USF1 Promotes Lung Adenocarcinoma Progression by Regulating Neurotrophin Signaling Pathway

Yu Wang
Shandong Provincial Hospital

Yunxia Zhao
Shandong Provincial Hospital

Xiangwei Zhang
Shandong Provincial Hospital

Yuanzhu Jiang
Shandong Provincial Hospital

Wei Ma
Shandong Provincial Hospital

Lin Zhang
Shandong Provincial Hospital

wei dong (✉ dr.dongwei@sdu.edu.cn)
Shandong Provincial Hospital

Research Article

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Abstract

Background: We aimed at investigation of the effect and the underlying neurotrophin signaling pathway of the upstream transcription factor 1 (USF1) in lung adenocarcinoma (LUAD).

Methods: The Cancer Genome Atlas (TCGA) database was used to analyze USF1 expression data and to extract patients’ clinical records. Immunohistochemical assay and Western blotting (WB) were used to determine the expression levels of USF1 in LUAD. The neurotrophin signaling pathway was analyzed by bioinformatic analysis while the expression of all related proteins was determined by WB. In addition cellular viability, proliferation, migration and invasion potential were investigated by the CCK-8, colony formation, wound healing and transwell. Meanwhile, the effect of USF1 in LUAD progression was investigated in a mouse model. The link between USF1 and UGT1A3 (UDP Glucuronosyltransferase Family 1 Member A3) was studied by the dual-luciferase reporter assay.

Results: We have detected a high expression level of USF1 in LUAD, which was associated with advanced tumor stage, nodal metastasis, and poor patient’s survival rate. The knockdown of USF1 inhibited cellular viability, proliferation, migration and invasion. Meanwhile, USF1 knockdown inhibited tumor growth in a mouse model. Besides, USF1 targeted UGT1A3, which was proven by the fact that the USF1 knockdown decreased the expression level of UGT1A3, whereas the upregulated expression of UGT1A3 increased cellular viability and proliferation. We have proved that the neurotrophin signaling pathway in LUAD was activated by USF1 and UGT1A3. The expression of the related proteins was also inhibited by the USF1 knockdown, while the overexpression of IRAK increased cancer cells’ migration and invasion potential.

Conclusion: USF1 was highly expressed in LUAD and promoted LUAD progression by regulating the neurotrophin signaling pathway. These findings provide a new theoretical data that could serve as a good foundation for the treatment of LUAD.

Introduction

Lung cancer is the most common malignant tumor and the main cause of death worldwide. At present, lung cancer ranks first in the mortality rate of malignant tumors in China, which is a serious threat to public health [1]. Lung adenocarcinoma (LUAD) is a type of non-small-cell lung carcinoma (NSCLC), which accounts for about 80 ~ 90% of NSCLC worldwide [2]. LUAD is divided into adenocarcinoma in situ, microinvasive adenocarcinoma, and invasive adenocarcinoma [3–5]. LUAD is more likely to occur in women, and the main risk factor is smoking. Approximately 70% of LUAD patients have local progression or metastasis at the time of diagnosis [6]. The patient was first diagnosed as locally advanced or too advanced for surgical treatment. Although LUAD conditions are controlled by medical treatments such as radiotherapy and chemotherapy, the results are yet pessimistic [7]. The average age of diagnosis and the average 5-year survival rate of LUAD are 71 years and 18%, respectively [8]. Therefore, there is an urgent need to develop an effective prognostic biomarker to predict the LUAD cancer type and to provide adequate and modern treatment.
Human upstream transcription factor 1 (USF1), a member of the helix-loop-helix leucine zipper family, is located in the region q22.3 of chromosome 1. It contains 11 exons and has a total length of 6.73 kb. USF1 is a ubiquitously expressed transcription factor that regulates gene transcription by binding to the E-box motif of target genes [9]. USF1 regulates genes involved in lipid and sugar metabolism [10, 11]. Furthermore, it was found to be a potential marker of patient’s susceptibility to gastric carcinogenesis [12] and to promote glioma cell invasion and migration [13]. Previous studies show that USF1-induced overexpression of the long noncoding RNA WDFY3-AS2 promoted LUAD progression [14]. To date, the specific molecular mechanism by which USF1 exerts its effects on LUAD are yet incomplete and need further study.

