

Periprostatic adipose tissue inhibits tumor progression by secreting apoptotic factors: A natural barrier induced by the immune response during the early stages of prostate cancer

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Abstract. Prostate cancer (PCa) is the second most prevalent malignancy in men worldwide. The risk factors for PCa include obesity, age and family history. Increased visceral fat has been associated with high PCa risk, which has prompted previous researchers to investigate the influence of body composition and fat distribution on PCa prognosis. However, there is a lack of studies focusing on the mechanisms and interactions between periprostatic adipose tissue (PPAT) and PCa cells. The present study investigated the association between the composition of pelvic adipose tissue and PCa aggressiveness to understand the role played by this tissue in PCa progression. Moreover, PPAT-conditioned medium (CM) was prepared to assess the influence of the PPAT secretome on the pathophysiology of PCa. The present study included 50 patients with localized PCa who received robot-assisted radical prostatectomy. Medical records were collected, magnetic resonance imaging scans were analyzed and body compositions were calculated to identify the associations between adipose tissue volume and clinical PCa aggressiveness. In addition, CM was prepared from PPAT and perivesical adipose tissue (PVAT) collected from 25 patients during surgery, and its effects on

the PCa cell lines C4-2 and LNCaP, and the prostate epithelial cell line PZ-HPV-7, were investigated using a cell proliferation assay and RNA sequencing (RNA-seq). The results revealed that the initial prostate-specific antigen level was significantly correlated with pelvic and periprostatic adipose tissue volumes. In addition, PPAT volume was significantly higher in patients with extracapsular tumor extension. PCa cell proliferation was significantly reduced when the cells were cultured in PPAT-CM compared with when they were cultured in control- and PVAT-CM. RNA-seq revealed that immune responses, and the cell death and apoptosis pathways were enriched in PPAT-CM-cultured cells indicating that the cytokines or other factors secreted from PPAT-CM induced PCa cell apoptosis. These findings revealed that the PPAT secretome may inhibit PCa cell proliferation by activating immune responses and promoting cancer cell apoptosis. This mechanism may act as a first-line defense during the early stages of PCa.

Introduction

Prostate cancer (PCa), the second most prevalent malignancy in men worldwide, caused ~34,500 deaths in the United States in 2022 (1), and research has indicated that an estimated 15% of men receive a diagnosis of PCa during their lifetime (2). Obesity influences the pathogenesis of prostate disease, including benign prostate hyperplasia (BPH) and PCa (3-5). Notably, obesity can lead to alterations in endocrine status, including increased levels of estradiol due to the expression of the enzyme P450 aromatase in adipose tissue, which converts androgens to estrogen; this results in gonadotropin suppression and favors the development of BPH (6). Obesity also enhances sympathetic nervous activity, which impacts the severity of urinary voiding dysfunction, contributing to BPH (7,8). Additionally, obesity induces inflammatory processes, promoting microvascular disease and leading to

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tissue ischemia and oxidative stress, creating a favorable intra-prostatic environment for hyperplastic growth and potential precancerous transformation (9).

Low serum testosterone levels are associated with aggressive PCa, since testosterone has a regulatory role in maintaining normal prostate cell growth (10), and men with low testosterone levels often exhibit a more aggressive PCa phenotype (11). Trials of 5 α -reductase inhibitors, which inhibit the conversion of testosterone into dihydrotestosterone (a hormone that plays a crucial role in prostate growth), have shown a decreased overall risk of PCa but a higher Gleason score, reflecting the association between low serum testosterone and aggressive PCa (12). Insulin resistance, which is commonly associated with obesity, has been shown to promote PCa by increasing circulating levels of bioactive IGF-1, a growth factor implicated in numerous types of cancer (13). Among adipokines, leptin, which is elevated in central obesity, promotes angiogenesis in human PCa cell lines, thereby supporting cancer growth (14). Conversely, adiponectin, which has antitumor effects by inhibiting cancer cell proliferation and metastasis, has been detected at reduced levels in central obesity (15). Notably, multiple studies have reported an association between PCa and obesity (16,17), and three meta-analyses have reported a positive correlation between the incidence of PCa and obesity (18-20). It has also been indicated that obesity can affect the outcomes of PCa. A systematic review and meta-analysis identified that for every 5-kg/m² increase in the body mass index (BMI) of patients undergoing radical prostatectomy, the risk of biochemical recurrence of PCa increased by 20% and the risk of PCa-specific mortality increased by 15% (21).

