Mechanism of Ferritin Heavy Chain in the Proliferation and Migration of Prostate Cancer Cells

Cuixiu Lu

Peking University Ninth School of Clinical Medicine

Huijun Zhao

Capital Medical University Affiliated Beijing Shijitan Hospital

Man Zhang (✉ zhangman@bjsjth.cn)

Capital Medical University Affiliated Beijing Shijitan Hospital <https://orcid.org/0000-0001-5166-6804>

Research Article

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Abstract

Ferritin is an important molecule in the regulation of cell proliferation, growth inhibition escape, cell death inhibition and angiogenesis. More and more studies have given evidence of abnormal iron metabolism in tumors. Whether ferritin can be a new type of marker for early diagnosis of tumorous diseases remains to be explored. In this study, we verified the expression level of ferritin heavy chain (FTH) in benign prostatic hyperplasia epithelial cell (BPH-1) and three kinds of PCa cells by Western blotting. Lentivirus was used to construct FTH gene overexpression vector, then construct FTH overexpression PCa cell lines. Meanwhile, LNCaP cells and DU145 cells FTH silent expression prostate cancer cell lines were constructed using Crispr Cas9 technology. Next, clone proliferation experiments, transwell experiments, and scratch experiments were used to verify the cell proliferation, invasion, and migration capabilities of each group of cells with different FTH expression levels. The FTH knockout PC-3 cell (LV-shFTH PC3) has been established in our laboratory. Make full use of the existing results from Isobaric Tag for Relative and Absolute Quantitation (iTRAQ) mass spectrometry technology to analyze differentially expressed protein analysis between LV-shFTH PC3 cells and control cells. Combine GO analysis to select the target protein for subsequent cell protein expression level verification. Our results show that FTH overexpression can promote the proliferation, migration and invasion of prostate cancer cell lines, while FTH knockout causes the opposite result. Combined with mass spectrometry (MS) detection, it is speculated that FTH may trigger changes in cell behavior by regulating the expression of the following proteins: *S100A4*, *CRABP1*, *S100A2*, *S100P*, *GDF15*, *FAM84B*, *KRT75*, *LYRM7*, *OCLN*, *LCP1*, *F2RL1*. (The study is not a clinical trial, no registration number and registration date are required)

Introduction

Ferritin is a highly symmetrical and stable iron storage protein. It was found to be crystallized and named 80 years ago in 1937[1, 2]. It is a nanoprotein composed of 24 subunits variably assembled including ferritin heavy chain (FTH) and ferritin light chain (FTL)[3]. One of the main characteristics of ferritin is its ability to absorb iron ions and induce its mineralization by utilizing its ferrioxidase activity and chemical properties specific to the cavity environment[4]. The two subunits of ferritin: the heavy chain (FTH) has ferrioxidase and antioxidant activity, which can convert toxic ferrous ions into less toxic iron ions[5, 6]. Although light chain (FTL) does not have iron oxidase activity, it can modify the cell microenvironment, promote the long-term storage of iron, and provide an effective place for the formation of iron nuclei, iron nucleation and mineralization[7, 8]. The presence of FTL may also promote the conversion rate of the FTH ferrite enzyme active center[7]. Despite extensive research, the special chemical and biological properties of various ferritins still attract researchers.

In the previous research in our laboratory, urine samples from patients with prostate cancer and benign prostatic hyperplasia and healthy volunteers were screened for differential proteins[9]. The results confirmed that Ferritin heavy chain (FTH) is highly expressed in prostate cancer patients. Further experiments proved that low expression of FTH can reduce the migration and proliferation ability of prostate cancer cell PC-3 cells[10]. However, the molecular mechanism of how FTH triggers changes in

cell behavior is not yet known. According to the analysis results of iTRAQ mass spectrometry technology, compared with the NC group cells, the top twelfth expression differences among down-regulated genes in FTH knockout PC3 cells are S100A4, CRABP1, S100A2, S100P, GDF15, FAM84B, KRT75, LYRM7, OCLN, LCP1, NDUFAB1, F2RL1. Among them, the connection between S100 family members and FTH and their possible effects on PC3 cells have been discussed in our previous studies[11]. In this study, the remaining other proteins will be further discussed and verified. These gene expression products are involved in cell signal transduction, cell proliferation and cell migration.

