

ZFC3H1 provides a reference to evaluate the prognosis and treat prostate adenocarcinoma: A study based on TCGA data

Shengye Chen

Wenzhou Medical University First Affiliated Hospital

Haokai Xu

Bengbu Medical College

Ping Li

Wenzhou Medical University First Affiliated Hospital

Xueting Ye

Wenzhou Medical University First Affiliated Hospital

Wei Chen

Wenzhou Medical University First Affiliated Hospital

Wei Chen

zhejiang cancer institue of integrated traditional Chinese and Western Medicine

Zhongqiu Lu

Wenzhou Medical University First Affiliated Hospital

Hang Huang (✉ huanghang2017@163.com)

Wenzhou Medical University First Affiliated Hospital

Research article

Keywords: ZFC3H1, Prostate adenocarcinoma, Computational Biology, Cell migration, Cell invasion, Prognosis

Posted Date: May 27th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-23147/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

Prostate adenocarcinoma (PRAD) is a common tumor with a high mortality rate, and advanced PRAD remains incurable. Exploring biomarkers related to PRAD prognosis could provide valuable information for clinical treatment. Zinc finger C3H1 domain-containing protein (ZFC3H1) is a large protein (1989 amino acids) that is expressed in many human tumors, playing an important role in RNA turnover and translation. In the present study, we first evaluated the expression profile of ZFC3H1 using bioinformatic analysis of public datasets from The Cancer Genome Atlas database. We then predicted the target genes of ZFC3H1, performed gene ontology enrichment analysis, and constructed a protein–protein interaction network. We also assessed the expression profiles of these genes and evaluated their prognostic significance. We also transfected a ZFC3H1 small interfering RNA (siRNA) into PRAD cells and investigated their migration and invasive capabilities through wound healing and transwell invasion assays.

Results

We found that the expression levels of ZFC3H1 in many cancers, including PRAD, were notably lower than in corresponding non-cancerous tissues, and patients in the high ZFC3H1-expression group showed poor survival. We hypothesized that the low expression of ZFC3H1 in tumor tissue might have been an inhibitory effect on the autoimmune system. The results also showed that the expression levels of *MPHOSPH6* (encoding M-phase phosphoprotein 6) and *MRPS31* (encoding mitochondrial ribosomal protein S31) were lower in PRAD tissues than in non-cancerous tissues, and the survival time of patients with high MPHOSPH6 and MRPS31 expression was poor. To further demonstrate the role of ZFC3H1 in PRAD, we did RNA transfection. Knockdown of *ZFC3H1* significantly inhibited PRAD cell migration and invasion.

Conclusion

ZFC3H1 is closely related to PRAD's migration and invasion ability, and could represent a new marker for PRAD prognosis and provide a reference for the development of new therapies to treat PRAD.

Background

Cancer is the second most common cause of death (8.97 million deaths) after ischemic heart disease [1]. Prostate adenocarcinoma (PRAD) is the second most common cancer causing cancer mortality in men [2]. The 5-year survival rate of PRAD is about 70–100%; however, for patients with distant metastatic cancer, the 5-year survival rate is only 30% [3]. Among the most prevalent solid tumors with advanced disease, PRAD has the fewest therapeutic options, and advanced PRAD remains incurable [4, 5]. Therefore, accurately evaluating the prognosis of PRAD is vital.

Zinc finger C3H1 domain-containing protein (ZFC3H1), also known as CCDC131 (coiled-coil domain containing protein), or PSRC2 (proline/serine-rich coiled-coil protein), is a large protein (1989 amino acids), is a large proteins with a C3H1-type zinc finger as the central, including five reversed transcribed peptide (TRP) repeats and six half-reversed transcribed peptide (HAT) repeats in the region of C-terminal. It is a central factor in the retention and degradation of polyadenylated RNA, and is involved in the processing of a wide range of RNAs, playing a crucial role in the degradation of nuclear RNAs [6–9]. Garland et al. reported a disruptive relationship between excess RNA and polycomb repressive complex 2 (PRC2) upon depletion of ZFC3H1 in mouse ESCs [10]. In addition, there are also reports that the translation was significantly inhibited by depletion of ZFC3H1 [11]. The reason may be that ZFC3H1 plays an important role in promoting turnover of unstable nuclear RNAs and preventing their cytoplasmic transport and global translational repression. When its activity decreases, normally unstable RNAs accumulate and are transported to the cytoplasm, thus inhibiting translation globally [12]. ZFC3H1 is also reported to play an important role in regulating cytokine production and RNA decay [13–15]. Considering that advanced PRAD depends on protein synthesis to maintain its survival and accelerate metabolism to promote growth [16], we hypothesized that ZFC3H1 would correlate with the prognosis of PRAD and play an active role in the progression of PRAD.

In the present study, based on The Cancer Genome Atlas (TCGA) database, we aimed to analyze the expression levels of *ZFC3H1* and its target genes in PRAD, explore the role of ZFC3H1 in PRAD, and finally determine whether ZFC3H1 could provide a reference to evaluate PRAD prognosis and treatment by detecting its influence on the migration and invasion of PRAD cells.

Method

ZFC3H1 expression in PRAD samples from the TCGA database

The TCGA is a central repository of multidimensional experimental cancer data, comprising data pertaining to more than 30 types of human tumors. We obtained the expression profile of *ZFC3H1* in different human cancers and corresponding non-cancerous tissues, including PRAD, as well as its correlation with the prognosis of patients with PRAD, via the Gene Expression Profiling Interactive Analysis (GEPIA) tool (<http://gepia.cancer-pku.cn/>), which is based on the TCGA database.

