549, and 255 genes (181 up-regulated and 74 down-regulated genes) differentially expressed in GSE71576 (Figure 1A–D). We conducted an overlapping analysis to clarify the exactly down-regulated or up-regulated DEGs. As shown in Figure 1E, a total of 99 DEGs were identified to be associated with BLCA tumor grade in two datasets, with 29 down-regulated and 70 up-regulated overlapped DEGs.

GO and Signaling Pathway Enrichment Analysis of DEGs

Then, 99 co-DEGs were used for further GO and KEGG pathway enrichment analysis. GO analysis included three different domains, named biological process (BP), cellular component (CC) and molecular function (MF). First, we analyzed all co-DEGs, genes associated with BP were mainly involved in nuclear division. The most aspects of CC were related to spindle and chromosome region. Moreover, DEGs in MF were significantly enriched in histone kinase activity (Figure 2A). Then we analyzed 70 up-regulated DEGs, and genes associated with BP, CC and MF were mainly involved in chromosome segregation, spindle and chromatin binding, respectively (Figure S1A).

The KEGG pathway enrichment analysis was conducted to further investigate the functional implication of DEGs. As shown in Figure 2B, DEGs were enriched in several tumor-related pathways. The most significantly enriched key pathway was cell cycle. Cellular senescence and p53 signaling pathway are also important pathways between different grades of bladder cancer. Meanwhile, KEGG enrichment analysis including 70 DEGs showed that cell cycle, cellular senescence and p53 signaling pathway were highly associated with up-regulated DEGs (Figure S1B).

PPI Networks Construction and Cluster Identification

To clarify the interaction of DEGs data, PPI network construction was conducted by using STRING database and visualized using Cytoscape software. The PPI network included 98 nodes and 1422 interactions (Figure 3). The top 10 hub nodes were identified, including CDKN3, ANLN, MELK, BUB1, CCNB2, NEK2, MAD2L1, CEP55, CDK1, UBE2C.

External Validation of the Genes in TCGA

We further analyze 99 overlapped DEGs by using the TCGA database. The clinicopathologic characteristics of 405 patients were shown in Supplementary Table 1. The median age (range) of BLCA patients in TCGA was 68.08 (50–90). One hundred and six patients were female and 299 were male. Tumor grade was low in 21 patients and
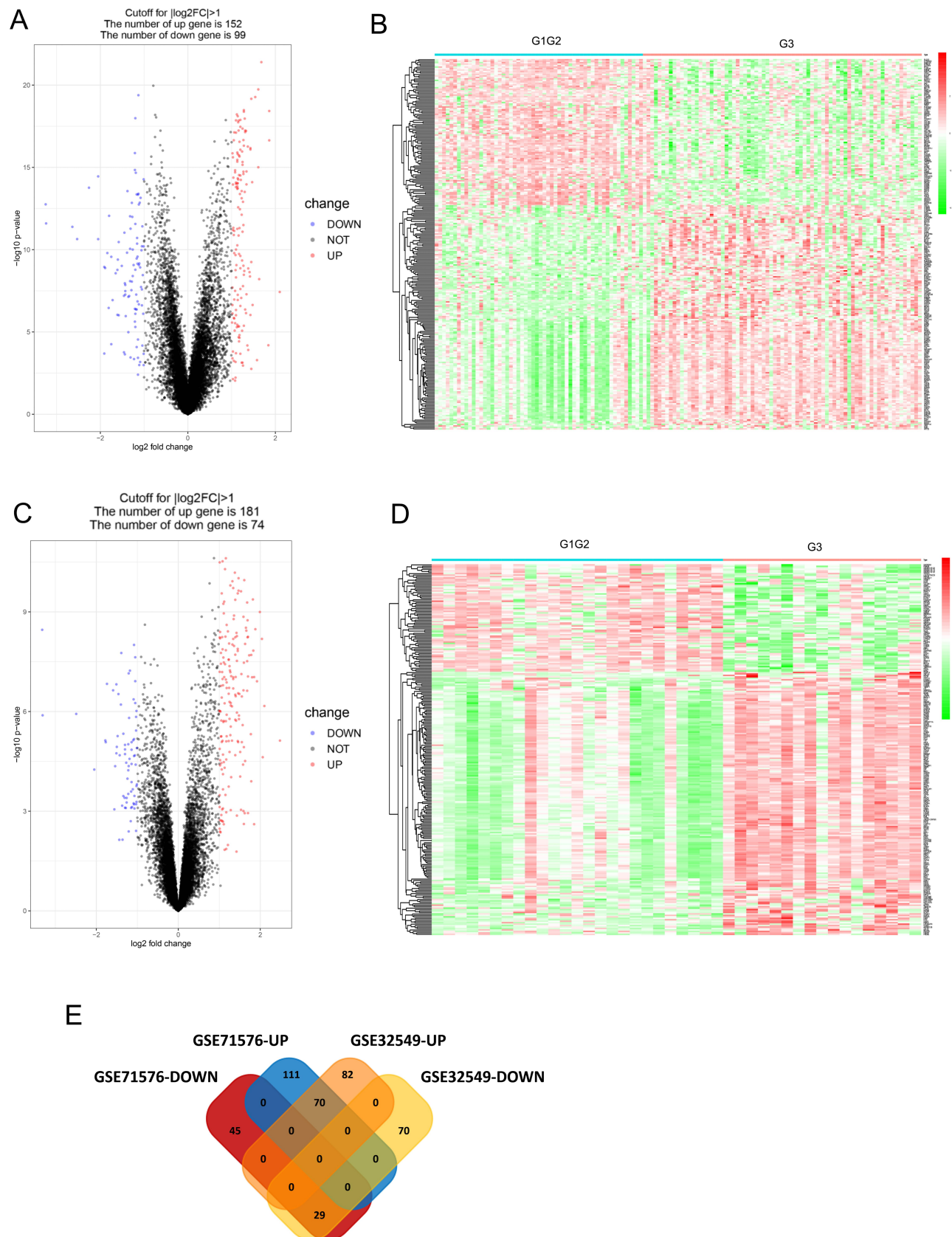
A

