Dietary palmitic acid promotes tumor growth and epithelial-mesenchymal transformation in prostate cancer

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Research

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Abstract

Background

High-fat diet (HFD) is a major risk factor for prostate cancer (PCA). Palmitic acid (PA) is the main constituent of HFD, and the main end product of fatty acid synthase (FASN). However, the effect of dietary intake of PA on the occurrence and development of PCA is rarely reported. This study aims to explore the effect of PA on PCA in mice.

Methods

Twelve nude mice were divided into two groups, one group was fed with a high-PA diet and the other group was fed with a normal control (NC) diet. Twelve c-Myc T mice were treated the same as the nude mice. The following analyses were performed: measuring tumor weight and size, H&E staining, immunohistochemical (IHC) staining and western blot assay detecting the expression of Ki67, E-cadherin, N-cadherin, TGF-β1, as well as searching and analyzing of the gene data from TCGA database.

Results

FASN is positively related to the malignancy of PCA. PA diet stimulated the progression of PCA, induced epithelial-mesenchymal transition (EMT), and activated transforming growth factor-β1 (TGF-β1) signal both in nude mice and c-Myc T mice. The expression levels of CD36 were positively associated with the expression of TGF-β1, and these two genes were related to poor prognosis of patients with PCA.

Conclusion

PA promotes PCA formation and activates TGF-β1 signal, inducing EMT in PCA. TGF-β1 is a potential target for the treatment of PCA.

Introduction

Prostate cancer (PCA) is the second most common carcinoma among men in the world[1], ranking seventh in the incidence and tenth in the mortality among male malignant tumors in China[2]. Diagnosis of PCA involves determining the presence of serum prostate-specific antigen (PSA), and locating tumors by ultrasound-guided biopsy. This can lead to problems like overdiagnosis and false negatives[3]. Despite robots have been used in prostatectomy, the curative effect of PCA need to be further improved[4]. In order to reduce the incidence of PCA and improve the therapeutic effect, it is imperative to further studying the pathogenesis of PCA.
High-fat diet (HFD) and blood lipid levels are considered important risk factors for PCA[5–7], suggesting a close relation between lipids and PCA progression. However, studies on the role of dietary lipids in cancer risk are not consistent[8]. For example, reports showed that omega-3 polyunsaturated fatty acids (PUFA) can inhibit tumor growth and metastasis[9, 10], while monounsaturated fatty acids, such as oleic acid, might increase cancer risk[11]. Short-term intake of dietary fatty acids could be balanced by regulating lipid metabolism in vivo[12]; however, the steady state may be disrupted by excessive intake of fatty acids or unhealthy lifestyle, leading to dyslipidemia, hyperglycemia, inflammatory reaction and so on[12]. Palmitic acid (PA) is the most common saturated fatty acid (SFA) in the diet and the main end product of fatty acid synthase (FASN). It has been recognized that FASN is positively correlated with the occurrence and development of various cancers[13–15]. However, the effects of dietary intake of PA on the progression of cancers (especially PCA) are rarely reported.

In recent years, metastasis has become the focus of most cancer research. The epithelial-mesenchymal transition (EMT) is a key procedure of tumor metastasis. In vitro studies, it has been proved that PA can increase the proliferation, migration and invasion of PCA cells[16]. Interestingly, CD36, as a receptor for long chain fatty acids, has been reported to promote tumor metastasis by inducing EMT[17, 18]. However, the effect of PA on PCA in vivo is still lack of studies and the mechanisms behind the PA-induced EMT in metastatic PCA have not been fully elucidated.

Therefore, it is crucial to explore the effect of PA on the development of PCA. In this study, we used nude mice with transplanted PCA tumor and c-MycT mice, and they were fed with a PA-rich diet or a normal diet to explore the effects of PA on PCA, aiming to establish a correlation between dietary PA and PCA, thus finding potential targets for PCA treatment.

**Materials And Methods**

**FASN gene expression analysis**

Gene expression data and clinical information from patients with PCA were obtained from The Cancer Genome Atlas (TCGA). Using the TCGA network, the differential expressions of FASN in PCA and normal prostate tissue were analyzed. The TCGA-PCA cohort consisted of 496 PCA tissue samples and 52 normal prostate tissue samples. The surgically resected PCA tissue and the non-tumor tissue with detailed clinicopathological parameters were collected from 243 patients who underwent surgical resection in the Affiliated Hospital of Jiangnan University from 2007 to 2018. For all patients, Gleason score (GS) were defined according to the GS systems revised in 1977 and published by the International Association of Urological Pathology (ISUP).

