Down-regulated ATF3 Promotes Renal Clear Cell Carcinoma Progression Through PAXIP1-AS2 and OIP5-AS1/ hsa-miR-221-3p/ATF3 Axis Regulation of Endoplasmic Reticulum Stress

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Article

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Abstract

Clear cell renal cell carcinoma (ccRCC) is the most invasive type with high metastasis risk and high recurrence rate in renal cell carcinoma and there is a pressing need to explore novel prognostic predictors and therapeutic targets for ccRCC. Activating transcription factor 3 (ATF3), an oncogene or a suppressor for tumor, has been poorly reported in ccRCC. Here, we comprehensively clarified the prognostic value and potential function of ATF3 in ccRCC. By analyzing ATF3 in ccRCC several TCGA-based online databases, we found that ATF3 expression is decreased in ccRCC and indicate that ATF3 is significantly associated with the prognosis of ccRCC patients. hsa-miR-221-3p might be the most potential regulatory miRNA of ATF3 in ccRCC. Prediction and analysis of upstream lncRNAs showed PAXIP1-AS2 and OIP5-AS1 might be the most potential upstream lncRNAs of hsa-miR-221-3p/ATF3 axis in ccRCC. GO and KEGG results implied that ATF3 is involved in the regulation of apoptotic signaling pathway in response to endoplasmic reticulum (ER) stress in ccRCC. Correlation analysis showed a positive correlation between ATF3 and ER stress. According to present study, down-regulated ATF3 promotes renal clear cell carcinoma progression through PAXIP1-AS2 and OIP5-AS1/ hsa-miR-221-3p/ATF3 axis regulation of endoplasmic reticulum stress.

Introduction

Renal cell carcinoma (RCC), including clear cell renal cell carcinoma (ccRCC), papillary renal cell carcinoma (pRCC) and chromophobe renal cell carcinoma (chRCC), is the most common solid lesion in kidney and accounts for approximately 90% of kidney malignancies and 3% of all cancers. In 2022, 79,000 new kidney cancer cases and 13,920 kidney cancer deaths are expected in the United States. Among these three subtypes, ccRCC is the main tissue subtype of renal cell carcinoma, accounting for 75% of all renal cell carcinoma diagnoses, and it is also the most invasive type with high metastasis risk and high recurrence rate. Studies have found that 30% of patients have tumor cells metastasis when they are diagnosed with ccRCC. About 25–40% of patients have metastasis after receiving treatment. In addition, conventional chemotherapy and radiotherapy are not effective in patients with metastatic ccRCC. Although extensive researches have been conducted on the mechanisms of cancer development and progression, the etiology and carcinogenesis of ccRCC remain unclear. Therefore, it is important to understand the molecular mechanism of ccRCC and there is a pressing need to explore novel prognostic predictors and therapeutic targets for ccRCC.

Activating transcription factor 3 (ATF3) is a stress-induced transcription factor that plays vital roles in modulating metabolism, immunity, and oncogenesis. ATF3 acts as a hub of the cellular adaptive-response network. Multiple extracellular signals, such as endoplasmic reticulum (ER) stress, cytokines, chemokines, and LPS, are connected to ATF3 induction. Numerous studies have demonstrated the roles of ATF3 as a regulator of prostate, breast, gastric and colon, lung, and liver cancers. Pelzer et al. found that the upregulated expression of ATF3 could be observed in prostate cancer in vivo and in vitro after androgen stimulation[22]. And MicroRNA301a3p overexpression promotes cell invasion and
proliferation by targeting runtrelated transcription factor 3 in prostate cancer. Rohini et al. showed that overexpression of miR-590-3p decreases proliferation and increases the apoptosis of breast cancer cells. In contrast, ATF3 promotes death receptor 5 (DR5) induction and apoptotic cell death upon zerumbone or celecoxib treatment in human p53-deficient colorectal cancer cells. Moreover, previous study has shown salermide triggers ER stress in human non-small cell lung cancer (NSCLC) cells, which modulates the induction of the ATF4-ATF3-C/EBP homologous protein (CHOP) axis that results in DR5-dependent apoptosis. These observations suggest that ATF3 acts as an oncogene or a suppressor for tumor. However, the role of ATF3 in ccRCC has been poorly reported. Recently, a literature reported ATF3 suppresses growth and metastasis of clear cell renal cell carcinoma by deactivating EGFR/AKT/GSK3β/β-catenin signaling pathway. But the upstream regulation mechanism of ATF3 remains unclear.

