FAM64A Promotes Prostate Cancer Growth In Vivo and In Vitro

Feilun Cui

Jiangsu University

Zhipeng Xu

Jiangsu University

Yumei Lv

Zhenjiang College

Jianpeng Hu (✉ hujianpeng99099@163.com)

Jiangsu University

Research

Keywords: prostate cancer, FAM64A, prognosis, proliferation, biomarker

Posted Date: June 15th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-33834/v1>

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

[Read Full License](#)

Abstract

Background Prostate cancer (PCa) is the most common type of human cancer in males. However, the mechanisms underlying PCa tumorigenesis remained unclear.

Methods The present study evaluated the expression levels of FAM64A in PCa by using 5 public datasets, including GSE8511, GSE45016, GSE55945, GSE38241 and GSE17951. Then, in vivo and in vitro assays were conducted to detect the biological functions of FAM64A in PCa. Microarray and bioinformatic analysis were carried out to detect the downstream targets and pathways regulated by FAM64A.

Results In this study, we for first time demonstrate FAM64A as a biomarker for PCa. FAM64A was found to be overexpressed in PCa compared to normal samples. Higher FAM64A expression were found in Gleason score (GS) ≥ 8 PCa compared to GS < 8 PCa samples, in N1 staging compared to N1 staging PCa samples, and T3/4 staging compared to T1 staging PCa. Moreover, higher FAM64A expression was correlated to shorter survival time in PCa. Knockdown of FAM64A significantly suppressed PCa cell proliferation and colony formation, however, induced PCa apoptosis in vivo and in vitro. Bioinformatics analysis combined with microarray analysis revealed FAM64A played crucial roles in regulating multiple cancer related pathways, including cell-matrix adhesion and cAMP signaling pathway.

Conclusions These results showed FAM64A could serve as a novel biomarker for PCa and will be helpful to understand the underlying FAM64A -related molecular mechanisms in the progression of PCa.

1. Introduction

As one of the most common types of human cancers in males worldwide, prostate cancer (PCa) had been a substantial economic and medical burden of both governments and households [1, 2]. In the past decades, researchers made great efforts to identify key regulators of PCa progression. AR signaling [3], Wnt signaling [4] and MAPK signaling [5, 6] were reported to be the most important pathways in PCa. Recently, emerging studies indicated non-coding RNAs, including miRNAs [7], lncRNAs [8, 9] and circRNAs [10], were also participated in the regulation of PCa related pathways. For instance, miR-183 promoted PSA synthesis in PCa [11], PCAT19 induced PCa growth and metastasis by interacting with hRNPAB to promote cell cycle regulators expression [12]. However, the mechanisms regulating PCa tumorigenesis and progression remained largely unclear. Therefore, identifying the drivers involved in PCa progression is critical to advance our understanding of PCa and to explore novel therapeutic targets for this disease.

FAM64A was identified as a CALM/AF10 interacting partner and a mitotic regulator which was involved in regulating metaphase-to-anaphase transition in cell cycle progression [13]. The abnormal cell cycle progression was considered as an important hallmark of cancer cells [14], suggested FAM64A may play an important role in human cancers. Consistent with this hypothesis, emerging studies showed FAM64A was overexpressed in leukemia [15], lymphoma [16] and breast cancer [17]. FAM64A was first found to play its roles in leukemogenesis by interacting with CALM/AF10 or antagonizing the transactivation activity of CALM/AF10 [15]. In cervical carcinoma HeLa cells FAM64A was involved in regulate

metaphase-to-anaphase transition but had no effect on cell proliferation [18]. Moreover, high expression of FAM64A was correlated to shorter survival time in triple-negative breast cancer, whose knockdown significantly suppressed cell growth in basal-like breast cancer cells [17]. However, the roles of FAM64A in the progression of prostate cancer remained to be further investigated.

Here, we compared the expression of FAM64A in normal prostate and PCa samples using public datasets. Using PCa cell lines DU145 and PC-3, we examined the effects of FAM64A on PCa growth in vivo and in vitro. We thought this study could provide evidences to validate FAM64A as a novel biomarker for PCa.