Prediction and screening of target genes

The target genes of ZFC3H1 were predicted using three databases: STRING (<https://string-db.org/>), BioGRID (<https://thebiogrid.org/>), and IntAct (<https://www.ebi.ac.uk/intact/>). To improve the accuracy of the prediction results and to construct Venn diagrams, we chose target genes that overlapped between at least two of the three databases.

Enrichment analysis of overlapping target genes in the ZFC3H1 signaling pathway

Enrichment analysis of overlapping target genes in the ZFC3H1 signaling pathway was performed using Metascape (<http://metascape.org/gp/index.html>). We analyzed the function of these genes in tumor biological processes via gene ontology (GO) enrichment analysis.

Identifying node degree genes via protein–protein interaction (PPI) network analyses of overlapping target genes

PPI analysis was performed using overlapping target genes of ZFC3H1 through Metascape, which identified 26 node degree genes.

Prognostic significance of the chosen node degree genes

To prevent any errors resulting from the use of a single database, the 26 node degree genes were analyzed using the starbase (<http://starbase.sysu.edu.cn/index.php>) tool to observe whether their expression levels varied between PRAD and corresponding non-cancerous tissues, and whether these genes had any influence on the prognosis of PRAD.

Cell culture

Human PRAD cells (22RV1, DU145) were obtained from American Type Culture Collection (Manassas, VA, USA). 22RV1 and DU145 cells were propagated in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Gibco) at 37 °C in a humidified atmosphere containing 5% CO₂.

RNA transfection

ZFC3H1 small interfering RNAs (siRNAs) were purchased from Genepharma Biotechnology (Shanghai, China). Lipofectamine 2000 was used to transfect the PRAD cells. The siRNA sequences were as follows: ZFC3H1-homo-2234: 5'- CCAAGAAGCAAUCAUAATT-3' and 5'-UUGAUAGAUUGCUUCUUGGTT-3'; ZFC3H1-homo-3986: 5'- GGAGUAAACAAAGAUCGAATT-3' and 5'- UUCGAUCUUUGUUUACUCCTT-3'; ZFC3H1-homo-5975: 5'-GCUGCUGAGAUUGUUCUAATT-3' and 5'- UUAGAACAAUCUCAGCAGCTT -3'; Negative control: 5'-UUCUCCGAACGUGUCACGUTT-3' and 5'-ACGUGACACGUUCGGAGAATT-3'.

Wound healing assay

Cells were plated in 6-well plates at 3×10^5 cells/well, then transfected with ZFC3H1 siRNA or negative siRNA. Using the tip of a micropipette to create a wound in the cell monolayer, the wound area was photographed under an inverted light microscope (Olympus IX51, Olympus, central valley, PA, USA) at 0 h and 48 h. Finally, ImagePro Plus v. 6.0 was used to quantify the wound area (Media Cybernetics, Bethesda, MD, USA) to evaluate the ability of cell migration.

Transwell invasion assay

The 24-well transwell chamber (8 μm ; Corning, Inc., Corning, NY, USA) were used for the invasion assays. Cells transfected for 48h were collected, and the cell concentration was adjusted to 10^5 cells/ml. 100ul cell suspension was inoculated in transwell chamber. After incubation for 24 h, the cells were fixed with methanol for 10 min and stained with 0.1% crystal violet for 10 min. The number of migrated cells was counted and photographed under an inverted phase contrast microscope (Olympus; $\times 40$ magnification).

Western blotting

Cell were lysed in lysis buffer (Cell Signaling Technology, Danvers, MA, USA) on ice and the concentration of protein was detected by a bicinchoninic acid protein assay kit (BCA, Sigma-Aldrich; Merck KGa). 40 μg /lane protein samples were separated on SDS-PAGE and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membranes were immunoprobed with antibodies. The proteins were examined using enhanced chemiluminescence reagents (Pierce, Rockford, IL, USA) and the band density of the immunoreactive protein was scanned using Image Lab 5.0 (Bio-Rad, Hercules, CA, USA).

Results

ZFC3H1 expression in PRAD samples from the TCGA database

As shown in Fig. 1A and Fig. 1B, the expression of ZFC3H1 was low in many human cancers, such as PRAD, adrenocortical carcinoma, colon adenocarcinoma, lung adenocarcinoma, etc, but showed high expression only in thymoma. However, unexpectedly, as shown in Fig. 1C, the survival time of patients with PRAD and high expression of ZFC3H1 was poor. These results indicated that ZFC3H1 might enhance the progression of PRAD.

Prediction and screening of target genes

Analysis using the STRING, BioGRID, and IntAct databases led to the identification of 71, 103, and 98 genes, respectively. We selected genes that were overlapping between at least two of the three databases as target genes, which produced a shortlist of 81 potential target genes of ZFC3H1. A Venn diagram was then constructed for these target genes (Fig. 2).

Enrichment analysis of overlapping target genes in the ZFC3H1 signaling pathway

GO enrichment analyses was performed to determine the functions of the 81 target genes in tumor biological processes (Fig. 3). The putative target genes of ZFC3H1 were found to be enriched in certain cell processes, such as 28S ribosomal subunit, mitochondrial, regulation of mRNA metabolic process, mRNA processing, chromosome separation, mRNA surveillance pathway, RNA 3'-end processing, cell cycle phase transition, nucleic acid transport, nucleotide-excision repair, etc.