**Animals and design**

All mice were maintained in an SPF environment at 22 ± 1°C and relative humidity of 55% ± 10% with a 12 h light-dark cycle. Twelve BALB/C nude mice (male, 6-7 weeks old) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd and evenly divided into two groups, one fed with a normal
diet (NC, total energy 3.6 kcal g\(^{-1}\), 10% kcal fat) and the other a high PA diet (containing 5% PA) designed and purchased from Trophic (Nantong, Jiangsu, China). A total of \(5 \times 10^5\) LNCaP cells were subcutaneously injected at left and right armpit site of each mouse. Tumor diameter was measured three times a week, and the size of xenograft tumor was calculated by the following standard formula: length \(\times\) width\(^2\) \(\times\) 0.5. Mice were sacrificed by neck breaking 4 weeks after injection. After that, the livers and tumors were excised for hematoxylin and eosin staining (H&E staining) and immunohistochemistry.

The c-Myc\(^ T\) mice were obtained from the Departments of Medicine, Urology, Molecular and Medical Pharmacology, University of California, Los Angeles[19]. The mice over-express the human c-Myc gene in prostatic epithelium. The PCR primer sequence of the c-Myc\(^ T\) gene is 5’ AAACATGATGACTACCAAGCTTGGC 3’ and 5’ TCGAGGTCTAGTTCTGTTGGTGA 3’. During the 48 weeks period, the control group was fed with normal control feed (NC, total energy 3.6 kcal g\(^{-1}\), 10% kcal fat), while the other groups were fed with a high PA diet (containing 5% PA) designed and purchased from Trophic (Nantong, Jiangsu, China). The body weight of each mouse was measured every two weeks. Mice were sacrificed by neck breaking at the age of 48 weeks, and the anterior, dorsolateral, and ventral prostate (AP, DL and VP) lobes were dissected, photographed, and weighed.

**Western blot**

Total proteins were extracted from tumor tissue in RIPA lysis buffer containing protease inhibitor and phosphatase inhibitor. Proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with 5% milk then incubated with primary antibodies at 4°C overnight. The primary antibodies used in the experiment were as follows: TGF-\(\beta\)1 (1:1000; Novus), E-cadherin (1:1000; Cell Signaling Technology), N-cadherin (1:1000; Cell Signaling Technology), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:1000; Cell Signaling Technology), FASN (1:1000; Cell Signaling Technology), and vimentin (1:1000; Cell Signaling Technology). After washing with 1 × Tris Buffered Saline with Tween 20 (TBST) three times, the membranes were incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit or anti-mouse IgG (1:2000; Abcam). Finally, the membranes were visualized using Immobilon™ Western Chemiluminescent HRP Substrate (Millipore). The protein bands were quantified by ImageJ software.

**Immunohistochemistry (IHC)**

The paraffin blocks of prostate tissue of patients with PCA were cut into 4 \(\mu\)m slices. The slices were deparaffinized with xylene, dehydrated with ethanol, washed with distilled water, permeabilized in 0.3% Triton X-100 for 20 minutes, and then treated with 0.3% hydrogen peroxide for 30 minutes. Then, the slices were then immersed in boiling citrate buffer solution for 10 minutes in medium and low fire. After cooling, the slices were placed in hydrogen peroxide and blocking solution. Then they were incubated with the primary antibody at 4°C overnight and treated with goat anti-rabbit secondary antibody for 10 minutes. The primary antibodies used in the experiment were as follows: TGF-\(\beta\)1 (1:500; Novus), E-
cadherin (1:300; Cell Signaling Technology), N-cadherin (1:400; Cell Signaling Technology). Then, the expression of the target molecule was observed with diaminobenzidine (DAB), and the slides were stained with hematoxylin. After washing in running water, the slides were dehydrated with gradient alcohol and xylene. Finally, the neutral balsam was mounted on the glass slide with a cover glass. Keep the slides in a cool and dry place for one week, and then scanned them with a Pannoramic MIDI slide scanner. ImageJ software was used to measure the positive cell (cells stained with brown) rate.