2. Materials And Methods

2.1 Cell culture.

DU145 and PC-3 were obtained from Chinese Academy of Sciences Cell Bank. DU145 and PC-3 cells were cultured in RPMI-1640 medium (HyClone Laboratories; USA) containing 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc. USA). DU145 and PC-3 cells were cultured at 37 °C in a humidified incubator with 5% CO₂.

2.2 Lentiviral constructs and transfections.

Lentiviral constructs and transfections were conducted according to our previous reports [19]. FAM64A shRNA-1 was CCGGCCCATGATTGACCAGTCAAATTTCAAGAGAATTTGACTGGTCAATCATGGGTTTTTG.

2.3 RT-qPCR.

RT-qPCR was conducted according to our previous reports [19]. The following primers were used for qPCR: FAM64A-F, 5'- CCAGAAAGCTAGGTCGTGGGT-3' and -R, 5'- TGGACTGATCGTGCTTCGTGT-3'; GAPDH-F, 5'-TGACTTCAACAGCGACACCCA-3' and -R, 5'-CACCTGTTGCTGTAGCCAAA-3'. the 2- $\Delta\Delta C_t$ method was conducted to calculate the relative expression levels of targets.

2.4 Cell cytometry and image analysis

Celigo® Image Cytometer (Nexcelom, Lawrence, MA, USA) was used to evaluate the number of cells by scanning green fluorescence daily for 5 days at room temperature according to our previous reports [19].

2.5 In vivo tumor metastasis assays

shFAM64A -transfected or ctrl PC-3 cells were suspended in phosphate-buffered saline (PBS) and injected subcutaneously into the backs of six-week-old male BALB/c nude mice (Shanghai SLAC Laboratory Animals Co., Ltd. Shanghai, China). Tumor growth was measured using calipers over the course of 49 days. Tumor volume was calculated according to the formula: volume = 0.5 × length × width². The mice were euthanized with CO₂ and sacrificed on day 49. Tumor weight was measured and

compared between two groups. All in vivo studies protocols were approved by the Shanghai Medical Experimental Animal Care Commission (Approval ID: ShCI-14-008).

2.6 Western blot analysis

In accordance with standard Western-blot protocols, proteins were separated in 10% SDS-PAGE and transferred to PVDF membrane through Bio-Rad systems (Bio-Rad, Hercules CA, USA). Rabbit anti-FAM64A, and mouse anti-GAPDH antibodies were used in this study. The Quantity One software package (Bio-Rad, USA) was used for the quantitation of signal intensities.

2.7 Statistical analysis

Each experiment was performed for three times. student' T-test was used to calculate statistical significance between two groups. For more groups, one-way ANOVA followed by Newman–Keuls posthoc test was used. P-values < 0.05 was determined as statistical significance. Statistical analysis was performed using the SPSS software package, version 15.0 (SPSS, Inc., Chicago, IL, USA).

3. Results

3.1 FAM64A was up-regulated in prostate cancer.

The expression of FAM64A in normal and PCa samples remained unknown. Here, we screened GEO datasets and downloaded 5 public datasets to investigate FAM64A levels between PCa and normal samples, including GSE8511, GSE45016, GSE55945, GSE38241 and GSE17951. As shown in Fig. 1, we observed FAM64A was significantly overexpressed in PCa compared to normal samples by analyzing these GEO datasets. Furthermore, we validate FAM64A expression profile in TCGA dataset, which was based on RNA-sequence method. We found FAM64A expression in PCa samples were higher than that in normal tissues by analyzing TCGA datasets (Fig. 1F).

3.2 Higher FAM64A expression was associated with shorter overall survival time in prostate cancer

Furthermore, we evaluated the correlation between FAM64A levels and clinical varies in PCa. We found FAM64A was up-regulated in Gleason score (GS) ≥ 8 PCa and T3 and T4 stage PCa compared to GS < 7 PCa and T2 stage PCa (Fig. 2A-B). Furthermore, our analysis showed FAM64A was overexpressed in N1 staging PCa samples in comparison with N0 staging PCa tissues (Fig. 2C). We also analyzed whether the dysregulation of FAM64A was correlated to overall survival time in prostate cancer using Kaplan-Meier method (Fig. 2D). These results suggested the dysregulation of FAM64A could serve as a novel biomarker for PCa.