In order to make the above conclusion more convincing, DU145 and LNCap cells were used in this experiment, and FTH-overexpression and FTH-knockout were performed on these two cells respectively. Western blots were also used to verify the protein expression levels of FTH in BPH cells and in three different PCa cell lines. The results showed that compared with BPH-1 cells, different PCa cell lines all have different degrees of increase in FTH expression. Western blot analysis showed that the down-regulated protein expression in FTH knockout PC3 cells showed a consistent trend in FTH-silenced LNCaP and DU145 cells. There are reasonable reasons to believe that ferritin plays an important role in the development of PCa. It is likely to be a potential biomarker for early diagnosis and treatment of PCa.

Materials And Methods

Materials and reagents

Human prostate cancer epithelial cells PC-3 cell, du145 cell and LNCap cell were purchased from the National Infrastructure of the Cell Line Resource (Beijing, China). Human benign prostate hyperplasia epithelial cells (BPH-1) were bought from the Germany DSMZ cell bank. Stable FTH silenced PC3 cells (LV-shFTH PC3) had already been established in our laboratory. RPMI-1640 and F-12 Kaighn Modification were purchased from Gibco Company, fetal bovine serum (FBS) from Zhejiang Tianhang Biotechnology Company, 0.05% trypsin from Genview Company, and puromycin from the Sigma Company. All materials required for transwell experiments come from Corning Company. The human FTH gene RNA was cloned into the GV358 vector, and its lentivirus was completed by Shanghai GeneChem Company. Lenti-CAS9-puro virus vector, expressing CAS9 protein, with puro resistance and the Lenti-FTH sgRNA-EGFP virus vector together with their control group were both completed by Shanghai GeneChem Company. Antibodies used for Western blot were from as the following: Anti-FTH polyclonal antibody (ab65080, Abcam), Anti-KRT75 (ab94853, Abcam), Anti-LYRM7 (ab151089, Abcam), Anti-GDF15 (A0185, Abclonal), Anti-LCP-1 (A5561, Abclonal), Anti-FAM84B (A18539, Abclonal), Anti-Occludin (A2601, Abclonal), Anti-CRABP1 (A8838, Abclonal), Anti-NDUFAB1 (A14657, Abclonal), Anti-F2RL1 (A8796, Abclonal). cellular total RNA extraction kit and SYBR Green fluorescent quantitative PCR kits were from Beijing TIANGEN Company.

Cell culture

PC3 Cells were cultured in F-12 Kaighn Modification supplemented with 10% FBS. The other three cells were cultured in RPMI-1640 with 10% FBS. All cell were grown in 5% CO₂ at 37°C, digested with 0.05% trypsin for subculturing.

Quantitative real-time PCR (qPCR)

RNA extraction kit (TIANGEN) was used to extract total RNA of cells following manufacturer's instructions. Reverse transcription kit was used to reverse-transcribe RNA samples. Use SYBR Green fluorescent quantitative PCR kit for qPCR analysis. For each sample, react at least three times in triplicate. The data was analyzed according to the classic 2 Ct method and normalized to the expression of GAPDH in each sample. Table 1 lists the primers were used.

Western blot

Total protein from treated cells was extracted in the role of RIPA lysis buffer supplemented with PMSF. All csystem was established using a Bis-Tris Gel system together with PVDF membranes following the manufacturer's instruction. The primary antibody was prepared at a dilution of 1:1000 in 5% blocking buffer, and incubated with the membrane overnight at 4°C, washed and incubated with the secondary antibody conjugated with horseradish peroxidase for 1 h. All experiments were repeated more than three times using β -actin as an internal reference protein.