UDP-glucuronosyltransferases (UGTs) are an important class of phase II drug metabolizing enzymes [15]. UGTs catalyze the glucuronidation of many important endogenous compounds such as bilirubin, bile acids, thyroid and steroid hormones, as well as a large number of carcinogenic exogenous substrates [16]. UGT enzymes are divided into two protein families, namely UGT1 and UGT2. UGT1A is one of the three protein subfamilies (UGT1A, UGT2A and UGT2B) [17] and is located on chromosome 2q37 and encodes 13 subtypes (UGT1A1-UGT1A13), which are specifically expressed in different tissues [18]. A growing amount of evidence suggest that they play important roles in the carcinogenesis of stomach and pancreatic cancers [16, 19]. Our previous results demonstrated that UGT1A3 was highly expressed in LUAD, and was associated with poor prognosis [20]. Therefore, it is prerequisite to investigate the relationship between UGT1A3 and USF1, and the underlying mechanism that accounts for its effects in LUAD.

In the current study, we have found that the upregulated expression of USF1 is associated with advanced stage, nodal metastasis, and poor survival rate in LUAD. While its knockdown leads to inhibition of tumor cells’ proliferation, migration and invasion potentials via downregulation of UGT1A3 and the neurotrophin signaling pathway. Our results indicate that USF1 could serve as a potential biomarker of LUAD, and provide a new theoretical ground for development of new treatment approaches for LUAD.

### Material And Methods

#### Cell culture

Human NSCLC cell lines A549, H1299, PC-9, and H1975 (Chinese Academy of Sciences Cell Bank, Shanghai, China) were cultured in RPMI-1640 medium containing 0.1% double antibodies (50 U/ml penicillin and streptomycin) and 10% fetal bovine serum, and were incubated at 37°C.

#### Tissue samples

Samples of NSCLC tissues and adjacent normal tissues were taken from patients who did not receive radio-, chemotherapy nor any other anti-tumor treatments at the Shandong Provincial Hospital affiliated to the Shandong University (Jinan, China). All specimens were confirmed as LUAD by HE staining. Informed written consent for scientific use of the biological material was obtained from each patient, and
this study was approved by the Ethics Committee of Cancer Institute of Shandong Province. All experiments were carried out in accordance with the Declaration of Helsinki.

**Transfection**

SiRNA1, siRNA2, and siRNA3 specifically targeting USF1 were synthesized and purified by RiboBio (Guangzhou, China). The USF1 specific short hairpin RNAs (shRNAs) and control shRNA were synthesized and produced by GenePharma (Shanghai, China). SiRNA specifically targeting UGT1A3 was synthesized and purified by RiboBio (Guangzhou, China). IRAK1 was cloned into pCDNA3.1 vector and an HA tag. Transfection was performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Transfection efficiency was proved by Western blotting (WB).

**Western blotting**

Protein samples were extracted and quantified by RIPA buffer (Biovision, American) and protein concentration detection kit (Guangzhou Yingdante Science & Technology Co., Ltd, Guangzhou, China). Then, proteins were separated by 10% SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore corp., Billerica, MA, USA). Non-specific sites were blocked with 5% milk powder diluted in TBS with 0.05% Tween 20 (TBST). After that, membranes were incubated overnight at 4°C with rabbit polyclonal antibody anti-USF1 (abs115735, 1:500 dilution, Absin, Shanghai, China), rabbit UGT1A3 polyclonal antibody (H00054659-A01, 1:500 dilution, Abnova, Wuhan, China), and rabbit P75 neurotrophic factor receptor (P75NTR) polyclonal antibody (TA328682, 1:200 dilution, OriGene Technologies, Rockville, US), rabbit receptor interacting serine/threonine kinase 2 (RIPK2) polyclonal antibody (abs130017, 1:500 dilution, Absin, Shanghai, China), rabbit interleukin-1 receptor-associated kinase 1 (IRAK1) polyclonal antibody (AF7290, 1:500 dilution, Beyotime Biotechnology, Shanghai, China), rabbit ikappaB kinase β (IKKβ) polyclonal antibody (AF7200, 1:300 dilution, Beyotime Biotechnology, Shanghai, China), while the rabbit anti-GAPDH polyclonal antibody (1:500, Cell Signaling Technology, Inc.; Shanghai, China) was used as a control. After washing of the membranes repeatedly with TBST, they were incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (#BHR101, 1:5000, Beijing Bersee Technology Co. LTD., Ltd., Beijing, China). Finally, the Western blots were assessed by enhanced chemiluminescence. The expression of the relative protein levels were quantified by densitometry using the Quantity One software (Bio-Rad, Hercules, CA, USA). Relative protein expression levels were normalized to GAPDH.