In this study, we performed expression analysis and survival analysis for ATF3 ccRCC. Moreover, the microRNAs (miRNAs) and long noncoding RNAs (lncRNAs)-associated regulation of ATF3 was also explored in ccRCC. Furthermore, we demonstrated ATF3 acts as a suppressor for ccRCC by regulating apoptotic signaling pathway in response to ER stress.

Results

The expression and prognostic values of ATF3 in ccRCC

By using the Tumor Immune Estimation Resource (TIMER) online database, the mRNA expression of ATF3 was explored in human pan-cancers. Decreased expression of ATF3 was observed in Bladder Cancer (BLCA), Breast Cancer (BRCA), Kidney Chromophobe (KICH), Kidney renal clear cell carcinoma (KIRC), Kidney renal papillary cell carcinoma (KIRP), Liver hepatocellular carcinoma (LICH), Lung adenocarcinoma (LUAD), Lung squamous cell carcinoma (LUSC), Prostate adenocarcinoma (PRAD), Rectal Cancer (READ), Thyroid Cancer (THCA) and Uterine Corpus Endometrial Carcinoma (UCEC) compared with the corresponding normal tissues. (Fig. 1A). But the results of survival analysis have shown there were no statistical significance of ATF3 for predicting prognosis of patients in BLCA, BRCA, KICH, KIRP, LICH, LUAD, LUSC, PRAD, READ, THCA and UCEC by using the UALCAN databases (Supplement Fig. 1A-K). Moreover, ccRCC patients with downregulated ATF3 expression exhibited poor overall survival (OS) according to the Kaplan-Meier plotter database (Fig. 2A), the UALCAN databases (Fig. 2B) and the HPA database (Fig. 2C). Based on the above results, the role of ATF3 in ccRCC will be explored. The protein expression of ATF3 was further investigated in ccRCC by using the HPA database, and we found that the ATF3 protein level was obviously decreased in ccRCC compared with normal tissues (Fig. 1B). In addition, ATF3 expression in ccRCC samples and adjacent normal tissues was analyzed using data directly obtained from The Cancer Genome Atlas (TCGA). ATF3 expression was significantly decreased in ccRCC tissues (Fig. 1C). Furthermore, a marked decrease in ATF3 expression in ccRCC was observed in tumor samples compared with normal samples (Fig. 1D). Consistently, we also found that lower mRNA of ATF3 was expressed in KIRC than in normal in the UALCAN databases.
(Fig. 1E). These findings illustrate that ATF3 expression is decreased in ccRCC and indicate that ATF3 is significantly associated with the prognosis of ccRCC patients.

ATF3 expression and clinical parameters of ccRCC patients

ATF3 expression among groups of patients according to different clinical parameters were investigated by using the UALCAN databases. According to cancer stage, a significant decrease in ATF3 expression was observed in ccRCC patients in stages 1, 2, 3 and 4 compared to the corresponding normal controls (Fig. 3A). Based on nods metastasis status, ATF3 expression was lower in patients with ccRCC classified as N0 and N1 (Fig. 3B). Downregulation of ATF3 expression was observed in grade 2, 3 and 4 ccRCC patients compared to normal controls (Fig. 3C). In terms of gender, ATF3 expression was significantly lower in ccRCC samples from males and female (Fig. 3D). Regarding age, the ATF3 level was significantly downregulated in patients from different age groups in ccRCC (Fig. 3E). Based on ccRCC subtypes, the ATF3 level was significantly downregulated in patients with ccA subtypes and ccB subtypes (Fig. 3F). These results were further confirmed the ATF3 expression level is intimately related to ccRCC progression and metastasis.

Prediction and analysis of upstream miRNAs of ATF3

It has been widely acknowledged that ncRNAs are responsible for the regulation of gene expression. To ascertain whether ATF3 was modulated by some ncRNAs, we first predicted upstream miRNAs that could potentially bind to ATF3 and finally found 28 miRNAs (Fig. 4B). The expression correlation analysis showed ATF3 was significantly negatively correlated with hsa-miR-221-3p and has-miR-378a-3p and positively correlated with has-miR-494-3p in ccRCC (Fig. 4A). Based on the action mechanism of miRNA in regulation of target gene expression, there should be negative correlation between miRNA and ATF3. Thus, we investigated the expression and prognostic values of hsa-miR-221-3p and has-miR-378a-3p in ccRCC by using starBase database. The results have shown that hsa-miR-221-3p was obviously increased in ccRCC compared with normal tissues but has-miR-378a-3p was decreased in ccRCC (Fig. 4C). The results of survival analysis indicated ccRCC patients with upregulated hsa-miR-221-3p expression exhibited poor overall survival (OS) but no statistical significance of has-miR-378a-3p for predicting prognosis of patients in ccRCC (Fig. 4D). All these findings suggest that hsa-miR-221-3p might be the most potential regulatory miRNA of ATF3 in ccRCC.

