Original Research Article

Technology in Cancer Research & Treatment
Volume 23: 1-16
© The Author(s) 2024
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/15330338241255585
journals.sagepub.com/home/tct

LncRNA AP000842.3 Triggers the Malignant Progression of Prostate Cancer by Regulating Cuproptosis Related Gene NFAT5

Gaobo Zhou, MS[1], Chaoqian Chen, MS[1], Hongjian Wu, MS[1], Jiao Lin, MS[1], Hang Liu, MS[2], Yiran Tao, MS[1], and Bin Huang, MS[1]

Abstract

Objectives: Prostate cancer (PRAD) is a highly malignant disease with poor prognosis, and its development is regulated by a complex network of genes and signaling pathways. LncRNAs and miRNAs have significant regulatory roles in PRAD through the ceRNA network. Cuproptosis is a unique form of programmed cell death that is involved in various signaling pathways and biological processes related to tumor development. Nuclear factor of activated T cells 5 (NFAT5), a transcription factor that activates T cells, has been implicated in cuproptosis. However, the regulatory mechanism by which NFAT5 is involved in the ceRNA network in PRAD remains unclear. **Methods:** Through bioinformatics analysis, we found the ceRNA axis that regulates cuproptosis. By performing ROS assay and copper ion concentration assay, we demonstrated that inhibiting NFAT5 can increase the sensitivity of PRAD to cuproptosis inducers. By using luciferase assay, we discovered that AP000842.3 acts as the ceRNA of miR-206 to regulate the expression of NFAT5. **Results:** In this study, we found that lncRNA AP000842.3, as a ceRNA of miR-206, was involved in the regulation of levels of the transcription factor NFAT5 associated with cuproptosis in PRAD. First, knocking down NFAT5 can increase the sensitivity of PRAD to cuproptosis inducers. Meanwhile, changes in the expression of AP000842.3 and miR-206 could affect the proliferation of PRAD by regulating NFAT5. Mechanistically, AP000842.3 acts as the ceRNA of miR-206 to regulate the expression of NFAT5. In addition, the effects of lncRNA AP000842.3 on malignant progression of PRAD and NFAT5 were partially dependent on miR-206. **Conclusion:** Taken together, our study reveals a key ceRNA regulatory network in PRAD and can be regarded as a new potential target for PRAD diagnosis and treatment.

Keywords

prostate cancer, cuproptosis, CeRNA, NFAT5, proliferation

Received: December 27, 2023; Revised: April 23, 2024; Accepted: April 25, 2024.

Introduction

Prostate cancer is a type of cancer affecting men's prostate gland.[1] It is considered highly malignant because it has the potential to spread or metastasize to other parts of the body, if not detected and treated early.[2] Prostate cancer diagnostic techniques encompass prostate-specific antigen testing, MRI scans, and biopsies of prostate tissue.[3] Among them, the actual effective diagnosis can only rely on prostate biopsy. While the majority of prostate cancers exhibit a slow growth rate and are characterized by low-grade features, posing a relatively low risk and limited aggressiveness, the absence of initial or early symptoms is common in most instances.[4] The prognosis for prostate cancer is generally considered poor when it is diagnosed at an advanced stage or has already metastasized.[5] The cancer may have spread beyond the prostate gland in advanced cases, making treating it more challenging. The present options

[1] Department of Urology, Taizhou Hospital of Zhejiang Province affiliated to Wenzhou Medical University, Taizhou, China
[2] Department of Urology, Yubei District Hospital of Traditional Chinese Medicine, Chongqing, China

Gaobo Zhou, Chaoqian Chen and Hongjian Wu have equal contribution to this study.

Corresponding Authors:
Yiran Tao, Department of Urology, Taizhou Hospital of Zhejiang Province affiliated to Wenzhou Medical University, No. 150, Xi Men Street, Taizhou 317000, China;
Email: 8911@enzemed.com

Bin Huang, Master's degree, Department of Urology, Taizhou Hospital of Zhejiang Province affiliated to Wenzhou Medical University, No. 150, Xi Men Street, Taizhou 317000, China.
Email: huangb17@tsinghua.org.cn

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (https://creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access page (https://us.sagepub.com/en-us/nam/open-access-at-sage).

for managing prostate cancer consist of various treatments such as surgical intervention, chemotherapy, radiotherapy, immunotherapy, and targeted therapy.[6] Nevertheless, despite the existence of multiple treatment alternatives, prostate cancer remains an incurable condition. Therefore, finding new ways to diagnose and treat prostate cancer is urgent.

MicroRNAs (miRNAs) and long non-coding RNAs (lncRNAs) are categorized as non-coding RNAs (ncRNAs) that are recognized as pivotal regulators of cancer.[7] MiRNAs, compact single-stranded RNAs, are fulfill crucial functions in biological processes including development, stem cell differentiation, and tumorigenesis.[8] MiRNAs exert post-transcriptional regulation over protein expression by either degrading mRNA or impeding mRNA translation processes.[9] Hsa-miR-206 is a novel factor in tumor regulation, and miR-206 can inhibit breast tumor initiation and metastasis,[10] but its precise role and signaling mechanisms in prostate cancer remain elusive. LncRNAs can act as precursors of miRNAs, establishing a direct association with the functional properties of miRNAs.[11] LncRNAs can also work as miRNA sponges, impeding the degradation of mRNAs that are targeted by miRNAs.[12] In prostate cancer, both lncRNAs and miRNAs perform in multiple processes, encompassing cell proliferation, apoptosis, invasion and metastasis.[13,14] LncRNAs have also been shown to affect molecular pathways critical for prostate cancer proliferation and metastasis.[15] Thus, lncRNAs are pivotal regulators of molecular pathways and mechanisms, and modulating their expression levels can potentially influence the progression of prostate cancer cells.[16] Competing endogenous RNAs (ceRNAs) are RNA molecules that possess the ability to competitively bind miRNAs and sequester them away to avoid target transcripts' degradation or expression inhibition.[17] Studies have shown that lncRNA-regulated ceRNA networks have significant regulatory effects in prostate cancer.[18] Research suggests that lncRNAs serve as key orchestrators in the cancer development by supporting tumor cell growth, evading growth suppressors and facilitating metastasis.[19]

Cuproptosis is a cellular process observed in tumors where an excessive accumulation of copper ions induces programmed cell death.[20,21] It represents a unique kind programmed cell death which sets it apart from other pathways such as apoptosis and ferroptosis.[22] Copper is closely linked to numerous signaling pathways and biological behaviors relevant to tumor development.[20] The role of copper homeostasis and cuproptosis in human cancers, including prostate cancer, is an active area of research.[23] Cuproptosis demonstrates a strong association with cellular metabolism, particularly in cancer types characterized by elevated aerobic respiration levels, such as melanoma, breast cancer, and leukemia.[20] Studies have been conducted in prostate cancer to annotate the involvement of cuproptosis and unravel its potential regulatory mechanisms.[24] A study developed a prediction model for prostate cancer prognosis depended on cuproptosis-related genes (CRGs) with high accuracy in forecasting.[25] Another study sought to establish a unique CRGs signature and investigate its underlying molecular role in prostate cancer.[26] While the extract function of cuproptosis in prostate cancer is still under investigation, it has been observed to exhibit characteristics related to the tumor microenvironment and demonstrate potential as a predictor of therapeutic sensitivity and prognosis.[25] Copper chelators and copper ionophores are suggested as potential therapeutic targets for treating cancer.[27] Understanding cuproptosis opens avenues for developing therapeutic strategies that exploit copper-induced cytotoxicity as a targeted approach to eliminate cancer cells while minimizing harm to healthy cells.

NFAT5, recognized as the Nuclear Factor of Activated T-cells 5, is a transcription factor in controlling genes expression implicated in osmoregulation and inflammation.[28,29] It belongs to NFAT family, which is activated by calcium signaling pathways and is involved in regulating immune response, cell differentiation, and proliferation.[30] NFAT5 regulates gene expression in the response to hypertonic stress.[29] It has shown that NFAT5 is critical in cancer progression and metastasis.[31] NFAT5 regulates genes associated with cell cycle progression, apoptosis, and angiogenesis, thereby promoting cancer cell proliferation and migration.[32] NFAT5 additionally facilitates the epithelial-mesenchymal transition, a pivotal part in the cancer metastatic process.[33] While no empirical investigations have yet established a direct association between NFAT5 and cuproptosis, extant research suggested that a signature of lncRNAs associated with cuproptosis may serve as a prognostic indicator for head and neck carcinoma. This signature appeared to regulate the miR-140-5p/NFAT5/Wnt/β-catenin pathway axis, thereby potentially modulating the progression of the disease.[34] The link between NFAT5 and cuproptosis may be related to immune infiltration.[34] There is no direct study on NFAT5 in prostate cancer, but NFATc1, one of the NFAT family, is overexpressed and associated with recurrence risk after radical prostatectomy.[35] The regulatory mechanism of NFAT5's receipt of ncRNA in prostate cancer remains to be elucidated. Further research is needed to gain comprehensive functions of NFAT5 in cancer and devise novel therapeutic approaches targeting this transcription factor.

In this study, we identified the involvement of lncAP000842.3 as a ceRNA for miR-206 in regulating cuproptosis-related transcription factor NFAT5 level in prostate cancer. We verified that knocking down NFAT5 can increase the sensitivity of PRAD to cuproptosis inducers. Furthermore, we confirmed the binding of lncAP000842.3 to miR-206, acting as a molecular sponge to modulate NFAT5. Notably, the influence of lncAP000842.3 on the prostate cancer malignant progression and NFAT5 was partially reliant on miR-206.

Materials and Methods

Data Collection and Differential Gene Expression Analysis

RNA-seq and miRNA-seq data and corresponding clinical information were downloaded from the prostate cancer dataset of TCGA database (https://portal.gdc.cancer.gov/), including 57 tumor samples and 52 normal samples. With *P*

values ≤ 0.05 and logFC > 1 as screening conditions. In this study, the differential expression of miRNAs, mRNAs, and lncRNAs between prostate cancer and normal samples was analyzed using the "limma" software package. To visualize the differential genes, heat maps and volcano maps were generated using the "ggplot2" and "pheatmap" packages, respectively.

Gene Set Enrichment Analysis

To explore the possible biological functions and potential signaling pathways of these differentially expressed genes involved in prostate cancer, "clusterProfiler" in the R package was used to perform gene set enrichment analysis of the identified DEGs. The screening criteria for GO enrichment analysis were $P < 0.05$ and FDR < 0.25. Finally, bubble plots were drawn to visualize the enrichment results, while chordplots were drawn using the "circlize" package to display the mRNAs in each pathway.

