Differential expressions of integrin-linked kinase, β-parvin and cofilin 1 in high-fat diet induced prostate cancer progression in a transgenic mouse model

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Abstract. High-fat diet induced obesity was associated with more aggressive prostate cancer. Recent research has demonstrated that integrin-linked kinase (ILK), β-parvin and downstream cofilin 1 jointly affected cancer progression. Meanwhile, these proteins were also involved in energy metabolism. Therefore, the present study was conducted to investigate the potential function of ILK, β-parvin and cofilin 1 in the high-fat diet-induced progression of prostate cancer. Transgenic mice with prostate cancer were employed, fed with different diets and sacrificed at 20 and 28 weeks. Tumor differentiation, extracapsular extension and metastasis were compared between the groups. Expression levels of ILK, β-parvin and cofilin 1 in prostate were evaluated by immunohistochemical analysis and determined by an immunoreactivity score. Public databases were applied for analysis and validation. It was detected that high-fat diet feeding promoted cancer progression in transgenic mice with prostate cancer, with increased expressions of β-parvin (P=0.038) and cofilin 1 (P=0.018). Higher expressions of ILK, β-parvin and cofilin 1 were also associated with poorer cancer differentiation. Additionally, higher mRNA levels of *CFL1* were correlated with a worse disease-free survival in patients of certain subgroups from The Cancer Genome Atlas database. Further studies were warranted in discussing the potential roles of ILK, β-parvin and cofilin 1 in high-fat diet feeding induced progression of prostate cancer.

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Introduction

Prostate cancer (PCa) recently ranked as the second most diagnosed malignancy and the fifth leading cause of cancer death in men globally (1). Its development and progression were closely associated with another global epidemic, obesity, as extensive evidence showed up demonstrating various epidemiological and biological associations (2). Obesity was proved to be associated with more aggressive PCa, e.g., higher pathological grade (3), higher recurrence rate after definitive therapy (4), and higher cancer-specific mortality (5). However, the exact molecular mechanisms contributing to obesity induced PCa progression remained largely unclear.

The progression and metastasis of cancer required cell mobilization and epithelial mesenchymal transition (EMT), involving the filopodium-like protrusions (FLPs) of cancer cells that interacted productively with surrounding microenvironment (6). The researchers identified that the activation of integrin-linked kinase (ILK), β-parvin, cofilin pathway could promote caner progression, via enhancing the formation of FLPs and maintaining its existence, which raised a brand new perspective in cancer researches (6). Meanwhile, the signaling of ILK, β-parvin or cofilin were also involved in obesity and energy metabolism as recently proposed (7-9). Liu *et al* (7) discovered that oleic acid, with high levels in sera of obese patients, would activate ILK signaling pathway and therefore promote proliferation of renal cell carcinoma. It was also demonstrated that ILK might promote diet-induced insulin resistance in obese mice, by impairing insulin signaling and insulin perfusion through capillaries via ILK-PINCH-parvin complex (IPP) (8). Besides, Cofilin 1 (*CFL1*) gene expression was proved to be markedly elevated in patients with metabolic syndrome in Turkish population (9). Despite the complicated crosstalk and molecular network, these findings guided us to explore whether ILK/β-parvin/cofilin pathway played a role in obesity induced cancer progression.

To better elucidate the effect of obesity on development and progression of PCa, we applied high-fat diet (HFD) to induce obesity in transgenic adenocarcinoma of mouse prostate (TRAMP) animal model, which was considered the best model to resemble the natural process of PCa progression in obese subjects (10). We aimed to verify the hypothesis that

ILK/β-parvin/cofilin pathway would affect the cancer progression in obese patients. With public database, we endeavored to further validate the differential expressions of ILK, β-parvin and cofilin in PCa, investigate their roles in cancer survival, and explore the possible molecular network.

Materials and methods

Animals and diets. The present study was carried out in strict accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications no. 8023, revised 1978). The protocol was approved by the Institutional Animal Care and Use Committee from Department of Laboratory Animal Science, Fudan University (20160816A197). The TRAMP mice were obtained from Jackson Laboratory (Bar Harbor, Maine, USA), bred and maintained under specific pathogen free conditions (SPF, Grade III) at Department of Laboratory Animal Science, Fudan University (Shanghai, China, certificate no. SCXK-HU-2014-0004). Each mouse was kept separately in a cage, bedding of cork dust, with a 12-hour light-dark cycle. All male TRAMP mice were selected by genotyping, and randomly admitted to two groups where the mice were fed with micronutrients-matched control diet (CD) or HFD *ad libitum* at 5 weeks of age as previously described (11). CD (16% calories from fat) and HFD (40% calories from fat) was supplied by Puluteng Bio-technology, Shanghai, China. TRAMP mice were sacrificed at 20 or 28 weeks of the age, resulting in a total of four groups in the present study (CD-fed 20-week TRAMP, n=12; HFD-fed 20-week TRAMP, n=12; CD-fed 28-week TRAMP, n=12; HFD-fed 28-week TRAMP, n=12). The maximum diameter of tumor allowed was 1.5 cm, and none of tumors in the present study reached this diameter.