Most studies on obesity define the condition and measure its degree using BMI (17-20); however, BMI is an inaccurate measure because it fails to directly account for the amount of adipose tissue in the body. Thus, researchers are increasingly considering the role of body composition, including the distribution of fat and lean tissue, in PCa. Several small-scale studies have implicated visceral and subcutaneous fat in the initiation and progression of PCa. Duong *et al* (22) demonstrated that adipocytes, the main cellular component of adipose tissue, were involved in solid tumor progression. Adipose tissue is considered to be more metabolically active than other tissues and to serve a prominent role in prostate tumorigenesis. Visceral fat cells produce multiple hormones and cytokines, including interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), leptin and adiponectin (2,23). von Hafe *et al* (24) used computed tomography (CT) to determine that visceral obesity may be a risk factor for PCa, which could be explained by the activities of adipokines secreted by visceral fat cells, as well as the elevated levels of insulin and disturbances in steroid hormone homeostasis associated with visceral obesity. Furthermore, Zimmermann *et al* (25) demonstrated that patients with higher visceral fat volumes and densities exhibited more favorable biochemical outcomes after radical prostatectomy and postoperative radiotherapy.

Periprostatic adipose tissue (PPAT), a type of visceral adipose tissue, serves a key role in PCa; the extracapsular extension of cancer cells into PPAT has been reported to be associated with poor prognosis (26). PPAT is anatomically defined as the local adipose tissue that surrounds the prostate

gland in the pelvic cavity. Spatial imaging, such as CT or magnetic resonance imaging (MRI), enables the measurement of distinct areas of adipose tissue (27). In addition to secreting multiple hormones and other protein factors, including adiponectin, leptin and IL-6, similar to general visceral adipose tissue, PPAT has been found to be correlated with PCa aggressiveness (28). van Roermund *et al* (28) demonstrated that fat-related parameters of PPAT, including area and density, were directly correlated with the aggressiveness of PCa and may serve as better markers of obesity than BMI. Multiple studies have also reported that PCa cells may alter adipocyte biology around the prostate gland, while *in vivo* studies have demonstrated the crosstalk between tumors and adipocytes (29-34).

The current medical treatments for PCa primarily target the hormonal pathway, with the immune checkpoint and homologous recombination pathways serving as secondary targets. Although current medical treatments have demonstrated promising efficacy, they face some limitations (35,36). Notably, current treatments for localized PCa, such as radical prostatectomy or radiation therapy, can achieve a high cure rate and low PCa-related mortality rate; however, there has been limited advancement in curing advanced PCa. Targeted therapies, such as androgen receptor (AR)-signaling inhibitors (ARSI), poly (ADP-ribose) polymerase (PARP) inhibitors and radiopharmaceuticals [prostate-specific membrane antigen (PSMA) Lu-177 or radium-223], are novel treatments for advanced metastatic PCa aimed at disease control rather than cure (37,38). Notably, the treatment efficacy of these novel agents for advanced or metastatic PCa is limited by their targeting mechanisms. PARP inhibitors are effective in PCa with germline or somatic homologous recombination repair deficiencies; however, these mutations, including *BRCA2*, *ATM*, *CDK12* and *CHEK2*, only account for one in four patients (39). ARSIs can achieve better chemical castration levels than conventional androgen deprivation therapy (ADT) with gonadotropin-releasing hormone agonist or antagonist, but patients may eventually progress to castration refractory PCa (CRPC), mainly due to AR mutation, splice variant formation, amplification, or progression to neuroendocrine PCa (40). Regarding radiopharmaceuticals, the effectiveness of PSMA-based treatments depends on PSMA expression in the tumor; however, the innate heterogeneity of PSMA expression is a notable limitation. Furthermore, downregulation of PSMA expression is widespread in patients with advanced metastatic CRPC, primarily due to lineage plasticity (41,42). The treatment efficacy of radium-223 is limited to metastatic bone lesions only, due to its bone-seeking calcium mimetic nature (43). Therefore, novel treatment strategies are warranted. Elucidating the interactions between PPAT and PCa may enable the design of novel anticancer strategies targeting different pathways.