3.3 Knockdown of FAM64A suppresses cell proliferation in prostate cancer

Our above analyses suggested that FAM64A may serve as an oncogene in PCa. Aiming to explore the functions of FAM64A in PCa, we knockdown its expression in PCa cells using a specific shRNA against

FAM64A. After transfecting PC-3 and DU145 with shFAM64A, we observed the expression of FAM64A was remarkably decreased compared to control groups. Meanwhile, western blot assay showed FAM64A protein level was reduced after transfection in DU145 cells (Fig. 3A-D).

Then, we measured the effect of FAM64A on PCa growth using Celigo cell counting assay for five days. Compared with the control group, knockdown of FAM64A significantly reduced cell proliferation in PC-3 and DU145 by 3.76- and 6.41-fold, respectively (Fig. 3E-F). Next, a CCK-8 assay was conducted. Consistent with above finding, we found knockdown of FAM64A suppressed PC-3 and DU145 cell proliferation rate (Fig. 4A-B).

3.4 Silencing of FAM64A inhibits prostate cancer cell colony formation

Furthermore, the cell colony formation assay was conducted to determine the roles of FAM64A in regulating PCa growth. As shown in Fig. 4, FAM64A knockdown remarkably inhibited the ability of PC-3 and DU145 cells to form colonies. The cell colonies in FAM64A knockdown group were significantly decreased by 75 percent and 85 percent, respectively.

3.5 Knockdown of FAM64A induced cell apoptosis in prostate cancer.

Deregulation in apoptotic pathways had been considered as a hallmark of human cancers. In present study, we accessed cell apoptosis in FAM64A knockdown group and control group by staining V-APC, which was analyzed by flow cytometry. Three days' post transfection, the number of PC-3 and DU145 apoptotic cells in FAM64A knockdown group were increased by 50% and 60% compared to control group, respectively. Taken together, these results showed FAM64A acted as an oncogene by inducing cell proliferation and reducing cell apoptosis.

3.6 Knockdown of FAM64A suppressed prostate cancer growth in vivo

The effects of FAM64A knockdown on tumor growth were analyzed in vivo using nude mouse models. The luciferase-expressing DU145 cells were constructed to trace tumors in live animals. By using an IVIS system, we found luciferase signaling in FAM64A knockdown group was decreased compared to control group. In vivo tumor growth curve showed that knockdown of FAM64A significantly suppressed PCa growth in nude mouse. The mice were euthanized with CO₂ and sacrificed on day 34. Tumor weight was measured and the results showed the tumor weight in FAM64A knockdown group was less than that in negative control group (Fig. 6).

3.7 Identification of FAM64A downstream targets in PCa using microarray analysis

To understand the mechanisms of FAM64A in regulating PCa progression, we used a gene expression microarray to identify FAM64A targeting genes in DU145 cells. A total of 259 genes (including 75 up-regulated and 184 down-regulated genes) were identified as downstream targets of FAM64A in PCa. DAVID system was used to predict the potential functions of FAM64A in PCa. GO analysis showed FAM64A was involved in regulating cell-matrix adhesion, chondrocyte proliferation, cell division, type I

interferon signaling pathway, extracellular matrix organization, apoptotic process, cellular response to insulin stimulus, autophagy, fatty acid metabolic process, and regulation of translation. KEGG analysis showed FAM64A was associated with the regulation of Pertussis, Complement and coagulation cascades, Hematopoietic cell lineage, Arginine biosynthesis, and cAMP signaling pathway (Fig. 7).

A FAM64A related protein-protein interaction network was also constructed using FAM64A targets. As shown in Fig. 7, 109 proteins and 232 edges were included in this PPI network. MAPK8, PRKACA, MAPK9, MX1, APP, GBP1, GNAI1, ITGA2, and ITGA6 were identified as key regulators in FAM64A related PPI network (Fig. 7F).

