Induction of neuroendocrine differentiation in castration resistant prostate cancer cells by adipocyte differentiation-related protein (ADRP) delivered by exosomes

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ABSTRACT

Although overall mortality rate of prostate cancer (PCa) declines in recent years, castration-resistant prostate cancer (CRPC) remains incurable. Clinical evidence indicates that CRPC recurred from hormonal therapy exhibits neuroendocrine differentiated (NED) phenotypes, which could contribute to therapeutic resistance and poor survival. Understanding the onset of NED could lead us to develop new therapeutic strategies for CRPC. Although PCa is known as a lipid-enriched tumor, its role in CRPC development is not fully understood. In this study, we demonstrated that IL-6 or androgen deprivation therapy (ADT)-induced lipid accumulation is associated with NED phenotypes. IL-6 or ADT can induce NED in PCa cells via peroxisome proliferator-activated receptor γ (PPARγ, a major lipogenic transcription factor) and adipocyte differentiation-related protein (ADRP, a major component of adiposome). In addition, ADRP protein can be detected in exosomes released from these cells and these exosomes are capable of inducing NED of PCa cells in a paracrine fashion. Understanding the role of PPARγ/ADRP in NED could provide new target(s) for CRPC therapy.

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Introduction

Prostate cancer (PCa) is the most common cancer among men in the United States [1]. The detection of early disease with local therapies has certainly improved the overall survival of PCa patients; however, the majority of mortality is caused by metastatic CRPC [2]. Current treatments such as androgen deprivation therapy (ADT) or chemotherapy for these patients are still unsatisfactory. Obviously, characterizing cell features of CRPC could provide new strategies of eradicating this disease. Recent clinical findings of CRPC recurred from ADT [3,4] indicate that some carcinoma cells exhibit neuroendocrine phenotypes with neuronal markers expression and neuronal factors secretion in an endocrine fashion [5], which is considered as neuroendocrine differentiation NED in PCa. NED can be classified into two categories in PCa: small cell carcinoma of prostate with neuroendocrine phenotypes (SCCP, accounts for 1% of the prostatic malignancies) and the recurrent carcinoma displaying NE features [6]. In advanced PCa, NED is frequently associated with highly proliferative area of tumor mass [7]: NED is known to increase chemo- or radio-resistance in PCa cells [8,9]. Thus, understanding the onset of NED can certainly lead us to develop new therapeutic strategies for CRPC.

In CRPC, the mechanisms leading to NED is still poorly understood. Nevertheless, some factors such as cytokines (IL-1β, IL-6, IL-8), neuromediators (such as calcitonin, serotonin) and cAMP are able to induce NED [6]. Elevated serum IL-6 levels are...
associated with CRPC [10–12] and its receptor is also highly expressed in PCa tissues [13]. Although IL-6-induced NED is thought to be clinically relevant, the underlying mechanism is not clearly defined.

In PCa, elevated lipogenic enzymes, such as ATP citrate lyase (ACLY), fatty acid synthase (FASN) and stearoyl CoA desaturase 1 (SCD1), have been observed in clinical specimens [14]. Lipogenesis is mediated by a series of enzymes in converting Acetyl-CoA to fatty acids. These fatty acids are then stored in the form of lipids such as triacylglycerols (TGs). It is believed that the main energy source in growing tumor cell is de novo lipogenesis [15]. Indeed, these enzymes are able to promote the growth of PCa cells by either inhibiting apoptosis or promoting cell cycle [16–18]. In addition to lipogenic enzymes, adiposomes (also called lipid droplets or lipid bodies; the storage organelle for TGs) are commonly found in several cancers [19]. For example, colon and gastrointestinal cancer tissues with enlarged size and increased number of adiposomes [20,21], however little is known in PCa.

 Peroxisomal proliferator-activated receptor γ (PPARγ) is a master regulator for lipogenesis. To date, accumulating clinical evidence suggests PPARγ can function as a tumor promoter. PPARγ protein level is found significantly higher in advanced PCa than localized PCa or benign prostate hyperplasia [22,23]. Higher protein expression of PPARγ is also associated with shorter patient survival [24]. Recently, Ahmad and his colleagues demonstrated that PPARγ is critical for PCa metastases in Pten-null mice [25]. However, the relationship between adipose accumulation and NED in CRPC cells remains unclear. In this study, we presented evidence that IL-6-elicited NED is mediated by PPARγ leading to elevated adipocyte differentiation-related protein (ADRP) associated with adipose accumulation. Noticeably, ADRP can be released into exosome from PCa cells and induce NED of adjacent cells in a paracrine fashion. Taken together, this study provides new therapeutic targets in NED CRPC.