Identifying node degree genes via PPI network analyses of overlapping target genes

To explore the interaction between the 81 overlapping target genes, a PPI network was constructed using Metascape (Fig. 4). From the PPI network, 26 node degree genes were identified: *APP*, *C1D*, *CDC5L*, *CSNK2A1*, *CUL7*, *EXOSC1*, *EXOSC5*, *EXOSC7*, *HNRNPK*, *MOV10*, *MPHOSPH6*, *MRPS11*, *MRPS14*, *MRPS23*, *MRPS24*, *MRPS25*, *MRPS31*, *MRPS35*, *MRPS5*, *MRPS7*, *MTREX*, *PTCD3*, *RALY*, *SRRT*, *UPF1*, and *ZC3H3*.

Prognostic significance evaluation of the twenty-six node degree genes

The twenty-six node degree genes were analyzed using the starbase tool to determine whether there were differences in their expression levels between PRAD and corresponding non-cancerous tissues. The expression levels of *APP*, *CDC5L*, *MPHOSPH6*, *MRPS31*, and *MTREX* in PRAD tissues were observed to be lower than those in corresponding non-cancerous tissues (Fig. 5A). In addition, we found that the overall survival time of patients in the *MPHOSPH6* and *MRPS31* high expression groups was lower than that of patients in the respective low expression groups (Fig. 5B).

ZFC3H1 siRNA reduced PRAD cell migration and invasive capability

There has been much evidence that ZFC3H1 is associated with cell mRNA translation. To verify whether silencing *ZFC3H1* could reduce the capability of migration and invasion in PRAD cells, western blotting determined the interference efficiency of ZFC3H1 siRNA (Figure 6C). The experimental results of wound healing showed that, compared with that of the negative control siRNA, *ZFC3H1* knockdown reduced PRAD cell migration and motility markedly (Figure 6A). The transwell assay also showed the number of invaded was significant decrease following transfected with ZFC3H1 siRNA (Figure 6B). These results indicated that inhibiting ZFC3H1 suppressed PRAD cell migration and invasion significantly.

Discussion

Given the high mortality and intractability of advanced PRAD, it is necessary to develop tools or markers to evaluate the prognosis of PRAD. The majority of PRAD initially depends on the androgen receptor (AR) pathway; therefore, the targeted inhibition of androgen biosynthesis therapy has been widely used. However, due to the frequent occurrence of drug resistance and progression of PRAD, new treatments need to be developed [16]. ZFC3H1, as a central factor in the retention and degradation of polyadenylated RNA, is involved in the processing of a wide range of RNAs, and plays a crucial role in the degradation of nuclear RNAs [6-9]. In addition, the depletion of ZFC3H1 resulted in a significant inhibition of translation [11]. Thus, the above studies show that ZFC3H1 has marked research value. To further understand the correlation between ZFC3H1 and the prognosis of PRAD, in the present study, we carried out bioinformatic analyses based on data from the TCGA database, and validated the results using human PRAD cells.

First, we found that ZFC3H1 expression levels in many cancers, including PRAD, were significantly lower compared with those in corresponding non-cancerous tissues. In addition, the survival time of patients with PRAD with high ZFC3H1 expression groups was shorter than that in patients with low ZFC3H1 expression.

Second, we predicted 81 target genes of ZFC3H1 and overlapping target genes were examined via GO enrichment analyses to provide clues to functional roles of these genes in the biological processes of PRAD. A PPI network was constructed, and the most significant node degree genes (n = 26) were selected. We found that the expression levels of *APP*, *CDC5L*, *MPHOSPH6*, *MRPS31*, and *MTREX* in PRAD tissues were notably lower than those in corresponding non-cancerous tissues, and the patients in the *MPHOSPH6* and *MRPS31* high-expression groups showed much shorter overall survival than patients in the respective low-expression groups. These findings supported the hypothesis that ZFC3H1 plays an active role in PRAD development.

To further demonstrate the effect of ZFC3H1 on the prognosis of PRAD, PRAD cells were transfected with an siRNA targeting ZFC3H1. Western blotting showed that the interference effect is remarkable. Subsequently, wound healing and transwell invasion assays showed that *ZFC3H1* knockdown significantly inhibited PRAD cell migration and invasion abilities.

Conclusions

In summary, we reported that ZFC3H1 is closely related to the migration and invasion ability of PRAD. ZFC3H1 or its regulated genes might provide new biomarkers for PRAD prognosis and provide a reference for the development of new therapies to treat PRAD. However, the low expression of ZFC3H1 in PRAD has not yet been explained and further studies are warranted.

Abbreviations

Prostate adenocarcinoma (PRAD)

Zinc finger C3H1 domain-containing protein (ZFC3H1)

coiled-coil domain containing protein (CCDC131)

proline/serine-rich coiled-coil protein (PSRC2)

five tetratricopeptide (TRP)

six half-tetratricopeptide (HAT)

polycomb repressive complex 2 (PRC2)

androgen receptor (AR)

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

All authors agreed on the manuscript.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Funding

This research was supported by Zhejiang Provincial Natural Science Foundation of China under Grant (No. LQ20H050001(Recipient: Hang Huang): collection, analysis, and (LY20H160013(Recipient: Wei Chen): writing the manuscript); Wenzhou Science and Technology Project ((Y20180676) (Recipient: Hang Huang): interpretation of data).

Authors' contributions

HH, ZQ and WC conceived the idea; SY and HK performed the experiments; PL, WC and XT analyzed the data; HH wrote the manuscript. All authors have read and approved the final version of the manuscript.