4. Discussion

PCa is the most common type of human cancer in males. However, the mechanisms underlying PCa tumorigenesis remained unclear. In this study, we for first time demonstrate FAM64A as a biomarker for PCa. FAM64A was found to be overexpressed and correlated to shorter survival time in PCa. Knockdown of FAM64A significantly suppressed PCa cell proliferation and colony formation, however, induced PCa apoptosis in vivo and in vitro. Bioinformatics analysis combined with microarray analysis revealed FAM64A played crucial roles in regulating multiple cancer related pathways, including cell-matrix adhesion and cAMP signaling pathway.

Previous studies indicated FAM64A was involved in regulating cancer progression. FAM64A was a CALM/AF10 co-factor, which regulated cell cycle processes in cells. FAM64A was up-regulated in a few types of cancers, such as leukemia and breast cancer. For instance, FAM64A overexpression was correlated to shorter survival time in triple-negative breast cancer. In this study, we found FAM64A was significantly overexpressed in PCa compared to normal samples by analyzing GEO datasets and TCGA database. Moreover, we found PCa was up-regulated in advanced N staging and T staging PCa samples. Higher FAM64A was associated with shorter disease-free survival time in PCa. These results suggested FAM64A could act as a biomarker for PCa prognosis.

The functions of FAM64A in PCa remained unknown. Loss of function assay showed knockdown of FAM64A suppresses cell proliferation and colony formation in PCa. However, silencing of FAM64A induced cell apoptosis in PCa. Of note, nude mouse models were used to evaluate the effects of FAM64A on PCa growth in vivo. The results showed knockdown of FAM64A suppressed prostate cancer growth in vivo. Furthermore, bioinformatics analysis and microarray analysis were conducted to explore the mechanisms of FAM64A in regulating PCa progression. Bioinformatics analysis showed FAM64A was involved in regulating cell-matrix adhesion, chondrocyte proliferation, cell division, type I interferon signaling pathway, extracellular matrix organization, apoptotic process, cellular response to insulin stimulus, autophagy, fatty acid metabolic process and cAMP signaling pathway.

By constructing FAM64A related PPI network, we identified MAPK8, PRKACA, MAPK9, MX1, APP, GBP1, GNAI1, ITGA2, and ITGA6 as key mediators of FAM64A regulating PCa progression. MAPK8 and MAPK9 were reported to regulate autophagy in non-small cell lung cancer [20]. PRKACA restored anti-apoptotic

signaling in breast cancer [21]. ITGA2 was found to be dysregulated in various cancers, including Gastric Cancer [22], pancreatic cancer [23], breast cancer [24] and prostate cancer [25]. Knockdown of ITGA2 promotes Gastric Cancer apoptosis and suppressed cell migration [22]. In prostate cancer samples, the methylation levels in the ITGA2 promotes were significantly decreased compared to normal tissues. ITGA6 was a hypoxia-responsive gene, which enhanced cancer stem cell activity and metastatic phenotypes in breast cancers [26]. These reports together with our finding showed FAM64A could serve as a novel biomarker for PCa and will be helpful to understand the underlying FAM64A-related molecular mechanisms in the progression of PCa.

Abbreviations

HD: Huntington's disease; WM: white matter; CC: corpus callosum; NSCs: neural stem cells; OPCs: oligodendrocyte progenitor cells; OCT4: octamer-binding transcription factor 4; SVZ: subventricular zone; PBS: phosphate-buffered saline, AAV9: adeno-associated virus serotype 9; CMV: cytomegalovirus, vg: viral genomes; LV: lateral ventricle; AP: anteroposterior; ML: mediolateral; DV, dorsoventral; qRT-PCR: quantitative reverse transcription polymerase chain reaction; TEM: transmission electron microscope; MRI: magnetic resonance imaging; FA: fractional anisotropy; RD: radial diffusivity; AD: axial diffusivity; PFA: paraformaldehyde, IP: intraperitoneal; BrdU: 5-bromo-2'-deoxyuridine; NG2: neural/gial antigen 2; Olig2: oligodendrocyte transcription factor; PDGFR α : platelet-derived growth factor receptor alpha; Wnt3: Wnt family member 3; MYRF: myelin regulatory factor; GDNF: glial cell-derived neuroprotective factor; β III-tubulin: neuron-specific class III beta-tubulin; NeuN: neuronal nuclei; GAD65: glutamic acid decarboxylase 65; DARPP32: dopamine- and cAMP-regulated neuronal phosphoprotein

Declaration

Acknowledgments

We thank Medical Illustration & Design, part of the Medical Research Support Services of Yonsei University College of Medicine for all artistic supports related to this work, Eun Jin Kim, part of the electron microscopy of Yonsei Biomedical Research Center, and Dr. Chan Gyu Joo, Severance Biomedical Science Institute of Yonsei University College of Medicine, for technical supports, and Soonil Pyo for proofreading the manuscript.