Generation of stable cell lines

Cell suspension was prepared with a density of $3 \sim 5 \times 10^4$, inoculating it in 24-well plate, 500ul per well. When the cell confluence reaches 30%, add the corresponding virus according to the cell MOI and virus titer for infection. After incubation at 37°C, 5%CO₂ for 12–16 hours, the culture was continued with complete medium. Pay attention to the cell infection efficiency and cell status during culture. The Crispr Cas9 technology contains two vectors, the infection of CAS9 vector is performed first, and then the infection of LV-FTH-sgRNA vector is performed. The operation is consistent with the previous procedure. Sequences of LV-FTH-sgRNA 452, LV-FTH-sgRNA 453, LV-FTH-sgRNA 454 are shown in Table 2.

Colony formation assay

Cells were treated with trypsin, resuspend and seeded in a 6-well plate with a density of 200 cells per well. After the cells were incubated for 10 days, the cell clumps were counted. The colonies were fixed with paraformaldehyde and stained with crystal violet for 20 min. After washing the cells with phosphate buffer, the cell clusters were imaged and the number of colonies was counted.

Scratch experiment

When the cells in the petri dish are cultured to a confluence of more than 95%, use a marker to draw straight lines on the back of the petri dish. Use the pipette tip to make the scratch as perpendicular to the horizontal line on the back as possible. The tip should be vertical and not tilted. Wash the cells three times with phosphate buffer to remove floating cells and add serum-free medium. Cells was incubated at

37°C, 5%CO₂. The healing rates of different groups of cells were recorded at 0h, 24h, 48h and 72h after the scratch treatment.

Statistical analysis

All data collected from independent experiments are expressed as mean standard deviation (SD). GraphPad Prism 6.0 statistical software was used for statistical analysis. SPSS 25.0 software was used to perform student t test for the differences between the experimental group and the control group. One-way ANOVA was used to compare the groups. $P < 0.05$ indicates that the difference is statistically significant.

Results

1. Ferritin heavy chain is highly expressed in prostate cancer cells.

In order to explore the relationship between the level of ferritin heavy chain and prostate cancer cells, avoiding the contingency of one cell line, this experiment selected three kinds of prostate cancer cell lines (DU145, LNCaP, PC3 cells) and benign prostatic hyperplasia epithelial cell (BPH-1). Western blot was used to directly observe the protein expression level of FTH in cells. Results showed that compared with BPH-1, the FTH protein expression levels in the three prostate cancer cells were significantly increased (Fig. 1 $P < 0.05$).

2. results of differentially expressed proteins in FTH knockout PC3 cells (LV-shFTH PC3)

The previous research of our laboratory performed iTRAQ mass spectrometry analysis on FTH knockout PC3 cells (LV-shFTH PC3) and the negative control group[11]. The results of mass spectrometry analysis showed that a variety of proteins were differentially expressed. The differentially expressed proteins are sorted by the amount of differentially expressed proteins, and the top twelve are shown in Table 3.

3. Successfully constructed CRISPR/CAS9 dual vector lentivirus-mediated FTH silent LNCap and DU145 cell lines.

This experiment successfully constructed a lentiviral vector: Lenti-sgRNA-EGFP virus, which expressed the target gene sgRNA sequence and the control sgRNA sequence. Table 2 show the synthetic oligo information and sequencing results. Performing qPCR to verifies the FTH-knockout efficiency of the three FTH-sgRNAs. Results show that the FTH-sgRNA 453 is the most efficient (Fig. 2), and lenti-FTH-sgRNA 453 vector was selected for subsequent transfection experiments. The FTH silence were named DU145-FTH-ko453, LNCap-FTH-ko453. The control group was named as DU145-FTH-nc, LNCap-FTH-nc. Results

of qRT-PCR showed that mRNA levels in DU145-FTH-ko453, LNCaP-FTH-ko453 cells were significantly decreased compared to the control group respectively (Fig. 3), and both cells met the requirements of the following experiments (Fig. 3).