Acknowledgements

Not applicable

References

1. Mattiuzzi C, Lippi G: Current Cancer Epidemiology. *Journal of epidemiology and global health* 2019, 9(4):217-222.
2. Venturini NJ, Drake CG: Immunotherapy for Prostate Cancer. *Cold Spring Harbor perspectives in medicine* 2019, 9(5).
3. Clancy TE: Surgery for Pancreatic Cancer. *Hematol Oncol Clin North Am* 2015, 29(4):701-716.
4. Kumar C, Bagga J, Chiliveru S, Kohli S, Bharadwaj A, Jain M, Inamdar S, Sharan B: Substantial remission of prostate adenocarcinoma with dendritic cell therapy APCEDEN((R)) in combination with chemotherapy. *Future science OA* 2019, 5(10):Fso435.
5. Yunger S, Bar El A, Zeltzer LA, Fridman E, Raviv G, Laufer M, Schachter J, Markel G, Itzhaki O, Besser MJ: Tumor-infiltrating lymphocytes from human prostate tumors reveal anti-tumor reactivity and potential for adoptive cell therapy. *Oncoimmunology* 2019, 8(12):e1672494.
6. Silla T, Karadoulama E, Makosa D, Lubas M, Jensen TH: The RNA Exosome Adaptor ZFC3H1 Functionally Competes with Nuclear Export Activity to Retain Target Transcripts. *Cell reports* 2018,

23(7):2199-2210.

7. Chlebowski A, Lubas M, Jensen TH, Dziembowski A: RNA decay machines: the exosome. *Biochimica et biophysica acta* 2013, 1829(6-7):552-560.
8. Meola N, Domanski M, Karadoulama E, Chen Y, Gentil C, Pultz D, Vitting-Seerup K, Lykke-Andersen S, Andersen JS, Sandelin A *et al*: Identification of a Nuclear Exosome Decay Pathway for Processed Transcripts. *Molecular cell* 2016, 64(3):520-533.
9. Lee NN, Chalamcharla VR, Reyes-Turcu F, Mehta S, Zofall M, Balachandran V, Dhakshnamoorthy J, Taneja N, Yamanaka S, Zhou M *et al*: Mtr4-like protein coordinates nuclear RNA processing for heterochromatin assembly and for telomere maintenance. *Cell* 2013, 155(5):1061-1074.
10. Garland W, Comet I, Wu M, Radzishewska A, Rib L, Vitting-Seerup K, Lloret-Llinares M, Sandelin A, Helin K, Jensen TH: A Functional Link between Nuclear RNA Decay and Transcriptional Control Mediated by the Polycomb Repressive Complex 2. *Cell reports* 2019, 29(7):1800-1811.e1806.
11. Ogami K, Manley JL: Mtr4/ZFC3H1 protects polysomes through nuclear RNA surveillance. *Cell cycle (Georgetown, Tex)* 2017, 16(21):1999-2000.
12. Ogami K, Richard P, Chen Y, Hoque M, Li W, Moresco JJ, Yates JR, 3rd, Tian B, Manley JL: An Mtr4/ZFC3H1 complex facilitates turnover of unstable nuclear RNAs to prevent their cytoplasmic transport and global translational repression. *Genes & development* 2017, 31(12):1257-1271.
13. Chen CY, Gherzi R, Ong SE, Chan EL, Raijmakers R, Pruijn GJ, Stoecklin G, Moroni C, Mann M, Karin M: AU binding proteins recruit the exosome to degrade ARE-containing mRNAs. *Cell* 2001, 107(4):451-464.
14. Tomita T, Ieguchi K, Coin F, Kato Y, Kikuchi H, Oshima Y, Kurata S, Maru Y: ZFC3H1, a zinc finger protein, modulates IL-8 transcription by binding with celestramycin A, a potential immune suppressor. *PloS one* 2014, 9(9):e108957.
15. Silla T, Schmid M, Dou Y, Garland W, Milek M, Imami K, Johnsen D, Polak P, Andersen JS, Selbach M *et al*: The human ZC3H3 and RBM26/27 proteins are critical for PAXT-mediated nuclear RNA decay. *Nucleic acids research* 2020, 48(5):2518-2530.
16. Low JY, Sirajuddin P, Moubarek M, Agarwal S, Rege A, Guner G, Liu H, Yang Z, De Marzo AM, Bieberich C *et al*: Effective targeting of RNA polymerase I in treatment-resistant prostate cancer. *The Prostate* 2019, 79(16):1837-1851.