Cutoff for |log2FC|>1
The number of up gene is 152
The number of down gene is 99

B

G1G2   G3

C

Cutoff for |log2FC|>1
The number of up gene is 181
The number of down gene is 74

D

G1G2   G3

E

GSE71576-UP   GSE32549-UP
GSE71576-DOWN   GSE32549-DOWN

111   82
0   70   0
45   0   0   70
0   0   0
0   0
29

Figure 1 Identification of expression differences between high grade and low grade tumors. (**A**) Volcano plot of the differential mRNA expression analysis in GSE32549. (**B**) Heat-map overview of the differentially expressed genes in GSE32549. Red: up-regulation; Green: down-regulation. (**C**) Volcano plot of the differential mRNA expression analysis in GSE71576. (**D**) Heat-map overview of the differentially expressed genes in GSE71576. Red: up-regulation; Green: down-regulation. (**E**) The result of Venn diagram. The cross areas represented the commonly changed DEGs.
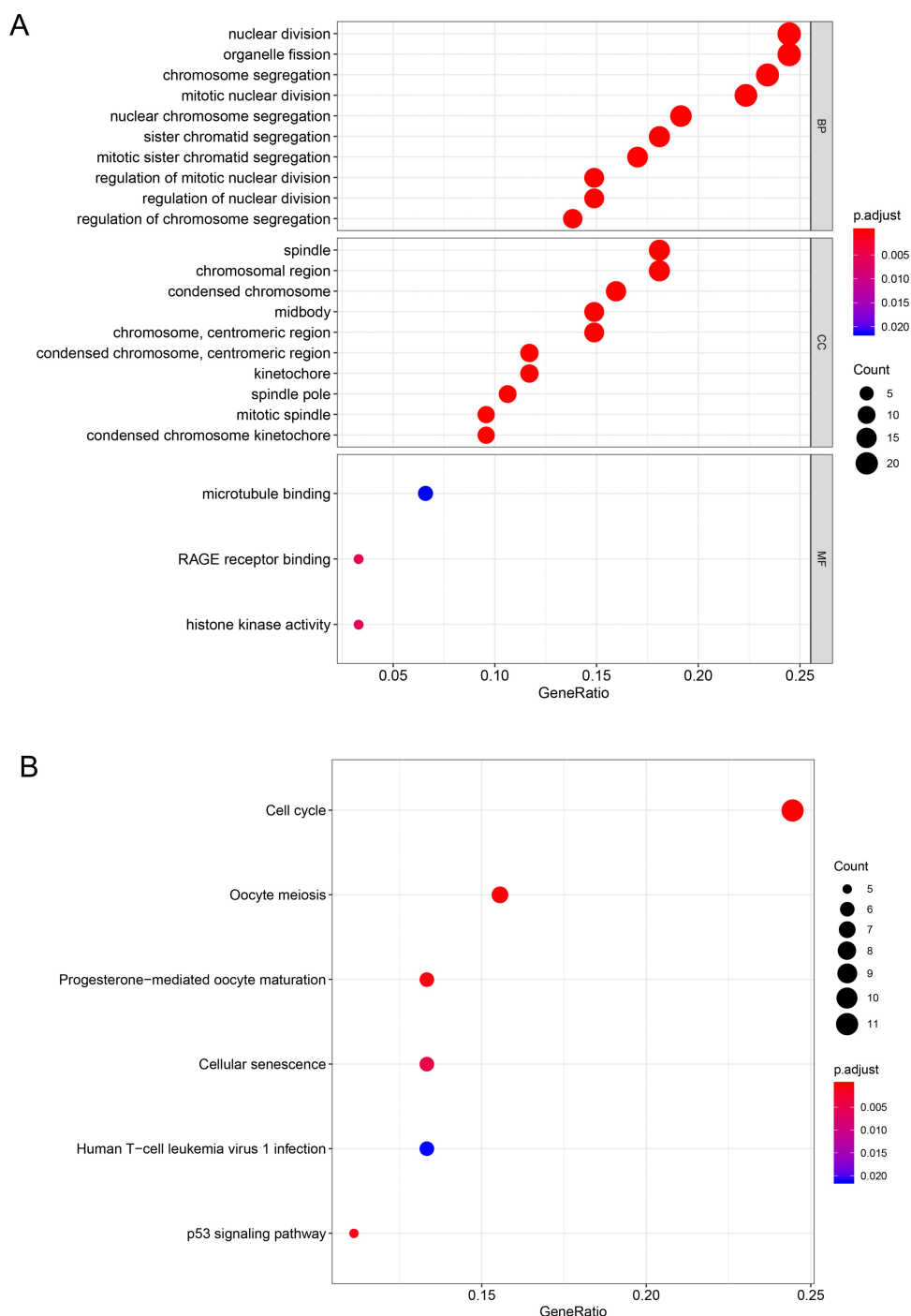
Figure 2 The functional analysis of integrated DEGs. (**A**) Gene Ontology analysis and significant enriched GO terms related to DEGs. (**B**) KEGG pathway enrichment terms related to DEGs.