4. Successfully constructed lentivirus-mediated FTH overexpression cell lines (DU145-FTH⁺, LNCaP-FTH⁺) and negative control groups (DU145-FTH^{NC}, LNCaP-FTH^{NC}).

The LV-FTH1 lentiviral vector was transfected into DU145 cells and LNCaP cells respectively, to construct FTH overexpression cell lines, which is denoted as DU145-FTH⁺ and LNCaP-FTH⁺ respectively. At the same time, the corresponding control cells named as DU145-FTH^{NC} and LNCaP-FTH^{NC} transfected with the negative vector were constructed respectively. Compared with the control group, the mRNA and protein levels of FTH in DU145-FTH⁺ and LNCaP-FTH⁺ cells were significantly increased (Fig. 4A, Fig. 4B).

5. The influence of FTH gene differential expression on cell proliferation ability

The plate clone formation experiment was used to evaluate the changes in the proliferation ability of the two prostate tumor cells after different treatments. The results show that the proliferation ability of different cells is different, even after the same type of cells are treated with different FTH gene expression interventions, they also show significant differences (Fig. 5). The proliferation ability of prostate cancer cell lines (DU145-FTH⁺, LNCaP-FTH⁺) in the FTH overexpression group was promoted to varying degrees, while the proliferation ability of FTH silent expressing cells (DU145-FTH-ko and LNCaP-FTH-ko) decreased significantly. The specific colony formation rate of each cell group: DU145 cell control group was 0.25 ± 0.01 , compared with 0.50 ± 0.01 for DU145-FTH⁺ cells and 0.06 ± 0.01 for DU145-FTH-ko cells ($P < 0.05$); LNCaP cell control The group was 0.31 ± 0.01 , compared with 0.63 ± 0.02 for LNCaP-FTH⁺ cells and 0.053 ± 0.02 for LNCaP-FTH-ko cells ($P < 0.05$).

6. The influence of FTH gene differential expression on cell migration ability

Scratch experiment was used to detect the effect of FTH expression differences on the migration ability of PCa cells. The healing rates of different groups of cells were recorded at 0h, 24h, 48h and 72h after the operation. Among them, the healing rates of DU145-FTH⁺ cells at 24h, 48h, and 72h were 19%, 70%, and 94%, respectively. The healing rates of cells in the control group were 12%, 34%, and 80% at 24h, 48h, and 72h, respectively. The healing rate of DU145-FTH-ko cells at 24h, 48h and 72h is only 2%, 8%, and 12%. The same trend is reflected in the LNCaP cell line: the healing rate of LNCaP-FTH⁺ cells at 24h, 48h, and 72h is 27%, 45%, and 77%. And the rate of the control group at 24h, 48h, and 72h is 9 %, 22%, 51%. The rates of LNCaP-FTH-ko cells were only 2%, 10%, and 23%. It can be seen that up-regulation of FTH gene expression can promote cell migration, while down-regulation of FTH expression will inhibit different cell

migration capabilities (Fig. 6). A close relationship between FTH expression and PCa cell migration can be seen. Changing the FTH expression of cells will directly affect the metastasis and invasion ability of tumor cells.

7. Validation of mass spectrometry results in three kinds of cells

In order to better explain the reasons why cell proliferation, migration and invasion ability of FTH knockout PC3 cells were reduced, western blot experiments were performed in three FTH knockout PCa cell lines to verify the differential expressed proteins according to the results of MS. Among them, S100A4, S100A2, and S100P have been verified in the previous article. The results of this experiment confirmed that the other 8 down-regulated proteins were not only down-regulated in FTH knock-out PC3 cells, but also in FTH knock-out LNCaP cells and DU145 cells, there was a consistent trend in protein expression levels (Fig. 7). However, the protein expression level of NDUFB1 was not detected in this experiment.