Authors' contributions

JHY and B-GN equally contributed to this study. JHY performed most of the experiments, analyzed data, and wrote the manuscript; B-GN performed most of molecular study, analyzed data, and wrote the manuscript; MGK performed experiments, and wrote manuscript; JWS performed animal experiments; S-RC developed the study concept and design, wrote the manuscript, and supervised the project. All authors read and confirmed the manuscript.

Funding

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (grant number : 2019R1I1A1A01057970) and the Korean Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number : HI16C1012)

Availability of data and materials

All relevant data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval

All animals were housed in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC 2016-0298).

Consent for publication

Not applicable.

Competing interests

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

Author details

¹Department and Research Institute of Rehabilitation Medicine, Yonsei University College of Medicine, Republic of Korea

²Brain Korea 21 PLUS Project for Medical Science, Yonsei University, Republic of Korea

³Graduate Program of Nano Science and Technology, Yonsei University, Republic of Korea

References

1. Ross CA, Aylward EH, Wild EJ, Langbehn DR, Long JD, Warner JH, et al. Huntington disease: natural history, biomarkers and prospects for therapeutics. *Nat Rev Neurol*. 2014;10(4):204-16.
2. Gatto RG, Ye AQ, Colon-Perez L, Mareci TH, Lysakowski A, Price SD, et al. Detection of axonal degeneration in a mouse model of Huntington's disease: comparison between diffusion tensor imaging and anomalous diffusion metrics. *Magma*. 2019;32(4):461-71.
3. Gregory S, Cole JH, Farmer RE, Rees EM, Roos RA, Sprengelmeyer R, et al. Longitudinal Diffusion Tensor Imaging Shows Progressive Changes in White Matter in Huntington's Disease. *J Huntingtons Dis*. 2015;4(4):333-46.