The present study investigated the association between pelvic adipose tissue (PAT) distribution and PCa aggressiveness, as well as the underlying mechanisms. MRI was used to evaluate clinical morphological characteristics. In addition, PPAT collected during robot-assisted radical prostatectomy (RaRP) was used to prepare conditioned medium (CM), the effects of which were investigated on two PCa cell lines and one prostate epithelial cell line.

Patients and methods

Patient data and tissue collection. Between January 2009 and December 2021, patients were consecutively enrolled at a single medical center, Linkou Chang Gung Memorial Hospital (Taoyuan, Taiwan). A total of 50 patients with localized PCa who underwent RaRP were included. All patients were reviewed and discussed at a multidisciplinary uro-oncological meeting. RaRP was indicated for patients with localized or selected locally advanced PCa. Before surgery, shared decision-making about the treatment plan, along with other alternative modalities, was discussed with the patients. Patients that had previously been treated with ADT, radiation therapy, chemotherapy or pelvic surgery were excluded, as were patients who were not willing to provide informed consent. Patients underwent pelvic MRI, with the results used to determine cancer stage and treatment plan before surgery. All treatments were administered in accordance with relevant regulations and guidelines. The medical records of the patients were retrospectively reviewed to obtain data regarding their general characteristics. PCa-related data were also collected, including the serum level of initial prostate-specific antigen (iPSA), Gleason score and pathological stage (44).

To standardize the study, only patients harboring tumors that were pathologically staged as T2 were selected. A total of 1 g each of PPAT and perivesical adipose tissue (PVAT) were obtained during RaRP and served as pericancerous adipose tissue and normal adipose tissue, respectively. For studying the characteristics of PPAT, only patients with pathological T2 stage were selected because T3 stage represents extracapsular invasion, meaning that tissue collected from patients with T3 stage or higher may have potential PCa cell contamination in the PPAT specimens. According to the final pathological report of the prostate specimen, T2 refers to organ-confined disease, whereas T1 stage is not applicable for PCa based on the 8th edition of the American Joint Committee on Cancer (AJCC) TNM Staging System for Prostate Cancer (44). This is because the clinical T1 stage indicates a clinical inapparent tumor that is not palpable. However, in the AJCC guideline for pathological T staging, only T2 or higher stages are defined. All adipose tissues were collected under the premise of noninterference with pathological diagnoses. Only paired PPAT and PVAT tissues were used for the preparation of CM and further studies, whereas patients with insufficient or inadequate tissue quality were excluded. The present study was approved by the Chang Gung Medical Foundation Institutional Review Board. Written informed consent was obtained from the patients for tissue collection.

Image analysis. Body composition, including the volume of PAT, PPAT and perirectal adipose tissue (PRAT), was determined from the MRI scans used for cancer staging by a single radiologist who was blinded to the clinical information of the patients. All MRI scans were performed using a 1.5-T or 3-T system according to the method described by Chien *et al* (45). The MRI results were analyzed using OsiriX (OsiriX MD, v10.0; Pixmeo SARL) by a single radiologist who was informed that the patients had PCa and underwent subsequent RaRP.

From the axial T1-weighted images of the pelvis, the pelvic cavity, bladder, prostate gland, seminal vesicles,

rectum and perirectal space were manually segmented from the level of the prostate base to the apex. The representative estimated volumes of the regions are depicted in Fig. 1. Subsequently, PAT, PRAT and PPAT volumes were calculated using the following formulae: i) PAT volume=(pelvic cavity volume)-(bladder volume)-(prostate volume)-(seminal vesicle volume)-(rectum volume); ii) PRAT volume=(perirectal space volume)-(rectum volume); iii) PPAT volume=(pelvic cavity volume)-(bladder volume)-(prostate volume)-(seminal vesicle volume)-(perirectal space volume).