Prediction and analysis of upstream lncRNAs of hsa-miR-221-3p

A total of 61 possible lncRNAs were predicted using starBase database and a lncRNA- hsa-miR-221-3p regulatory network was constructed by cytoscape software (Figure S2). Firstly, the expression correlation between the lncRNAs and hsa-miR-221-3p in ccRCC was detected using starBase database. Among all the 61 lncRNAs, there were 15 lncRNAs which are negatively correlated with hsa-miR-221-3p and 16 lncRNAs which are positively correlated with hsa-miR-221-3p (Table 1, Supplement Table 1). According to
the competing endogenous RNA (ceRNA) hypothesis, IncRNA could increase mRNA expression by competitively binding to shared miRNAs. Therefore, there should be negative correlation between IncRNA and miRNA or positive correlation between IncRNA and mRNA. Therefore, we explored correlation between 15 IncRNAs, negatively correlated with hsa-miR-221-3p and ATF3. To predict possible IncRNAs that could potentially regulated ATF3, we predicted the correlation between 15 IncRNAs and ATF3 using starBase database and GEPIA database. Only the predicted IncRNAs that have a significant correlation in more than one database as mentioned above were included for subsequent analyses. As the Table 2 shown, only GAS5, AC095055.1, PAXIP1-AS2, ZEB1-AS1, AP000766.1, OIP5-AS1 and TUG1 were positively correlation with ATF3. Next, the expression of GAS5, AC095055.1, PAXIP1-AS2, ZEB1-AS1, AP000766.1, OIP5-AS1 and TUG1 in ccRCC were performed using starBase database. The results demonstrated PAXIP1-AS2, AP000766.1 and OIP5-AS1 were downregulated in ccRCC compared with normal tissues but GAS5 was upregulated in ccRCC (Fig. 5A-C, Supplement Fig. 3A-D). The results of survival analysis showed ccRCC patients with downregulated PAXIP1-AS2 and OIP5-AS1 expression exhibited poor overall survival according to the starBase database (Fig. 5D, F), but not AP000766.1 (Fig. 5E). These findings indicated PAXIP1-AS2 and OIP5-AS1 might be the most potential upstream IncRNAs of hsa-miR-221-3p/ATF3 axis in ccRCC.
Table 1
Correlation analysis between IncRNA and hsa-miR-221-3p in ccRCC.

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<th>IncRNA</th>
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Table 2
Correlation analysis between lncRNA and ATF3 in ccRCC.

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<th>GEPIA database</th>
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<td>0.03</td>
<td>4.91E-01</td>
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<tr>
<td>ADD3-AS1</td>
<td>0.04</td>
<td>3.59E-01</td>
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<td>AP000766.1</td>
<td>0.097</td>
<td>2.53E-02</td>
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<tr>
<td>DHRS4-AS1</td>
<td>0.008</td>
<td>8.51E-01</td>
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<td>TYMSOS</td>
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<td>TUG1</td>
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Identification of ATF3-interacting genes and proteins and Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of ATF3-interacting genes

Using the GeneMANIA database and the STRING online database, we found a total of 20 genes and proteins that interact with ATF3 (Fig. 6A-B). In order to further clarify the role of ATF3 in the
In order to verify whether ATF3 is closely related to ER stress, we explored the correlation between ATF3 and key genes regulating ER stress using TIMER database. The results showed that ATF3 were positively correlated with SERP1, SERP2, IRE1α, ATF6 and PERK (Fig. 7A). Moreover, the expression of SERP1, SERP2 and ATF6 was significantly decreased in ccRCC. The IRE1α and PERK expression in ccRCC was not different from that in normal (Fig. 7B). These results suggest that the anticancer effect of ATF3 may be achieved through the regulation of apoptotic signaling pathway in response to ER stress in ccRCC.