high in 384. Patients of clinical stage from I to IV were 2, 129, 139, 135, respectively. Kaplan-Meier plotter was applied to evaluate the prognostic value of DEGs, the results showed that 14 DEGs were associated with overall survival for BLCA patients (Supplementary Table 2). The Kaplan-Meier plotter of top 5 DEGs were shown in Figure 4. Low expression level of FADS1 and TM4SF1 was

associated with longer OS, while high expression level of CRTAC1, HSD17B2 and MST1R was associated with longer OS. As shown in Figure 5 and Supplementary Table 3, FADS1 and TM4SF1 expression level was up-regulated in high grade, while CRTAC1, HSD17B2 and MST1R expression levels were down-regulated in high grade.
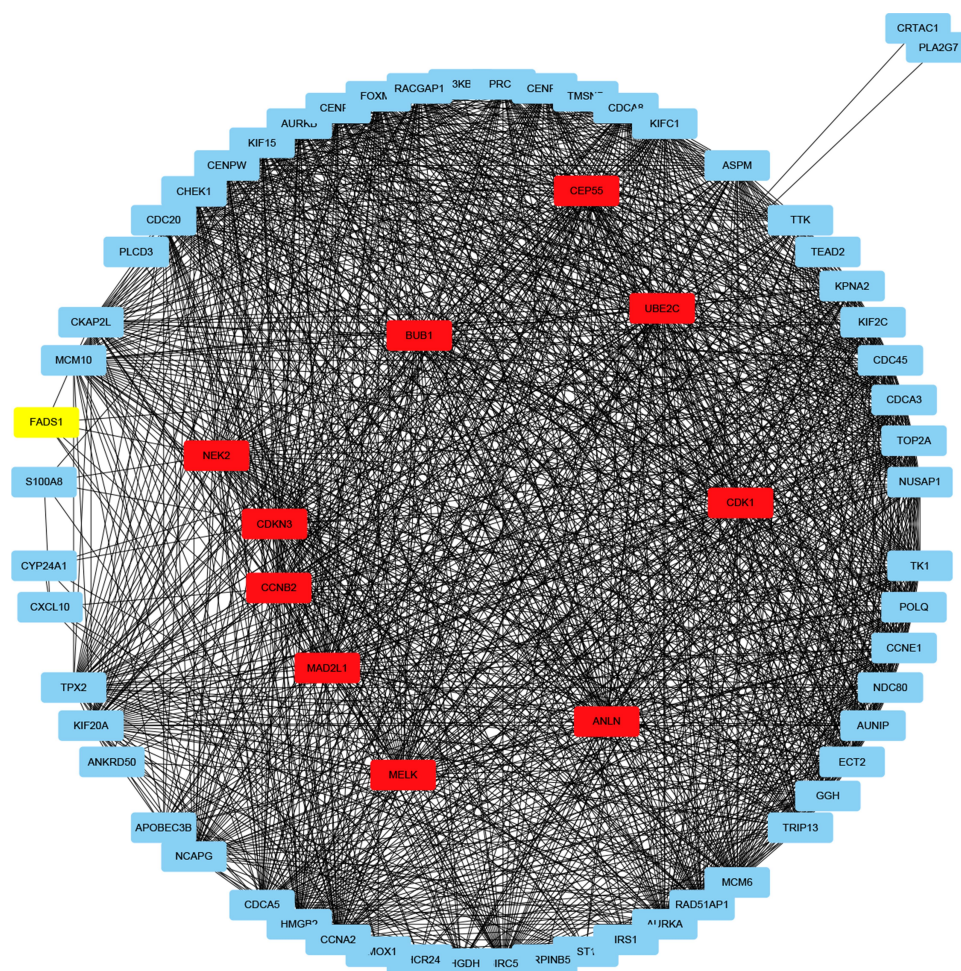
Figure 3 DEGs were analyzed and constructed a PPI network. Red nodes represent the top 10 hub genes.

In univariate analysis, no survival difference was found in gender and CRTAC1 expression level. Age, tumor stage, FADS1, TM4SF1, HSD17B2 and MST1R expression level were significantly associated with OS in BLCA patients (Supplementary Table 4). To clarify the independent prognostic value of FADS1, multivariate Cox hazards regression analysis revealed that FADS1 was an independent predictor for OS (hazard ratio [HR]=1.03, 95% confidence interval [CI], 1.01–1.05, p=0.014) (Figure 6A, Supplementary Table 4).

Identification of FADS1 Associated Biological Pathways

To determine biologic characteristics according to the different FADS1 expression levels by GSEA analysis, a robust computational method that determines whether the defined set of genes shows statistically significant differences. Cell cycle-related, DNA replication and

mismatch repair biological processes and pathways were significantly enriched (Figure 6B), indicating that FADS1 have a potential marked influence on these biological processes and pathways in BLCA.