Primary adipose tissue cultures and preparation of CM. The collected PPAT or PVAT specimens were first washed three times with prechilled phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.8 mM KH₂PO₄, pH 7.4) to eliminate cell debris, and were then weighed. The tissue specimens were minced using sterile scissors and incubated in a T75 culture flask for 1 h at 37°C in 5% CO₂ with M199 culture medium (Gibco; Thermo Fisher Scientific, Inc.; 1 g tissue in 10 ml medium) supplemented with gentamicin (50 µg/ml). After 1 h, the medium was discarded, and the minced tissue was incubated in fresh M199 medium for a further 24 h. The medium in the flask was collected and centrifuged 5 min at 400 x g at 4°C to remove cell pellets and debris. The resulting supernatant was labeled as CM and stored at -80°C. In total, 25 pairs of PPAT and PVAT were collected for CM preparation. Additionally, a control was created by collecting serum-free M199 medium after 24 h of incubation without adipose tissue in a T75 flask at 37°C in 5% CO₂.

Cell lines and cell culture. The human PCa cell lines LNCaP and C4-2 were obtained from the Bioresource Collection and Research Centre (BCRC) and the American Type Culture Collection, respectively. The human prostate epithelial cell line PZ-HPV-7 was obtained from the BCRC. The LNCaP cells were maintained in RPMI 1640 medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 1 mM sodium pyruvate and 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.). The C4-2 cells were maintained in DMEM/F12 (4:1; Gibco; Thermo Fisher Scientific, Inc.) supplemented with 0.1 µg/ml insulin (Gibco; Thermo Fisher Scientific, Inc.), 275 ng/ml triiodothyronine (Sigma-Aldrich; Merck KGaA), 88.6 ng/ml apo-Transferrin (Sigma-Aldrich; Merck KGaA), 4.9 ng/ml d-Biotin (Sigma-Aldrich; Merck KGaA), 251.8 ng/ml adenine (Sigma-Aldrich; Merck KGaA) and 10% FBS. The PZ-HPV-7 cells were maintained in keratinocyte serum-free medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 0.05 ng/ml bovine pituitary extract (Gibco; Thermo Fisher Scientific, Inc.), 5 ng/ml epidermal growth factor (Gibco; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin G and 100 µg/ml streptomycin. All cells were cultured at 37°C in a humidified atmosphere of 5% CO₂.

Cell proliferation and cell apoptosis assay. The cells were seeded in 6-well plates (Corning, Inc.) at a density of 3x10⁵ cells/well and were incubated at 37°C for 24 h in complete medium. Subsequently, the spent medium was replaced with fresh serum-free medium containing 50% control-CM, PPAT-CM (tumor) or PVAT-CM (normal) and

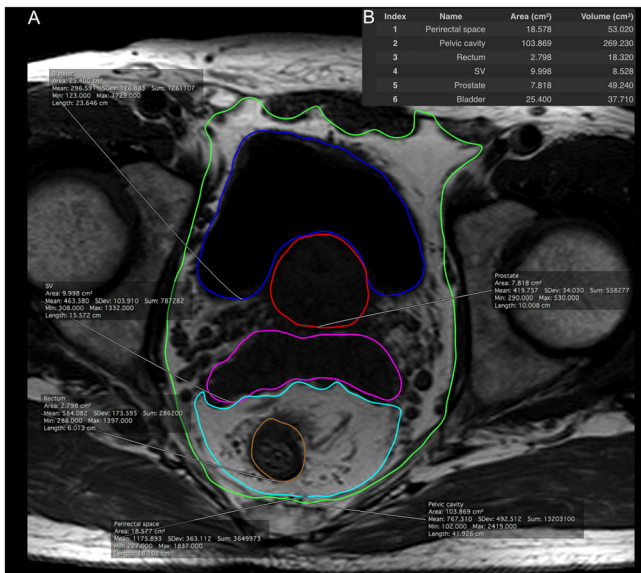


Figure 1. Adipose tissue distribution in the pelvic cavity was measured from T1-weighted magnetic resonance imaging scans. (A) Each area is illustrated with a different color: perirectal space (1, cyan), pelvic cavity (2, green), rectum (3, orange), SV (4, pink), prostate (5, red) and bladder (6, blue). (B) Measurement was performed from the level of the prostate base to the apex. SV, seminal vesicles.