Discussion

As an essential element for cell growth, iron is involved in DNA synthesis, energy metabolism and other important cellular processes[1, 12]. It is important for proteins containing iron-sulfur clusters (such as DNA helicase involved in DNA replication), factors involved in electron transport, and elements related to oxidative phosphorylation[13]. Compared with normal cells, tumor cells have a greatly increased demand for iron, cell activity including DNA replication and energy metabolism[14]. The metabolism of iron in the body depends on the role of ferritin[3]. More and more data show that many types of cancer, including prostate cancer, are accompanied by iron disorders[15]. As a storage form of iron in the body, ferritin has many new functions in addition to the function of storing iron. For example, ferritin is involved in angiogenesis[16]. And the ferritin receptor, transferrin receptor 1 (TfR1) and serum ferritin (SF) levels have been found to be elevated in tumors[17, 18]. It is known that elevated SF is caused by a variety of different diseases. Researchers investigated 627 patients with $SF \geq 1000 \text{ ug/l}$ in the outpatient and hospitalization of tertiary medical centers with significantly increasing ferritin levels. It was found that the most common cause of increased ferritin was malignant tumors, then is iron overload[17]. studies using FTH to synthesize biomimetic magnetic ferritin nanoparticles. It can specifically target the protein shell of the tumor and the iron oxide core that catalyzes the peroxidase substrate to produce a color reaction, allowing the visualization of tumor tissue. FTH was found to bind specifically to nine of the most common solid tumors. In addition, a large number of studies have also given the differential expression of tumor cells related to iron metabolism by ferritin[15].

In this research, in order to make the research results more convincing and avoid the chance of experimental results caused by only one cell line, this research selected three PCa cells (PC3, DU145, LNCaP) as the experimental objects, looking for ferritin, especially Ferritin heavy chain-Evidence of the effect of FTH on the proliferation and migration ability of PCa cells. The results of Western blot

experiments showed that compared with BPH-1 cells, FTH expression increased in different degrees in the three PCa cells. This finding indicates that FTH is likely to be an important marker for identifying benign prostatic hyperplasia and prostate cancer. The FTH gene mediated by CRISPR/CAS9 dual vector lentivirus was used to construct FTH silent expression LNCaP and DU145 cell lines, and FTH overexpression LNCaP and DU145 cell lines were constructed at the same time. Using clonal proliferation experiments, transwell experiments, and scratch experiments, it can be proved that FTH has the ability to promote tumor cell proliferation, migration and invasion for different types of PCa cells, which is consistent with the previous research results of our laboratory, FTH knockout can inhibit PC3 cell proliferation and promote apoptosis. In this experiment, by adding different cell lines and increasing FTH overexpression group, the original conjecture was better verified, indicating that ferritin, especially heavy chain FTH, is in the prostate. Cancer will play a major role in the occurrence and development of cancer, which can be roughly divided into: promoting tumor cell proliferation and inhibiting cell apoptosis, promoting cell metastasis and generating greater invasiveness, making tumor cells more prone to metastasis and triggering a malignant prognosis.

With the help of existing data in Pubmed, Uniprot, KEGG and other databases, we roughly classify the 12 proteins verified in this experiment as follows.

Proteins related to tumor cell proliferation

Growth differentiation factor 15 (GDF15) is a secreted dimeric protein with the characteristic structure of a cytokine in the TGF- β superfamily[19]. GDF15 is also known as PDF (prostate differentiation factor), PLAB (placental bone morphogenetic protein), PTGF- β (placental transforming growth factor- β) and NAG-1 (non-steroidal anti-inflammatory drug activating gene-1). Existing studies have shown that GDF15 is closely related to the development of a variety of tumors: GDF-15 contributes to the proliferation and immune escape of immune host malignant gliomas[20]; The traditional non-steroidal anti-inflammatory drug sulfamate inhibits the growth of ovarian cancer cells by regulating the expression of NAG-1/MIC-1/GDF-15[21];The expression of GDF-15 is related to the apoptosis of intestinal cells, and is related to the pathogenesis of colorectal cancer and the anti-tumor effect of NSAIDs[22].