**H&E staining**

After deparaffinization and dehydration (these procedures are the same with IHC staining), the slices were stained with hematoxylin for 20 seconds, washed with running water for 15 minutes, and then rapidly immersed in eosin staining solution, 95%, 70% and 80% ethanol for 50 seconds, and then soaked with 100% ethanol for 2 minutes. Next, the slices were incubated with xylene for 2 minutes twice in total. Finally, the neutral balsam was mounted on the glass slide with a cover glass. The slides were kept in the cool and dry place for one week, and then scanned with Pannoramic MIDI slide scanner. Image-Pro Plus 6.0 software was used to measure the area of adipose cells.

**Patient survival analysis**

Raw counts of RNA-sequencing data (level 3) and corresponding clinical information from PCA were obtained from TCGA database. For Kaplan-Meier curves, \( P \)-values and hazard ratios (HRs) with 95% confidence intervals (CIs) were generated by log-rank tests and univariate Cox proportional hazards regression. The Kaplan-Meier survival analysis with log-rank test was used to compare the survival differences between the two groups. For the expression distribution of TGF-\( \beta \)1 in PCA samples, the tumoral RNA-sequence data were downloaded from the Genomic Data Commons (GDC) data portal (TCGA).

**Statistical analysis**

All results were expressed as the means ± SD using GraphPad Prism 7.0 (La Jolla, California, CA). For two-group analysis, datasets were compared using unpaired two-tailed Student’s t-tests. For the analysis of three or more groups, one-way analysis of variance (ANOVA) was performed. Two-way ANOVA was used to analyze datasets with two independent factors. ImageJ software was used to analyze the results of western blot and IHC staining. The area of adipose cells was measured by Image-Pro Plus 6.0. \( P < 0.05 \) was considered to be statistically significant.

**Results**

**FASN is highly expressed in human PCA tissues and positively associated with Gleason score (GS)**

With data from the TCGA network, we found that FASN was up-regulated in PCA tissue (\( n = 496 \)) compared with non-tumor tissue (\( n = 52 \)) \( (P < 0.0001) \) (Figure 1A). The result of IHC staining was consistent with the above finding, as FASN expression was higher in PCA tissue (\( n = 176 \)) compared with
the non-tumor prostate tissue (n = 67) \((P < 0.001)\) (Figure 1B). Gleason score (GS) is one of the methods for PCA diagnosis. The increase of GS is related to the improvement of invasiveness and survival rate in PCA cells[20]. Therefore, to understand the relationship between FASN expression and malignant progression of PCA, the FASN-expression level and GS of each PCA specimens were analyzed. IHC staining showed that high FASN expression levels were associated with a high grade of GS; specifically, the expression levels of FASN in PCA specimens with a GS of 8 or 9 were significantly higher than that with a GS of 7 \((P < 0.05)\) (Figure 1C). These data indicate that FASN expression is positively associated with PCA progression.

**PA diet promotes tumor growth in a PCA xenograft model**

Because HFD is the key risk factor of PCA[5, 21] and PA is the main end product of FASN, this study discussed whether PA is responsible for PCA progression. After the construction of the PCA xenograft model, we provided a normal for the normal-control (NC) group and a PA diet for the PA group. In the first three weeks, the size of tumor in PA group was not significantly different from the NC group. However, three weeks later, tumors grew dramatically faster in the PA group (Figure 2A). In NC group, the tumor cells were complete, arranged orderly and sparsely, while the tumor in PA group has obvious pathological changes, such as disordered and close arrangement of tumor cells (Figure 2B). Both the size and weight of the xenografted tumors were greater in the PA group than in the NC group, which included 1 mouse that did not develop tumors \((P < 0.05)\) (Figure 2C-D). Therefore, PA can promote PCA cells to form tumors in nude mice.