4. Jin J, Peng Q, Hou Z, Jiang M, Wang X, Langseth AJ, et al. Early white matter abnormalities, progressive brain pathology and motor deficits in a novel knock-in mouse model of Huntington's disease. *Hum Mol Genet.* 2015;24(9):2508-27.
5. Teo RT, Hong X, Yu-Taeger L, Huang Y, Tan LJ, Xie Y, et al. Structural and molecular myelination deficits occur prior to neuronal loss in the YAC128 and BACHD models of Huntington disease. *Hum Mol Genet.* 2016;25(13):2621-32.
6. Beglinger LJ, Nopoulos PC, Jorge RE, Langbehn DR, Mikos AE, Moser DJ, et al. White matter volume and cognitive dysfunction in early Huntington's disease. *Cogn Behav Neurol.* 2005;18(2):102-7.
7. Faria AV, Ratnanather JT, Tward DJ, Lee DS, van den Noort F, Wu D, et al. Linking white matter and deep gray matter alterations in premanifest Huntington disease. *Neuroimage Clin.* 2016;11:450-60.
8. Franklin RJ. Why does remyelination fail in multiple sclerosis? *Nat Rev Neurosci.* 2002;3(9):705-14.
9. Imitola J, Snyder EY, Khoury SJ. Genetic programs and responses of neural stem/progenitor cells during demyelination: potential insights into repair mechanisms in multiple sclerosis. *Physiol Genomics.* 2003;14(3):171-97.
10. Dehghan S, Hesarakı M, Soleimani M, Mirnajafi-Zadeh J, Fathollahi Y, Javan M. Oct4 transcription factor in conjunction with valproic acid accelerates myelin repair in demyelinated optic chiasm in mice. *Neuroscience.* 2016;318:178-89.
11. Seo JH, Lee MY, Yu JH, Kim MS, Song M, Seo CH, et al. In Situ Pluripotency Factor Expression Promotes Functional Recovery From Cerebral Ischemia. *Mol Ther.* 2016;24(9):1538-49.
12. Kim JB, Zaehres H, Arauzo-Bravo MJ, Scholer HR. Generation of induced pluripotent stem cells from neural stem cells. *Nat Protoc.* 2009;4(10):1464-70.
13. Kim JB, Sebastiano V, Wu G, Arauzo-Bravo MJ, Sasse P, Gentile L, et al. Oct4-induced pluripotency in adult neural stem cells. *Cell.* 2009;136(3):411-9.
14. Kim JB, Greber B, Arauzo-Bravo MJ, Meyer J, Park KI, Zaehres H, et al. Direct reprogramming of human neural stem cells by OCT4. *Nature.* 2009;461(7264):649-3.
15. Cho SR, Suh H, Yu JH, Kim HH, Seo JH, Seo CH. Astroglial Activation by an Enriched Environment after Transplantation of Mesenchymal Stem Cells Enhances Angiogenesis after Hypoxic-Ischemic Brain Injury. *Int J Mol Sci.* 2016;17(9).
16. Ge X, Cho A, Ciol MA, Pettan-Brewer C, Snyder J, Rabinovitch P, et al. Grip strength is potentially an early indicator of age-related decline in mice. *Pathobiol Aging Age Relat Dis.* 2016;6:32981.
17. Wilkins A, Majed H, Layfield R, Compston A, Chandran S. Oligodendrocytes promote neuronal survival and axonal length by distinct intracellular mechanisms: a novel role for oligodendrocyte-derived glial cell line-derived neurotrophic factor. *J Neurosci.* 2003;23(12):4967-74.
18. Ertle B, Schlachetzki JCM, Winkler J. Oligodendroglia and Myelin in Neurodegenerative Diseases: More Than Just Bystanders? *Mol Neurobiol.* 2016;53(5):3046-62.
19. Gao X, Deng L, Wang Y, Yin L, Yang C, Du J, et al. GDNF Enhances Therapeutic Efficiency of Neural Stem Cells-Based Therapy in Chronic Experimental Allergic Encephalomyelitis in Rat. *Stem Cells Int.*

- 2016;2016:1431349.
20. Gonzalez-Perez O, Alvarez-Buylla A. Oligodendrogenesis in the subventricular zone and the role of epidermal growth factor. *Brain Res Rev.* 2011;67(1-2):147-56.
 21. Ihrie RA, Alvarez-Buylla A. Lake-front property: a unique germinal niche by the lateral ventricles of the adult brain. *Neuron.* 2011;70(4):674-86.
 22. Falcão AM, Marques F, Novais A, Sousa N, Palha JA, Sousa JC. The path from the choroid plexus to the subventricular zone: go with the flow! *Front Cell Neurosci.* 2012;6:34.
 23. Maki T, Liang AC, Miyamoto N, Lo EH, Arai K. Mechanisms of oligodendrocyte regeneration from ventricular-subventricular zone-derived progenitor cells in white matter diseases. *Front Cell Neurosci.* 2013;7:275.
 24. El Waly B, Macchi M, Cayre M, Durbec P. Oligodendrogenesis in the normal and pathological central nervous system. *Front Neurosci.* 2014;8:145.
 25. Lu QR, Sun T, Zhu Z, Ma N, Garcia M, Stiles CD, et al. Common developmental requirement for Olig function indicates a motor neuron/oligodendrocyte connection. *Cell.* 2002;109(1):75-86.
 26. Lo Nigro A, de Jaime-Soguero A, Khoueiry R, Cho DS, Ferlazzo GM, Perini I, et al. PDGFR α (+) Cells in Embryonic Stem Cell Cultures Represent the In Vitro Equivalent of the Pre-implantation Primitive Endoderm Precursors. *Stem Cell Reports.* 2017;8(2):318-33.
 27. Zawadzka M, Rivers LE, Fancy SP, Zhao C, Tripathi R, Jamen F, et al. CNS-resident glial progenitor/stem cells produce Schwann cells as well as oligodendrocytes during repair of CNS demyelination. *Cell Stem Cell.* 2010;6(6):578-90.
 28. Huang B, Wei W, Wang G, Gaertig MA, Feng Y, Wang W, et al. Mutant huntingtin downregulates myelin regulatory factor-mediated myelin gene expression and affects mature oligodendrocytes. *Neuron.* 2015;85(6):1212-26.
 29. Pierpaoli C, Barnett A, Pajevic S, Chen R, Penix LR, Virta A, et al. Water diffusion changes in Wallerian degeneration and their dependence on white matter architecture. *Neuroimage.* 2001;13(6 Pt 1):1174-85.
 30. Rosas HD, Lee SY, Bender AC, Zaleta AK, Vangel M, Yu P, et al. Altered white matter microstructure in the corpus callosum in Huntington's disease: implications for cortical "disconnection". *Neuroimage.* 2010;49(4):2995-3004.
 31. Bohanna I, Georgiou-Karistianis N, Sritharan A, Asadi H, Johnston L, Churchyard A, et al. Diffusion tensor imaging in Huntington's disease reveals distinct patterns of white matter degeneration associated with motor and cognitive deficits. *Brain Imaging Behav.* 2011;5(3):171-80.
 32. Delmaire C, Dumas EM, Sharman MA, van den Bogaard SJ, Valabregue R, Jauffret C, et al. The structural correlates of functional deficits in early huntington's disease. *Hum Brain Mapp.* 2013;34(9):2141-53.