As a branch member of the TGF- β family, GDF-15 has attracted more and more attention. It has been proposed that this stress-induced cytokine has an immunomodulatory function[23], and its high expression is usually associated with the progression of cancers including prostate cancer (PCa)[24]. However, the research on GDF15's involvement in signal transduction, cell interaction, cancer progression and therapeutic function has not yet been fully clarified. In this study, we proved that the PCa cell line after FTH knockout will be accompanied by the down-regulation of GDF-15. Combining with the existing research, it has been proved that GDF-15 has a promoting effect on PCa cell proliferation. It is speculated that GDF-15 may be involved Knockout of FTH caused changes in cell proliferation. Although the specific mechanism is unknown, it is certain that there is a certain association between GDF-15 and FTH. Research on this association is likely to bring new opportunities for the early diagnosis and treatment of PCa.

Protein FAM84B promotes prostate cancer through a complex network that predicts the recurrence of prostate cancer[25]. In this study, knockout of FTH caused the down-regulation of FAM84B expression, linking FTH and FAM84B in the process of tumorigenesis and development. This will bring new discussion points to FAM84B.

Cellular retinoic acid-binding protein 1 (CRABP-1) is a member of the CRABP family. The member of this family, CRABP2 plays significant role in tumors which has been verified by numerous studies[26, 27]. However, the role of CRABP-1 in tumors is still unclear. Some studies have pointed out that the expression of CRABP-1 in cancers, including ovarian cancer, thyroid cancer, and esophageal cancer, is reduced, which is mainly due to this gene is high methylation[28, 29]. But there are also studies using immunohistochemical analysis to show that it increases in ovarian cancer tissues. We confirm that the FTH knockout PCa cell lines were accompanied by the down-regulated expression of CRABP-1. Combining with the existing research, it has been clear that CRABP-1 regulates the response of cells to RA, and the latter can be regulated by regulating the transcriptional activity of multiple target genes[30]. Cell proliferation and differentiation, it is speculated that the down-regulation of CRABP-1 expression after FTH knockout affects the role of RA in the cell, thereby weakening the cell proliferation ability. Although this study did not in-depth study the specific mechanism of CRABP-1 regulated by FTH, this will be a question worthy of discussion in the future.

Proteins related to tumor cell migration and invasion

In situ prostate tumor cells are not lethal. However, once the cells are invasive and metastatic, it will seriously threaten the life and health of the patient[31]. Therefore, exploring the molecular mechanism that triggers the invasion and migration of PCa cells is very important for the early diagnosis and intervention of PCa patients. After consulting a large amount of literature, a preliminary conclusion was drawn: Among the 12 down-regulated proteins verified in this experiment, the protein S100A4, S100A2, S100P, F2RL1, OCLN, and LCP-1 were likely to be the key molecule that FTH knockout causes the decline in the migration and invasion ability of PCa cells.

Protein S100A4, S100A2, S100P belong to the S100 protein family, which is composed of 21 members and has a high degree of structural similarity to each other[32]. Members of this family participate in the migration/invasion process of cells and participate in the regulation of cytoskeletal transition[33]. For example, epithelial mesenchymal transition (EMT) is a classic process involving members of the S100 family. The molecular mechanism of these three proteins in FTH-induced changes in the migration ability of PCa cells has been discussed in our previous studies[11], so we will not discuss them here.