Systemic evaluation and tissue preparation. All TRAMP mice received body weight and blood glucose examinations before sacrifice. Then, the mouse underwent general anesthesia with intraperitoneal injection of pentobarbital (50 mg/kg), scanned by GE eXplore Locus micro-CT scanner (GE Healthcare Biosciences, Chicago, IL, USA) for systemic evaluation, and euthanized by asphyxiation of CO_2 (flow rate at 1.5 l/min). The prostate tumor, genitourinary tract, epididymal fat, enlarged lymph nodes, liver and lung were removed from the mouse, weighed, and fixed for further analysis.

Pathological and immunohistochemical analysis. Prostate and other prepared tissues were fixed in 10% buffered formalin, processed in an alcohol-xylene series, and embedded in paraffin. A series of sections were prepared with hematoxylin and eosin staining, for evaluation of tumor differentiation, extracapsular extension and confirmation of distant metastasis. The IHC was performed in mouse prostate. All prostate sections were dewaxed, rehydrated and incubated in 3% hydrogen peroxide for 10 min at room temperature to block the activity of endogenous peroxidase. These sections were then incubated overnight at 4˚C with rabbit monoclonal Anti-ILK, Anti-B-Parvin and Anti-Cofilin antibody, respectively (dilution, 1:50; Abcam, Cambridge, MA, USA). Staining was detected using Envision detection kit (Dako, Hamburg, Germany) and diaminobenzidine (DAB) as the chromogen, according to the manufacturer's instructions. In each mouse, three sections of the prostate were included for further evaluation and analysis.

Evaluation of sections were accomplished by two pathologists at our institute. Immunoreactivity score (IRS) category, determined by the multiplication of score of staining intensity and score of percentage of positive cells, was applied to assess the immunohistochemistry (IHC) sections as previously described (12). In specific, intensity of staining was classified as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong), and percentage of positive cells was classified as 0 (<1%), 1 (1-25%), 2 (26-50%), 3 (51-75%), and 4 (76-100%). IRS category was thus determined as score 0 (negative, multiplication values 0), score 1 (weakly positive, multiplication values 1, 2), score 2 (moderately positive, multiplication values 3, 4, 6) and score 3 (strongly positive, multiplication values 8, 9, 12).

Public database analysis and validation. The molecular and clinical data of prostate adenocarcinoma patients (499 samples) were obtained from The Cancer Genome Atlas (TCGA) database that generated by TCGA Research Network (http://cancergenome.nih.gov/). Using cBioPortal for Cancer Genomics (http://www.cbioportal.org) (13,14), these data were further analyzed in Oncoprint, disease-free survival (DFS), and biological neighborhood networks, to validate and explore the expressions of ILK, β-parvin and cofilin in PCa patients.

Statistical analysis. The results were presented as mean ± standard error. TRAMP mice sample size was determined by previous experiments on the characterization of the mouse model, and all mice were randomized to different groups in a blinded manner. Unpaired two-tailed Student's t-test, analysis of variance, Fisher exact test, Pearson's correlation test or Kaplan-Meier curves with log-rank test were conducted, as appropriate, using SPSS Statistics version 24 (IBM, Armonk, NY, USA) and Prism version 6.0c (GraphPad Software, La Jolla, CA, USA). A two-tailed $P<0.05$ was considered to indicate a statistically significant difference in all analyses.

Data availability. The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Results

HFD feeding stimulated fat accumulation and promote PCa progression in TRAMP mice. HFD feeding stimulated adipose tissue accumulation and increased body weight in TRAMP mice, as presented in Table I. In 28-week group, the body weight of mice fed with HFD was higher than that fed with CD (34.2 g vs. 28.2 g, $P<0.001$). Meanwhile, the genitourinary weight was also higher in HFD-fed mice in 28-week group (1.54 g vs. 1.21 g, $P<0.05$). The epididymal fat weight was higher in HFD-fed mice in both 20-week (1.05 g vs. 0.53 g, $P<0.01$) and 28-week group (1.32 g vs. 0.58 g, $P<0.01$).

We further determined the development and progression of PCa, by evaluating the tumor differentiation, extracapsular extension and metastasis (Table I). PCa formation was detected from all TRAMP mice in the present study. Compared with

Table I. Comparisons of systemic characteristics and tumor progression between control diet-fed and high-fat diet-fed TRAMP mice.