### Material and methods

**Cell culture, chemicals, and treatment condition**

Human prostate cancer cell lines, DU145 and LNCaP, were purchased from American Type Culture Collection (Manassas, VA). C4-2, a subline derived from DU145, was obtained from Dr. Chung, Cifer-Sina Medical Center [26]. C4-2B and C4-2B MDVR (maintained with 20 μM Enzalutamide) were kindly provided by Dr. Gao, UC-Davis [27]. All these cells were grown in RPMI-1640 medium (Sigma Aldrich, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin. RWPE-1, a normal human prostate epithelial cell line immortalized by human papillomavirus 18, was obtained from ATCC and maintained in Keratinocyte-SFM medium (Waltham, MA) supplemented with 10% FBS and penicillin/streptomycin. Cells were incubated in a humidified 5% CO2 incubator at 37 °C. Experiments were performed when cell confluence reached 80%. Enzalutamide was purchased from Selleck Chemicals (Houston, TX, USA).

Cells were seeded onto either 6-well (5 × 104) or 10-cm plate (1 × 105) for 24 h prior to treatment. For IL-6 (Bioviosion, Milipitas, CA) treatment, cells were starved for another 24 h then incubated with IL-6 (in RPMI medium containing 5% FBS). For Enzalutamide treatment, different concentrations of Enzalutamide were added into medium containing 10% FBS. Medium was changed every two days.

**Cell proliferation**

Briefly, cells were seeded in a 96-well plate (4 × 103 cells/well) 24 h before drug treatment. Total cell number was assessed using a 3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyltetrazolium bromide (MTT) assay [Roche, Indianapolis, IN] according to the manufacturer’s instructions.

**Oil red O staining**

Lipid accumulation was measured by Oil red O staining. The working solution of Oil red O was prepared as described with modification [28]. Cells (1 × 105) were washed with PBS and fixed with 4% (v/v) paraformaldehyde solution for 1 h at 4 °C, then stained with Oil red O working solution for 15 min at room temperature. After PBS washing, Oil red O was extracted from adipose using 150 μL isopropanol and subjected to OD510 nm measurement.

**Western blot**

Total proteins were extracted from cells by using ice-cold lysis buffer [150 mmol/L NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 mmol/L Tris (pH 8.0), protease inhibitor cocktail [Roche]]. The extracted proteins were subjected to SDS-PAGE and immunoblotted with antibodies against PPARγ, ADRF, Chromogranin A (CgA), Neuron-specific enolase (NSE), synaptophysin (SYP) and GAPDH (Santa Cruz Biotechnology, Dallas, TX, Actin (Sigma). Appropriate secondary antibodies conjugated with horseradish peroxidase and signals were detected by enhanced Chemiluminescent (ECL) substrate kit (Thermo Fisher Scientific, Waltham, MA).

**Real-time reverse-transcription polymerase chain reaction (qRT-PCR)**

Total RNA was extracted with Maxwell System (Promega Corporation, Madison, WI). RNA (1 μg) was reverse transcribed with iScript DNA synthesis kit (Bio-Rad, Hercules, CA). qRT-PCR analysis was carried out with the SYBR Green Supermix kit (Life Technologies) in MyIQ thermal cycler (Bio-Rad). The relative level of target mRNA was determined by normalizing 18S rRNA. All experiments were performed in triplicates.

**Plasmid transfection**

Small hairpin RNA (shRNA) constructs against human PPARγ or ADRP were purchased from the National Core Facility, Academia Sinica, Taiwan. Target sequence for PPARγ is CAGCATTTTTCCTCCCATTA or ATGAGTCCACGACATT, and for ADRP is CTTCTAGAGCAGCTGTT or GCACCATGCTAGCATT. PPARγ expression plasmid was a kind gift from Dr. Howard Li, University of Colorado [29]. ADRP expression plasmid was purchased from Sino Biological Inc. (Beijing, China). Briefly, cells were transiently transfected with plasmids or empty vector by using Xfect transfection reagent (Takara Bio USA, Mountain View, CA) according to manufacturer’s instruction.