Proteinase-activated receptor 2 (PAR2, F2RL) Protease activated receptors(PARs) belong to the family of G protein-coupled receptors and have been confirmed to be involved in a variety of tumor development processes[34]. The PARs family includes 4 members: PAR-1, also known as thrombin receptor, whose main function is to activate platelets, which has been confirmed to be related to tumor cell metastasis. PAR-1 binds to thrombin, and PC3 cells stimulated by thrombin show elevated urokinase, which is the main mediator of cancer cell invasion. PAR-2, this protein cannot be activated by thrombin, but can be

activated by trypsin[34, 35]. It plays a vital role in regulating the inflammatory response and wound healing process. Elevated expression of PAR-1 and PAR-2 can be seen in a variety of tumors, and the elevated level is related to the stage of tumor development[36–38]. The specific functions of other PAR-3 and PAR-4 need to be further studied and will not be discussed here. Existing studies have proved that there is an up-regulation of PARs protein expression in LNCap cells, and the up-regulated PAR-1 is associated with a higher tumor biochemical recurrence rate. Stimulating PAR-1 and PAR-2 can significantly enhance the migration ability of LNCap cells[38]. In our experiment, FTH knockout will inhibit cell migration and invasion, and it has been verified that FTH knockout will cause down-regulation of PAR-2 expression. Therefore, it is speculated that there is a regulatory axis between FTH, PAR-2 and cell migration, and FTH can regulate the migration ability of PCa cells.

Occludin (OCLN), Plastin-2 (LCP-1) can regulate cytoskeleton transformation[39]. The promotion effect of these two kinds of cells on tumor cell migration and invasion has been confirmed by many studies[40]. Research on lung cancer points out that occludin can produce tumor promoters and promote the role of transfer factors[41]. Besides, Antisense L-Plastin gene can suppress invasion of PCa cell[42]. our experiment confirmed that FTH knockout can cause the expression of both of them to be down-regulated in PCa cells, suggesting that FTH knockout triggers cells. The molecular mechanism of reduced migration and invasion ability is closely related to these two proteins. The molecular mechanism by which FTH induces the down-regulation of these proteins remains to be further studied.

Conclusion And Prospect

Compared with BPH1 cells, the expression level of ferritin heavy chain protein in different prostate tumor cell lines (PC3, DU145, LNCaP) has different degrees of increase. This reveals that FTH has great potential as a diagnostic marker of PCa for disease diagnosis and monitoring. The results of plate clone proliferation experiments, scratch experiments, and transwell experiments can prove that in different prostate cancer cell lines, overexpression of FTH can promote cell proliferation, migration and invasion to varying degrees. Silencing FTH expression will cause the cells to show the opposite results. It reminds us that regulating the expression of FTH is likely to become a new and potential target for PCa therapy.

We believe that FTH may be a new target for the treatment of primary and metastatic prostate diseases. Efforts will continue to elucidate the specific molecular mechanisms by which FTH gene expression differences trigger changes in cell behavior, such as the molecular regulatory mechanism between it and S100 family members. In addition, further experiments will be considered, such as establishing tumor xenograft models in nude mice. In addition, the relationship between other differentially expressed proteins and ferritin after FTH knockout and their effects on PCa cells need to be further confirmed.

Declarations

Ethics approval and consent to participate:

This study didn't contain any researches on human participants or animals.

Consent for publication:

Not applicable.

Availability of data and material:

All the data and materials we provide are available.

Conflicts of interest:

Cuixiu Lu, Huijun Zhao, Man Zhang declare that they have no conflicts of interest.

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Authors' contributions:

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Cuixiu lu, Huijun Zhao and ManZhang. The first draft of the manuscript was written by Cuixiu lu and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Ethical standards

Conflict of interest:

All authors declare that they have no conflict of interest.

Ethical approval:

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Consent for publication:

All authors knew and agreed to publish this article.