FADS1 is Overexpressed and Positively Correlated with the Tumor Grade in BLCA

To assess the roles of FADS1 in BLCA, the expression level of FADS1 was detected in five human bladder cancer cell lines, T24 (grade 3), 253J (grade 3), 5637 (grade 2), RT4 (grade 1), and an immortalized human urothelial cell, SV-HUC-1. As shown in Figure 7A and B, FADS1 protein level and mRNA level had a similar tendency, highest expression in T24 and 253J, and lowest expression in SV-HUC-1. Moreover, immunohistochemistry staining was used to examine the FADS1 expression level in tumor specimens. Only 2 low grade and 3 high-grade tumor
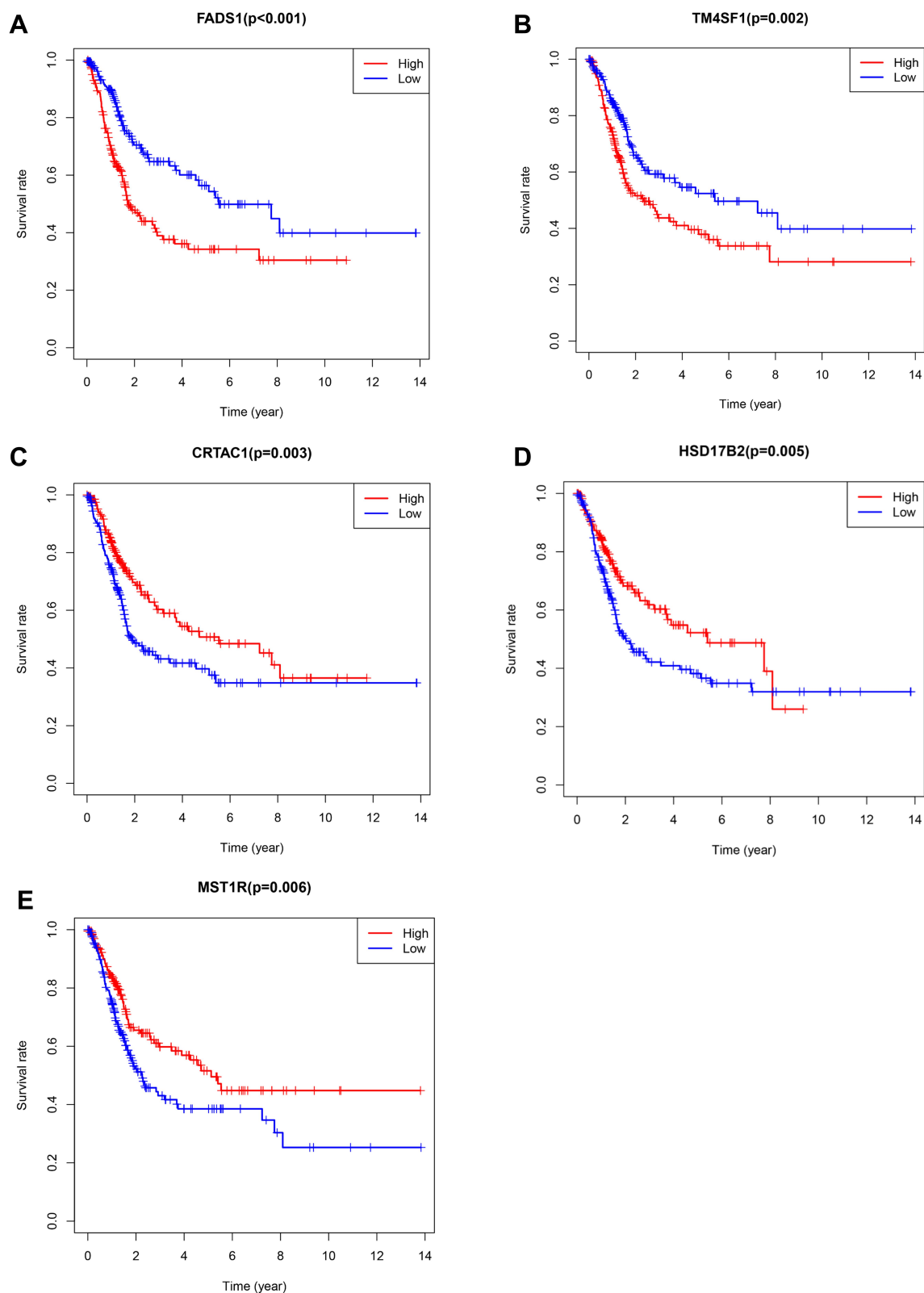