**PA accelerates PCA progression in c-Myc T mice**

To determine the effect of PA on the progression of primary PCA, mice with stable over-expression of the c-Myc gene were selected for the study and evenly divided into PA group and NC group. The c-Myc gene is a recognized oncogene exert a significant effect in driving the development of PCA[22, 23]. After 48 weeks, the average weight of the mice in the PA group was significantly higher than that of NC group \((P < 0.05)\) (Figure 3A). It should be noted that the relative weight of the prostate (expressed as mg/25g body weight) in PA group was significantly higher than that of NC group \((P < 0.01)\) (Figure 3B). Prostate lobes from PA-fed mice were larger than that from NC group (Figure 3C). Notably, anterior prostate lobes (AP) and dorsolateral prostate lobes (DL) grew larger than the ventral prostate lobes (VP) in the PA group (Figure 3C). H&E staining of liver, brown adipose tissue (BAT), beige adipose tissue (MAT), and white adipose tissue (WAT) showed increased lipid deposition in mice fed with PA (Figure 3D). In NC group, the structure of hepatocytes was complete, arranged orderly, and the cell size was uniform (Figure 3D). However, the liver in PA group has obvious pathological changes (serious damage), such as disordered arrangement of hepatocytes and irregular lipid droplet vacuoles. These changes suggested that there was PA-induced lipid rearrangement. IHC analysis showed that the Ki67 staining in prostate tissue of mice fed with a PA diet was about 20% higher than that of mice fed with a normal diet. (Figure 3E). Therefore, these results suggested that a PA diet can fuel PCA progression in c-Myc T mice.
PA promotes tumor metastasis by inducing EMT

To determine the metastasis-promoting ability of PA during PCA development, the role of PA on EMT was explored by detecting the expression of N-cadherin and E-cadherin in nude mice and c-Myc\textsuperscript{T} mice. IHC analysis demonstrated that transplanted tumors of mice in the PA group had higher expression levels of N-cadherin ($P < 0.001$) and lower expression levels of E-cadherin compared with that in the NC group ($P < 0.001$) (Figure 4A). Consistent with the results of IHC, the protein expression levels of E-cadherin decreased in the PA group, while the protein expression levels of vimentin and N-cadherin increased in the PA group ($P < 0.05$) (Figure 4B). In c-Myc\textsuperscript{T} mice, the expression levels of N-cadherin were significantly higher in prostate tissue derived from PA-fed mice ($P < 0.0001$), while the expression levels of E-cadherin were noticeably lower compared with the NC group ($P < 0.0001$) (Figure 4C). Therefore, these data imply that PA exerts its effect on inducing EMT in PCA.

PA upregulates TGF-$\beta_1$ expression

As TGF-$\beta_1$ is a prominent inducer of EMT\cite{24}, we assessed its expression levels in tumors from nude mice and prostates from c-Myc\textsuperscript{T} mice. Our results showed that nude mice fed with PA had a significant increase in TGF-$\beta_1$ protein levels, which were detected by IHC staining ($P < 0.05$) (Figure 5A) and western blot ($P < 0.01$) (Figure 5B). TGF-$\beta_1$ staining in prostate tissue of c-Myc\textsuperscript{T} mice fed with PA was more intense compared with that in the NC group ($P < 0.05$) (Figure 5C).

CD36 is positively associated with TGF-$\beta_1$ expression in PCA.

CD36, a receptor for fatty acids\cite{25, 26}, is an initiating factor for metastasis\cite{17, 27}. The survival curves in Figure 6A show that patients with high expression levels of TGF-$\beta_1$ have a significantly lower survival rate compared with those who showed low expression of TGF-$\beta_1$ ($P = 0.001$), suggesting that TGF-$\beta_1$ is associated with poor prognosis in PCA. Moreover, high expression levels of CD36 are accompanied by high expression of TGF-$\beta_1$ ($P < 0.001$) (Figure 6B), implying that CD36 is positively related to TGF-$\beta_1$ signaling. The analysis of univariate and multivariate Cox regression showed that CD36, TGF-$\beta_1$ and TGF-$\beta$R1 were risk factors for PCA (HR > 1) (Figure 6C). TGF-$\beta_1$ was also an independent prognostic factor to assess patient outcomes (Figure 6C).