Variables	20-week		28-week	
	Control diet (N=12)	High-fat diet (N=12)	Control diet (N=12)	High-fat diet (N=12)
Body weight[a] (g)	20.8±0.52	22.3±1.1	28.2±0.61	34.2±1.44[f]
Blood glucose[a] (mmol/l)	15.1±0.95	15.2±1.41	14.8±1.07	18.8±1.85
Genitourinary weight[a] (g)	0.86±0.06	1.02±0.08	1.21±0.07	1.54±0.13[d]
Epididymal fat weight[a] (g)	0.53±0.09	1.05±0.14[e]	0.58±0.1	1.32±0.22[e]
Tumor diameter[a] (mm)	6.17±0.19	6.08±0.17	7.67±0.16	8.17±0.25
Tumor differentiation (%)				
Well	50.0	41.7	8.3	8.3
Moderate	25.0	33.3	33.3	16.7
Poor	25.0	25.0	58.3	75.0
Extracapsular extension (%)	8.3	16.7	50.0	66.7
Extracapsular extension[a,b] (positive margins per mouse prostate)	0.08±0.08	0.33±0.22	0.75±0.25	1.83±0.46[d]
Metastasis (%)	0	0	25.0	41.7
Metastasis[a,c] (sites per mouse)	0	0	0.33±0.19	2.75±1.09[d]

[a]Data presented as mean ± standard error of the mean. [b]Extracapsular extension was quantitatively evaluated based on the average number of margins that was invaded in mouse prostate (upper, lower, anterior, posterior, left, right). [c]Metastasis was quantitatively evaluated based on the average number of sites that was metastasized in distant organs. [d]P<0.05, [e]P<0.01 and [f]P<0.001 high-fat diet vs. control diet fed TRAMP mice from the same week sub-group. TRAMP, transgenic adenocarcinoma of mouse prostate.

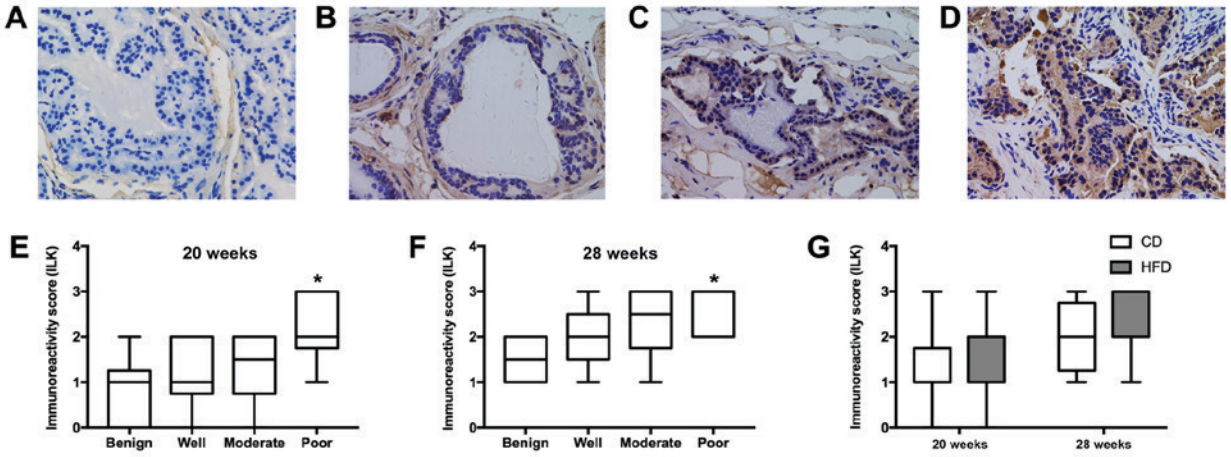

Figure 1. Representative IHC staining demonstrating ILK expression of different IRS category in TRAMP prostate and ILK levels compared among different prostate tissue and between different diet feeding. (A-D) Representatives images showing (A) negative (IRS category=0), (B) weak (IRS category=1), (C) moderate (IRS category=2) and (D) strong (IRS category=3) expressions of ILK in prostate specimens (magnification, x400). (E and F) Boxplots graphs showing the IRS category of ILK expression across benign prostate tissue, well-differentiated, moderately-differentiated and poorly-differentiated prostate cancer in 20-week and 28-week TRAMP mice, respectively. The horizontal line indicates the median and the central box indicates the inter-quartile range, with whiskers indicate the lowest and highest results. *P<0.05, significant differences are indicated as compared with benign subgroup. (G) Boxplots graphs showing the IRS category of ILK expression in prostate cancer between CD-fed and HFD-fed TRAMP mice of 20 and 28 weeks of age. The horizontal line and central box shows the median and inter-quartile range, with whiskers indicating the lowest and highest result. ILK, integrin-linked kinase; TRAMP, transgenic adenocarcinoma of mouse prostate; IHC, immunochemistry; CD, control diet; HFD, high-fat diet; IRS, immunoreactivity score.

CD-fed mice, there was a trend towards poorer PCa differentiation in HFD-fed mice, whereas no statistical significance was detected. Moreover, HFD-fed mice suffered higher rates of extracapsular extension (20-week, 16.7% vs. 8.3%; 28-week, 66.7% vs. 50.0%), as well as higher rates of distant metastasis, eg, retroperitoneal lymph nodes or lung metastasis (28-week, 41.7% vs. 25.0%). In quantitative analysis, the average positive margins of extracapsular extension and average sites of metastasis were both significantly higher in 28-week HFD-fed mice.