Figures

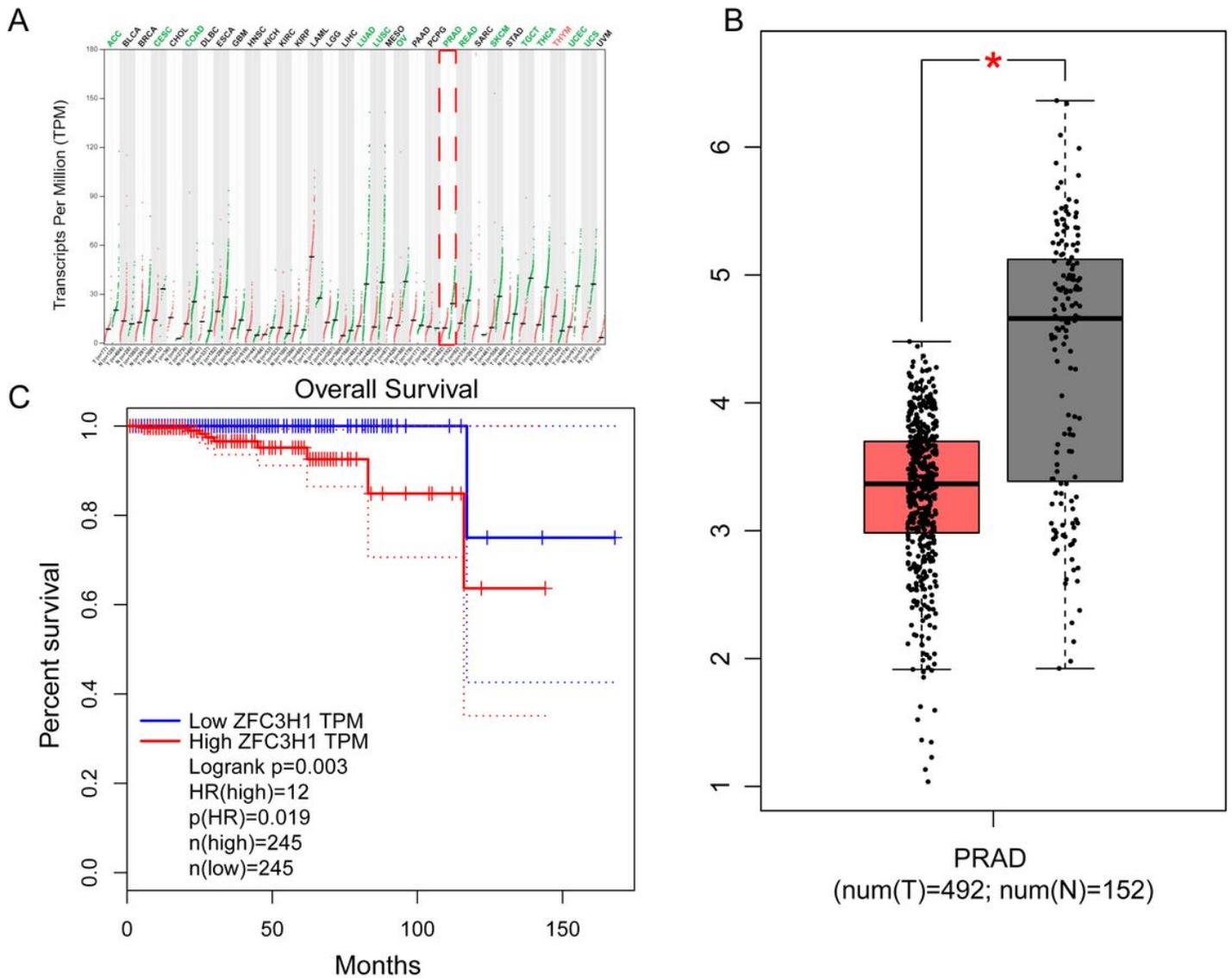


Figure 1

. The correlation between ZFC3H1 expression levels and overall survival of patients with PRAD. A, ZFC3H1 expression profile across all tumor samples and paired normal tissues. B, The expression level of ZFC3H1 in PRAD tissues and adjacent non-tumor tissues. C, The overall survival of human PRAD patients in relation with high or low expression levels of ZFC3H1.