Figure 4 Kaplan–Meier plotters and Log rank tests for validation the prognostic value of top 5 DEGs by TCGA database. (**A**) FADS1; (**B**) TM4SF1; (**C**) CRTAC1; (**D**) HSD17B2; (**E**) MST1R.
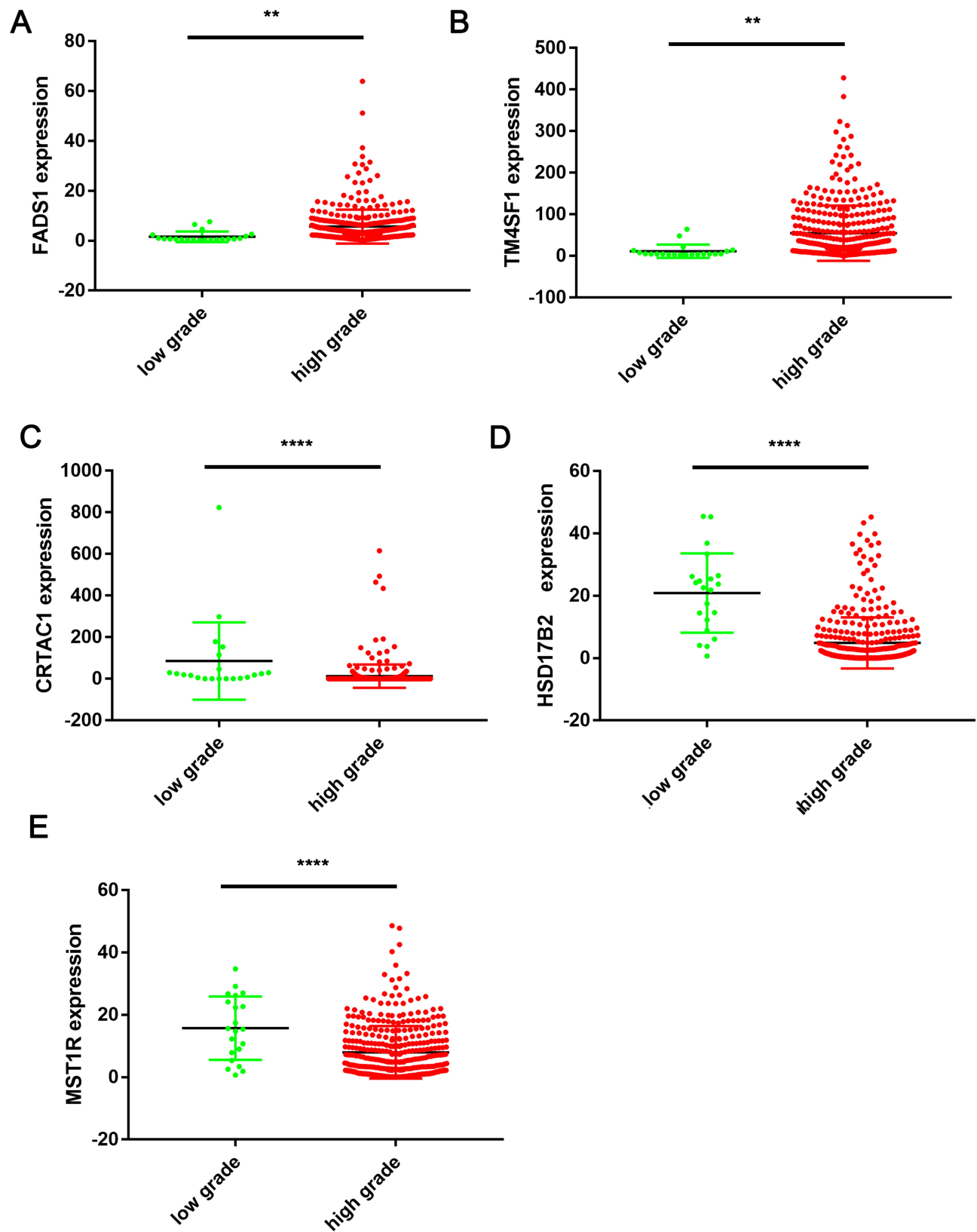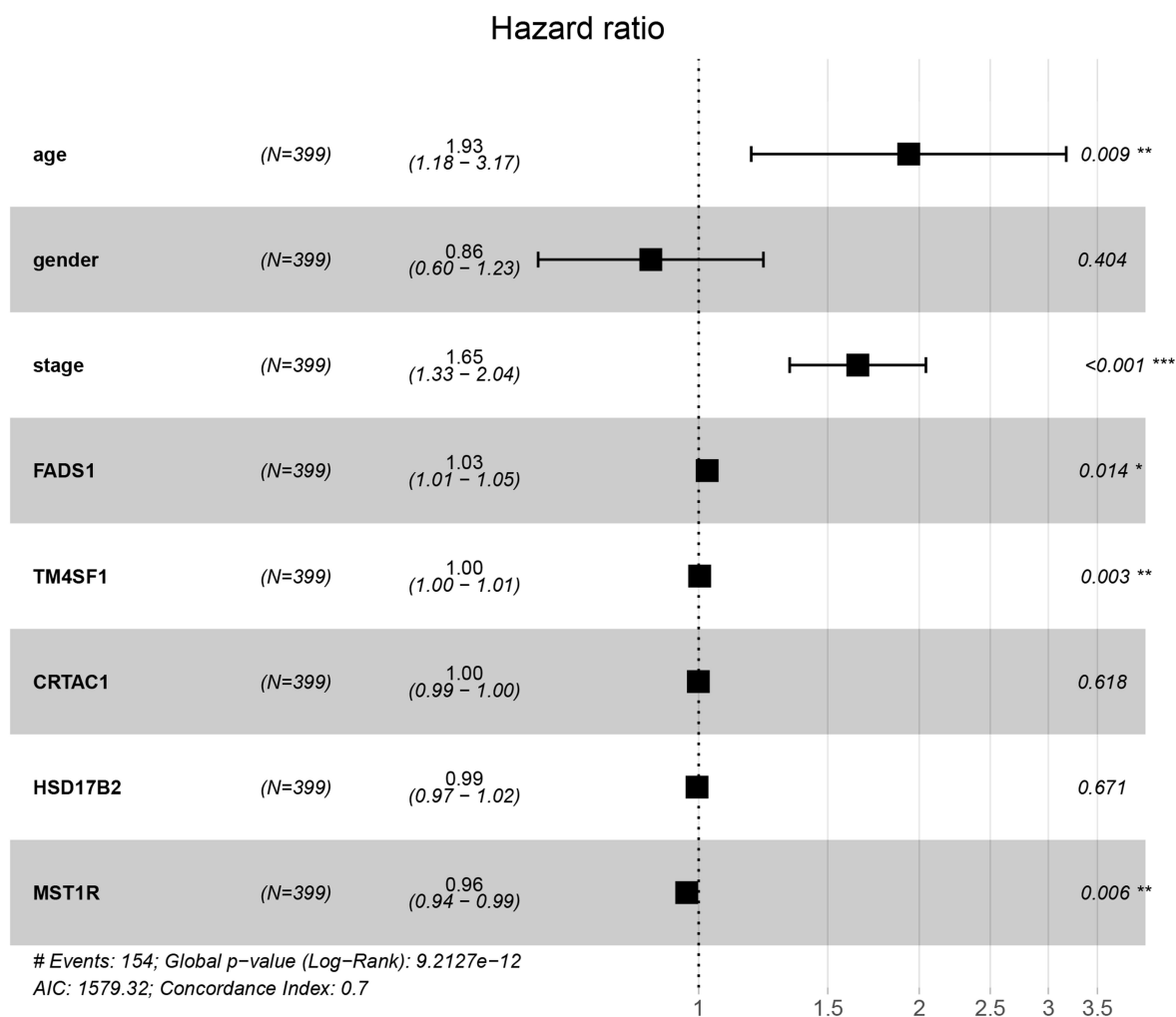
Figure 5 Top 5 DEGs expression levels in TCGA database between low grade and high grade BLCA tissues. (**A**) FADS1 (LG (Mean ± SEM): 1.601 ± 0.4624; HG (Mean ± SEM): 5.679 ± 0.3447; (**B**) TM4SF1 (LG (Mean ± SEM): 11.3 ± 3.483; HG (Mean ± SEM): 55.45 ± 3.411); (**C**) CRTAC1 (LG (Mean ± SEM): 85.54 ± 40.5; HG (Mean ± SEM): 12.54 ± 2.837); (**D**) HSD17B2 (LG (Mean ± SEM): 20.91 ± 2.782; HG (Mean ± SEM): 4.88 ± 0.4152); (**E**) MST1R (LG (Mean ± SEM): 15.74 ± 2.216; HG (Mean ± SEM): 7.921 ± 0.4288); **p < 0.01, ****p<0.0001, based on student's *t*-test.