Protein expression of ILK, β-parvin and cofilin 1 in HFD-induced PCa progression. The representative IHC images of ILK, β-parvin and cofilin 1 in prostate specimens

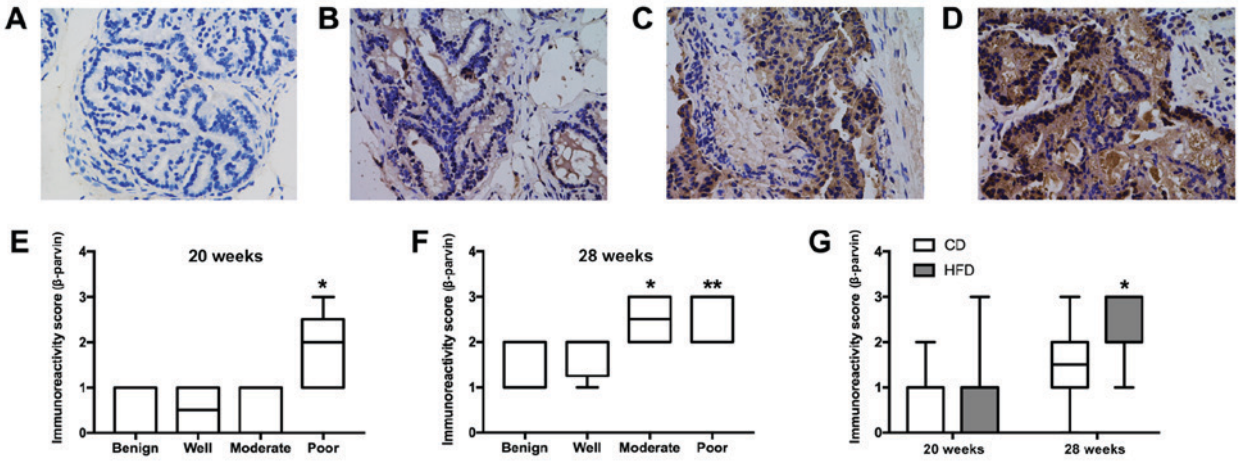

Figure 2. Representative IHC staining demonstrating β-parvin expression of different IRS category in TRAMP prostate and β-parvin levels compared among different prostate tissue and between different diet feeding. (A-D) Representatives images showing (A) negative (IRS category=0), (B) weak (IRS category=1), (C) moderate (IRS category=2) and (D) strong (IRS category=3) expressions of β-parvin in prostate specimens (magnification, x400). (E and F) Boxplots graphs showing the IRS category of β-parvin expression across benign prostate tissue, well-differentiated, moderately-differentiated and poorly-differentiated prostate cancer in 20-week and 28-week TRAMP mice, respectively. The horizontal line indicates the median and the central box indicates the inter-quartile range, with whiskers indicate the lowest and highest results. *P<0.05, **P<0.01, significant difference is indicated as compared with benign subgroup. (G) Boxplots graphs showing the IRS category of β-parvin expression in prostate cancer between CD-fed and HFD-fed TRAMP mice of 20 and 28 weeks of age. The horizontal line and central box shows the median and inter-quartile range, with whiskers indicating the lowest and highest result. *P<0.05, significant difference is indicated as compared with control-diet fed mice of the same week subgroup. TRAMP, transgenic adenocarcinoma of mouse prostate; IHC, immunochemistry; CD, control diet; HFD, high-fat diet; IRS, immunoreactivity score.