**Immunohistochemistry assay**

The specimens were routinely embedded in paraffin, section thickness was 3μm, and routine dewaxing was performed. EDTA (pH 8.0) was used for microwave repair. After that, the endogenous peroxidase was blocked by adding 3% hydrogen peroxide in distilled water after natural cooling, incubated at room temperature for 10 min, and rinsed three times with phosphate buffer (PBS) for 2 min. The specimens were covered with 1:100 diluted MAGE-A3 monoclonal antibody, incubated for 1 h at 37°C, and rinsed three times with PBS for 5 min each. HRP-labeled secondary antibody was added to cover the specimen, incubated for 20 min at 37°C, and rinsed with PBS. The HRP-labeled secondary antibody was added to
cover the specimen and was incubated for 20 min at 37°C. Three times rinsing with PBS followed for 5 min each. Diaminobezidin (DAB) was added to control the color development under the microscope. Rinsing thoroughly with tap water, re-stained with hematoxylin, dehydrated, and observed under the microscope after the tablets were sealed.

**Cell counting kit-8 (CCK-8)**

Firstly, log phase cells were seeded in 96-well plate and incubated at 37°C with 5% CO₂. Then, 10 µl of CCK-8 solution was added to each well and incubation for 1 to 4 hours followed. Finally, the 96-well plate was placed on the enzyme-linked immunoassay instrument, and then measured the absorbance at 450 nm.

**Colony forming assay**

Cells were inoculated into a 6-well plate (500 cells in each well), the medium was changed every 2 days. After 2 weeks, the culture medium was discarded, and cells were fixed with 10% formaldehyde, stained with crystal violet for 15 min. Then, the staining solution was discarded and cells were washed thoroughly with PBS. Finally, the growth status was observed under the microscope ((Leica Microsystems, Wetzlar, Hesse, Germany).

**Wound healing assay**

Cells were inoculated into a 6-well plate (2×10⁵ cells per well). 24 hours post- incubation, the cells were scratched with a pipette tip and were washed 3 times with PBS to remove the scratched cells. The remaining monolayer was then incubated at 37°C with 5% CO₂ with serum-free medium, and at the time points: 0 h and 48 h photos were taken to measure the distance, i.e. the wound healing potential.

**Transwell migration assay**

30 µl low-concentration matrix glue (BD Matrigel) diluted matrix glue was applied to each transwell chamber prior to cell inoculation. The Transwell plate was then placed in the cell incubator for 4 h and cells (1.333×10⁵/ml) were inoculated after the matrix gel solidified. After inoculation, the cells were inoculated and placed into a cell incubator. At 24 h, the compartment was taken and after staining was photographed (Carl ZEISS, Jena, German).

For the migration assay, logarithmic phase cells were digested and inoculated to the bottom of the transwell chamber (1 × 10⁵). The cells were then cultured for 24–48 h, the Transwell chamber was taken out and the cells in the chamber and the remaining matrigel glue were wiped with a cotton swab, and washed 3 times with PBS. After fixation and staining with polyoxymethylene and crystal violet, microscopic observations followed (Carl ZEISS, Jena, German) for data quantitation.

**Mice model and transfection**

C57BL/6 mice were purchased from Jinan Xingkang Biotechnology Co., Ltd. (Jinan, China). The animal house was maintained at a temperature of 22 ± 2°C with relative humidity of 50 ± 15% and 12 h dark/light
cycle. Xenograft tumors were established by subcutaneous injection of H1299 cells into 6-week-old male C57BL/6 mice. Sh-USF1 group mice were transfected with sh-USF1 tail-vein injection. The tumor was monitored by Vernier caliper while the tumor volume was indicated by a × b²/2 (a for long diameter; b for short diameter) every 5 d after injected 10 d. After 30 d, mice were anesthetized with 1% pentobarbital sodium (35 mg/kg, Dainippon Sumitomo Pharma) and fixed on the plate. The tumors were then removed weighed and analyzed after the mice were euthanized by slow release of carbon dioxide in a box. All experimental procedures were approved by the Ethics and Scientific Committees of our institution (No. 2017440) performed following the Guide for the Care and Use of Laboratory Animals.