Discussion

ccRCC, with a mortality rate of 30–40% compared to bladder and prostate cancers which have a mortality rate of about 20%, is a complex and heterogeneous disease with different clinical features. Despite the increase in the total incidence over the past three decades, especially in developed countries, due to early diagnosis and therapy, the mortality rate of ccRCC has decreased rapidly. However, despite the progress in disease control and survival, locally advanced disease and distant metastases still occur in many patients. Therefore, elucidating the molecular mechanism of ccRCC carcinogenesis may provide key clues for developing effective therapeutic targets or seeking promising prognostic biomarkers. It has been reported that ATF3 is a marker of nerve injury and significantly changed in animal models of osteoarthritis. Nowadays, increasing evidence has demonstrated that ATF3 plays key roles in initiation and progression of multiple human cancers. However, the knowledge of ATF3 in ccRCC remains inadequate and needs to be further investigated.

In present study, we demonstrated ATF3 was obviously decreased in ccRCC compared with normal lung tissues both in protein level and mRNA level. And ccRCC patients with downregulated ATF3 expression exhibited poor overall survival. Furthermore, downregulation of ATF3 expression was observed in grade 2, 3 and 4 and nods metastasis ccRCC patients compared to normal controls. Gao et al. demonstrated that ATF3 suppresses growth and metastasis of clear cell renal cell carcinoma. This report together with our analytic results showed ATF3 acts as a suppressor in ccRCC.

It has been shown that ncRNA, including miRNA, IncRNA and cyclic RNA (circRNA), participate in the regulation of gene expression through the interaction of ceRNA mechanism. Overexpression of the clustered mRNAs hsa-miR-221 and hsa-miR-222 is reported to directly lead to increase the growth and...
tumorigenesis of oral carcinoma cells\textsuperscript{27}. In this study, 28 miRNAs were finally obtained and hsa-miR-221-3p was significantly negatively correlated with ATF3. Moreover, hsa-miR-221-3p was obviously increased in ccRCC compared with normal lung tissues and correlated with poor overall survival of ccRCC patients. Previous studies have documented that miR-221 and miR-222 play a key role in modulating the clinical outcome in cancer patients in both solid and hematological malignancies\textsuperscript{28–30}. All these findings combined with our data suggest that hsa-miR-221-3p might be the most potential regulatory miRNA of ATF3 in ccRCC.

IncRNAs play significant roles in the regulation of various biological functions, including epigenetic regulation and posttranscriptional regulation\textsuperscript{31}. To explore the upstream regulatory IncRNAs of hsa-miR-221-3p/ATF3 axis, we predicted a total of 64 IncRNAs associated with hsa-miR-221-3p using starBase database. By conducting expression analysis, survival analysis, and correlation analysis, PAXIP1-AS2 and OIP5-AS1 might be the most potential upstream IncRNAs of hsa-miR-221-3p/ATF3 axis in ccRCC. PAXIP1-AS2 is a long non-coding antisense RNA, which had no known function. Recent study had reported that PAXIP1-AS2 led each to reduced amounts of the RAD18 protein and DNA polymerase η, leading to reduced translesion DNA synthesis (TLS) and PAXIP1-AS2 were considered to be a new TLS regulator\textsuperscript{32}. Nevertheless, OIP5-AS1 has been reported to function as oncogenes in multiple malignancies\textsuperscript{33–35}, but it plays a protective role in esophageal squamous carcinoma cells\textsuperscript{36} and esophageal carcinoma\textsuperscript{37}. Taken together, PAXIP1-AS2 and OIP5-AS1/ hsa-miR-221-3p/ATF3 axis were identified as potential regulatory pathways in ccRCC.