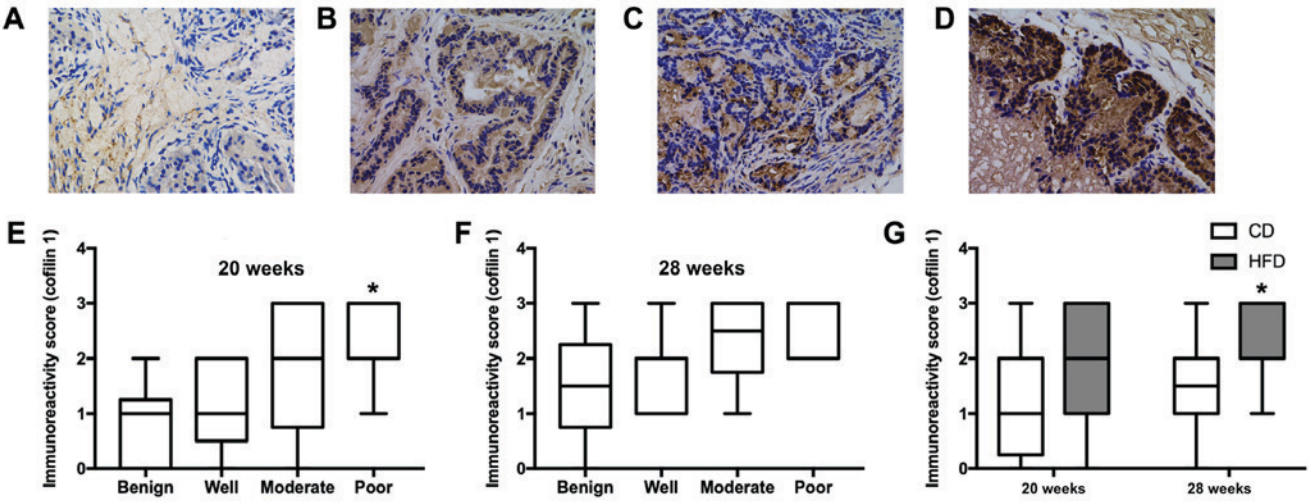

Figure 3. Representative IHC staining demonstrating cofilin 1 expression of different IRS category in TRAMP prostate and cofilin 1 levels compared among different prostate tissue and between different diet feeding. (A-D) Representatives images showing (A) negative (IRS category=0), (B) weak (IRS category=1), (C) moderate (IRS category=2) and (D) strong (IRS category=3) expression of cofilin 1 in prostate specimens (magnification, x400). (E and F) Boxplots graphs showing the IRS category of cofilin 1 expressions across benign prostate tissue, well-differentiated, moderately-differentiated and poorly-differentiated prostate cancer in 20-week and 28-week TRAMP mice, respectively. The horizontal line indicates the median and the central box indicates the inter-quartile range, with whiskers indicate the lowest and highest results. *P<0.05, significant differences are indicated as compared with benign subgroup. (G) Boxplots graphs showing the IRS category of cofilin 1 expression in prostate cancer between CD-fed and HFD-fed TRAMP mice of 20 and 28 weeks of age. The horizontal line and central box shows the median and inter-quartile range, with whiskers indicating the lowest and highest result. *P<0.05, significant difference is indicated as compared with control-diet fed mice of the same week subgroup. TRAMP, transgenic adenocarcinoma of mouse prostate; IHC, immunochemistry; CD, control diet; HFD, high-fat diet; IRS, immunoreactivity score.

(including benign prostate tissue and PCa), with IRS category ranging from 0-3 were presented as reference, respectively (Figs. 1-3). The staining for ILK, β-parvin and cofilin 1 was mainly located at cytoplasm.

Compared to benign prostate tissue, the ILK immunoreactivity was stronger in poorly-differentiated PCa in both 20-week and 28-week mice (Fig. 1E and F). Meanwhile, the expression of ILK presented with a slight increase in 28-week HFD-fed mice (IRS category=2.50±0.67 vs. 2.00±0.74, P=0.242) (Fig. 1G) as compared with CD-fed mice.

Immunoreactivity of β-parvin also increased steadily as PCa progressed, and was higher in poorly differentiated-PCa than that in benign prostate tissue (Fig. 2E and F). In 28-week mice, the β-parvin expressions were also higher in HFD-fed group (IRS category=2.25±0.62 vs. 1.50±0.80, P=0.038) (Fig. 2G).