**Exosome purification and characterization**

Cells were grown in medium containing exosomes-depleted FBS (SBL, Palo Alto, CA) and seeded in 10-cm plates then treated with IL-6 or Enzalutamide for 4 days. Exosomes were isolated from the cell culture supernatant by using exoEasy kit (Qiagen, Valencia, CA) according to manufacturer’s instruction. Isolated exosomes were then characterized by Zetaview (Particle Matrix, Mebane, NC) and determined their number by using Exoquant quantitation kit (SBI). The purified exosomes were further concentrated by using ultrafiltration tube of 100,000 MWCO (Spin-XR UF 500, Corning, Corning, NY), recovered in PBS and ready for cell treatment.

**Statistical analysis**

Student two-tailed t-test was used for the determination of statistical relevance between groups, and < 0.05 was considered statistically significant. All statistical analyses were performed with GraphPad Prism software.

### Results

**IL-6 increases adipose accumulation and NED in PCa cells**

Increased serum level of IL-6 has been associated with PCa patients and IL-6 is known as a potent inducer of NED of PCa cells [30,31]. On the other hand, PCa is characterized to have high lipid accumulation and elevated lipogenesis enzymes have shown to promote PCa cell growth [16–18]. Some evidence also indicates that IL-6 can induce lipogenesis enzymes in tissues other than prostate. However, the relationship between NED and lipogenesis induced by IL-6 in PCa cells is largely unknown. As shown in Fig. 1A and B, CRPC cells such as DU145 and C4-2 exhibited higher accumulation of adiposomes (lipid droplets) with Oil Red O staining than control after IL-6 treatment. Meanwhile, significantly increased length of dendrite-like extension, a typical phenotype of neuroendocrine cells, was also induced in both cell lines after IL-6 treatment (Fig. 1A, C). Noticeably, dendrite-like extension is accumulated with adiposomes (Fig. S1B) suggesting adipose may be critical for dendrite-like extension. In contrast, LNCaP (an androgen-responsive PCa) and RWPE-1 (an immortalized normal prostate epithelium) did not show any accumulation of adipose as well as dendrite-like extension (Fig. 1A–C). Particularly, RWPE-1 exhibits a typical epithelial morphology; no dendrite-like extension can be detected before or after IL-6 treatment (Fig. 1A).
Fig. 1. Determination of IL-6-induced NED and lipid accumulation in DU145 and C4-2 cells. Cells (5 × 10^3) were seeded onto 6-well plates for 24 h for attachment. After another 24 h of serum starvation, cells were treated with or without IL-6 (50 ng/ml in 5% FBS RPMI medium) for 4 days. At the end of incubation time, cells were fixed with 4% paraformaldehyde for 1 h at 4 °C and stained with Oil red O. (A) The degree of lipid accumulation and dendrite-like extension were observed and photographed under phase contrast microscope. (B) Lipids were quantified as described in “Materials and Methods” Section. (C) Dendrite-like extension was quantified by using Image J software. Data represent mean ± S.D. in triplicates from each group. *Significant difference compared with control group (p < 0.05).

Fig. 2. Determination of IL-6-induced PPARγ, ADRP and CgA expression in DU145 and C4-2 cells. (A) Cells (5 × 10^4) were seeded onto 6-cm dishes for 24 h for attachment. After another 24 h of serum starvation, cells were treated with or without IL-6 (indicated doses in RPMI medium supplemented with 5% FBS) for 2 days (DU145) or 1 day (C4-2). (B) Cells were treated with or without IL-6 (50 ng/ml in 5% FBS RPMI medium) and the expression levels of PPARγ, ADRP and CgA protein were determined by Western blot. Actin was used as the loading control.
Fig. 3. The role of PPARγ and ADRP in NED of DU145 cells. (A) PPARγ was knocked down in DU145 and C4-2 cells. Forty-eight hours after transfection, the expression levels of PPARγ, ADRP, and CgA protein were determined by Western blot. GAPDH was used as the loading control. (B) ADRP was knocked down in DU145 cells. Cell morphology was observed under phase contrast microscope and lipid accumulation was determined and quantified. (C) ADRP-knockdown DU145 cells were treated with IL-6 (50 ng/ml in 5% FBS RPMI medium) for 4 days and lipid accumulation and dendrite-like extension were determined. The expression levels of ADRP or CgA protein were determined by Western blot. GAPDH was used as the loading control. (D) PPARγ and ADRP were overexpressed in RWPE-1 cells. The expression levels of PPARγ, ADRP, CgA and NSE protein were determined by Western blot. GAPDH was used as the loading control. Lipid accumulation and dendrite-like extension were determined. Data represent mean ± S.D. in triplicates from each group. *Significant difference compared with control group (p < 0.05).
We further investigated the relationship between lipid accumulation and NED biochemically. As shown in Fig. 2A and B, the expression levels of PPARγ (a master lipogenesis regulator), ADRP (a major key component in adiposome) and Chromogranin A (CgA, a well-known NED marker) were significantly increased by IL-6 in a dose- and time-dependent manner. Also, IL-6 increased neuron specific enolase (NSE, another NED marker) in DU145 and C4-2 cells (Fig. S1C). Since, Synaptophysin (SYP) is barely detected in both cells, IL-6 effect is less significant on the expression of this gene (Fig. S1C).