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Tables

Table 1

The primers used in realtime quantitative PCR (qRT-PCR)

Gene	Forward primer	Reverse primer
FTH	5'- CATCAACCGCCAGATCAAC - 3'	5'- GATGGCTTTACCTGCTCAT - 3'
GAPDH	5'- TTTGGTATCGTGGAAGGACT - 3'	5'- AGTAGAGGCAGGGATGATGT - 3'

Table 2

The sequences of NC, three sgRNA against FTH

Gene	Target sequence (5'-3')
FTH-sgRNA 452	CACCGCTGAGTCCTGGTGGTAGTTC
FTH-sgRNA 453	CACCGTGGCGGCCTCTGAGTCCTGG
FTH-sgRNA 454	CACCGCGTAGGAGGCGTAGAGCTCC
NC	CGCTTCCGCGGCCCGTTCAA

Table 3
 FTH vs PC3

Gene Name	Description	Ratio
S100A4	Protein S100-A4	0.319177
CRABP1	Cellular retinoic acid-binding protein 1	0.362891
S100A2	Protein S100-A2	0.402769
S100P	Protein S100P	0.456751
GDF15	Growth/differentiation factor 15	0.497500
FAM84B	Protein FAM84B	0.518719
KRT75	Keratin, type II cytoskeletal 75	0.520150
LYRM7	Complex III assembly factor LYRM7	0.535401
OCLN	Occludin	0.536313
LCP-1	Plastin-2	0.542544
NDUFAB1	Acyl carrier protein, mitochondrial	0.543570
F2RL1	Proteinase-activated receptor 2	0.545129

Figures

Figure 1

Differential expression of FTH in PC-3, DU145, and LNCaP cells and BPH-1 cells. A representative image of the Western blot show that Compared with BPH-1 cells, protein expression was significantly increased in three breast cancer cells (DU145, LNCaP and PC3 cells). *: $P < 0.05$ **: $P < 0.01$.

Figure 2

Verification of three sgRNA-mediated FTH knockout efficiency at the cellular RNA expression level A representative image of the qPCR graph shows that FTH1-sgRNA (08453-1) has the highest knockout efficiency. This lentiviral vector was selected for cell infection in subsequent experiments

Figure 3

Differential expression of FTH in FTH knocked-out DU145 cells and LNCap cells. (A) A representative image of the Western blot and qPCR graph show that the FTH transcription level and protein expression level in the cells of the DU145-FTH-ko453 group are significantly reduced, compared to DU145-FTH-nc. (B) A representative image of the Western blot and qPCR graph show that the FTH transcription level and protein expression level in the cells of the LNCap-FTH-ko453 group are significantly reduced, compared to LNCap-FTH-nc.

FTH过表达组

Figure 4

Differential expression of FTH in FTH overexpression DU145 cells and LNCap cells. (A) A representative image of the Western blot and qPCR graph show that the FTH transcription level and protein expression level in the cells of the DU145-FTH⁺ group are significantly increased, compared to DU145-FTH^{NC}. (B) A representative image of the Western blot and qPCR graph show that the FTH transcription level and protein expression level in the cells of the LNCaP-FTH⁺ group are significantly increased, compared to LNCaP-FTH^{NC}.

Figure 5

The influence of FTH gene differential expressed on cell proliferation ability (A) Proliferation ability of FTH gene differential expressed DU145 cells—DU145-FTH⁺ > NC > DU145-FTH-ko453 (B) Proliferation ability of FTH gene differential expressed LNCaP cells—LNCaP-FTH⁺ > NC > LNCaP-FTH-ko453.

Figure 6

The influence of FTH gene differential expressed on cell migration ability (A) Migration ability of FTH gene differential expressed DU145 cells (DU145-FTH+ > NC > DU145-FTH-ko453) (B) Migration ability of FTH gene differential expressed LNCaP cells (LNCaP-FTH+ > NC > LNCaP-FTH-ko453).

Figure 7

Verification of protein expression level of down-expressed protein in FTH-knocked out PCa cells and the NC group. Western blot results showed that the proteins CRABP1, GDF15, FAM84B, KRT75, LYRM7, OCLN, LCP1, and F2RL1 were down-regulated in three different FTH knockdown PCa lines, which was consistent with the results of mass spectrometry.