A

Hazard ratio

age	(N=399)	1.93 (1.18 – 3.17)		0.009 **
gender	(N=399)	0.86 (0.60 – 1.23)		0.404
stage	(N=399)	1.65 (1.33 – 2.04)		<0.001 ***
FADS1	(N=399)	1.03 (1.01 – 1.05)		0.014 *
TM4SF1	(N=399)	1.00 (1.00 – 1.01)		0.003 **
CRTAC1	(N=399)	1.00 (0.99 – 1.00)		0.618
HSD17B2	(N=399)	0.99 (0.97 – 1.02)		0.671
MST1R	(N=399)	0.96 (0.94 – 0.99)		0.006 **

 # Events: 154; Global p−value (Log−Rank): 9.2127e−12
AIC: 1579.32; Concordance Index: 0.7
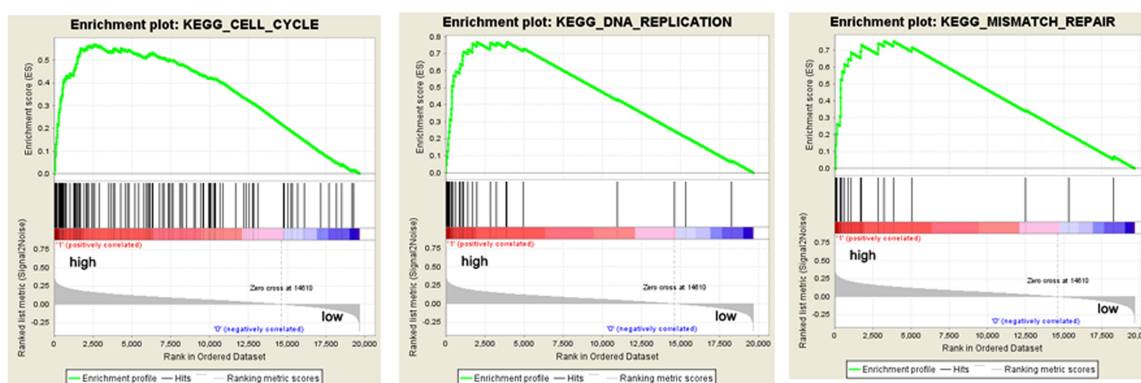
B

Figure 6 The prognostic value and three most significant enrichment of FADS1. (**A**) The multivariate Cox hazards regression analyses. (**B**) GSEA plot showed FADS1 expression associated with cell cycle-related, DNA replication and mismatch repair processes; *p < 0.05; **p < 0.01, ***p<0.001.

and adjacent tissues were collected. FADS1 staining intensity seemed positively associated with tumor stage (Figure 7C and D). Together, these results showed FADS1 is overexpressed in BLCA, and its mRNA and protein expression level are positively associated with tumor grade.
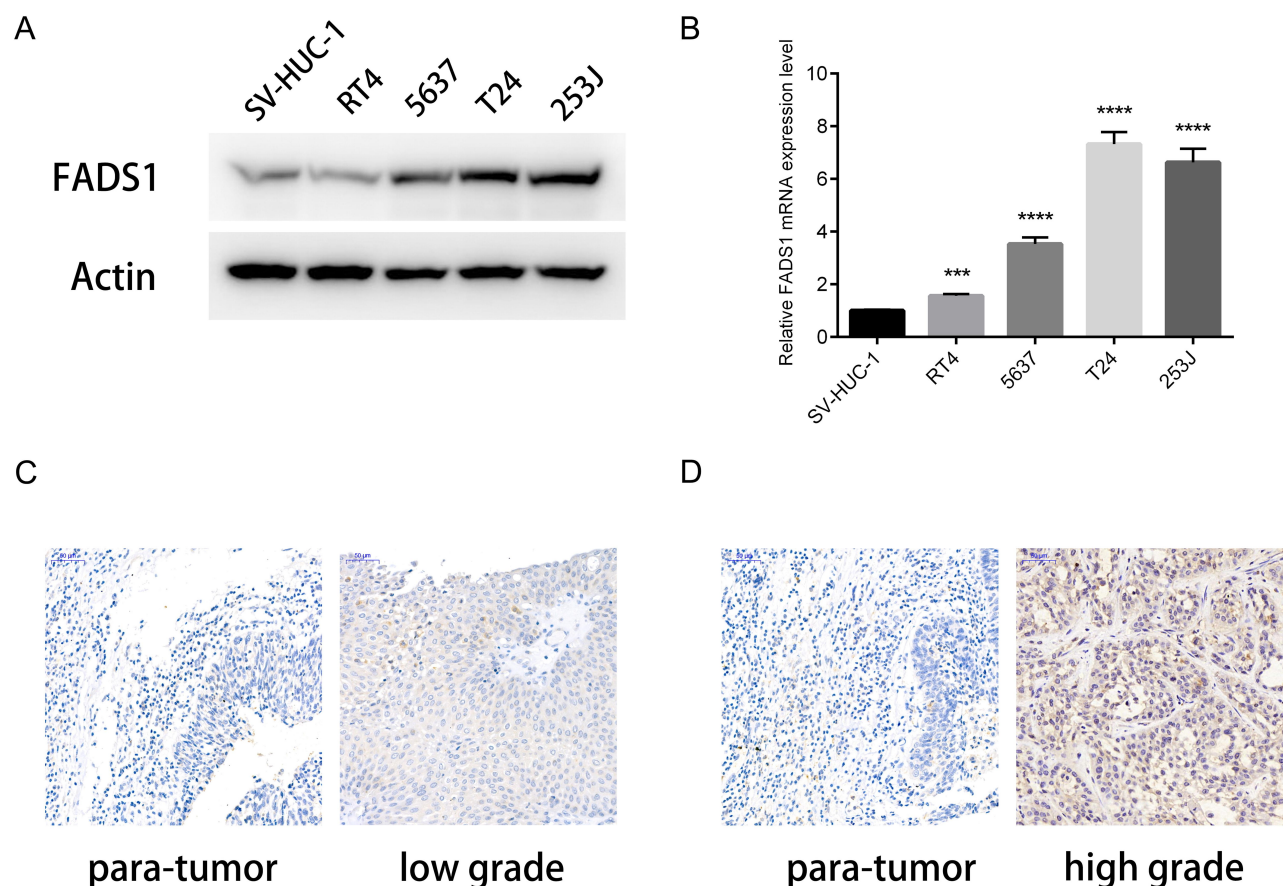
A

B

C

para-tumor   low grade

D

para-tumor   high grade

Figure 7 FADS1 expression positively correlated with tumor grade in BLCA. (**A**) The protein expression levels were determined by Western blot. (**B**) The mRNA expression levels were determined by RT-PCR. (**C, D**) Representative staining of FADS1 in BLCA tissue by IHC. C. low grade of BLCA and its para-tumor tissue; D. high grade of BLCA and its para-tumor tissue. ***$p<0.001$, ****$p<0.0001$, based on student's t-test.