Discussion

PCA is a major disease threatening men’s health. In recent years, HFD has become an important risk factor for many cancers, especially PCA\cite{5, 28, 29}. A case-control study of 1,300 men under 60 years old in Britain showed that men with higher fat intake had a higher risk of PCA\cite{30}. Moreover, epidemiological studies have shown that high intake of SFA and dairy products are easy to increase the risk of PCA\cite{31}. Our results indicated that FASN was positively related to the malignant progression of PCA. Rich intake of PA in diet can promote the development of PCA, induce EMT and activate TGF-$\beta_1$ signal. Moreover, our results suggest that CD36 is positively related to TGF-$\beta_1$, and both are risk factors for PCA.
Gleason score is firmly established as prognostic indicators in PCA[32]. The higher the score, the worse the prognosis of PCA. FASN is a key enzyme in de novo fatty-acid synthesis and is involved in the process of the initial phases of prostate tumorigenesis and cancer metastasis[33-35], which is consistent with our study. We identified that FASN expression is positively correlated with Gleason scores. These suggest that endogenous synthetic SFA is associated with PCA development.

In previous studies, a HFD can promote cancer progression[5, 28, 29], but it remains unclear whether exogenous SFAs are responsible for this effect. HFD has been proved to increase cell proliferation and promotes tumorigenesis via metabolic alterations[5]. Our study focused on the effect of PA, one of the main components of lipids, on PCA development. Earlier articles indicated that PA could increase invasiveness of cancer cells[17, 36, 37]. Our research showed that PA induced tumor growth in a PCA xenotransplantation model and accelerated PCA progression in c-Myc<sup>T</sup> mice. Notably, the increase of PA intake has been directly associated with aberrant lipid accumulation in c-Myc<sup>T</sup> mice. Overexpression of c-Myc can lead to an increase of SFA uptake in PCA model mice[5], and our results confirm that overexpression of c-Myc can promote PCA progression via increasing PA intake.

Interestingly, our results showed increased lipid accumulation in c-Myc<sup>T</sup> mice of PA group. Given that HFD may induce tumor metastasis through increased lipid accumulation[38], we proposed that PA promotes tumor metastasis by inducing lipid rearrangement in c-Myc<sup>T</sup> mice. Earlier studies showing that the CD36 mediates PA-initiated metastasis in gastric cancer[39], our finding provides significant evidence that a PA-rich diet can activate EMT, which is characterized by up-regulation of mesenchymal markers such as N-cadherin and vimentin, down-regulation of epithelial markers, such as E-cadherin. Notably, CD36 is positively related to TGF-β<sub>1</sub> expression, and these two genes are associated with poor prognosis of PCA. Therefore, we hypothesize that PA may promote PCA metastasis by combining with the CD36 receptor to induce EMT process via activating TGF-β signal, which needs further research.

Exogenous SFA was shown to enhance tumor growth in our study, but we did not exclude the effects of endogenous SFA, which may require further investigation. Moreover, TGF-β<sub>1</sub> was up-regulated in mice fed a diet rich in PA. However, the direct effect of PA on the TGF-β signaling pathway was not clear and may vary with cell types. Thus, the exact mechanism of PA-induced EMT in PCA remains undefined. Anyway, our study elucidated a possible target for the prevention and treatment of metastatic PCA, that was by inhibiting TGF-β<sub>1</sub> signaling.

**Conclusions**

Our study showed that FASN expression was positively correlated with the progression of PCA and that exogenous PA could activate TGF-β<sub>1</sub> and enhance EMT in PCA progression. Furthermore, CD36 and TGF-β<sub>1</sub> were positively associated with poor prognosis of PCA. The interaction between endogenous SFA and exogenous SFA on the occurrence and development of PCA, and the specific mechanism of PA-induced EMT can be further explored. Our research suggests that reducing PA intake in daily diet or targeting TGF-β<sub>1</sub> can help prevent or treat PCA.
Abbreviations

HFD: High-fat diet; PCA: Prostate cancer; PA: Palmitic acid; FASN: Fatty acid synthase; NC: Normal control; EMT: Epithelial-mesenchymal transformation; IHC: Immunohistochemical; TGF-β: Transforming growth factor-β; H&E: Hematoxylin-eosin; PSA: Prostate-specific antigen; PUFA: Polyunsaturated fatty acid; SFA: Saturated fatty acid; TCGA: The Cancer Genome Atlas; GS: Gleason score; ISUP: International Association of Urological Pathology; SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis; PVDF: polyvinylidene fluoride; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; TBST: Tris Buffered Saline with Tween; HRP: Horseradish peroxidase; DAB: Diaminobenzidine; HR: Hazard ratios; CI: confidence intervals; GDC: Genomic Data Commons; SD: Standard Deviation; ANOVA: Analysis of variance; AP: Anterior prostate lobes; DL: Dorsolateral prostate lobes; VP: Ventral prostate lobes; BAT: Brown adipose tissue; MAT: Beige adipose tissue; WAT: white adipose tissue.