Consistent Cluster Analysis

"ConsensusClusterPlus" R package was used to perform consensus clustering on the expression matrix of mRNA and expression profile of cuproptosis-related genes, and the samples were divided into k groups (k = 2-9), which were repeated 1000 times to ensure the stability of classification. To determine the optimal number of clusters in this study, the consensus matrix and cumulative distribution function (CDF) were utilized. Additionally, the Kaplan-Meier method was employed to estimate differences in overall survival (OS) between different clusters. Furthermore, *Chi*-square tests were utilized to compare the distribution of categorical data among clusters.

Construction of the lncRNA-microRNA-mRNA Network

Firstly, the miRcode database was used to predict the interaction between lncRNA and miRNA. After that, the miRNA-mRNA interactions were searched using TargetScan, mirTarBase, and miRDB databases. This study aimed to investigate the potential association between targeted miRNAs and lncRNAs at the expression level. The analysis was conducted to identify lncRNAs that could be more appropriate for inclusion in the ceRNA network. Finally, the identified co-expression competing triplets were used to construct mRNA-miRNA-lncRNA networks. The ceRNA network was visualized using Cytoscape v3.7.2 in this study.

DAVID Enrichment Analysis

DAVID is a biological information database designed to provide annotated information on biological functions for establishing functional enrichment in different diseases. In this study, the differential gene list was uploaded to the database to explore the GO terms and KEGG pathways related to prostate cancer. The cutoff value was set to $P < 0.05$, where a smaller P value indicates more abundant GO term and KEGG pathways. The enrichment results were visualized using bubble plots.

Cell Culture and Cell Transfection

Human prostate cancer cell lines (LNCaP and 22RV1) were purchased from the National Certified Cell Culture Preservation Center (Shanghai, China). The cells were cultured in RPMI-1640 medium (Gibco, China) supplemented with 10% fetal bovine serum (Gibco, China) and 1% penicillin/streptomycin (Gibco, China) and placed in an incubator at 37°C and 5% CO_2, and the solution was changed every one day. Lipofectamine 2000 (Invitrogen, MA, USA) transfection reagent was used for transfection, and all mimics, inhibitors and their NC were designed and constructed by Geneseed (Guangzhou, China). Oligostranded RNA and empty vector were transfected into prostate cancer cells, and 48 h after transfection, cells were harvested for further experiments.

CCK8

Briefly, cells in the logarithmic growth phase were seeded in 96-well plates at a density of 1000 cells per well, and were then incubated at 37°C for 48 h. Following this period, 10 µL of CCK-8 reagent was added to each well and incubated for an additional 2 h. The absorbance value at 450 nm was then measured using a microplate reader (Bio-Tek, USA). The experiment was repeated at least three times.

RNA Extraction and qRT-PCR

Total RNA was isolated from cells using TRIZOL reagent (TaKaRa, Japan) and 1µg RNA was synthesized into cDNA according to manufacturer's instructions. Secondly, cDNA was amplified using SYBR Premix Ex Taq kit (TaKaRa, Japan) and RNA expression was measured by qRT-PCR. β-actin was used as the internal reference gene of mRNA. The experiment was repeated at least three times.

Protein Extraction and Western Blotting

Cells were harvested and lysed in RIPA lysis buffer (Beyotime, China), and the supernatant was collected after centrifugation. Protein concentrations were measured using the BCA kit (Beyotime, China). Proteins were separated on 10% SDS-PAGE gels and transferred to PVDF membranes, which were blocked in 5% skim milk powder for 2 h at room temperature. The PVDF membrane was incubated with the primary antibody at 4°C overnight, washed with TBST for 3 times, and the secondary antibody was closed for 1 h. Finally, the images were examined using a gel imaging system (BIO-RAD, USA). The antibodies used include: NFAT5 (CST, #5861, 1:1000) and β-actin (CST, #4967, 1: 1000). The experiment was repeated at least three times.

Luciferase Assay

To test the binding specificity of lncRNA AP000842.3 and miR-206, we constructed a vector containing the wild-type AP000842.3 and the corresponding mutant AP000842.3, and the plasmids were co-transfected into LNCaP cells for 24 h with the indicated luciferase reporter and Renilla luciferase reporter using liop2000. Following this incubation period, luciferase activity was measured using the dual luciferase assay kit (FR201-01, TransGen, China), with Renilla luciferase activity used to standardize for transfection efficiency. The experiment was repeated at least three times.

Reactive Oxygen Species Detection

Prostate cancer cell lines (22RV1 and LNCaP) in logarithmic growth phase were seeded into 12-well plates and cultured until the cell density reached 40%-50%. Cells were treated with an appropriate concentration of the cupredoxin inducer Elesclomol for 24 h. During the last 20 min of the cell treatment, a 10 μM reactive oxygen species (ROS) fluorescent probe was added. The probe specifically reacts with ROS and emits a fluorescent signal. The cells were further incubated at 37 °C for 20 min to allow sufficient reaction between the ROS fluorescent probe and intracellular ROS. Fluorescent signals were detected using a flow cytometer with an excitation wavelength of 488 nm, which excites the fluorescence signal generated by the ROS fluorescent probe. The experiment was repeated at least three times.

Intracellular Copper Ion Concentration Detection

The prostate cancer cell lines (22RV1 and LNCaP) were seeded into a 6-well plate and cultured overnight. Subsequently, the cells were treated with $CuCl_2$ and the cupredoxin inducer Elesclomol for 24 h. After treatment, the cells were collected and resuspended in 120 μL of water. The cells were then sonicated to disrupt the cell membranes and release the intracellular copper ions. Finally, the intracellular copper ions were detected following the instructions provided in the manual (E-BC-K775-M, Elabscience, USA). The experiment was repeated at least three times.

Transwell Assay

Prepare pre-chilled Matrigel matrix gel and mix it with serum-free culture medium in a 1:5 ratio. Add 50 μL of the mixture to each well and incubate overnight at 37°C to allow gel solidification. Retrieve pre-treated cells, resuspend in 1% serum-containing medium, and add 100 μL of cell suspension to the upper chamber. Add 10% serum-containing medium to the lower chamber and place in a 24-well plate. Incubate for 24 h, remove chambers, discard medium, and wash with PBS. Fix cells with formaldehyde for 10 min, stain with 0.1% crystal violet for 10 min, gently remove cells, wash with PBS, randomly select and observe cells in five fields, count and capture images.

Statistical Analysis

All the data in this study were statistically analyzed using GraphPad Prism 8 software. For comparisons between two groups, a two-tailed Student's t-test was employed, while Spearman correlation analysis was utilized for gene correlation studies. A significance level of $P < 0.05$ was employed to determine statistical significance.

Results

To Screen the lncRNA, microRNA and mRNA Related to Prostate Cancer Progression

The expression levels of tumor markers vary significantly during the occurrence and development of tumors. A significant proportion of these markers act as oncogenes and tumor suppressor genes, regulating the malignant progression of tumors. However, the role of these genes in the ceRNA axis of prostate cancer remains unclear. To investigate this mechanism, gene expression and clinical information data of 57 prostate cancer patients and 52 normal samples were downloaded from the TCGA database. The study identified a total of 2135 differentially expressed mRNAs, of which 866 were up-regulated and 1269 were down-regulated. Similarly, 1416 differentially expressed lncRNAs were identified, with 548 up-regulated and 868 down-regulated. Additionally, 43 differentially expressed microRNAs were screened, with 26 up-regulated and 17 down-regulated. These results are shown in the volcano map and heat map (Figure 1A-B and supplementary figure 1A-B). To further explore the importance of these genes in prostate cancer, GSEA enrichment analysis was performed for the differentially expressed genes. Enrichment analysis of the differential genes in this study revealed that they were primarily associated with the cell cycle and cell proliferation in prostate cancer (Figure 2A-B). These findings suggested that genes involved in these processes may play a key role in the development and progression of the disease.

Consensus Cluster Analysis of Genes Associated with Cuproptosis

Cuproptosis is a cellular process observed in tumors where an excessive accumulation of copper ions induces programmed cell death. Emerging evidence suggests that cuproptosis may play a critical role in the development and progression of prostate cancer. In order to divide prostate cancer patients into groups with different cuproptosis levels, cluster analysis was carried out according to the expression matrix of 13 genes related to cuproptosis and mRNA data of PRAD from the TCGA database. These data were divided into k groups ($k = 2$-9), and the optimal classification was obtained when $k = 4$ (Figure 3A and supplementary figure 2A-C). The difference in overall survival between cluster 1 and cluster 3 when $k = 4$ was explored using Kaplan-Meier analysis and found that cluster 1 (G1) had a significantly better survival status than