FADS1 Promotes Proliferation in BLCA Cells

MTT and colony formation assays showed that over-expressed FADS1 increased, while FADS1 knockdown decreased cell proliferation ability (Figure 8A–E and Figure S2A–B). Consistently, EdU incorporation assay showed the role of FADS1 in proliferation (Figure 8F and G; Figure S2C–D). Cell cycle measured by flow cytometry showed that over-expressed FADS1 did not significantly influence the cell cycle. However, the FADS1 knockdown cells were markedly arrested at the G1 phase (Figure 8H and I; Figure S2E–F). Taken together, these results indicated that FADS1 could influence tumor growth by arresting cell cycle.

Discussion

In the present study, we first performed a bioinformatics analysis to explore the potential crucial genes associated with tumor grade and prognosis of BLCA. Two GEO datasheets were used to analyze DEGs by GEO2R, 70 up-regulated and 29 down-regulated DEGs were found in both databases. GO and KEGG analysis showed cell proliferation could play an important role in different tumor grades. Meanwhile, TCGA database was used for external validation, and survival analysis revealed that high-level expression of FADS1 was associated with poor prognosis of BLCA patients. And multivariate Cox analysis revealed that FADS1 was an independent predictor for OS in BLCA. To further explore the function of FADS1 in BLCA, over-expressed and knockdown FADS1 cells were used to determine the changes in proliferation ability, and knockdown FADS1 significantly arrested cell cycle at the G1 phase.

BLCA is a highly immunogenic malignant tumor,[20] and high tumor grade is strongly associated with poor prognosis (especially progression-free survival and cancer-specific survival).[4,21] Cell cycle is a highly conserved process which is essential for mammalian cell growth and development. Loss of normal cell-cycle control is a hallmark of
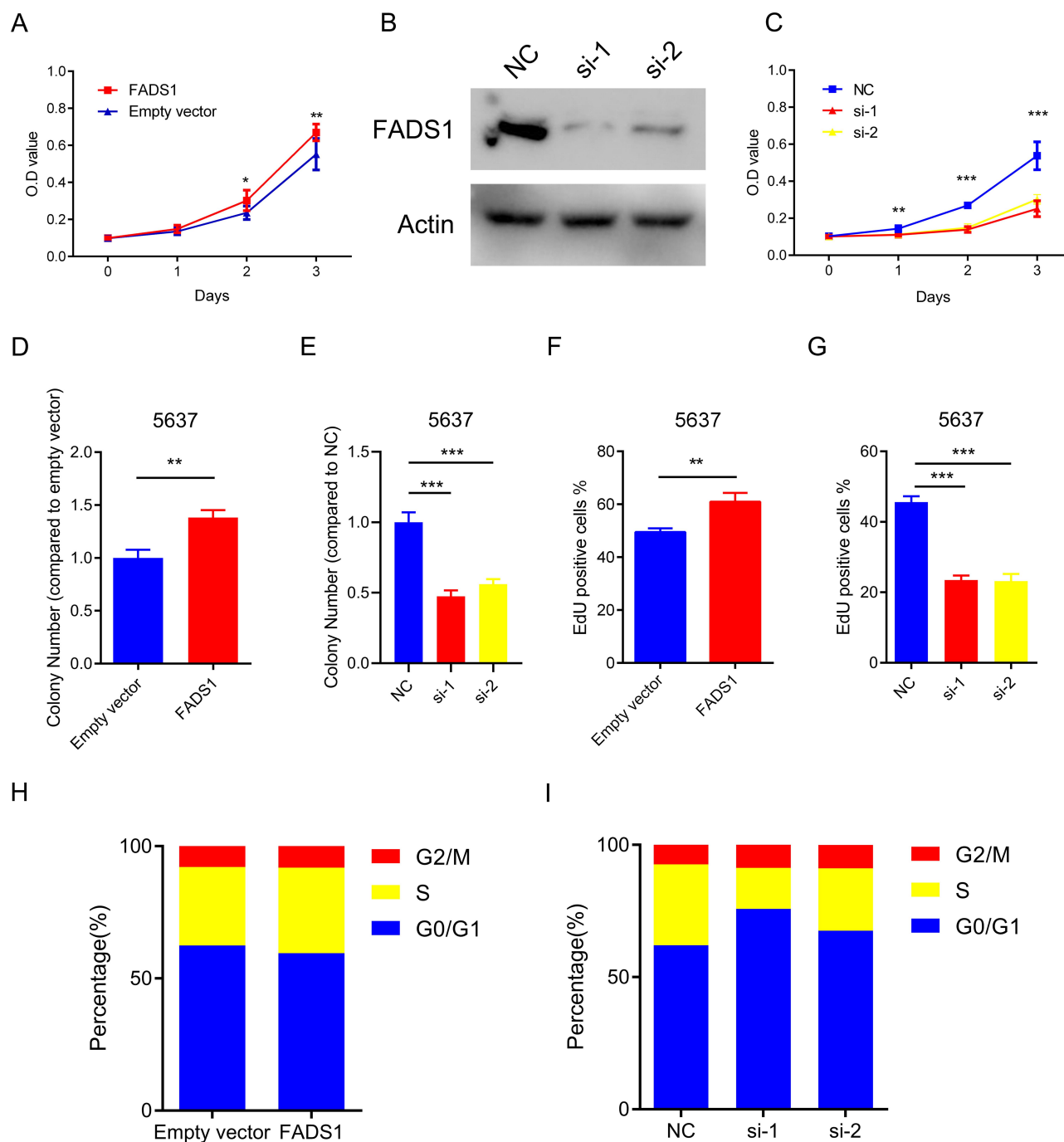
Figure 8 FADS1 promotes proliferation in BLCA cells. (**A**) Cell survival of 5637 cells was assessed with MTT assay for 0, 1, 2 and 3 days. (**B**) The protein expression levels of FADS1 by siRNA interference in 5637 cells were determined by Western blot. (**C**) Cell survival of 5637 cells was assessed with MTT assay for 0, 1, 2 and 3 days. (**D, E**) Colony-formation assays were conducted to determine the proliferation ability of FADS1. Data are mean ± SD values from three experiments, each performed in triplicate. (**F, G**) EdU assays were performed to examine the proliferation ability of FADS1. Data are mean ± SD values from three experiments. (**H, I**) Cell cycle analysis was determined by flow cytometry. *p < 0.05; **p < 0.01, ***p<0.001.