IL-6-induced NED depends on PPARγ and ADRP

To unveil the regulatory network underlying IL-6-induced adiposome accumulation and NED, we knocked down PPARγ and ADRP mRNA expression in DU145 and C4-2 cells using gene specific shRNA. It appears that PPARγ is critical for regulating ADRP levels in adiposomes; knocking down PPARγ in DU145 and C4-2 cells resulted in a significant reduction of ADRP and CgA protein levels (Fig. 3A), implying that lipogenesis may contribute to NED in PCa cells. Furthermore, knocking down ADRP in DU145 cells (Fig. 3B, Left panel) caused the morphologic changes from spindle-like to cuboidal-like epithelium (Fig. 3B, Middle panel); these cells became more adherent to each other. Similarly, lipid accumulation in ADRP knockdown cells was significant lower than the control cells (Fig. 3B, right panel). As expected, in ADRP knockdown cells by two different shRNA constructs, IL-6 treatment failed to induce lipid accumulation and dendrite-like extension (Fig. 3C and S2).

The presence of ADRP in exosomes induces NED in adjacent cells

It is well documented that NED in PCa cells can be induced by extracellular secretory factors via their membrane receptors-activating signal transduction [6]. However, it is less known whether NED can be induced by intracellular factors. Thus, we examined whether IL-6-induced PPARγ, ADRP or CgA can be delivered to adjacent cells via exosomes. As shown in Fig. 4A, IL-6 had no impact on the cell growth of DU145 and C4-2 and did not change the size and number of exosomes isolated from both cells.
Fig. 6. The role of exosomes released from C4-2B MDVR cells in a paracrine induction of NED. (A) Exosomes were isolated from C4-2B MDVR cells with or without Enzalutamide treatment for 2 or 4 days. The number of isolated exosomes was determined by using Exocet kit. Cell number after Enzalutamide treatment was determined by Trypan blue exclusion assay. (B) The expression levels of PPARγ, ADRP, CgA protein from exosomes or cell lysate were determined by Western blot. (C) Exosomes were added into the culture medium of C4-2B MDVR cells for 4 days. Dendrite-like extension was determined and quantified. ADRP protein level was determined 3 h after exogenous exosome treatment. Data represent mean ± S.D. in triplicates from each group. *Significant difference compared with control group (p < 0.05).

Fig. 5. The role of ADRP in NED of C4-2B MDVR cells. (A) C4-2B MDVR cells were treated with or without Enzalutamide for 2 and 4 days and the expression levels of PPARγ, ADRP and CgA protein were determined by Western blot. GAPDH was used as the loading control. (B, C) C4-2B MDVR cells were treated with 80 μM Enzalutamide for 4 days and lipid accumulation and dendrite-like extension were determined. (D) ADRP was knocked-down in C4-2B MDVR cells. Cell morphology was observed under phase contrast microscope. (E) ADRP knockdown C4-2B MDVR cells were further treated with 80 μM Enzalutamide for 4 days and the expression levels of CgA protein were determined by Western blot. Lipid accumulation and dendrite-like extension were also determined. GAPDH was used as the loading control. Data represent mean ± S.D. in triplicates from each group. *Significant difference compared with control group (p < 0.05). #Significant difference compared with IL-6-treated shCON group (p < 0.05).
with or without IL-6 treatment for 4 days. Interestingly, only ADRP protein was detected in exosomes from IL-6–treated cells (Fig. 4B). To determine the effect of these exosomes on inducing NED in adjacent cells, exosomes were added to DU145 or C4-2 cells under serum-starvation for 24 h and the result clearly demonstrated that PKH26-labeled exosomes were able to enter cells (Fig. 4C). Consistently, the result from immunofluorescent staining of ADRP (Fig. 4D) indicated that more ADRP-positive cells were detected in cells incubated with exosome from IL-6 treatment than the control. Indeed, these exosomes derived from IL-6-treated cells contained ADRP protein and were able to induce dendrite-like extension in PCa cells and normal cell such as RWPE-1 (Fig. 4E and F). Taken together, these data indicate NED of PCa can be induced by delivery of ADRP in a paracrine manner via exosome.