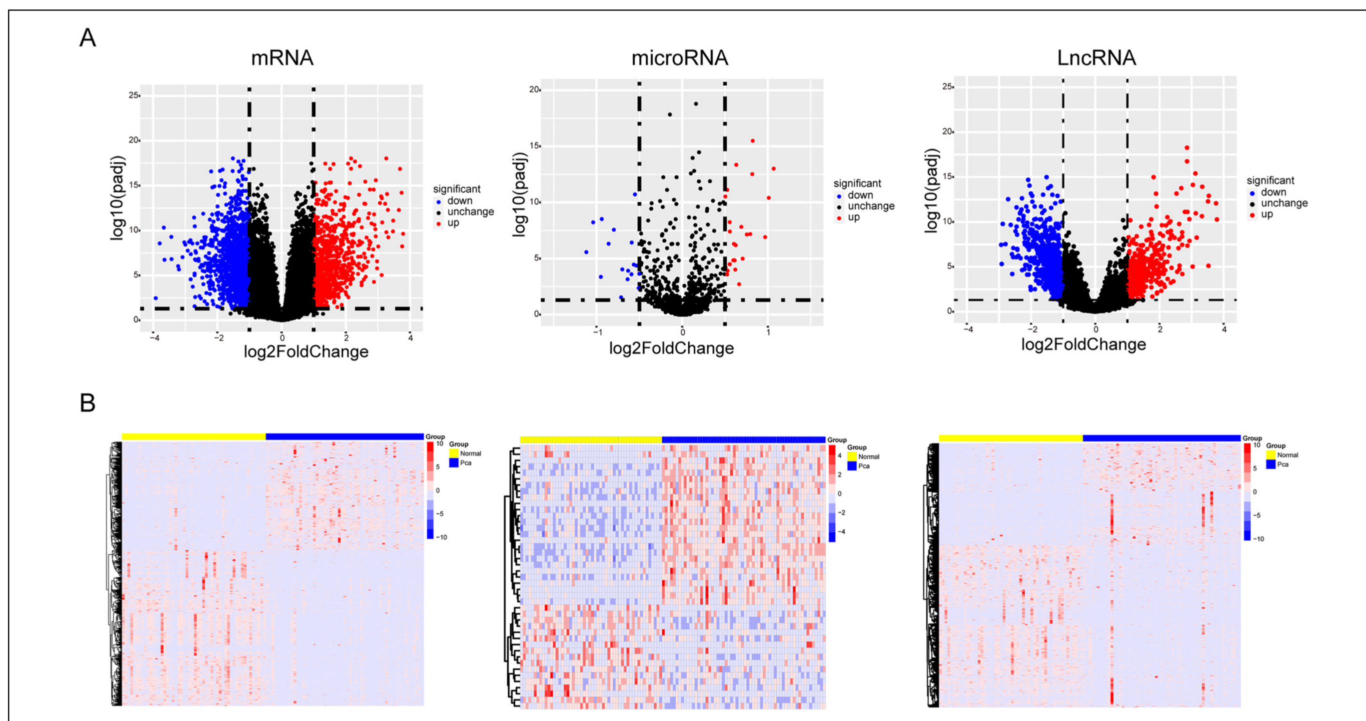

Figure 1. To screen the lncRNA, microRNA and mRNA related to prostate cancer progression. (A) Heatmap representing differentially expressed mRNAs, miRNAs and lncRNAs between prostate cancer patients (n = 57) and normal samples (n = 52). Red indicated increased expression and blue indicated decreased expression. (B) The volcanic map showed differentially expressed mRNAs, miRNAs and lncRNAs.

cluster 3 (G2) (Supplementary figure 3D, $P = 0.049$). Following the clustering analysis, the "limma" package was employed to conduct differential analysis between the two identified clusters, and a total of 1439 up-and 6 down-regulated differential genes were screened out (Figure 3B-C, G3 group was the control group). To better understand the mechanism of these 1445 differentially expressed genes in prostate cancer, David enrichment analysis was performed, and the results showed that these genes were mainly enriched in cell cycle and apoptosis (Figure 3D).

NFAT5/miR-206 May Be Important ceRNA Axis Regulation of Prostate Cancer

To further elucidate the mechanism by which miRNAs affect the occurrence and progression of prostate cancer, we constructed a ceRNA regulatory network regulating the malignant progression of prostate cancer through differentially expressed lncRNAs and miRNAs and mRNA after cuproptosis cluster analysis. Firstly, the regulatory relationship between lncRNA and miRNA was obtained by bioinformatics software. Secondly, the downstream target mRNA corresponding to miRNA was predicted by Targetscan, mirdb, and mirtarbase databases. Finally, Cytoscape 3.9.0 software was utilized to create a visual representation of a triple regulatory network comprising of 2 miRNAs, 9 mRNAs, and 33 lncRNAs, which resulted in a total of 201 interaction axes. The mulberry diagram (Figure 4A-B and supplementary figure 3A) depicts this network. Although NFAT5, a T cell transcription factor, is known to play a crucial role in the development of tumors, its role in prostate cancer has not been fully understood. Thus, our study specifically focuses on investigating the role of NFAT5 in prostate cancer.

Inhibiting NFAT5 Can Increase the Sensitivity of PRAD to Cuproptosis Inducers

In order to investigate the role of NFAT5 in cuproptosis, the IC50 values of Elesclomol (a cuproptosis inducers) in 22RV1 and LNCaP cells were determined by using CCK-8 assay (Figure 5A). Subsequently, low concentrations (10 nM and 20 nM) of Elesclomol were used to treat the control group and NFAT5 knockdown group in both cell lines. Flow cytometry analysis revealed a significant reduction in ROS levels in the NFAT5 knockdown group compared to the control group (Figure 5B), indicating that NFAT5 knockdown alleviated oxidative stress response. To further validate the relationship between changes in ROS levels and cuproptosis, the Cu^{2+} concentrations were measured in both cell groups, and the results showed an increase in Cu^{2+} concentration in the NFAT5 knockdown group (Figure 5C). In conclusion, knocking down NFAT5 can increase the sensitivity of PRAD to cuproptosis inducers.

LncRNA AP000842.3 May Regulate the Nucleic Acid and Protein Levels of NFAT5

In order to explore the major lncRNAs that regulate NFAT5, we screened the top 10 lncRNAs that are highly expressed in

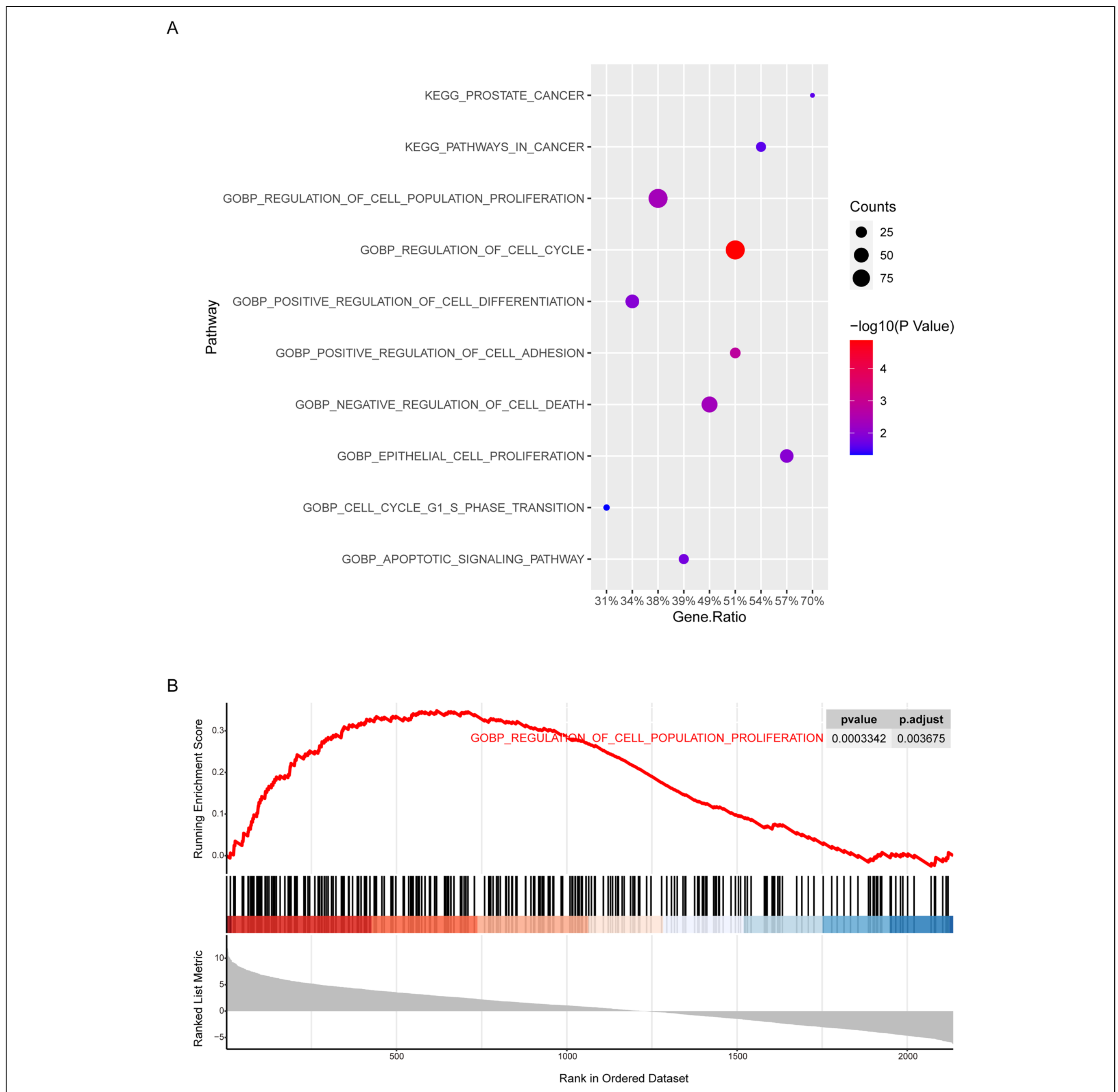

Figure 2. Pathway enrichment of genes associated with prostate cancer progression. (A-B) GSEA enrichment analysis of differential genes. The size of the bubble area represents the number of enrichment genes, and the color of the bubble represents the enrichment significance.

ceRNA network, and the results showed that lncRNA AL121790.1, lncRNA AP000842.3, lncRNA AL031123.2, lncRNA AC141930.1 and lncRNA AP000696.1 were significantly highly expressed in prostate cancer (Figure 6A and supplement Figure 4A). Our results suggested that these lncRNAs may be responsible for the elevated expression of NFAT5 in prostate cancer. Additionally, AUC curve analysis was conducted which revealed that AP000842.3 and AL031123.2 had high AUC values (Figure 6B and supplement Figure 4B). ASO was utilized to downregulate the expression of lncRNA AP000842.3 and lncRNA AL031123.3 in LNCaP and 22Rv1 cells to detect the alteration levels of nucleic acid of NFAT5. The findings revealed that the expression of NFAT5 was significantly decreased after knocking down AP000842.3 and AL031123 (Figure 6C). Next, we examined the protein expression of NFAT5 after knockdown of these five lncRNAs by ASO using western blotting, and the results showed that the expression of NFAT5 was most significantly decreased after knockdown of AP000842.3 (Figure 6D). Based on the available information, it can be