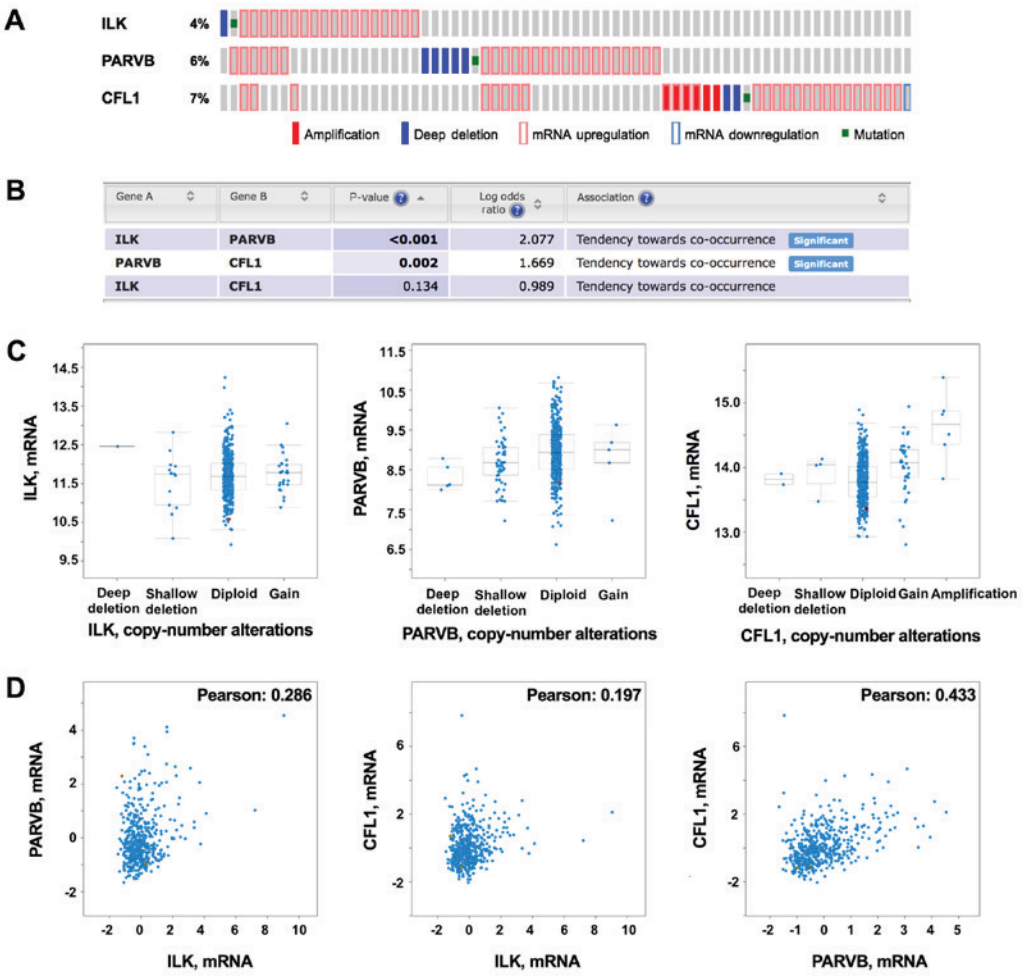

Figure 4. Protein and genetic expression profiles of *ILK*, *PARVB*, *CFL1* in prostate adenocarcinoma from TCGA database. (A) Oncoprint of *ILK*, *PARVB* and *CFL1* genetic alterations, with the specific alteration categories presented in the lower panel. (B) The tendency towards co-occurrence among *ILK*, *PARVB* and *CFL1*. (C) Plots showing the associations between DNA copy number alterations and related mRNA abundance in the gene *ILK* (left panel), *PARVB* (middle panel) and *CFL1* (right panel) in prostate adenocarcinoma. Blue dots indicate individual cases. (D) Plots demonstrating the mRNA expression correlations in *PARVB* vs. *ILK* (left panel), *CFL1* vs. *ILK* (middle panel) and *CFL1* vs. *PARVB* (right panel). Correlations are determined by Pearson coefficient with P-value. Blue dots indicate individual cases. TCGA, The Cancer Genome Atlas.

As for cofilin 1 expression, we identified a higher IRS category in poorly-differentiated PCa in 20-week mice (Fig. 3E), while no significant difference was detected in 28-week mice (Fig. 3F). Besides, the cofilin 1 expressions were higher in 28-week HFD-fed group (IRS category=2.50±0.67 vs. 1.50±0.80, P=0.018) (Fig. 3G).

ILK, PARVB and CFL1 jointly participated in PCa progression and correlated with worse disease-free survival within a public database. The genetic alterations in *ILK*, *PARVB* and *CFL1* were evaluated in TCGA database from 499 PCa samples. In total, ~17% of PCa patients exhibited alterations (mainly in mRNA upregulation and amplification) in either *ILK*, *PARVB* or *CFL1* levels (Fig. 4A), with both mRNA and protein expression Z-score threshold ±2. Both *ILK-PARVB* and *PARVB-CFL1* gene pairs showed tendencies towards co-occurrence (Fig. 4B). We further discovered that upregulation of gene *ILK* and *PARVB*, as well as the upregulation and amplification of gene *CFL1* were all correlated with an increase in the corresponding mRNA (Fig. 4C). Besides, slight to moderate positive correlations were detected in three gene pairs (*ILK-PARVB*, Pearson 0.286, P<0.001; *ILK-CFL1*, Pearson 0.197, P<0.001; *PARVB-CFL1*, Pearson 0.433, P<0.00) (Fig. 4D).

In the analysis of DFS, a total of 499 PCa patients from TCGA provisional database were enrolled. Among them, 92 (18.4%) patients suffered disease recurrence and progression. DFS was compared between subgroups expressing higher vs. lower levels of mRNA (75th and 25th percentile as cutoff) (Fig. 5). In the whole cohort, a tendency towards worse DFS was observed in the patients with higher *CFL1* mRNA expression (Log-Rank P=0.083) (Fig. 5A). In subgroup analysis, higher mRNA expression in *CFL1* was correlated with worse DFS (Log-Rank P=0.048) (Fig. 5B) in patients with stage III and IV PCa. Meanwhile, in patients with Gleason score≥7, trends towards worse DFS were also detected in patients with higher mRNA expression of *PARVB* (Log-Rank P=0.095) (Fig. 5C) and CFL1 (Log-Rank P=0.064) (Fig. 5C).