**Dual-luciferase reporter assay**

The sequences of the wild type or mutant USF1 3’-UTR including UGT1A3 binding sites were sub-cloned into the pMIR-luciferase reporter construct and each construct was co-transfected with UGT1A3 into the cells using Lipofectamine3000 (Invitrogen, USA). After the cells were cultured at 37°C for 48 h, the cells were evaluated by a Dual-Luciferase Reporter Assay System following to the manufacturer’s instructions. The luminescence values of cells in every group were detected using Renilla luminescence activity as an internal reference.

**Data sources and bioinformatics analysis**

The Cancer Genome Atlas (TCGA) data were downloaded at the website of the University of California Santa Cruz (UCSC) cancer browser (http://genome-cancer.ucsc.edu). Difference in gene expression was analyzed by Next-generation sequencing (NGS) and Venn. We used the DEseq of R software to analyze the difference in gene expression. KEGG analysis was performed on the common differentially expressed genes (DEGs) of each dataset using R software. The String11.0 database (https://stringG db.org/) was used to analyze the interactions of common DEGs encoded proteins.

**Statistical analysis**

Data in the present study were presented as means ± standard deviation (SD). Statistical analysis was performed using SPSS 22.0 (Chicago, IL, U.S.A.) and R software. One-way ANOVA or two-tailed Student’s t-test were used for comparisons between groups. Survival analysis was performed using Kaplan–Meier method. The relationship between the variables and patient’s survival status was performed by Multivariate Cox proportional hazards method. P< 0.05 was considered statistically significant.

**Results**

**USF1 is overexpressed in LUAD**

TCGA dataset was used to analyze the relation between the USF1 expression data and LUAD patients’ clinical records. As indicated in Fig. 1A, the USF1 demonstrated higher expression levels in the primary tumors than in the normal control group (Fig. 1A). As shown in Fig. 1B, the expression of USF1 was markedly increased in the advanced stages of LUAD, especially in the stage 1 (Fig. 1B). The expression of
USF1 was increased in the nodal metastases status especially in nodal metastases 3 (Fig. 1C). Interestingly, the higher USF1 expression was associated with a lower survival rate (Fig. 1D). Taken together our results show that the USF1 had a higher expression in LUAD, especially in the stage 1 and nodal metastases 3, which was correlated with a poorer survival rate than the LUAD patients with low USF1 expression.

**USF1 was upregulated in LUAD tissues and cells**

The expression of USF1 in LUAD tissues was determined by immunohistochemical assays. The obtained results indicated that the USF1 had a higher expression in LUAD tissues than in the normal ones (Fig. 2A). Besides, the results of WB indicated that the USF1 had a higher expression in LUAD cells lines (A549, H1299, and PC-9 cells lines) (Fig. 2B). Collectively, USF1 was upregulated in LUAD. Moreover, the following small interfering RNAs: si-USF1-1, si-USF1-2 and si-USF1-3 were transfected into the studied H1299 cell lines. The results of the WB showed that the USF1 mRNA levels were downregulated in the cells transfected with si-USF1, compared with the si-NC group, especially in the cells transfected with si-USF1-3 (Fig. 2C), indicating that the cells were transfected with si-USF1-3 successfully.

The CCK-8 assay was used to determine cellular viability. The obtained results showed that the cells in si-USF1 group had a lower viability than of the si-NC group (Fig. 2D). In addition, the results from the colony forming assays showed that the proliferation of the cells in the si-USF1 group was decreased significantly in comparison with the si-NC group (P < 0.05, Fig. 2E&2F). Taken together, USF1 was upregulated in LUAD, while the knockdown of USF1 inhibited cellular viability and proliferation.

**USF1 knockdown inhibited the migration and invasion of the studied LUAD cells**

Cellular migration and invasion were determined by the wound healing and transwell migration assays. As shown in Fig. 3A, the wound healing area in the cells transfected with si-USF1 was significantly decreased in comparison with the si-NC group (P < 0.05, Fig. 3A&3B). Meanwhile, results of the transwell assays indicated that the number of migrated and invaded cells was significantly decreased in the si-USF1 group compared with the si-NC group (P < 0.05, Fig. 3C&3D). Taken together these results indicate that the knockdown of USF1 inhibited cells migration and invasion.