AR antagonist induces NED of CRPC via PPARγ-mediated adiposome accumulation

Recent clinical observation indicates that NED is associated with CRPC recursed from second-line of ADT. Therefore, we hypothesize that AR antagonist is able to increase PPARγ-mediated adiposome accumulation leading to NED of CRPC cells by using an Enzalutamide-resistant cell line (C4-2B MDVR) [27]. We found that both 40 μM and 80 μM Enzalutamide significantly inhibited the cell growth of C4-2B cells but less effect on C4-2B MDVR cells after 48 h incubation. Under the same condition, Enzalutamide could induce the levels of PPARγ, ADRP and CgA protein expression in a dose-dependent manner in resistant cells (Fig. 5B). As expected, both adiposome and dendrite-like extension in C4-2B MDVR cells were increased by Enzalutamide in a dose-dependent manner (Fig. 5C and D). By knocking down ADRP in C4-2B MDVR cells, it appeared that cell–cell adhesion was enhanced (Fig. 5E). Also, Enzalutamide-induced CgA expression was inhibited in ADRP knockdown cells treated with Enzalutamide (Fig. 5F). Similarly, Enzalutamide-induced lipid accumulation and dendrite-like extension were also suppressed (Fig. 5F). The results suggest that Enzalutamide can induce NED via PPARγ and ADRP in C4-2B MDVR cells.

ADRP released in exosomes also induces NED in adjacent C4-2B MDVR cells

Though Enzalutamide did not change the size of exosomes (Fig. S5), Fig. 6A shows that Enzalutamide was able to induce more exosomes released from C4-2B MDVR cells since the growth of these cells was inhibited. Similar to our previous results in DU145 and C4-2 cell models, ADRP protein can be detected in exosomes released from Enzalutamide-treated cells (Fig. 6B). These exosomes also increased ADRP protein in adjacent C4-2B MDVR cells and induced the dendrite-like extension (Fig. 6C). All the data indicate that Enzalutamide can induce NED in a paracrine manner through ADRP-containing exosomes.

Discussion

For patients manifested with systemic PCa, ADT is considered as a gold standard of regimen; this treatment paradigm has been in places for many decades based on androgen as an exclusive growth factor for these tumor cells. Inevitably, PCa becomes a lethal disease once it acquires castration resistant phenotypes. Increasing evidence from the characterization of clinical CRPC specimens indicates that these cells could undergo genomic reprogramming to turn on specific genes associated with different cell lineages. For example, neuroendocrine (NE) cells are different cell type from epithelial cell in prostate gland and they express potent neuropeptides that activate different pathways and biological processes such as cell growth and transformation [5,6]. In most cancers, the NE component co-exists with non-NE component, suggesting NE may play a supporting role for carcinoma cell. Increasing attention has been focused on NED for CRPC patients [4]. For example, IL-6, frequently detected in the serum of CRPC patients, is known to induce NE-specific gene expression associated with NED [6]. In addition, our data in this study and others have similar findings that Enzalutamide can induce NED of CRPC cells [32]. Thus, the identification of the NED inducers with underlying mechanisms and their potential interaction will be helpful mainly for tailoring therapeutic strategies of CRPC, increasing survival rates and ameliorating quality of life.

Some reports have defined PPARγ clinically relevant, however, the regulation of PPARγ in PCa cells is not well characterized. In this study, our data indicate that the elevation of PPARγ gene is not seen in both PPARγ database (Fig. S3B) as well as IL-6 treatment (Fig. S3C), which imply the post-transcriptional regulation may be an underlying mechanism; more detailed studies are expected in the future. PPARγ has been shown to regulate ADRP in mouse adipocytes [33]. Several clinical studies demonstrate that elevated PPARγ is associated with high tumor grade and poor overall survival of PCa patients [23–25]. Our data (Fig. 3) provide new evidence that PPARγ promotes NED of PCa cells by increasing the accumulation of adiposome in the dendrite-like extension of these cells. These observations suggest a potential inductive relationship between adiposome accumulation and NED of CRPC cells exposed to IL-6 or acquired Enzalutamide resistance (Figs. 3 and 4). Certainly, PPARγ appears to be a potent factor in NED, which may explain the growth inhibitory role of PPARγ in PCa [34–36].