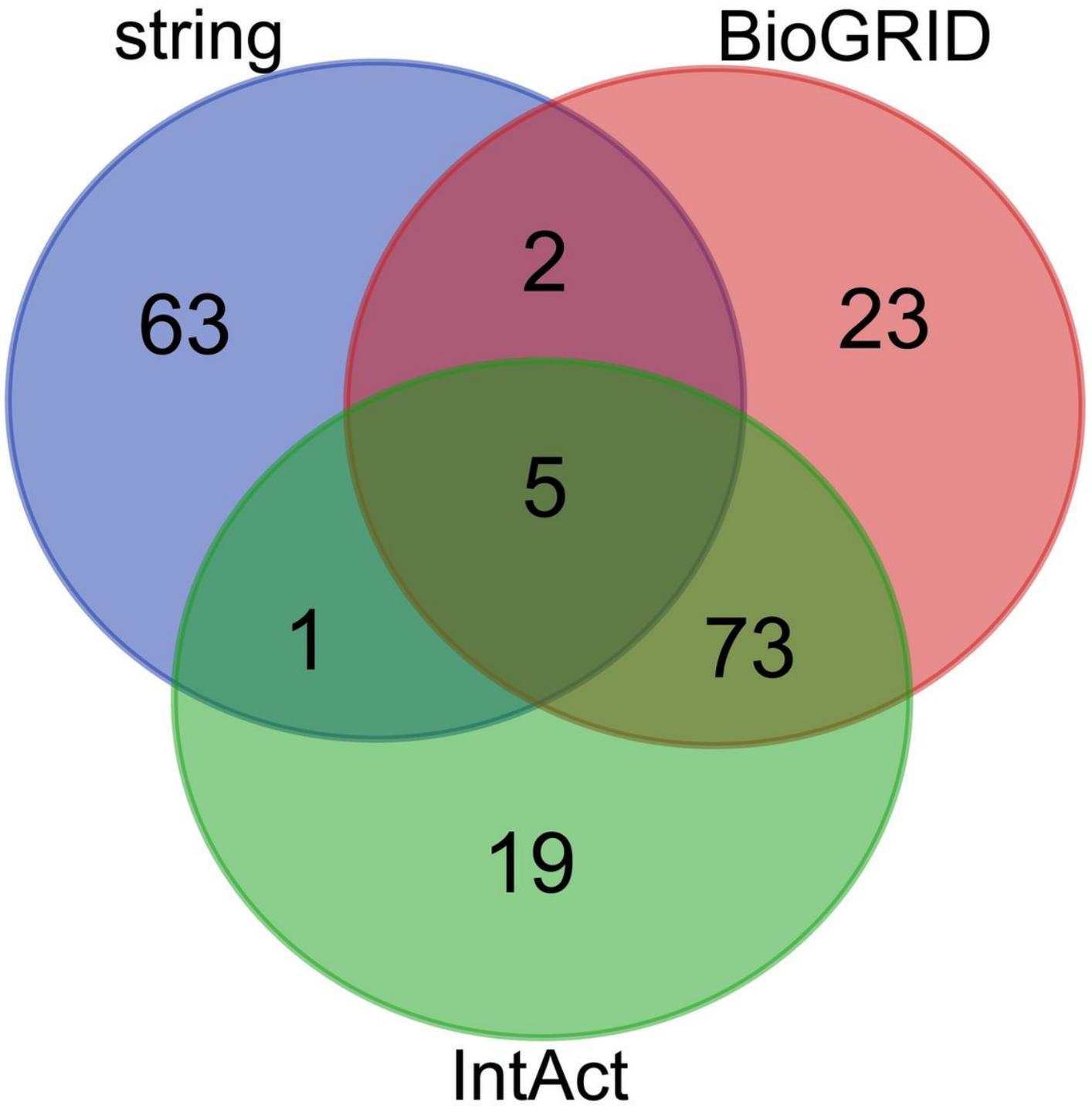


Figure 2

Venn diagram of predicted target genes from three databases

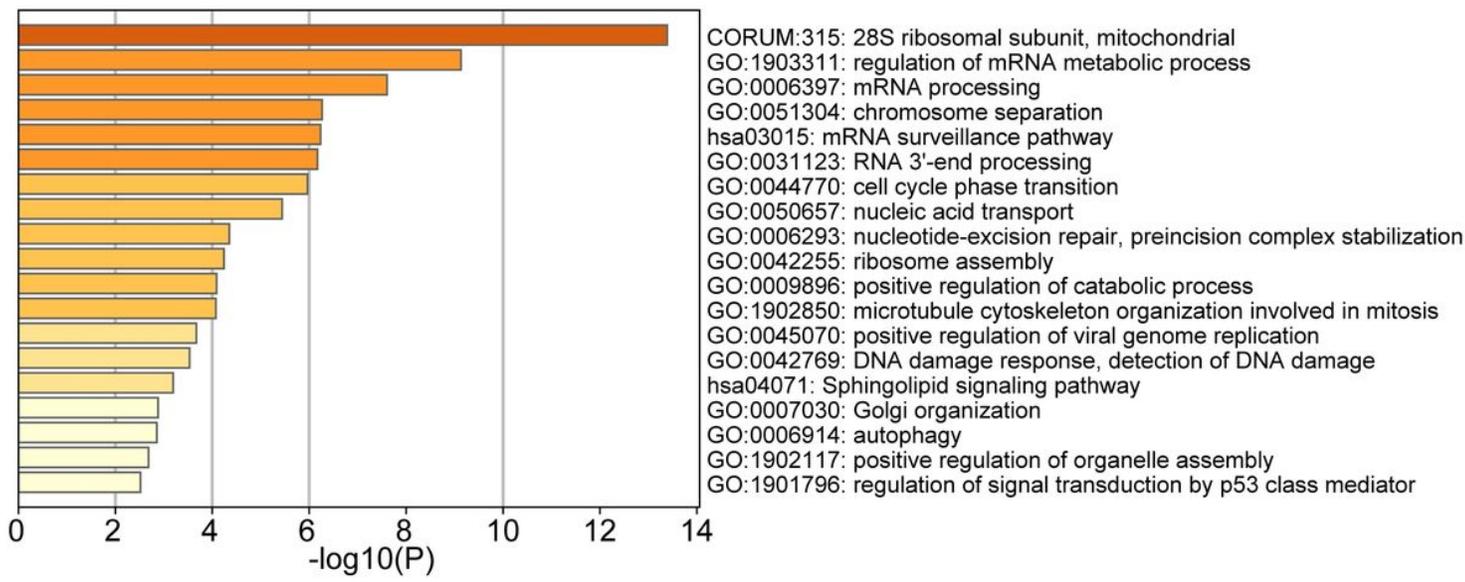


Figure 3

Gene ontology enrichment analysis of overlapping target genes

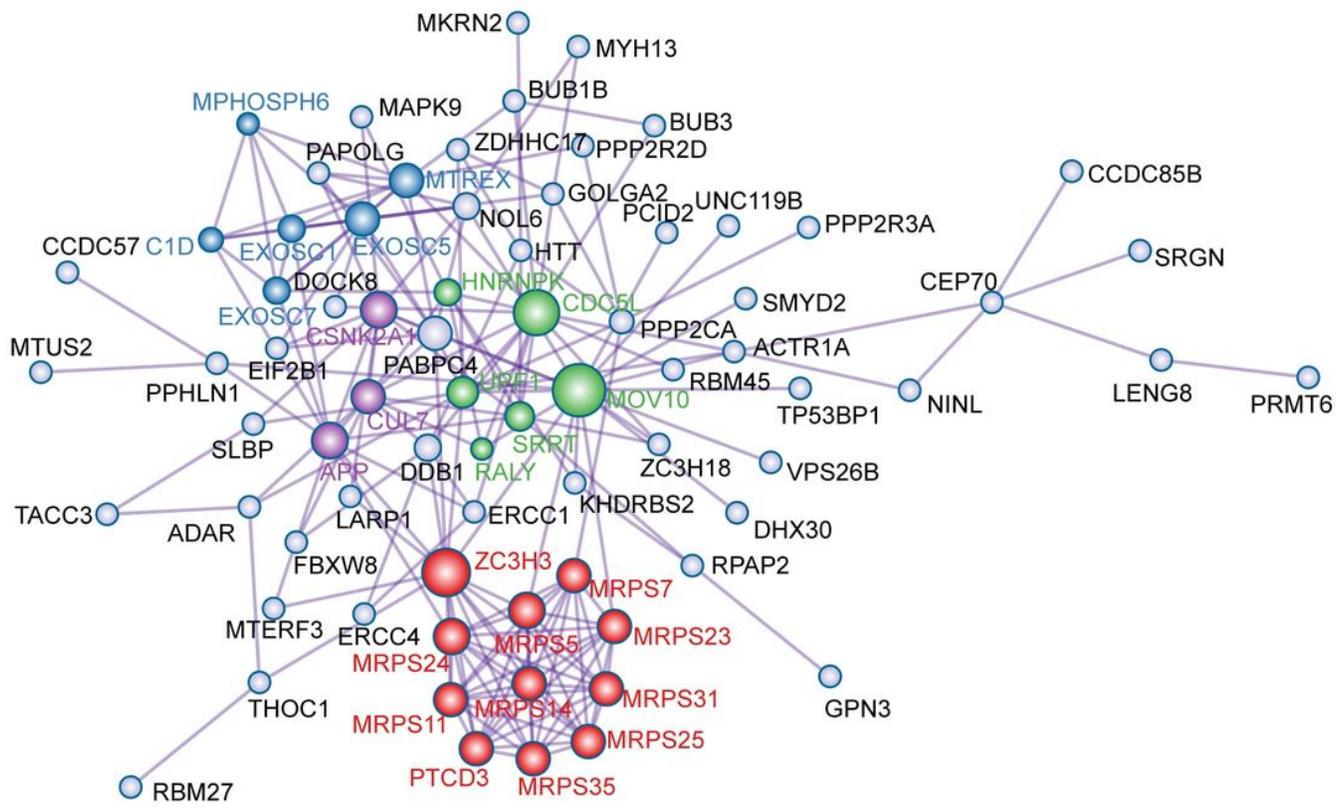


Figure 4

Protein-protein interaction network of overlapping target genes

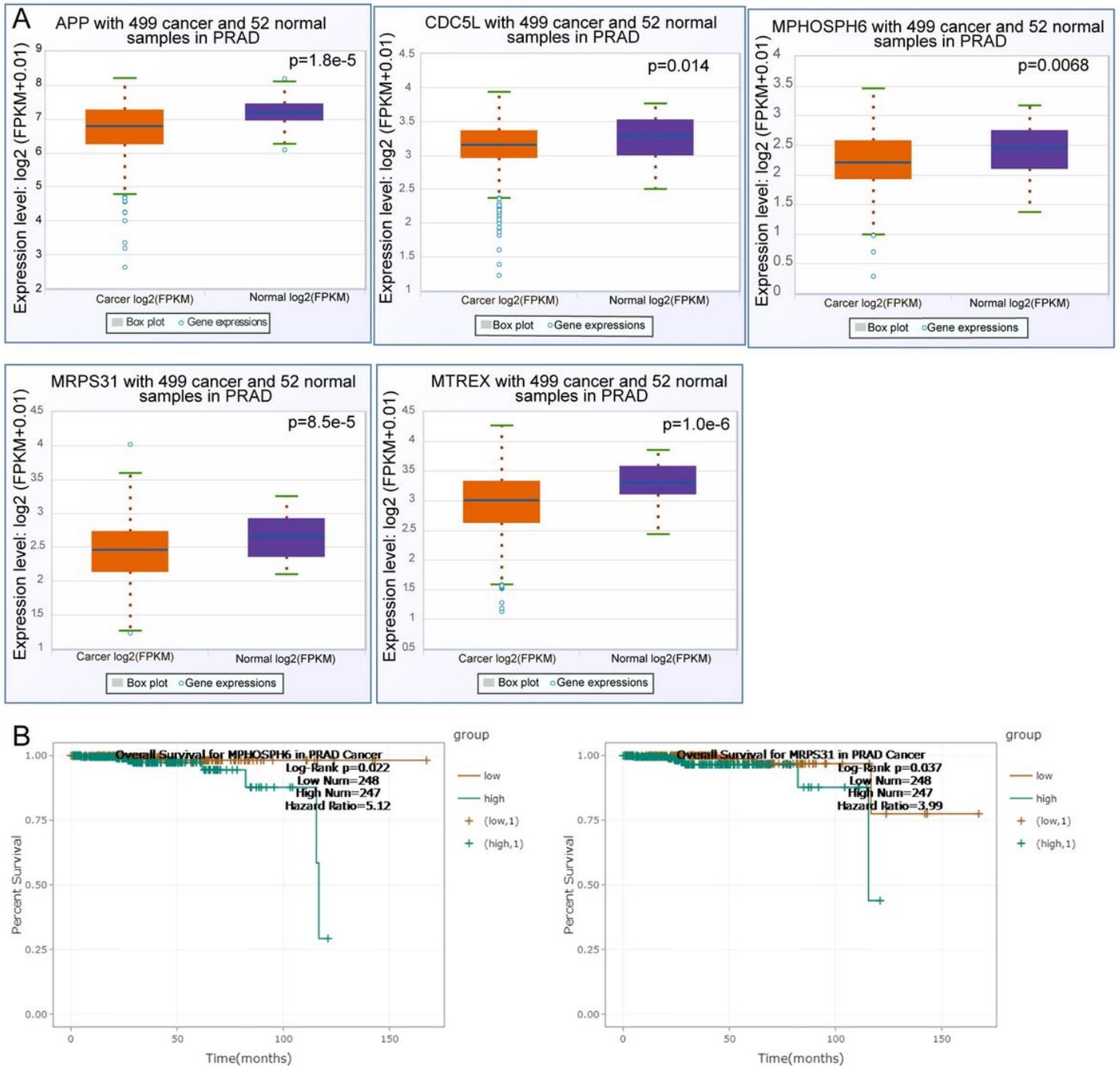


Figure 5