Figures

Figure 1

FAM64A was up-regulated in prostate cancer. (A-F) FAM64A was significantly overexpressed in PCa compared to normal samples by analyzing GSE8511, GSE45016, GSE55945, GSE38241, GSE17951 and TCGA datasets. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Figure 2

Higher FAM64A expression was associated with shorter overall survival and disease free time in PCa. (A-C) Higher FAM64A expression were observed in GS>7, N1 and T2/3 staging PCa samples. (D) Higher FAM64A expression was associated with shorter disease-free survival time in PCa by analyzing TCGA. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Figure 3

FAM64A knockdown inhibits cell proliferation in PCa cell lines. (A-B) Expression of FAM64A mRNA after transfection with shFAM64A in PC-3 and DU145 cells. (C-D) Expression of FAM64A protein after transfection with shFAM64A in DU145 cells. (E-H) The Celigo® system showed knockdown of FAM64A inhibited cell proliferation in PC-3 AND du145 cells. Significance was defined as $p < 0.05$ (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

Figure 4

FAM64A knockdown inhibits cell colony formation in PCa cell lines. (A-B) CCK-8 assay showed knockdown of FAM64A inhibited cell proliferation in PC-3 and DU145 cells. (C-D) knockdown of FAM64A inhibited cell colony formation in PC-3 cells. (E-F) knockdown of FAM64A inhibited cell colony formation in DU145 cells. The cell apoptosis analysis results presented as mean $\pm$ SD (n = 3). Significance was defined as $p < 0.05$ (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

Figure 5

FAM64A knockdown promotes apoptosis of prostate cancer cells. (A-D) Treatment with sh FAM64A promotes apoptosis of prostate cancer cells. The cell apoptosis analysis results presented as mean $\pm$ SD (n = 3). Significance was defined as $p < 0.05$ (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

Figure 6

FAM64A knockdown inhibits prostate cancer growth. Knockdown of FAM64A suppressed PCa growth in vivo. (A-C) FAM64A knockdown significantly inhibits PCa growth in vivo. (D-E) The luciferase signaling in FAM64A knockdown group was significantly lower than that in control groups. Data are presented as the mean $\pm$ SD ($n = 3$) *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Figure 7

Bioinformatics analysis revealed multiple functional roles of FAM64A in PCa. (A-C) Heat map shows differential genes expression after FAM64A knockdown. (D-E) GO and KEGG pathway analysis shows the potential pathways regulated by FAM64A. (F) Construction of FAM64A related protein-protein interaction network in PCa.

Supplementary Files

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

- [supplement11.docx](#)