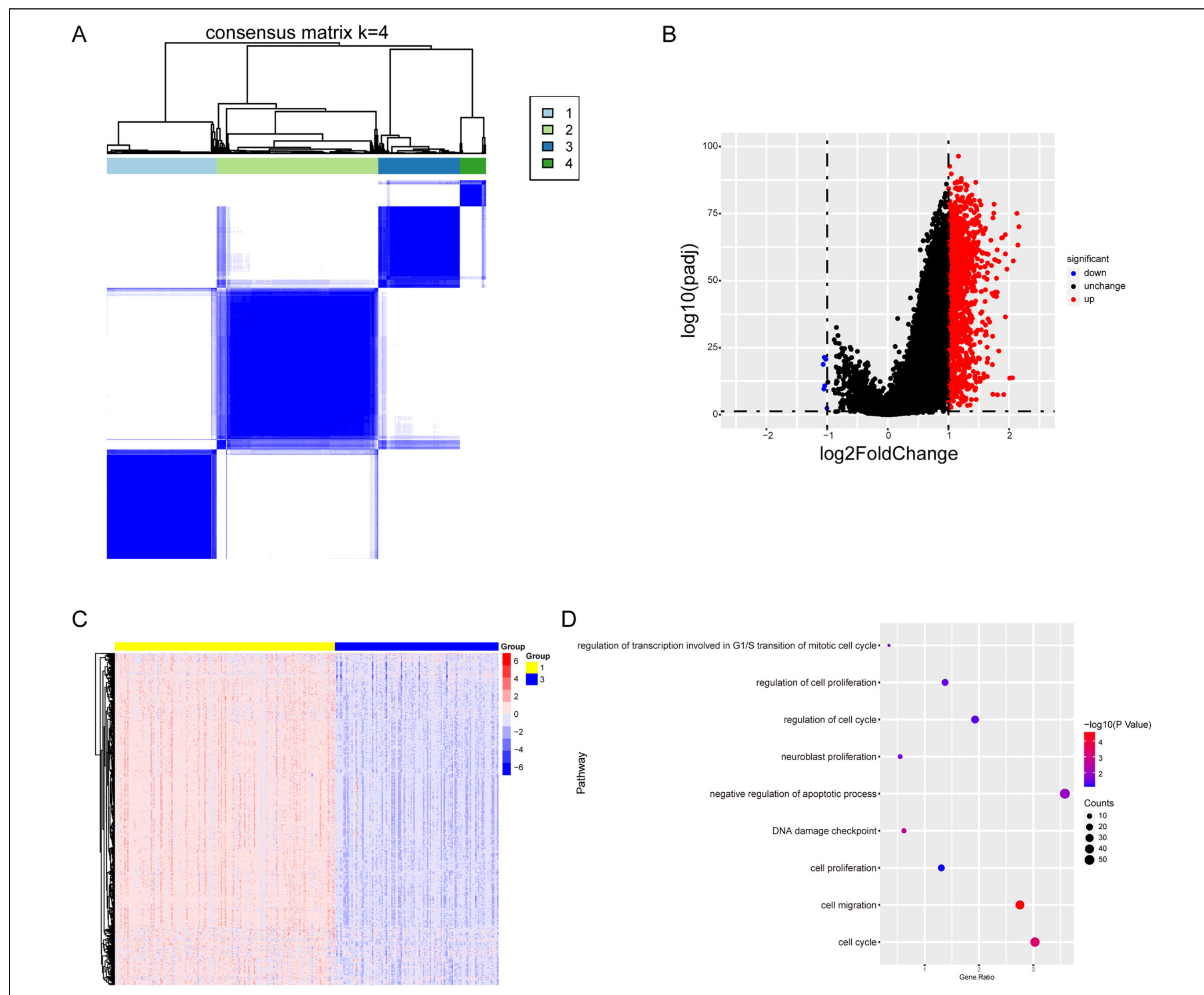

Figure 3. Consensus cluster analysis of genes associated with cuproptosis. (A) Consensus clustering heatmap of genes associated with cuproptosis with k = 4. (B) The volcanic map showed the differential genes of the two clusters. (C) Heatmap of differential genes. (D) Bubble plots showed GSEA enrichment of differential genes of the two clusters. The size of the bubble area represents the number of enrichment genes, and the color of the bubble represents the enrichment significance.

inferred that AP000842.3 may function as the primary lncRNA responsible for regulating NFAT5.

LncRNA AP000842.3, miR-206 and NFAT5 Regulate the Malignant Progression of Prostate Cancer

To verify the constructed ceRNA network, the AP000842.3/miR206/NFAT5 axis was selected for further investigation. The predicted results suggested that this axis may regulate the occurrence and development of PRAD, but its role in the malignant progression of PRAD remains unclear. In this study, prostate cancer samples were analyzed by dividing them into two groups based on AP000842.3 expression levels, namely the AP000842.3-high expression group and the AP000842.3-low expression group. Differential and enrichment analysis were performed on the groups with miR-206 and NFAT5 undergoing the same analysis (Figure 7A). The results showed that the regulatory genes of AP000842.3 were mainly enriched in the cell proliferation and migration of stem cells (Supplementary Figure 5 A-B). To further confirm the regulatory effects of the three genes on prostate cancer cell proliferation, CCK8 and clonal formation experiments were performed after inhibition of the three genes. The results showed that after knockdown of NFAT5 and AP000842.3 respectively, the proliferation ability of cells was decreased, and after inhibiting the expression of miR-206, the proliferation ability of cells was enhanced,

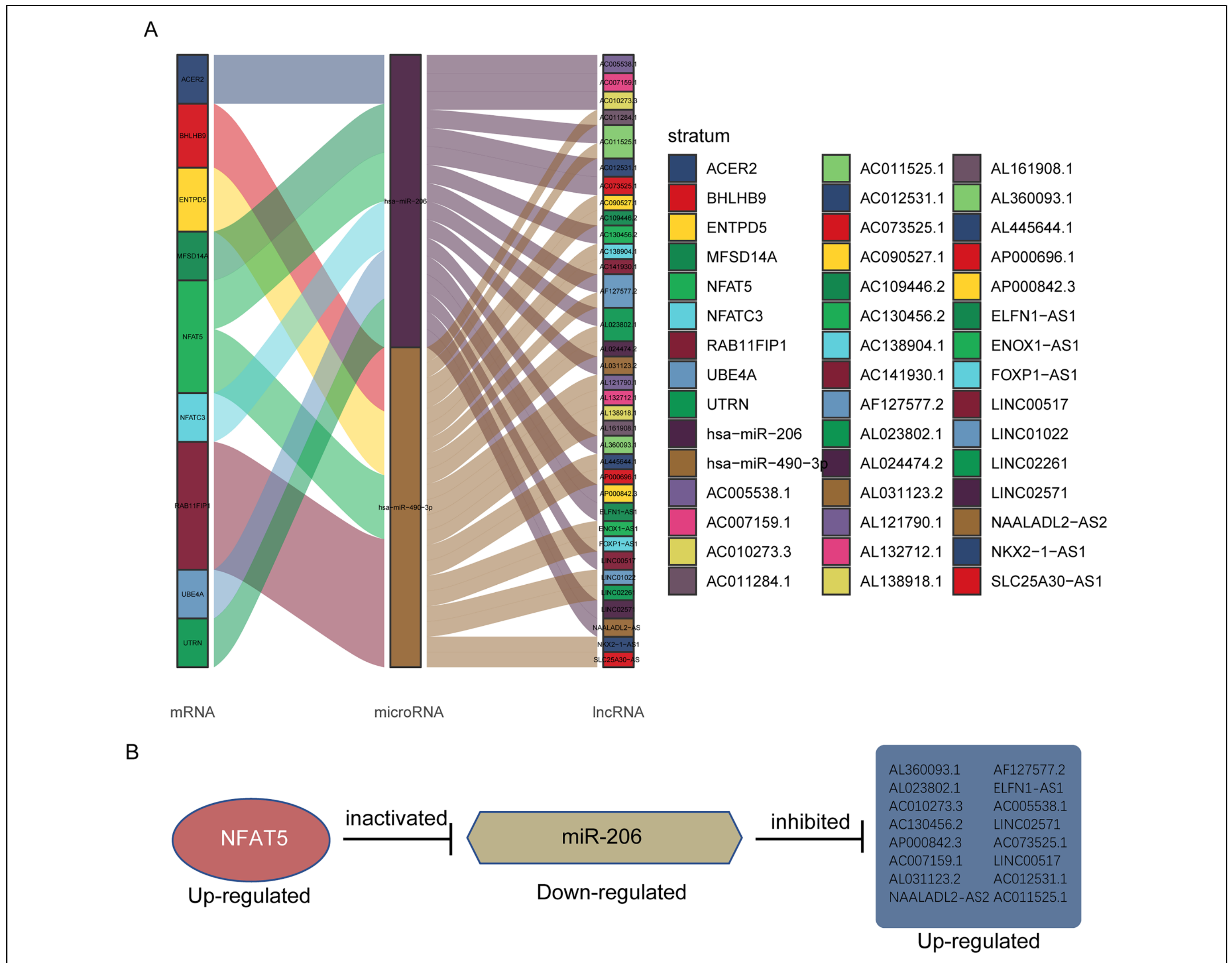

Figure 4. NFAT5/miR-206 may be important ceRNA axis regulation of prostate cancer. (A) Sankey diagram for the ceRNA network in prostate cancer. Each rectangle represents a gene, and the connection degree of each gene is visualized based on the size of the rectangle. (B) The mRNA-miRNA-LncRNA pattern diagram.

which was consistent with the previous enrichment results (Figure 7B-C). Transwell assay showed that knockdown of NFAT5 and AP000842.3 reduced the migration ability of cells, while inhibition of miR-206 enhanced the migration ability of cells (Figure 7D).Taken together, the results of this study suggested that three genes, namely miR-206, lncRNA AP000842.3, and NFAT5, may play important roles in the regulation of malignant progression in prostate cancer.

LncRNA AP000842.3 and miR-206 Could Regulate NFAT5 Protein in Prostate Cancer

To further investigate the functional relationship between lncRNA AP000842.3, miR-206, and NFAT5 proteins, antisense oligonucleotides (ASO) were used to knock down AP000842.3. The changes in miR-206 and NFAT5 protein expression were measured following knockdown of AP000842.3. The results demonstrated that knockdown of AP000842.3 led to the downregulation of NFAT5, while miR-206 expression was upregulated (Figure 8A). Subsequently, changes in the lncRNA AP000842.3 and NFAT5 protein pathways were evaluated following the overexpression or inhibition of miR-206, using the corresponding miR-206 mimic and inhibitor, respectively. These results demonstrated that lncRNA AP000842.3 and NFAT5 were negatively regulated by miR-206 (Figure 8B-C). In short, lncRNA AP000842.3 and miR-206 could regulate NFAT5 protein in prostate cancer, which was in line with the trend of ceRNA axis.