Discussion

Increasing evidence indicated a positive association between obesity and PCa incidence and aggressiveness (15). Generally,

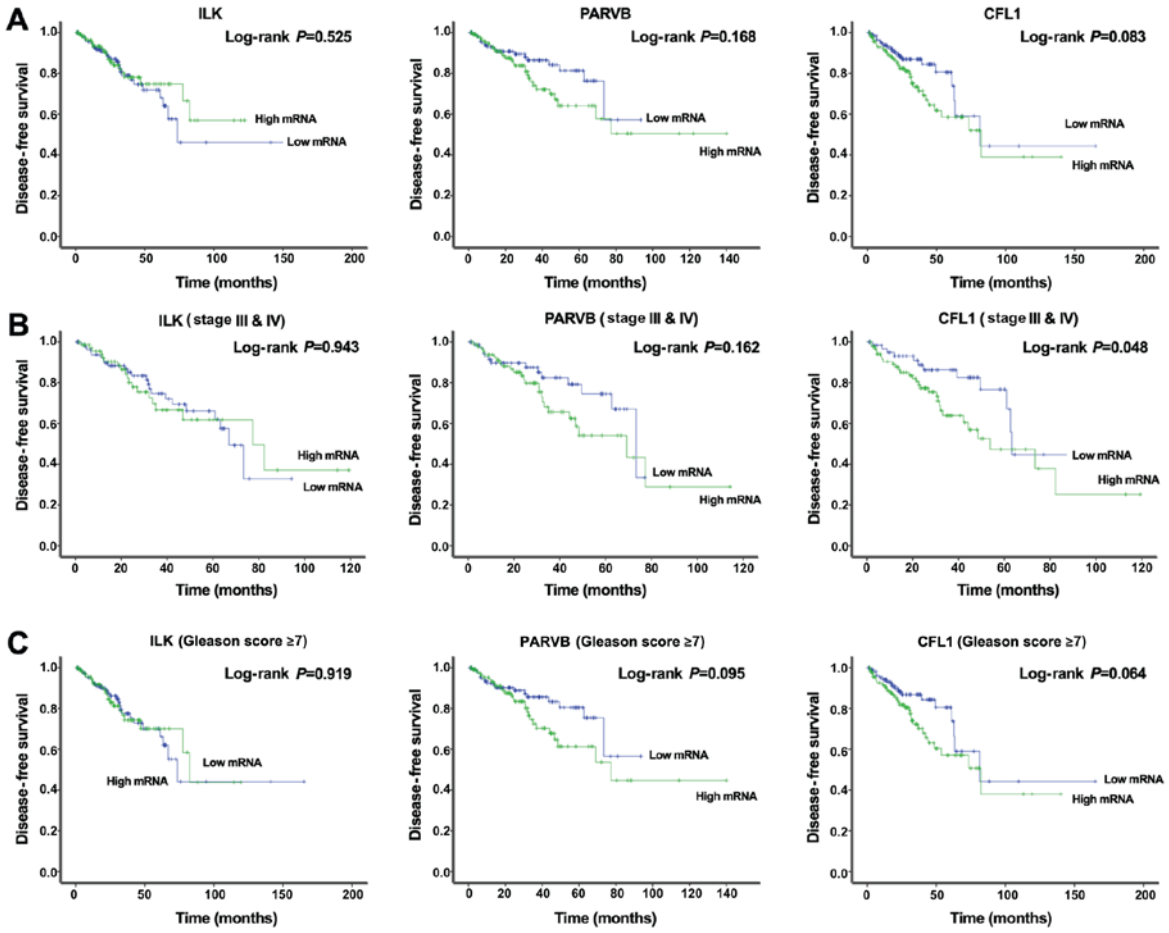

Figure 5. Effects of mRNA expression of *ILK*, *PARVB* and *CFL1* on the disease-free survival in TCGA prostate adenocarcinoma cohort. (A) Kaplan-Meier plots with log-rank test demonstrating the disease-free survival in patients expressing high vs. low mRNA levels of *ILK* (left panel), *PARVB* (middle panel) and *CFL1* (right panel). (B) Disease-free survival curves for stage III and stage IV cancer patients with high vs. low mRNA expression in *ILK* (left panel), *PARVB* (middle panel) and *CFL1* (right panel). (C) Disease-free survival curves for Gleason score ≥7 cancer patients with high vs. low mRNA expression in *ILK* (left panel), *PARVB* (middle panel) and *CFL1* (right panel). TCGA, The Cancer Genome Atlas.

the positive association was due to three major mechanisms, including decreased testosterone, adipokine alterations and insulin resistance (2,16). Recently, several studies reported the involvement of ILK, β-parvin and cofilin in patients with obesity or metabolic syndromes (7-9). Meanwhile, a pioneering research conducted by Shibue *et al* firstly identified that the activation of ILK/β-parvin/cofilin pathway was critical in the formation of FLPs and the progression of carcinomas (6). Therefore, the present study aimed to validate the expression of ILK, β-parvin and cofilin in PCa in TRAMP model, and to further explore the role of obesity induced protein expression alterations in the progression of PCa.