The endoplasmic reticulum (ER) is an important organelle for protein synthesis, folding and modification, lipid synthesis and calcium storage. ER stress is activated to restore homeostasis when intracellular homeostasis is disrupted and the demands on endoplasmic reticulum function exceed its capacity\textsuperscript{38}. ER stress is a double-edged sword. When disruptions in the internal environment are relatively mild, ER stress can reverse these disruptions. However, when the body is unable to correct these abnormalities, intense and chronic ER stress will induce associated apoptosis\textsuperscript{38, 39}. Numerous studies have documented that ER stress contributes to tumorigenesis in various tumors, including glioblastoma and carcinomas of the breast, stomach, colon, esophagus, lung, prostate, pancreas, and liver\textsuperscript{40}. But in RCC, ER stress can significantly inhibit tumor progression\textsuperscript{41}. Our results of GO and KEGG results strongly implied that ATF3 is involved in the regulation of apoptotic signaling pathway in response to ER stress in ccRCC. In addition, ATF3 were positively correlated with Genes involved in endoplasmic reticulum stress-related SERP1, SERP2, IRE1α, ATF6 and PERK. Furthermore, the expression of SERP1, SERP2 and ATF6 was significantly decreased in ccRCC. In present study, it was found that promoting ER stress can suppress renal cell carcinoma progression\textsuperscript{42}. And another study found that the expression of ATF6, PERK, but not IRE1α, was downregulated in ccRCC patients with advanced tumor stage and grade. This demonstrated ER stress was suppressed in progressive ccRCC patients\textsuperscript{43}. The above studies further confirm our conclusion antitumor effect of ATF3 may be achieved through the regulation of apoptotic signaling pathway in response to ER stress in ccRCC.
Conclusions

ATF3 was lowly expressed in ccRCC and negatively correlated with poor prognosis in ccRCC. PAXIP1-AS2 and OIP5-AS1/ hsa-miR-221-3p/ATF3 axis were identified as potential regulatory pathways in ccRCC. This anticancer effect may be achieved through the regulation of apoptotic signaling pathway in response to ER stress in ccRCC (Fig. 8). Although our study still needs to be validated by extensive basic research, it can also provide a new theoretical basis for ccRCC research.

Materials and Methods

UALCAN

UALCAN (http://ualcan.path.uab.edu/) was used to investigate ATF3 expression and the association between ATF3 and various clinicopathological parameters (cancer stage, nodes metastasis, tumor grade, gender, age, subtypes) of KIRC and survival analysis for ATF3 in KIRC, BLCA, BRCA, KIRC, KIRP, LIHC, LUAD, LUSC, PRAD, READ, THCA and UCEC.

The Human Protein Atlas (HPA)

The Human Protein Atlas (https://www.proteinatlas.org/) was used to explore the protein of ATF3 expression and survival analysis for ATF3 in ccRCC.

Gene Expression Profiling Interactive Analysis (GEPIA)

GEPIA (http://gepia.cancer-pku.cn/index.html) is a web portal for gene expression analysis based on TCGA and GTEx data. In the current study, overall survival (OS) analysis for ATF3 was evaluated using TCGA-KIRC datasets. And the expression correlation between the lncRNAs and ATF3 was explored by GEPIA database.

Analysis of ATF3-Interacting Genes and Proteins

The GeneMANIA database (http://www.genemania.org) was applied to construct the ATF3 interaction network. The STRING online database (https://string-db.org/) was applied to construct a protein-protein interaction (PPI) network of ATF3.

Gene Ontology (GO) Term and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment Analysis

DAVID (https://david.ncifcrf.gov/summary.jsp) online analytical tool was used to KEGG Pathway Enrichment Analysis and GO analysis including Biological Process (BP), Cellular Component (CC),
Molecular Function (MF) for genes interacted with ATF3. Enrichment analysis results through the http://www.bioinformatics.com.cn online tools for visualization.

**Tumor Immune Estimation Resource (TIMER)**

TIMER (https://cistrome.shinyapps.io/timer/) is a web server for comprehensive analysis of tumor-infiltrating immune cells. TIMER was used to analyze the correlation of ATF3 expression in pan-cancer and the correlation of ATF3 and key genes regulating ER stress in KIRC. TIMER was also applied to investigate the expression of the key genes regulating ER stress in KIRC.

**Kaplan-Meier Plotter Database Analysis**

KM Plotter (http://kmplot.com) was used to analyze the prognostic value of ATF3 in KIRC. The patient samples were separated into two groups by median expression (high expression and low expression) to analyze the overall survival (OS) with hazard ratios (HRs) with 95% confidence intervals (95% CIs) and log-rank p-values. The prognostic value of GAS5, DANCR and RASSF8-AS1 in KIRC were also estimated by Kaplan-Meier plotter.

**Candidate miRNA prediction**

Upstream binding miRNAs of ATF3 were predicted by several target gene prediction programs, consisting of PITA, RNA22, miRmap, microT, miRanda, PicTar, and TargetScan. Only the predicted miRNAs that commonly appeared in more than two programs as mentioned above were included for subsequent analyses. These predicted miRNAs were regarded as candidate miRNAs of ATF3.