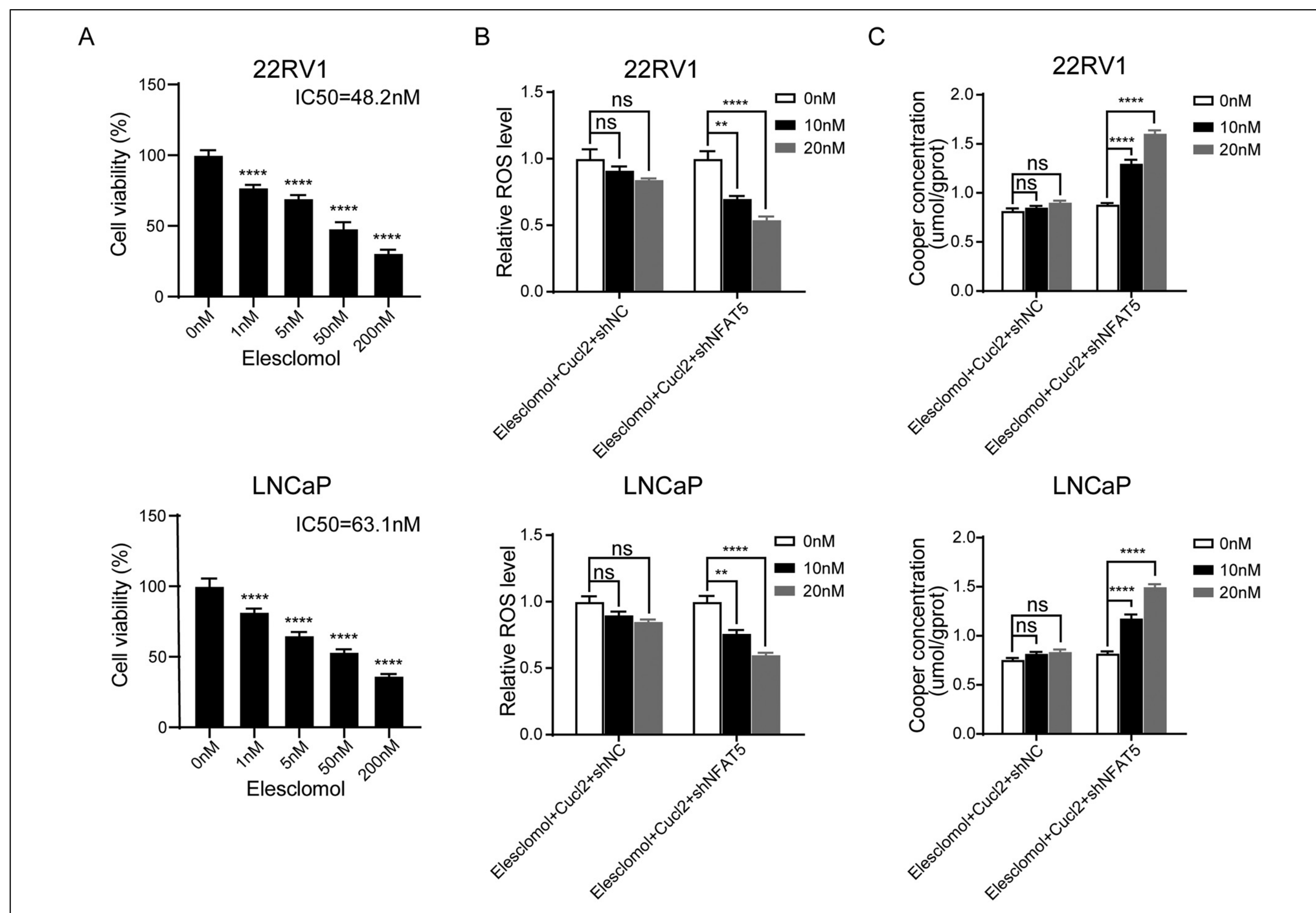

Figure 5. Inhibiting NFAT5 can increase the sensitivity of PRAD to cuproptosis inducers. (A) CCK8 assay was used to measure cell viability 24 h after exposure to increasing doses of Elesclomol. (B) NFAT5 knockdown cells and control groups cells were treated with increasing doses of Elesclomol for 24 h, and the levels of reactive oxygen species (ROS) were measured using flow cytometry. (C) NFAT5 knockdown cells and control groups cells were treated with increasing doses of Elesclomol for 24 h, and the concentration of copper (Cu^{2+}) was measured. Data were expressed as the mean ± standard deviation of three independent experiments (n = 3). *P < 0.05, ** P < 0.01, *** P < 0.001 and **** P < 0.0001.

As a ceRNA of miR206, lncRNA AP000842.3 Regulates NFAT5 Protein to Affect the Malignant Progression of Prostate Cancer

In order to determine whether lncRNA AP000842.3 acted as a ceRNA of miR206, we mutated the predicted miR-206 binding sites of AP000842.3 in a complementary manner (Figure 9A), and the luciferase activity was detected by reporter gene assay before and after the change of miR-206 level, which showed that the luciferase activity of the wild-type AP000842.3 was regulated by miR-206, while there was no statistically significant change in the mutant type (Figure 9B). To further explore the functional implications of this ceRNA axis, we predicted the enrichment of genes co-regulated by miR-206 and AP000842.3 in the malignant progression pathway of prostate cancer. The results showed that 185 genes were co-regulated, and these genes were mainly enriched in the proliferation pathway (Figure 9C-D). To verify these findings, we detected the proliferation of prostate cancer cells after knockdown AP000842.3 in controls and miR-206 mimic group. The results showed that knockdown of AP000842.3 inhibited tumor cell proliferation in the control group, while this inhibition was no longer observed in the mimic group (Figure 9E-F). These results suggested that lncRNA AP000842.3 as a ceRNA of miR-206, regulated NFAT5 protein and influenced the malignant progression of prostate cancer.

Discussion

Prostate cancer ranks among the prevalent malignant tumors affecting the male reproductive system within the urinary system. Prostate cancer in some patients has a high degree of malignancy and can develop and spread rapidly, causing death and poor prognosis. There is still a lack of fully effective treatments, so new targeted diagnostic and therapeutic strategies must be explored. LncRNAs and miRNAs, hold a vital significance in prostate cancer. LncRNAs exhibit the capability to

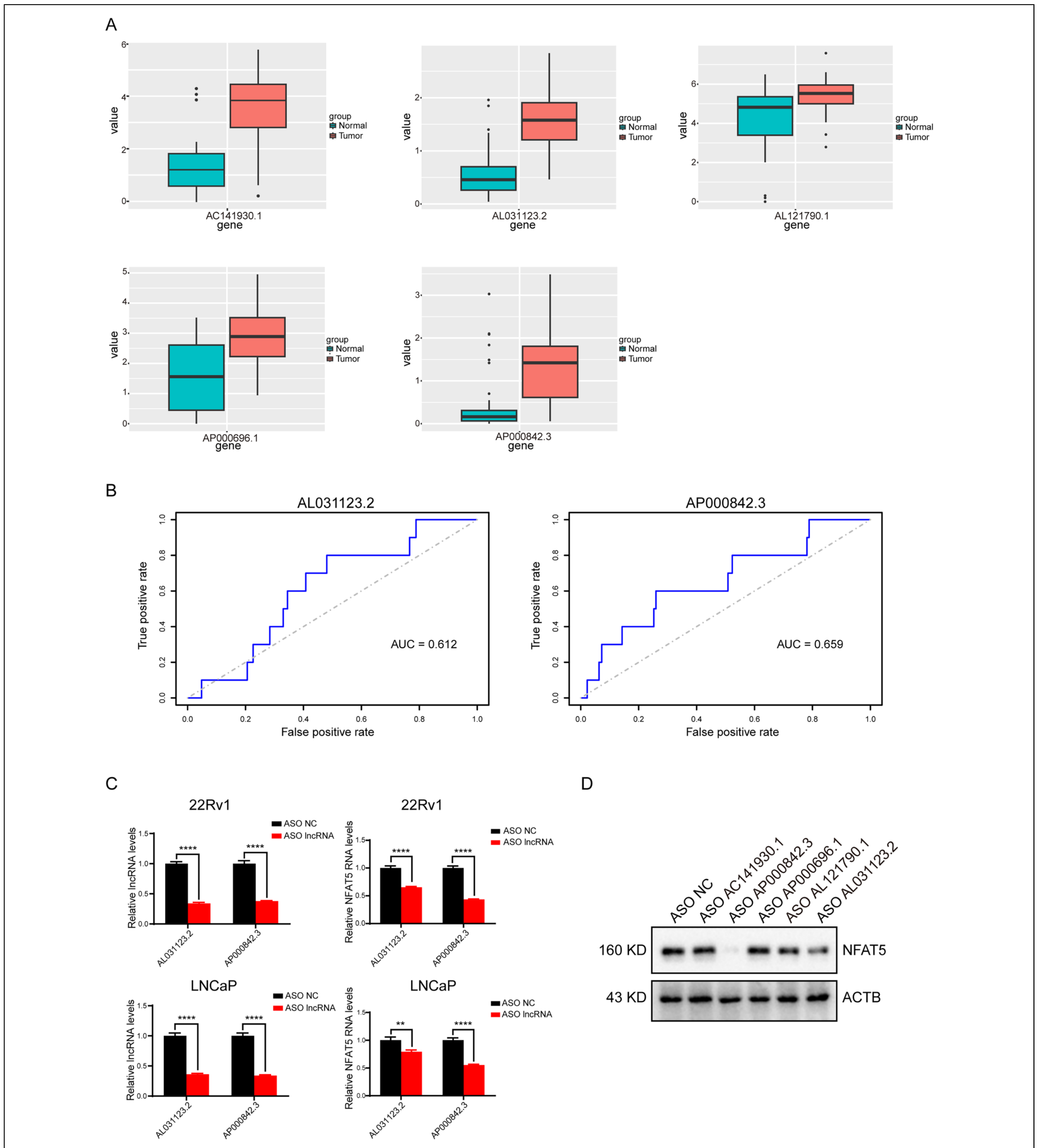

Figure 6. LncRNA AP000842.3 may regulate the nucleic acid and protein levels of NFAT5. (A) Expression of potential lncRNAs in tumor and normal tissues of prostate cancer patients. Normal tissue is shown in blue and tumor tissue is shown in red. (B) Analysis of AUC curve of lncRNA AL031123.2 and lncRNA AP000842.3. (C) After knocking down lncRNA AL031123.2 and lncRNA AP000842.3 using ASO, the nucleic acid expression level of NFAT5 was detected by qPCR. (D) After knocking down lncRNA AC141930.1, AP000842.3, AP000696.1, AL121790.1 and AL031123.2 using ASO, the protein expression level of NFAT5 was detected by western blot. Data were expressed as the mean ± standard deviation of three independent experiments (n = 3). *P < 0.05, ** P < 0.01, *** P < 0.001 and **** P < 0.0001.