**USF1 knockdown of inhibited the LUAD tumor growth**

To further confirm the effect of USF1 in LUAD, we constructed xenografts mice models. As indicated in Fig. 4A, the tumor size in the NC group was larger than that of the sh-USF1 group (Fig. 4A). The weight of the tumor was diminished in the sh-USF1 group compared with the NC group (P < 0.05, Fig. 4B&C). Besides, the results of the WB found that the expression levels of USF1 were decreased in mice transfected with sh-USF1 in comparison with the NC group (Fig. 4D). Collectively, these data show that first, the sh-USF1 transfection was successful in the mice and second he knockdown of USF1 inhibited the tumor growth in mice model.
UGT1A3 overexpression improved LUAD cell viability and migration

Based on the binding sites predicted by the Jaspar (http://jaspardev.genereg.net/) and ConSite (http://consite.genereg.net/) database, we found a Pax-2 motif (CACGTGT) at position 8993/8999 of the UGT1A3 promoter (Fig. 5A). To investigate whether the USF1 regulates the UGT1A3 promoter activity by binding to this motif, a luciferase reporter assay was performed. Obtained results showed that the USF1 targeted the UGT1A3 (P < 0.05, Fig. 5B). As indicated in Fig. 5B the knockdown of USF1 inhibited cellular viability, while the overexpression of UGT1A3 reversed this inhibition effect, compared with the NC (Fig. 5C). Besides, the knockdown of USF1 decreased the expression of USF1 and UGT1A3, while the overexpression of UGT1A3 increased USF1 and UGT1A3 expression (Fig. 5D). Furthermore, the knockdown of USF1 inhibited the migration of LUAD cells, while UGT1A3 overexpression reversed this inhibition effect (P < 0.05, Fig. 5E&F). These results show that the USF1 targeted UGT1A3, and the overexpression of UGT1A3 improved LUAD cells viability and migration.

Differentially expressed genes analysis of LUAD cells with knockdown of USF1 or UGT1A3

Results of NGS showed there were 326 DEGs in the cells with UGT1A3 knockdown (Fig. 6A). The NGS results showed there were 1029 DEGs in cells with USF1 knockdown (Fig. 6B). The Venn diagram showed that there 63 overlapping genes. The KEGG pathway enrichment analysis indicated that these DEGs were significantly enriched in 12 pathways, and the enrichment score of the neurotrophin signaling pathway was the paramount (Fig. 6C). The main signal elements involved were p75NTR, RIPK2, IRAK1, TRAF5, and IKKβ (Fig. 6D). These results indicated that the neurotrophin signaling pathway in LUAD was activated by the USF1 and UGT1A3.

USF1 knockdown inhibited LUAD cells proliferation, migration and invasion via downregulation of the neurotrophin signaling pathway

WB was used to assess the expression levels of the neurotrophin signaling pathway associated proteins. The WB indicated that the levels of p75NTR, RIPK2, IRAK1, IKKβ and USF1 were significantly decreased in the si-USF1 group when compared with the NC group (Fig. 7A&7B). Besides the expression level of IRAK1 in the H1299 cells was determined. As indicated in Fig. 7C and 7D, the level of IRAK1 was decreased in the cells transfected with sh-USF1, while the level of IRAK1 was increased in the cells transfected with sh-USF1 and pcDNA3.1-IRAK1 (P < 0.05, Fig. 7C&7D). Furthermore, our results found that the knockdown of USF1 inhibited LUAD cells proliferation, migration and invasion, while the IRAK1 overexpression reversed this inhibition (Fig. 7E&7F). Taken together these data show that the knockdown of USF1 inhibited LUAD cells proliferation, migration and invasion via a downregulation of the neurotrophin signaling pathway.

Discussion
LUAD originates from the bronchial mucosal epithelium and mucous glands [21]. It can be asymptomatic in the early stages with various unspecific symptoms in the later stage. The incidence and mortality of LUAD are increasing every year [22]. Currently, there are no specific tumor markers for LUAD, and multiple indicators are used for joint detection to increase the detection rate [23]. Therefore, at present, it is urgent to find better molecular biomarkers for LUAD diagnosis, treatment and prognosis, and to provide explanations for the formation and progression of this type of tumor. TCGA database stores more than 20 kinds of cancer genome data, including mutations, copy number variations, mRNA expression, miRNA expression, methylation data, etc., which are widely used in cancer research [24]. In the current study, based on TCGA data analysis, the USF1 was found to be highly expressed in LUAD. Meanwhile, the expression level of USF1 increased in cancer advanced stages and metastasis, and with the increase of the USF1 expression, the overall survival time was shorter. This allowed us to further investigate the effect of USF1 in vivo and in vitro.