**starBase database analysis**

starBase (http://starbase.sysu.edu.cn/) was introduced to perform expression correlation analysis for miRNA-ATF3, IncRNA- hsa-miR-221-3p, or IncRNA-ATF3 in KIRC. The expression and prognostic values of hsa-miR-221-3p and has-miR-378a-3p in KIRC was also analyzed by database. Besides, starBase was used to predict candidate IncRNAs that could potentially bind to hsa-miR-221-3p.

**Statistical analysis**

The statistical analysis in this study was automatically calculated by the online database mentioned above. p value < 0.05 or log rank p value < 0.05 was considered as statistically significant.

**Declarations**

**Acknowledgements**
This study was supported by the Hebei Health Commission Scientific Research Foundation Project (20220596).

**Authors’ contributions**

Conceptualization and data curation: Xinsheng Wang; formal analysis: Jingqi Li, Methodology: Dandan Xu, Zhichao Yang; Writing—original draft: Zhicong Yang; Writing—review & editing: Yongwang Hou.

**Data availability statement**


**Competing Interests Statement**

The author(s) declare no competing interests.

**Abbreviations**

ATF3, activating transcription factor 3; ccRCC, clear cell renal cell carcinoma; ncRNA, non-coding RNA; lncRNA, long non-coding RNA; miRNA, MicroRNA; ER, endoplasmic reticulum; OS, overall survival.

**Ethics approval and consent to participate**

Our study did not require an ethical board approval because it did not contain human or animal trials.

**References**


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Figures

Expression of ATF3 in ccRCC. (A) The mRNA of ATF3 expression in different types of cancer was investigated with the TIMER database. (B) The protein of ATF3 expression in ccRCC was examined by
using the HPA database. (C) TCGA database and statistical analyses of ATF3 expression in 72 pairs of ccRCC tissues and adjacent normal tissues. (D) Analysis of ATF3 expression in ccRCC and normal tissues in the TCGA database. (E) The mRNA of ATF3 expression in ccRCC was examined by using the UALCAN database. **p value < 0.01; ***p value < 0.001; ****p value < 0.0001.

Figure 2

Survival curve evaluating the prognostic value of ATF3 for ccRCC. (A) Survival curves using the Kaplan-Meier plotter is shown for OS. (B) Survival curves using the UALCAN database is shown for OS. (C) Survival curves using the HPA database is shown for OS.

Figure 3
Box plots evaluating ATF3 expression among different groups of patients based on clinical parameters using the UALCAN database. Analysis is shown for cancer stage (A), nodes metastasis (B), tumor grade (C), gender (D), age (E), subtypes (F). *p < 0.05, **p < 0.01, ***p < 0.001, ****p value < 0.0001.

**Figure 4**

Identification of hsa-miR-221-3p as a potential upstream miRNA of ATF3 in ccRCC. (A) The expression correlation between predicted miRNAs and ATF3 in ccRCC analyzed by starBase database. (B) The miRNA-ATF3 regulatory network established by Cytoscape software. (C) The expression of hsa-miR-221-3p and hsa-miR-378a-3p in ccRCC and control normal samples determined by starBase database. (D) The prognostic value of hsa-miR-221-3p and hsa-miR-378a-3p in KIRC assessed by starBase database.
Figure 5

Expression analysis and survival analysis for upstream lncRNAs of hsa-miR-221-3p in ccRCC. **(A–C)** The expression of PAXIP1-AS2, AP000766.1 and OIP5-AS1 in ccRCC and control normal samples determined by starBase database. **(D–F)** The OS analysis for PAXIP1-AS2, AP000766.1 and OIP5-AS1 in ccRCC by using the starBase database.
Figure 6

(A) The gene-gene interaction network of ATF3 was constructed using GeneMANIA database. (B) The PPI network of ATF3 was generated using STRING. (C, D) GO and KEGG enrichment analysis for ATF3.
Correlation analysis between ATF3 and genes of ER-stress and the expression of genes of ER-stress in ccRCC determined by TIMER database. (A) Correlation analysis among ATF3 and SERP1, SERP2, ATF6 and EIF2AK3. (B) The expression of SERP1, SERP2, ATF6 and EIF2AK3 in ccRCC.
Figure 8

The model of PAXIP1-AS2 and OIP5-AS1/hsa-miR-221-3p/ATF3 axis in carcinogenesis of ccRCC.

**Supplementary Files**

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- Supplementarymaterial.docx