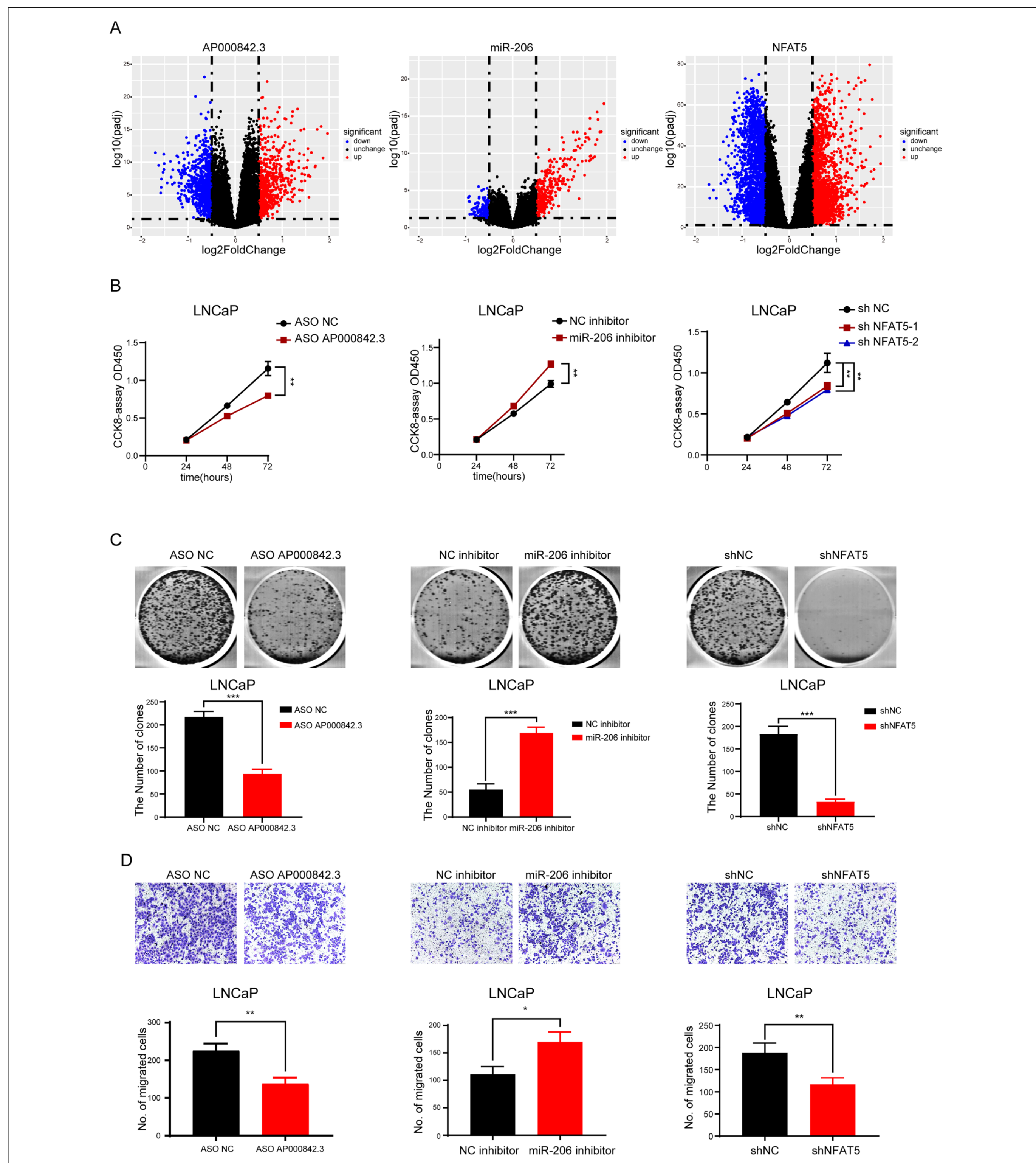

Figure 7. LncRNA-AP000842.3, miR-206 and NFAT5 regulate the malignant progression of prostate cancer. (A) Heatmap representing differentially expressed lncRNAs, miRNAs and mRNAs. (B) CCK8 assay was used to detect cell viability after knockdown of lncRNA AP000842.3, miR-206 and NFAT5 respectively. (C) Colony formation assay was used to detect the proliferation ability of cells after knocking down lncRNA AP000842.3, miR-206 and NFAT5, respectively. (D) Transwell assay was used to detect the migration ability of cells after knocking down lncRNA AP000842.3, miR-206 and NFAT5, respectively. Data were expressed as the mean ± standard deviation of three independent experiments (n = 3). *P < 0.05, ** P < 0.01, *** P < 0.001 and **** P < 0.0001.

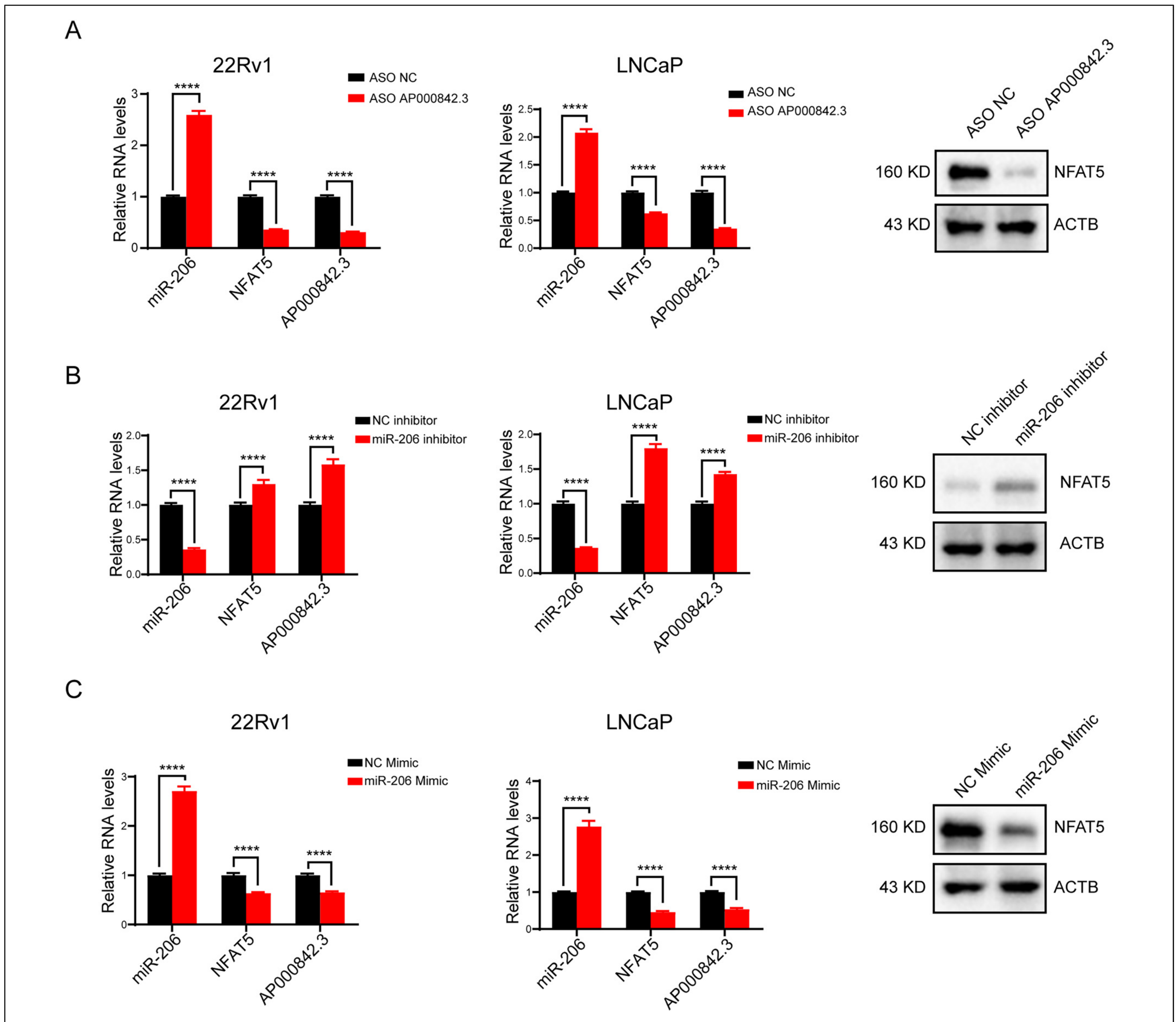

Figure 8. LncRNA AP000842.3 and miR-206 could regulate NFAT5 protein in prostate cancer. (A) RT-qPCR and western blot assay were used to detect the expression levels of miR-206 and NFAT5 after ASO knockdown of lncRNA AP000842.3. (B) RT-qPCR and western blot assay were used to detect the expression levels of lncRNA AP000842.3 and NFAT5 with adding miR-206 inhibitor. (C) RT-qPCR and western blot assay were used to detect the expression levels of lncRNA AP000842.3 and NFAT5 with adding miR-206 mimic. Data were expressed as the mean ± standard deviation of three independent experiments ($n = 3$). *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$.

attenuate the proliferation and invasiveness of cancer cells by sequestering oncogenic miRNAs. Furthermore, they regulate a multitude of processes, encompassing chromatin remodeling, histone modifications, miRNA sequestration, and mediation of complex formation. Similarly, miRNAs exert influence on drug resistance, tumor progression in prostate cancer. The overexpression of the lncRNA-NEAT1 has been implicated in the development of docetaxel resistance in prostate cancer. LncRNAs are known to function as competing endogenous RNAs, sequestering miRNAs that would otherwise hinder their target genes expression. This mechanism can foster the progression of neuroendocrine prostate cancer. These findings suggest that ncRNAs, particularly lncRNA and miRNA, are potential targets for drug resistance and other aspects of prostate cancer. Researches have revealed that lncRNAs can work as ceRNAs, sequestering miRNAs and participating in the regulation of target gene expression, thereby exerting a significant joint in tumor initiation and progression. Various regulatory factors such as TF, lncRNA, miRNA, and mRNA work together to affect prostate cancer, and some lncRNAs function as ceRNAs to regulate gene expression.[36,37] ceRNAs can potentially identify novel biomarkers for diagnosis and therapy in