a final concentration of 2% FBS. The cells were incubated at 37°C for 72 h, trypsinized and counted after trypan blue staining. The staining protocol included 0.4% trypan blue (Gibco; Thermo Fisher Scientific, Inc.), a staining duration of 3 min at room temperature, and visualization using a light microscope. Cell apoptosis was assessed using the Annexin V-PE/7-AAD apoptosis assay kit (BD Biosciences) with a BD LSRFortessa flow cytometer (BD Biosciences) in accordance with the manufacturer's instructions. FlowJo software (v10.4; FlowJo LLC) was used for data analysis.

RNA sequencing (RNA-seq) and pathway analysis. RNA was extracted from LNCaP cells cultured in control-CM, PPAT-CM or PVAT-CM for 72 h using the RNeasy mini kit (Qiagen, Inc.) in accordance with the manufacturer's instructions. The extracted RNA was sequenced to enable a comparison of the RNA expression between the three groups of cultured cells. RNA-seq libraries were prepared using 1 µg total RNA with the KAPA mRNA HyperPrep Kit (cat. no. 08098123702; KAPA Biosystems; Roche Diagnostics) following the manufacturer's recommendations, with index codes added to attribute sequences to each sample. Short double-stranded cDNA fragments were constructed and ligated to sequencing adaptors, and the library fragments were purified using the KAPA Pure Beads system (KAPA Biosystems; Roche Diagnostics). The library, carrying appropriate adapter sequences at both ends, was amplified using KAPA HiFi HotStart ReadyMix (KAPA Biosystems; Roche Diagnostics) along with library amplification primers. The strand marked with dUTP was not amplified, allowing for strand-specific sequencing. PCR products were purified using the KAPA Pure Beads system and assessed on the Qubit® 2.0 Fluorometer (Thermo Fisher Scientific, Inc.) and Agilent Bioanalyzer 5400 system (Agilent Technologies, Inc.). Finally, paired-end sequencing was performed using

the NovaSeq 6000 S4 Reagent Kit v1.5 (300 cycles; cat. no. 20028312; Illumina, Inc.) on the Illumina NovaSeq 6000 platform (cat. no. 20012850; Illumina, Inc.) with the type of sequencing being 150 bp paired-end. The loading concentration of the final library was 400 pM, with concentrations measured by Q-PCR and Qubit® 2.0 Fluorometer. Gene Set Enrichment Analysis (GSEA) was used to analyze the enriched pathways, with the C5 ontology gene sets in the Molecular Signatures Database (v7.5.1) serving as a reference (46-48).

Statistical analysis. Data are presented as the mean ± standard deviation. Pearson correlation analysis was used to analyze the correlation between PAT distribution and PCa aggressiveness. Intergroup differences were analyzed using one-way ANOVA, followed by a post hoc Bonferroni test, or an unpaired Student's t-test when appropriate. All statistical analyses were performed using SPSS software (v22.0; IBM Corporation). All tests were two-tailed, and $P \leq 0.05$ was considered to indicate a statistically significant difference. All *in vitro* experiments were performed as three independent replicates.

Results

Baseline characteristics. The average age of the 50 patients enrolled in the present study was 65.18 ± 5.94 years. Their mean body weight, BMI and serum iPSA levels were 70.39 ± 9.55 kg, 25.92 ± 3.17 kg/m² and 16.64 ± 12.84 ng/ml, respectively. The most common biopsy Gleason score was 7 (42%), followed by 6 (38%) and 9 (14%), and the most common pathological stage was T2c (46%), followed by T2a (24%) and T3a (20%). Detailed information is presented in Table I.