The protein encoded by the USF1 gene regulates the expression of multiple genes related to glucose and lipid metabolism [25]. Recent studies have found that USF1 is involved in regulating the development of various types of tumors, including oral cancer by regulating the expression of human telomerase gene [26]. USF1 is found highly expressed in glioma tissues and cell lines. The inhibition of its expression reduced the migration and invasion potential of the glioma cells [13]. In our study, we have detected that the USF1 was highly expressed in LUAD tissues and cell lines, which was in unison with the results of the TCGA data analysis. Furthermore, The USF1 knockdown was successful in mice and showed that LUAD cells had decreased viability, proliferation, migration and invasion. These results were analogous with the results indicating the same effect of USF1 in glioma cells [13]. Meanwhile, the effect of LUAD was studied in mice model. Results found that the USF1 knockdown inhibited the tumor volume and weight. Thus we prove that the USF1 knockdown inhibits LUAD progression.

The dual-luciferase assay also showed that the USF1 targeted UGT1A3. UGT1A3 plays an important role in intestinal and liver drug metabolism, participating in the metabolism of a variety of cancers, thus promoting the resistance of tumor cells to chemotherapy drugs [27, 28]. Meanwhile, the USF1 knockdown decreased the UGT1A3 expression level while the overexpression of UGT1A3 reversed the cellular viability and proliferation inhibition effect of USF1 in the studied models, which was consistent with other authors results [20].

NGS can sequence hundreds of thousands or even millions of DNA sequences at the same time, and can comprehensively analyze the overall transcriptome and genome of a certain species, and then screen and analyze DEGs [29]. Results of NGS found that there were 63 overlapping differentially expressed genes in cells with USF1 and UGT1A3 knockdown, respectively. Furthermore, KEGG pathway enrichment analysis indicated that the neurotrophin signaling pathway can be activated by USF1 and UGT1A3 in LUAD. Neurotrophins form a family of growth factors which play significant roles in neuronal development, such as survival, differentiation, axon outgrowth or apoptosis [30]. The neurotrophin signaling pathway was also proved to promote the growth and proliferation of glioma cell line [31]. PPI network showed P75NTR, RIPK2, IRAK1, TRAF5, and IKKβ were crucial proteins. Further study on this pathway related proteins was
investigated to verify that the neurotrophin signaling pathway in LUAD cells is important for tumor progression.

p75NTR, one of the cell surface receptors of neurotrophin, is a structural member of the Fas/TNF-R family [32]. IRAK1 was one of the commonly overexpressed genes in solid tumors [33, 34]. IRAK is recruited to the p75-NGF receptor [35]. In the present study, the neurotrophin signaling pathway related protein (P75NTR, RIPK2, IRAK1, TRAF5, and IKKβ) levels were decreased in the si-USF1 group, indicating that the USF1 knockdown inhibited the neurotrophin signaling pathway. In addition, the IRAK1 overexpression was achieved by transfection with IRAK1, while the overexpression of IRAK1 promoted cells migration and invasion. Collectively these data prove that the USF1 knockdown inhibited LUAD cells proliferation, migration and invasion via deregulating the neurotrophin signaling pathway.

**Conclusion**

USF1 was highly expressed in LUAD in all investigated samples including patients’ tissues, studied cells lines, and mice models. The knockdown of USF1 inhibited cells viability, proliferation, migration and invasion, and reduced the tumor volume. Notably, the USF1 targeted UGT1A3 through which and by regulating the neurotrophin signaling pathway USF1 promoted LUAD progression, thus providing novel data for the design of successful therapeutic approaches for treatment of LUAD.