Dietary high fat was proved to induce obesity by increasing fat deposit in body, and in turn, the excessive fat would further affect cancer progression (17). Several studies were conducted in murine xenograft (18,19) and genetically engineered animal models (20), and discovered that HFD-fed would cause obesity and promote PCa development and progression. Among them, the application of genetically engineered mouse (GEM) caught great attention from researchers. Till now, four kinds of GEM model (TRAMP, LADY, Hi-Myc, Pten-null) were widely used (21). Among them, the TRAMP model was the first and also one of the most widely used model. The TRAMP model could successfully recapitulate all the parameters of PCa progression in human, including formation and progression from prostatic hyperplasia, intraepithelial neoplasia, adenocarcinoma to metastatic PCa (22). Besides, the great frequency and breadth of metastases was another merit of TRAMP in the research of cancer aggressiveness. The TRAMP model was specifically appreciated in the research of tumor microenvironment-cancer progression relationship, as neoplastic prostates were marked by stromal remodeling (reactive stroma) (22). Consistent with previous studies, the present study demonstrated that HFD feeding increased body weight and adipose tissue deposit in TRAMP mice, and HFD-fed mice possessed PCa with poorer differentiation, higher rates of extracapsular extension and metastasis, especially in 28-week group.

In the present study, ILK (encoded by gene *ILK*) was proved to be overexpressed in PCa, especially in poorly-differentiated PCa, which was consistent with previous outcomes in breast cancer, melanoma, colon cancer and PCa (23). Several *in vivo* studies showed the role of ILK in different aspects of cancer progression, including cell growth, EMT, migration and invasion (24). We also detected a slight increase of ILK expression in HFD-fed mice, though without statistical significance, and the outcomes warranted further investigation.

β-parvin (encoded by *PARVB*) linked with ILK and constituted IPP complex, which was known to facilitate FLPs formation, promote cell motility and extracellular matrix adhesion (6,25). Of note, the present study for the first time described the expression of β-parvin in PCa, and identified its overexpression in poorly-differentiated PCa. Moreover, we identified that HFD feeding could increase the expression of β-parvin in PCa. From TCGA database, we further discovered a trend towards worse DFS in PCa patients (Gleason score ≥7) with higher mRNA expression of *PARVB*. Previous studies reported conflicting outcomes, as *PARVB* was downregulated in breast cancer (26) and urothelial cell carcinoma (27), while overexpressed and correlated with tumor progression in colorectal cancer (12) and tongue squamous cell carcinoma (28), which implied an organ-specific expression or function of β-parvin.

Cofilin 1 (encoded by *CFL1*), an actin binding protein that played an key role in actin filament dynamics, functioned in cancer cell migration, invasion and mitosis (29). The present study found that cofilin 1 was overexpressed in moderately and poorly-differentiated PCa of TRAMP mice, which was in consistency with previous study (30). From TCGA database, higher mRNA expression of *CFL1* was considered a risk factor for worse DFS in patients with Stage III or IV PCa. In HFD-fed group, we detected an increase in cofilin 1 expression.

In TCGA prostate adenocarcinoma database, *ILK*, *PARVB* and *CFL1* all presented mainly with mRNA upregulation. A tendency towards co-occurrence was also identified in gene pairs of *ILK-PARVB* and *PARVB-CFL1*. These observations implied close relationships among these genes and the possible mechanisms contributed to carcinogenesis in their neighborhood network.

Several limitations existed in the present study. First, the key objects (ILK, β-parvin and cofilin 1) was only examined and evaluated by IHC, which required further western blotting and reverse transcription polymerase chain reaction study to confirm. Second, the alterations in the level of these proteins might result from both the diet and the progression of PCa. The discrimination of these two factors was difficult. Third, the TCGA database did not contain obesity, BMI, or diet habit as a clinical parameter, so we failed to apply these factors in the stratified analysis. Besides, the K-M survival analysis from TCGA database could not take elemental parameters (age, race) into account during analysis, therefore the associations between the subject proteins and patient survival were warranted by more cohort studies.

In conclusion, the present study demonstrated that HFD feeding contributed to higher expressions of β-parvin and cofilin 1 in PCa tissue and promoted cancer progression in TRAMP mice. Besieds, higher expressions of ILK, β-parvin and cofilin 1 were associated with poorer cancer differentiation. In TCGA database, higher mRNA levels of CFL1 were identified to be correlated with worse disease-free survival in certain subgroups. Further studies were warranted in discussing the potential roles of ILK, β-parvin and cofilin 1 in HFD feeding induced progression of PCa.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

MBH, JMH, TY, WHZ, YH and XBW performed the animal studies and histological staining. LRJ performed the pathological analysis. MBH performed the general statistical analysis and drafted the manuscript. HWJ and QD conceived the study and participated in its design and coordination. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was performed in strict accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH publication no. 8023, revised 1978). The protocol was approved by the Institutional Animal Care and Use Committee from Department of Laboratory Animal Science, Fudan University (approval no. 20160816A197).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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