Declarations

Ethics approval and consent to participate

All animal procedures were performed accordance with the Guidelines for Care and Use of Laboratory Animals of Research Institute of Schistosomiasis Control in Jiangsu Province. All the animal experiments were approved by Laboratory Animal Management and Animal Welfare Ethics Committee of Jiangnan University. (JN. No20180915b04011020 [176]). Written informed consent was obtained from all participants before data collection.

Consent for publication

Not applicable.

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.

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Authors’ contributions
Conceptualization: Fengjiao Huang; methodology: Fengjiao Huang; software: Bingqian Sun; validation: Xiaoying Wang, Bingqian Sun and Xiao Jian; formal analysis: Fengjiao Huang; resources: Xiaoying Wang; data curation: Qindan Du; writing-original draft preparation: Fengjiao Huang; writing-review and editing: Fengjiao Huang; visualization: Jiayao Chen; supervision: Xiaoying Wang and Yong Q. Chen; project administration: Xiaoying Wang; funding acquisition: Yong Q. Chen and Xiaoying Wang. All authors have read and agreed to the published version of the manuscript.

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Figures
Figure 1

FASN expression is positively associated with the development of PCA. (A) TCGA database retrievals showing the relationship between FASN expression and PCA. (B) IHC staining showing FASN expression in human PCA and normal prostate tissue. Original magnifications are 20×. (C) FASN expression associated with Gleason score in PCA tumors. Original magnifications are 20×. (*, P < 0.05; **, P < 0.01; ****, P < 0.0001)
Figure 2

PA diet aggravates tumor development in a PCA xenograft model. (A) The tumor growth in PA group and normal-control (NC) group were monitored for 4 weeks. (B) H&E staining of tumor form nude mice. Original magnifications are 20×. (C-D) Photo-graphs of tumors and Relative tumor weights of mice in PA group and NC group. (*, P < 0.05)
Figure 3

PA promotes PCA progression in c-MycT mice. (A) Final body weights of PA group and NC group. (B) Relative prostate weight (expressed as mg/25g body weight) in PA group and NC group. (C) Photographs of anterior, dorsolateral, and ventral prostate (AP, DL, and VP) lobes after excision. (D) H&E staining of liver, brown adipose tissue (BAT), beige adipose tissue (MAT), and white adipose tissue (WAT) from c-MycT mice fed with either a PA-rich feed or normal feed. Original magnifications are 20× in liver tissue.
and 40× in adipose tissue. (E) IHC staining of Ki67 in the prostate tissue from mice fed a normal or PA diet. Original magnifications are 20×. (*, P < 0.05; **, P < 0.01; ***, P < 0.001, ****, P < 0.0001)

Figure 4

PA promotes EMT in PCA models. (A) IHC analysis of E-cadherin and N-cadherin in xenograft tumors from nude mice. Original magnifications are 5× and 20×. (B) The relative protein expression of vimentin, E-cadherin and N-cadherin in xenograft tumors from mice fed a normal or a PA diet. GAPDH served as an internal control. (C) IHC analysis of E-cadherin and N-cadherin in prostate tissue from c-MycT mice. Original magnifications are 20×. (*, P < 0.05; ***, P < 0.001, ****, P < 0.0001)
PA upregulated TGF-β1 expression. (A) IHC analysis of TGF-β1 in xenograft tumors from nude mice. Original magnifications are 5× and 20×. (B) The expression level of TGF-β1 in xenograft tumors from PA or NC group. GAPDH served as an internal control. (C) IHC analysis of TGF-β1 in prostate tissues of c-MycT mice. Original magnifications are 20×. (*, P < 0.05; **, P < 0.01)
Figure 6

CD36 is associated with TGF-β1 expression and PCA progression. (A) Kaplan Meier survival analysis for progression-free survival (PFS) of patients with PCA, stratified by TGF-β1 level. (B) The expression distribution of TGF-β1 in high-CD36-expressing and low-CD36-expressing PCA tissues. (C) Hazard ratio and P-value of genes involved in univariate and multivariate Cox regression. (***, P < 0.001)