Body composition and tumor aggressiveness. Serum iPSA levels were significantly correlated with the volumes of PAT (Pearson's $r=0.404$, $P=0.006$) and PPAT (Pearson's $r=0.436$, $P=0.003$), and were not significantly correlated with the volume of PRAT (Pearson's $r=0.280$, $P=0.062$) (Table II). Gleason scores were not correlated with any PAT-related factor. The volume of PPAT was significantly higher in patients exhibiting extracapsular extension (pT3 or higher stage, $P=0.031$; Table III).

Prostate cell proliferation in CM. Proliferation assays were performed on LNCaP and C4-2 cells, as well as on PZ-HPV-7 cells, cultured in control-CM, PVAT-CM (normal) or PPAT-CM (tumor) (Fig. 2). A total of 25 pairs of PPAT-CM and PVAT-CM were used in the experiment. Initially, morphological changes were observed after 3 days of culture in CM, with the cells cultured in PPAT-CM exhibiting the most severe shrinkage among the experimental groups (Fig. 2A). The relative proliferation rate of the LNCaP cells was significantly lower in the PPAT-CM group than in the control- and PVAT-CM groups ($P=0.0216$ and 0.0343 , respectively; Fig. 2B). Similar trends in proliferation rates were observed for C4-2 cells ($P=0.0074$ and 0.0266 compared with the control- and PVAT-CM groups, respectively) and PZ-HPV-7 cells ($P=0.0063$ compared with the control-CM group) cultured in PPAT-CM. When comparing the cell proliferation rates between the PVAT- and PPAT-CM groups, the cells in the PPAT-CM group showed a trend of slower growth, but the difference was not statistically

Table I. General characteristics of the patients (n=50).

Characteristic	Value
Age, years	65.18±5.94 (52-76)
Body weight, kg	70.39±9.55 (53.1-93.4)
BMI, kg/m ²	25.92±3.17 (20.8-34.6)
TRUS volume, cm ³	40.02±28.19 (13-137)
iPSA, ng/ml	16.64±12.84 (4.16-58.08)
Gleason score	
5	1 (2)
6	19 (38)
7	21 (42)
8	2 (4)
9	7 (14)
Clinical T stage	
2a	12 (24)
2b	1 (2)
2c	23 (46)
3a	10 (20)
3b	3 (6)
4	1 (2)
Pelvic adipose tissue volume, ml	114.05±48.31 (38.05-254.36)
Periprostatic adipose tissue volume, ml	69.78±31.48 (22.71-164.32)
Perirectal adipose tissue volume, ml	44.28±24.54 (1.16-119.61)

Values are expressed as the mean ± SD (range) or n (%). BMI, body mass index; iPSA, initial prostate-specific antigen; TRUS, transrectal ultrasound of prostate.

significant (Fig. 2C). These results indicated that PPAT may inhibit prostate cell proliferation, and such impacts appear to be more pronounced in PCa cells used in this study.

Prostate cell apoptosis in CM. To verify whether the attenuated proliferation rate in the PPAT-CM group was due to the induction of cell apoptosis, a cell apoptosis assay was performed using flow cytometry. The extent of apoptosis in all three cell lines was higher in the PPAT-CM group than in the control- and PVAT-CM groups (Fig. 2D), suggesting that some factors in PPAT may induce the apoptosis of prostate cells.

RNA-seq of PCa cells cultured in CM. GSEA was used to compare the RNA expression profiles of LNCaP cells cultured in control-CM, PVAT-CM and PPAT-CM, since LNCaP cells exhibit significantly differential responses to CM, particularly in cell apoptosis. The results, according to the C5 ontology gene sets, are presented in Fig. 3. The following pathways were more activated in the PPAT-CM-cultured LNCaP cells than in the control-CM- or PVAT-CM-cultured cells: 'Regulation of immune response' (P=0.00149), 'Leukocyte-mediated immunity' (P=0.00150), 'Cytokine-mediated signaling pathways' (P=0.00153), 'Innate immune response' (P=0.00151),

'Regulation of defense response' (P=0.00151), 'Positive regulation of cell death' (P=0.00149) and 'Apoptotic signaling pathway' (P=0.00151). These results indicate that the presence of factors in PPAT-CM that induce immune related responses and cell apoptosis. Specifically, several genes associated with cell death and cell apoptosis, such as *SOD2*, *FADD* and *CASP10*, were upregulated in the PPAT-CM-cultured LNCaP cells, as shown in the 'Positive regulation of cell death' and 'Apoptotic signaling pathway' (Fig. 3B and C).