The correlation between selected gene expression levels and overall survival of PRAD patients. A, The expression level of selected genes in PRAD tissues and adjacent non-tumor tissues. B, The overall survival of human patients with PRAD in relation with high or low expression levels of MPHOSPH6 and MRPS31

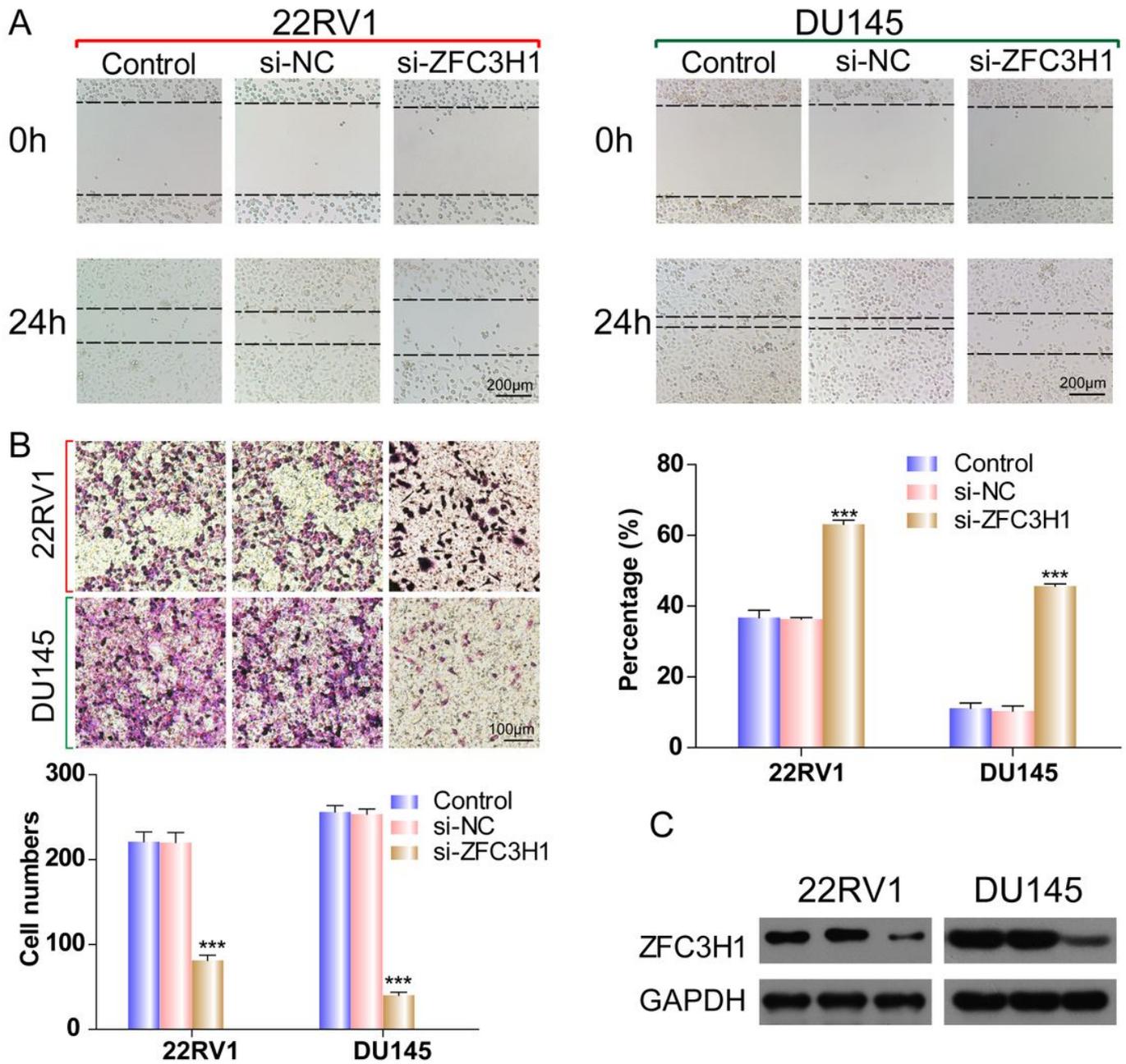


Figure 6

ZFC3H1 knockdown reduced PRAD cell invasive and migration capability. (A) Wound healing assay to determine the cell migration ability following transfection with the ZFC3H1 siRNA or negative siRNA. (B) Transwell assay showing the number of invaded cells following transfection with ZFC3H1 siRNA or negative siRNA. *** $P < 0.001$. (C) Western blotting showing the interference efficiency of ZFC3H1 siRNA. The full-length blots/gels are presented in Supplementary files.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementalfiles.zip](#)