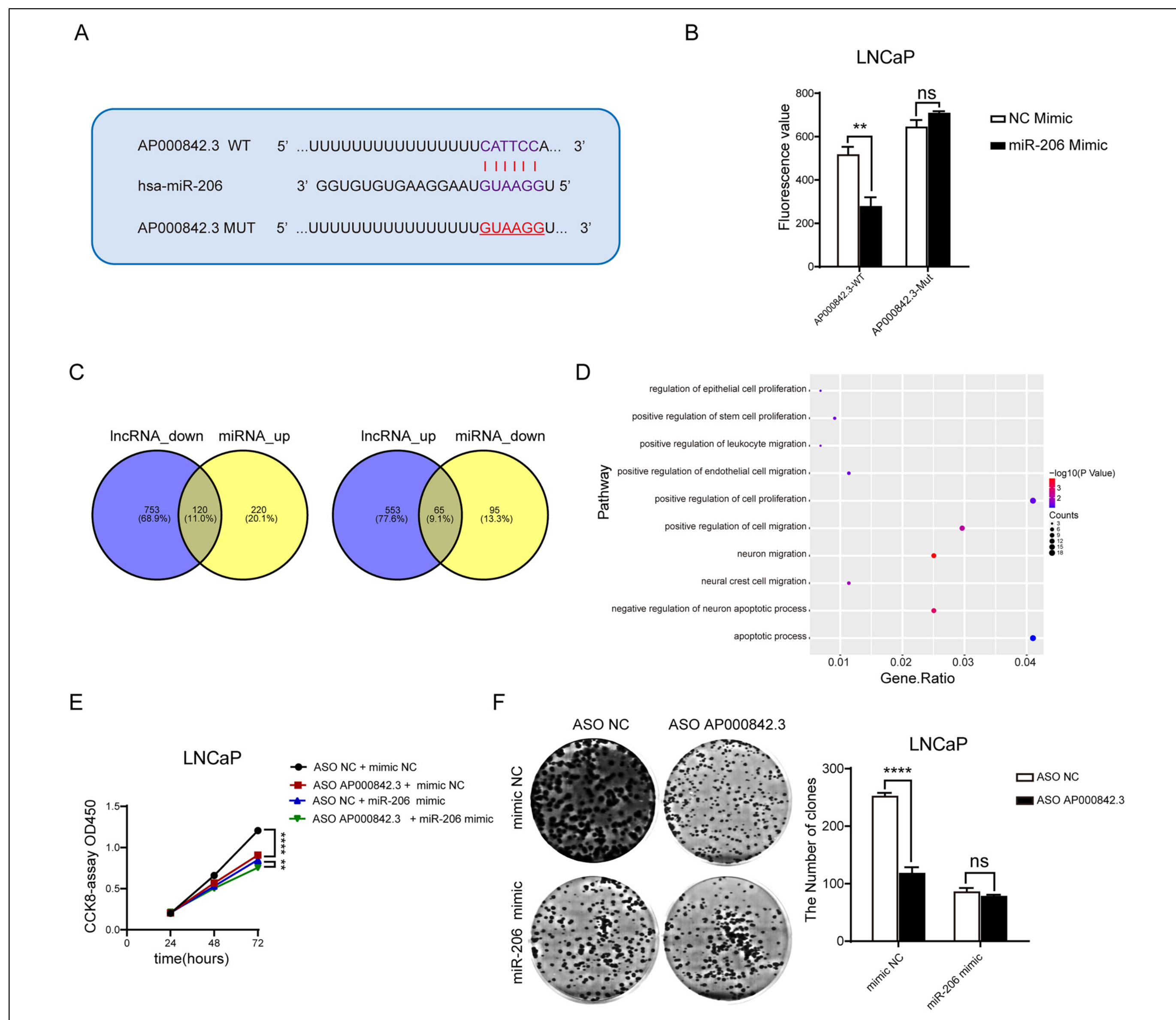

Figure 9. As a ceRNA of miR-206, lncRNA AP000842.3 regulates NFAT5 protein to affect the malignant progression of prostate cancer. (A) Predicted binding site between miR-206 and lncRNA AP000842.3. (B) Luciferase assays were performed to test the effect of miR-206 on wild-type or mutant lncRNA AP000842.3. (C) Venn diagram showed the overlapping genes of AP000842.3 groups and miR-206 groups. (D) Pathway enrichment of genes co-regulated by lncRNA AP000842.3 and miR-206. The size of the bubble area represents the number of enrichment genes, and the color of the bubble represents the enrichment significance. (E) The CCK-8 results of prostate cancer cells with knockdown AP000842.3 treated with NC mimic or miR-206 mimic. (F) The clonal formation results of prostate cancer cells with knockdown AP000842.3 treated with NC mimic or miR-206 mimic. Data were expressed as the mean ± standard deviation of three independent experiments (n = 3). *P < 0.05, ** P < 0.01, *** P < 0.001 and **** P < 0.0001.

prostate cancer. Herein, we established a specific ceRNA regulatory network in prostate cancer and validated the role of lncAP000842.3 as a ceRNA for miR-206 in modulating the malignant advancement of prostate cancer. This study greatly enriched the ceRNA regulatory network in prostate cancer.

Cuproptosis is a recently identified form of regulated cell death that occurs as a result of the direct attachment of copper ions to lipoylated components found in the tricarboxylic acid cycle. This attachment leads to the accumulation of lipoylated proteins and reduced expression of Fe-S cluster proteins, ultimately resulting in the initiation of proteotoxic stress responses and the subsequent demise of the cell.[38] In the context of cancer, cuproptosis can serve as a mechanism to eliminate cancer cells selectively. The accumulation of excessive copper ions within cancer cells generates oxidative stress and reactive oxygen species, triggering signaling pathways that activate the intrinsic apoptotic pathway.[39] The mechanisms and precise manifestations of cell death induced by copper have

long remained elusive. More studies have revealed that cuproptosis may be implicated in various cancers process, including prostate cancer, colorectal cancer, and others.[40–42] Cuproptosis can kill cancer cells and induce copper toxicosis to destroy them. By exploiting the vulnerability of cancer cells to copper-induced cytotoxicity, cuproptosis can inhibit tumor growth, invasion, and metastasis. Aberrant levels of copper have been observed in both the serum and tumor tissue of various cancer patients, including those with prostate cancer. Moreover, a recent study has shown that genes associated with cuproptosis may influence the prognosis of prostate cancer by modulating immune cell infiltration and the process of cancer cell mitosis. Here, we performed consistent clustering of prostate cancer samples based on 13 cuproptosis genes, divided the patients into two groups with different levels of cuproptosis, and conducted difference analysis to obtain CRGs in prostate cancer. In addition, we screened out the ceRNA axis regulating CRGs NFAT5, proving that this axis can regulate the malignant progression of PRAD. Recent studies of ceRNA axis and cuproptosis in PRAD have been reported, but our results have clarified the role of cuproptosis-related ceRNA axis in PRAD (Table 1). These findings announce the important clinical significance of CRGs and offer potential targets and new therapeutic strategies for targeting cuproptosis in the treatment of prostate cancer.

Transcription factors are critical in prostate cancer by controlling gene expression and influencing tumor progression.[43] Dysregulation of key transcription factors can promote abnormal cell growth and metastasis.[44] Tumor suppressor transcription factors, including p53, are often impaired in prostate cancer, leading to resistance to therapy and genomic instability.[45] Moreover, some studies have identified CRGs and constructed signatures to predict cancer prognosis.[46] The regulation of CRGs and pathways has been linked to transcription factors. One such example is the metal transcription factor 1, which has been identified as a regulator of CRGs in various cancers.[47] And CDKN2A, a gene associated with cuproptosis, shows a strong correlation with immune-regulatory genes and immune cell infiltration.[48] NFAT5, a transcription factor, controls gene expression during osmotic stress. It can promote proliferation and migration in multiple cancer types.[49] NFAT5 controls AQP5 expression in lung adenocarcinoma cells and plays a role in miR-194 mediated proliferation, migration, and invasion of lung cancer cells. NFAT1, from the same NFAT family as NFAT5, was shown to be critical for its phosphorylation in its ability to promote prostate cancer cell migration and invasion. NFAT5 has been shown to participate in the proliferation and invasion of cancer cells through the regulation of miR-194. Additionally, NFAT5 has been implicated in the competing ceRNA network of cancer cells, including glioblastoma.[50] However, the cuproptosis-related ceRNA network regulating NFAT5 has not been published in prostate cancer. We established a co-regulatory network of ceRNAs regulating NFAT5 in prostate cancer, providing a theoretical basis for targeting NFAT5 in diagnosis and therapy.

In this study, we found that knocking out NFAT5 can increase the sensitivity of prostate cancer to cupric inducers. We expect to further clarify the regulation of cuproptosis by NFAT5 and its specific mechanism through electron microscopy in the future. lncAP000842.3, as a molecular sponge of miR-206, competitively regulates the expression level of NFAT5, an important oncogene in prostate cancer. Despite the lack of animal experimental models, our research results can provide new potential targets for the diagnosis and treatment of prostate cancer.

Table 1. The Comparative Study and Others.

References	Compare
[24] Zhang L, Xu J, Chu X, et al	Experimental verification is lacking
[25] Cheng B, Tang C, Xie J, et al	The ceRNA axis is not involved
[26] Cheng X, Zeng Z, Yang H, et al	Experimental verification is lacking
[36] Taheri M, Safarzadeh A, Hussen BM, et al	Experimental verification is lacking; The ceRNA axis can't trigger regulated cell death

Conclusion

lncRNA AP000842.3, as a ceRNA of miR-206, was involved in the regulation of levels of the transcription factor NFAT5 associated with cuproptosis in prostate cancer. Knocking down NFAT5 can increase the sensitivity of prostate cancer to cuproptosis inducers. Meanwhile, the changes in the expression of AP000842.3 and miR-206 could affect the proliferation and migration of PRAD by regulating NFAT5. Mechanistically, AP000842.3 acts as the ceRNA of miR-206 to regulate the expression of NFAT5. In addition, the effects of lncRNA AP000842.3 on malignant progression of PRAD and NFAT5 were partially dependent on miR-206.

Acknowledgements

The authors are grateful to Department of Urology, Taizhou Hospital of Zhejiang Province affiliated to Wenzhou Medical University for providing the necessary resources and facilities to conduct this study.

Author's Contributions

Conceived and designed the analysis: Gaobo Zhou, Chaoqian Chen and Bin Huang

Collected the data: Hongjian Wu and Jiao Lin

Contributed data or analysis tools: Hang Liu and Yiran Tao

Performed the analysis: Yiran Tao and Hongjian Wu

Wrote the paper: Gaobo Zhou and Bin Huang

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethical Statement

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

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

ORCID iD

Bin Huang https://orcid.org/0009-0003-5449-7657

Supplemental Material

Supplemental material for this article is available online.