**Abbreviations**

Human upstream transcription factor 1 (USF1); lung adenocarcinoma (LUAD); The Cancer Genome Atlas (TCGA); Western blotting (WB); UDP Glucuronosyltransferase Family 1 Member A3 (UGT1A3); non-small-cell lung carcinoma (NSCLC); UDP-glucuronosyltransferases (UGTs); receptor interacting serine/threonine kinase 2 (RIPK2); interleukin-1 receptor-associated kinase 1 (IRAK1); ikappaB kinase β (IKKβ); horseradish peroxidase (HRP); Diaminobezidin (DAB); Cell counting kit-8 (CCK-8); University of California Santa Cruz (UCSC); Next-generation sequencing (NGS); differentially expressed genes (DEGs); standard deviation (SD)

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.
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Authors' contributions

WD and YW had full access to all of the data in the manuscript and take responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: all authors. Acquisition, analysis, and interpretation of data: all authors. Manuscript drafting: WD and YW. Critical revision of the manuscript for important intellectual content: all authors. Statistical analysis: YW and YXZ. Supervision: WD. All authors read and approved the final manuscript.

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Authors' information

1 Department of Oncology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, China

2 Department of Neurology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, China

3 Department of Thoracic Surgery, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, China

References


Figures

Figure 1

Relationship between the USF1 expression levels and patients’ clinical data in the TCGA database. (A) USF1 expression data in LUAD and normal tissues as reported in TCGA; (B) USF1 expression data in the
Investigation of the USF1 expression levels and study of the effect of its downregulation in LUAD. NC group, cells were transfected with si-NC; si-USF1 group cells were transfected with si-USF1; (A) The protein levels of USF1 in the studied tissues were determined by immunohistochemical assay; (B) The protein levels of USF1 in cells were determined by WB; (C) Transfection efficiency was proved by WB; (D)
Cells viability was detected by CCK-8 assay; (E) (F) cells proliferation potential was detected by the colony forming assay. *P<0.05 signifies statistically significant difference when compared with NC group.

Figure 3

Study of the effect of the USF1 knockdown on LUAD cells migration and invasion potentials. (A) Cells migratory potential was detected by wound healing assay; (B) Cellular migratory and invasion potentials were detected by the transwell assay; *P<0.05 signifies statistically significant difference when compared with NC group.
**Figure 4**

Effect of the USF1 knockdown on LUAD progression. Controls were designated as the NC group and represented mice transfected with sh-NC; while sh-USF1 group mice stood for those transfected with sh-USF1; (A) Measurement of the xenograft tumor size in different groups, n = 12; (B) The xenograft tumor growth curve; (C) The weight of the tumor xenograft; (D) Transfection efficiency, determined by WB. *P<0.05 signifies statistically significant difference when compared with NC group.
Figure 5

Study on the effect of the UGT1A3 overexpression on LUAD cell viability and migration. si-USF1+UGT1A3 group stood for cells, transfected with si-USF1 and pCDNA3.1-UGT1A3. (A) A schematic representation of the location of Pax-2 motif bound by USF1 at UGT1A3 promoter region. (B) The relationship of USF1 and UGT1A3 was studied by Dual-luciferase reporter assay; (C) Cells viability was detected by CCK-8 assay; (D) Transfection efficiency was determined by WB; (E) Cells migration was detected by wound healing assay. *P<0.05 signifies statistically significant difference when compared with NC group.
Figure 6

Identification and KEGG pathway enrichment analysis of DEGs. The si-UGT1A3, group cells were transfected with UGT1A3; (A) DEGs in cells knockdown of UGT1A3 determined by NGS; (B) DEGs in cells knockdown of USF1 determined by NGS; Venn diagram of the overlapping genes USF1 and UGT1A3; (C) KEGG pathways enriched by DEGs; (D) Pathway signature protein predicted by STRING.
Figure 7

Knockdown of USF1 inhibited cells proliferation, migration and invasion via deregulation of the neurotrophin signaling pathway. The sh-USF1, cells were transfected with sh-USF1; sh-USF1+pcDNA3.1, cells were transfected with sh-USF1 and pcDNA3.1; sh-USF1+ pcDNA3.1- IRAK1; (A) & (B) The expression levels of p75NTR, RIPK2, IRAK1, IKKβ, and USF1, detected by WB; (C) & (D) The levels of IRAK1 was detected by WB; (E) Cells migration was detected by wound healing assay; (F) Cells migration and
invasion were detected by transwell assay; *P<0.05 signifies statistically significant difference when a comparison was done with sh-USF1+ pcDNA3.1 group.