human cancer.[22] Chromosomal aberrant segregation or instability correlates with poor prognosis in various tumors, suggesting that genetic abnormalities contribute to tumorigenesis and progression.[23] Recently, more evidence had demonstrated that fatty acid metabolism was associated with cancer cell proliferation, and caused various abnormalities, and the consumption of dietary lipids had been regarded as a kind of cancer risk factor.[24,25] Multiple genes within the pathway of fatty acid metabolism could influence cancer initiation and progression.[26–28]

FADS1 was first cloned in 1999 for the first time and identified on chromosome 11q12-11q13.1 cluster in the human genome in 2000.[29] And FADS1 was mainly focused on its polymorphisms, and could alter fatty acid profiles or desaturase activity. FADS1 cluster showed a strong association with the n-6 and n-3 fatty acids in an analysis of single nucleotide polymorphisms (SNPs) of the FADS1 gene cluster,[30] and Genome wide association studies (GWAS)[31–33] have associated lower blood concentrations of LC-PUFAs with minor alleles of SNPs in the FADS1-FADS2 genes. Also, FADS minor alleles might present a lower desaturase activity. Meanwhile, the well-known biological function of FADS1 is a rate-limiting enzyme in the biosynthesis of long-chain polyunsaturated fatty acids (LC-PUFA) precursors of eicosanoids.[34,35] And it has been reported that FADS1 is associated with risk of metabolic syndrome (MetS). FADS1 and its homolog FADS1 variants located on chromosome 11q12-11q13.1 are likely to be linked to variations in serum phospholipids and erythrocyte membranes.[36] And aberrant FADS1 expression caused a variety of diseases, such as asthma, osteoarthritis, type 1 diabetes et al.[37–39]

Despite the functions of FADS1 in metabolism were explored deeply, the functions in tumorigenesis and progression were poorly understood. It's well known that the gene could act as a totally opposite function in different sites of the body. FADS1 was reported as an oncogene in osteosarcoma, and involved in linc00460, miR-1224-5p and FADS1 axis.[40] Moreover, Tang et al clarified that FADS1 was downregulated in both human vitiligo samples and vitiligo PIG3V melanocytes, and downregulation of FADS1 could reduce proliferation, induce reactive oxygen species (ROS) generation, apoptosis and cell cycle arrest in G1 stage.[41] A recent study has reported that FADS1 could activate AKT/mTOR signaling pathway to promote the progression of laryngeal squamous cell carcinoma.[42] However, FADS1 was identified as a tumor suppressor gene in non-small-cell lung cancer (NSCLC) and esophageal squamous cell carcinoma.[13,14] Decreased FADS1 expression correlated with a poor prognosis in esophageal squamous cell carcinoma and NSCLC patients. We identified FADS1 as an oncogene in BLCA patients, high expression level in tumor tissue, compared to the normal tissue. Meanwhile, FADS1 expression level was related to the tumor grade of BLCA. The FADS1 expression level could influence the cell proliferation ability and cell cycle. Thus, we speculate that FADS1 could be a causative effect in bladder carcinogenesis.

We hypothesize some possible mechanisms of FADS1 exerting its function. First, FADS1 could influence PUFA metabolites, including arachidonic acid (AA), and eicosapentaenoic acid (EPA), dihomo-gamma-linolenic acid (DGLA) and docosahexaenoic acid (DHA), which play an important role of inflammatory and tumor progression. AA could promote tumor progression through its metabolite, prostaglandin E2 (PGE_2), which is highly expressed in various kinds of human malignancies and predicts poor prognosis.[43–45] It has been reported that AA/DGLA could be enhanced by upregulated FADS1,[42] thus, FADS1 may promote tumor initiation and progression by disrupting the balance of fatty acid metabolism. In addition, several molecular pathways have been identified, such as the linc00460/miR-1224-5p/FADS1 axis and AKT/mTOR signaling pathway.[40,42] Thus, the potential mechanisms of FADS1 in BLCA will be explored in further studies.

However, there were several limitations in this study. First, the results above, including exploring the expression level of FASD1 in TCGA and GEO database, detecting FADS1 expression and promoting proliferation ability in vitro, were needed to be validated in vivo experiments, such as subcutaneous tumor model of nude mice, which would be conducted in our further studies. Second, limited cases of patients specimen were collected, and no clinicopathologic characteristics or K-M curves based on follow up were shown in this study, these will be analyzed in further studies.

Conclusion

In conclusion, we identified that FADS1 could be regarded as an independent prognostic factor in BLCA patients, and it could play a significant role in BLCA proliferation. FADS1 might be a potential therapeutic target for BLCA. However, more studies still are needed to further clarify the function of FADS1 in BLCA.

Funding

Disclosure

The authors report no conflicts of interest in this work.

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