ADRP is an important component of adiposome and also an indicator of adipocyte differentiation [37]. Although ADRP has been shown to be a potential diagnostic biomarker for breast, lung, colorectal, renal and esophageal cancers [38–42], the role of ADRP in PCa is poorly characterized. Our data provide new evidence that ADRP plays a key regulator in PPARγ-elicited adiposome accumulation and NED of CRPC cells by increasing CgA and NSE expression (Fig. 3). Noticeably, the induction of adiposome accumulation and NED can be mediated by exosomes isolated from either IL-6-treated cells or Enzalutamide-resistant cells (Figs. 4 and 6). Most interestingly, ADRP but not PPARγ was only detected in exosome, implying exosome may have specific function in protein sorting and package. These data suggest that detecting exosome containing specific regulatory protein in CRPC patients may be valuable for predicting disease progression.

In summary, we have provided new evidence to connect adiposome accumulation and NED of CRPC cells exposed to IL-6 and Enzalutamide in which PPARγ–ADRP pathway represents a key underlying mechanism that can be mediated by secretory exosomes in a paracrine manner. Based on this study, PPARγ can be a potential therapeutic target for CRPC as specific inhibitors of PPARγ are currently testing clinically for other disease [25,43,44]. Furthermore, by developing exosome-containing ADRP as a potential CRPC biomarker that can be detected using minimal invasive liquid biopsy, we believe that the future study of the correlation of ADRP with ADT resistance could establish ADRP as a new prognostic marker in CRPC progression.

Acknowledgements

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Conflict of interest statement

All the authors have no conflict of interest.
Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.canlet.2017.01.018.

References
Supplemental Figure legends

Figure S1 Enriched adiposomes in dendrite-like extension of PCa cells treated with IL-6. (A) Increased lipid accumulation in CRPC cells. Lipids were observed and quantified as previously described. (B) The association of adiposome and dendrite-like extension in DU145 or C4-2 cells after 4 days of IL-6 treatment (50 ng/ml in 5% FBS-supplemented RPMI medium). (C) Protein expressions of lipogenic proteins and NED markers were analyzed in DU145 or C4-2 cells after 4 days of IL-6 treatment (50 ng/ml in 5% FBS-supplemented RPMI medium). Data represent mean ± S.D. in triplicates from each group. * Significant difference compared with control group (p<0.05).

Figure S2 Effect of ADRP on lipid accumulation and dendrite-like extension in DU145 treated with IL-6. (A) ADRP-knockdown DU145 cells were treated with IL-6 (50 ng/ml in 5%FBS RPMI) for 4 days and the expression levels of ADRP or CgA protein were determined by Western blot. Actin was used as the loading control. (B, C) Dendrite-like extension or lipid accumulation was quantified. Data represent mean ± S.D. in triplicates from each group. * Significant difference compared with control group (p<0.05).

Figure S3 Expression profile of PPARγ and ADRP in PCa cell lines or tissues. (A) The expression levels of PPARγ and ADRP protein in PCa cell lines and immortalized normal prostate epithelial cell. (B) PPARγ and ADRP mRNA expressions in normal and prostate cancer tissues. (C) PPARγ and ADRP mRNA expressions in DU145 or C4-2 cells treated with IL-6 treatment (50 ng/ml in 5% FBS RPMI medium) for 1 day.

Figure S4 Effect of Enzalutamide on C4-2B and C4-2BMDVR. C4-2B and C4-2B MDVR Cells (2×10^3) were seeded onto 96-well plate for 24 h for attachment. Cells were treated with or without Enzalutamide at indicated time, cell growth was determined using MTT assay.

Figure S5 Size determination of exosome isolated from C4-2B MDVR treated with Enzalutamide. The size of exosomes from C4-2B MDVR cells was determined by Zetaview.
Supplemental Fig. 1
Supplemental Fig. 2
Supplemental Fig. 3

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| ![Image of Western Blots](image)

- PPARγ
- ADRP
- Actin

B

- PPARγ relative RNA levels
- ADRP relative RNA levels
- normal tissue
- cancer tissue

C

- DU145
- C4-2

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Relative gene expression
Supplemental Fig. 4
Supplemental Fig. 5