References

1. Wasim S, Lee S-Y, Kim J. Complexities of prostate cancer. *Int J Mol Sci.* 2022;23(22):14257.
2. Rycaj K, Li H, Zhou J, Chen X, Tang DG, editors. *Cellular determinants and microenvironmental regulation of prostate cancer metastasis*. Seminars in cancer biology; 2017: Elsevier.
3. Duffy MJ. Biomarkers for prostate cancer: Prostate-specific antigen and beyond. *Clin Chem Lab Med (CCLM)*. 2020;58(3): 326-339.
4. David MK, Leslie SW. *Prostate specific antigen*. StatPearls [Internet]. StatPearls Publishing; 2022.
5. Fizazi K, Tran N, Fein L, et al. Abiraterone Acetate plus prednisone in patients with newly diagnosed high-risk metastatic castration-sensitive prostate cancer (LATITUDE): Final overall survival analysis of a randomised, double-blind, phase 3 trial. *Lancet Oncol.* 2019;20(5):686-700.
6. Sekhoacha M, Riet K, Motloung P, Gumenku L, Adegoke A, Mashele S. Prostate cancer review: Genetics, diagnosis, treatment options, and alternative approaches. *Molecules*. 2022;27(17): 5730.
7. Sun B, Liu C, Li H, et al. Research progress on the interactions between long non-coding RNAs and microRNAs in human cancer. *Oncol Lett*. 2020;19(1):595-605.
8. Yao Q, Chen Y, Zhou X. The roles of microRNAs in epigenetic regulation. *Curr Opin Chem Biol.* 2019;51:11-17.
9. Kilav-Levin R, Hassan A, Nechama M, et al Post-transcriptional mechanisms regulating parathyroid hormone gene expression in secondary hyperparathyroidism. *FEBS J.* 2020;287(14):2903-2913.
10. Khasraghi LB, Nouri M, Vazirzadeh M, et al. MicroRNA-206 in human cancer: Mechanistic and clinical perspectives. *Cell Signal.* 2023;101:110525. doi:10.1016/j.cellsig.2022.110525
11. Zhou Z, Leng C, Wang Z, et al. The potential regulatory role of the lncRNA-miRNA-mRNA axis in teleost fish. *Front Immunol.* 2023;14:1065357.
12. Sun B, Liu C, Li H, et al. Research progress on the interactions between long non-coding RNAs and microRNAs in human cancer. *Oncol Lett.* 2020;19(1):595-605.
13. Hao H, Chen H, Xie L, Liu H, Wang D. LncRNA KCNQ1OT1 promotes proliferation, invasion and metastasis of prostate cancer by regulating miR-211-5p/CHI3L1 pathway. *Onco Targets Ther.* 2021;14:1659. doi:10.2147/OTT.S288785
14. Pan J, Xu X, Wang G. lncRNA ZFAS1 is involved in the proliferation, invasion and metastasis of prostate cancer cells through competitively binding to miR-135a-5p. *Cancer Manag Res.* 2020;12:1135. doi:10.2147/CMAR.S237439
15. Chen J, Wang F, Xu H, et al. Long non-coding RNA SNHG1 regulates the Wnt/β-catenin and PI3K/AKT/mTOR signaling pathways via EZH2 to affect the proliferation, apoptosis, and autophagy of prostate cancer cell. *Front Oncol.* 2020;10:552907.
16. Mirzaei S, Paskeh MDA, Okina E, et al. Molecular landscape of LncRNAs in prostate cancer: A focus on pathways and therapeutic targets for intervention. *J Exp Clin Cancer Res.* 2022;41(1):214.
17. Xu J, Xu J, Liu X, Jiang J. The role of lncRNA-mediated ceRNA regulatory networks in pancreatic cancer. *Cell Death Discov.* 2022;8(1):287.
18. Xu Y-H, Deng J-L, Wang G, Zhu Y-S. Long non-coding RNAs in prostate cancer: Functional roles and clinical implications. *Cancer Lett.* 2019;464:37-55.
19. de Oliveira JC, Oliveira LC, Mathias C, et al. Long non-coding RNAs in cancer: Another layer of complexity. *J Gene Med.* 2019;21(1):e3065.
20. Xie J, Yang Y, Gao Y, He J. Cuproptosis: Mechanisms and links with cancers. *Mol Cancer.* 2023;22(1):46.
21. Wang Y, Zhang L, Zhou F. Cuproptosis: A new form of programmed cell death. *Cell Mol Immunol.* 2022;19(8):867-868.
22. Li S-R, Bu L-L, Cai L. Cuproptosis: Lipoylated TCA cycle proteins-mediated novel cell death pathway. *Signal Transduction and Targeted Therapy*. 2022;7(1):158.
23. Ge EJ, Bush AI, Casini A, et al. Connecting copper and cancer: From transition metal signalling to metalloplasia. *Nat Rev Cancer.* 2022;22(2):102-113.
24. Jin L, Mei W, Liu X, et al. Identification of cuproptosis-related subtypes, the development of a prognosis model, and characterization of tumor microenvironment infiltration in prostate cancer. *Front Immunol.* 2022;13:974034.
25. Cheng B, Tang C, Xie J, et al. Cuproptosis illustrates tumor microenvironment features and predicts prostate cancer therapeutic sensitivity and prognosis. *Life Sci.* 2023;325:121659.
26. Cheng X, Zeng Z, Yang H, et al. Novel cuproptosis-related long non-coding RNA signature to predict prognosis in prostate carcinoma. *BMC cancer*. 2023;23(1):1-17. doi:10.1186/s12885-023-10584-0
27. Oliveri V. Selective targeting of cancer cells by copper ionophores: an overview. *Front Mol Biosci.* 2022;9:841814.
28. Lee N, Kim D, Kim W-U. Role of NFAT5 in the immune system and pathogenesis of autoimmune diseases. *Front Immunol.* 2019; 10:270.
29. Cen L, Xing F, Xu L, Cao Y. Potential role of gene regulator NFAT5 in the pathogenesis of diabetes mellitus. *J Diabetes Res.* 2020;2020. doi:10.1155/2020/6927429

30. Park YJ, Yoo SA, Kim M, Kim WU. The role of calcium-calcineurin-NFAT signaling pathway in health and autoimmune diseases. *Front Immunol*. 2020;11:195.
31. Yoshimoto S, Morita H, Matsuda M, Katakura Y, Hirata M, Hashimoto S. NFAT5 Promotes oral squamous cell carcinoma progression in a hyperosmotic environment. *Lab Invest*. 2021; 101(1):38-50.
32. Lin Y, Song Y, Zhang Y, Shi M, Hou A, Han S. NFAT Signaling dysregulation in cancer: Emerging roles in cancer stem cells. *Biomed Pharmacother*. 2023;165:115167.
33. Drak Alsibai K, Meseure D. Tumor microenvironment and non-coding RNAs as co-drivers of epithelial–mesenchymal transition and cancer metastasis. *Dev Dyn.* 2018;247(3):405-431.
34. Huang J, Xu Z, Yuan Z, Teh BM, Zhou C, Shen Y. Identification of a cuproptosis-related lncRNA signature to predict the prognosis and immune landscape of head and neck squamous cell carcinoma. *Front Oncol.* 2022;12:983956.
35. Liu Y, Liang T, Qiu X, et al. Down-regulation of Nfatc1 suppresses proliferation, migration, invasion, and warburg effect in prostate cancer cells. *Med Sci Monit: Int Med J Exp Clin Res*. 2019;25:1572.
36. Taheri M, Safarzadeh A, Hussen BM, Ghafouri-Fard S, Baniahmad A. LncRNA/miRNA/mRNA Network Introduces Novel Biomarkers in Prostate Cancer. *Cells*. 2022;11(23):3776. doi:10.3390/cells11233776
37. Zhong H, Lai Y, Ouyang W, et al. Integrative analysis of cuproptosis-related lncRNAs: Unveiling prognostic significance, immune microenvironment, and copper-induced mechanisms in prostate cancer. *Cancer Pathogenesis Ther*. 2024;02(00):E27-E70.
38. Chen L, Min J, Wang F. Copper homeostasis and cuproptosis in health and disease. *Signal Transd Target Ther*. 2022;7(1):378.
39. Emami MH, Sereshki N, Malakoutikhah Z, et al. Nrf2 signaling pathway in trace metal carcinogenesis: A cross-talk between oxidative stress and angiogenesis. *Comp Biochem Physiol C Toxicol Pharmacol*. 2022;254:109266.
40. Li Y, Wang R-y, Deng Y-j, Wu S-h, Sun X, Mu H. Molecular characteristics, clinical significance, and cancer immune interactions of cuproptosis and ferroptosis-associated genes in colorectal cancer. *Front Oncol.* 2022;12:975859.
41. Li C, Xiao Y, Cao H, Chen Y, Li S, Yin F. Cuproptosis regulates microenvironment and affects prognosis in prostate cancer. *Biol Trace Elem Res*. 2024;202(1):99-110.
42. Dong Q, Qiu H, Piao C, Li Z, Cui X. LncRNA SNHG4 promotes prostate cancer cell survival and resistance to enzalutamide through a let-7a/RREB1 positive feedback loop and a ceRNA network. *J Exp Clin Cancer Res.* 2023;42(1):209.
43. Vishnoi K, Viswakarma N, Rana A, Rana B. Transcription factors in cancer development and therapy. *Cancers (Basel)*. 2020;12(8):2296. doi:10.3390/cancers12082296
44. van Roey R, Brabletz T, Stemmler MP, Armstark I. Deregulation of transcription factor networks driving cell plasticity and metastasis in pancreatic cancer. *Front Cell Dev Biol*. 2021;9:753456.
45. Marei HE, Althani A, Afifi N, et al. P53 signaling in cancer progression and therapy. *Cancer Cell Int.* 2021;21(1):1-15. doi:10.1186/s12935-021-02396-8
46. Zhang G, Sun J, Zhang X. A novel cuproptosis-related LncRNA signature to predict prognosis in hepatocellular carcinoma. *Sci Rep.* 2022;12(1):11325.
47. Zhou C, Li C, Zheng Y, Huang X. Regulation, genomics, and clinical characteristics of cuproptosis regulators in pan-cancer. *Front Oncol.* 2022;12:934076.
48. Zhang D, Wang T, Zhou Y, Zhang X. Comprehensive analyses of cuproptosis-related gene CDKN2A on prognosis and immunologic therapy in human tumors. *Medicine (Baltimore).* 2023;102(14): e33468.
49. Wan L, Gu D, Li P. LncRNA SNHG16 promotes proliferation and migration in laryngeal squamous cell carcinoma via the miR-140-5p/NFAT5/Wnt/β-catenin pathway axis. *Pathol Res Pract*. 2022;229:153727.
50. Rahnama S, Bakhshinejad B, Farzam F, Bitaraf A, Ghazimoradi MH, Babashah S. Identification of dysregulated competing endogenous RNA networks in glioblastoma: A way toward improved therapeutic opportunities. *Life Sci.* 2021;277:119488.