Discussion

According to the literature, PPAT can markedly influence the progression of PCa. Woo *et al* (49) studied 190 patients with PCa who underwent MRI before radical prostatectomy and demonstrated that PPAT thickness, as determined through MRI, was significantly correlated with Gleason score and was an independent predictor of high-grade PCa. In addition, Zhang *et al* (50) discovered that periprostatic adiposity was significantly associated with Gleason scores and clinical stage after evaluating the MRI scans of 184 patients with PCa who underwent radical prostatectomy. Bhindi *et al* (51) demonstrated that PPAT volume, as estimated through transrectal ultrasound, can predict the presence and the grade of PCa. PPAT area and density have also been reported to be more strongly correlated with PCa aggressiveness than other obesity-related parameters, including waist circumference and BMI (28,49,50). In addition, the correlations of PPAT thickness with BMI and weight have been shown to be nonsignificant or weak (49-54). Therefore, measurements of PPAT may serve as independent predictors of PCa aggressiveness. In the current study, the volumes of PAT, PRAT and PPAT were measured using MRI to identify associations between PAT distribution and PCa aggressiveness. The present study revealed that serum iPSA levels were significantly associated with higher PAT and PPAT volumes. Furthermore, a higher PPAT volume was correlated with extracapsular extension, which is consistent with the findings of a study investigating the association between PPAT volume and tumor aggressiveness (55).

Although clinical findings have indicated an association between PPAT and PCa, the mechanisms underlying this association remain unclear. PPAT may provide a favorable microenvironment for aggressive PCa or may passively accumulate fat content in response to local PCa progression. Furthermore, PPAT may affect PCa progression and pathogenesis by providing cancer cells with fatty acids and other mitogens. A previous study identified factors secreted by both PPAT and PCa cells potentially capable of mediating bidirectional communication between them (56). Ribeiro *et al* (31) reported that PPAT-CM stimulated PC3 and LNCaP cell migration but inhibited LNCaP proliferation. Conversely, another study reported that PPAT-CM did not significantly affect the proliferation and motility of LNCaP or PC3 cells (57). In addition, a previous study indicated that the proliferation of PC3 cells was increased when cocultured with rat epididymal adipocytes (58), but these findings were not observed in a later study (33). These conflicting results are likely due to differences in the characteristics of the experimental methodologies and cell lines that were used.

Table II. Correlation between factors of prostate cancer aggression and pelvic adipose distribution (n=50).

Volume	iPSA		Gleason score	
	Pearson correlation coefficient	P-value	Pearson correlation coefficient	P-value
Pelvic adipose tissue volume	0.404	0.006 ^a	0.220	0.146
Periprostatic adipose tissue volume	0.436	0.003 ^a	0.175	0.251
Perirectal adipose tissue volume	0.280	0.062	0.202	0.184

^aP<0.01. iPSA, initial prostate-specific antigen.

Table III. Association of extracapsular extension with pelvic adipose distribution.

Variable	Mean volume, ml		P-value
	With extracapsular extension	Without extracapsular extension	
Pelvic adipose tissue	126.88	101.79	0.082
Periprostatic adipose tissue	80.16	59.86	0.031 ^a
Perirectal adipose tissue	46.73	41.93	0.518

^aP<0.05.

The present study used the LNCaP and C4-2 cell lines as the experimental models because the present clinical analysis focused on patients with T2 stage PCa, ensuring that the adipose tissue was free from potential contamination by PCa cells. LNCaP cells, known for their well-established use in PCa research, have lower malignancy and AR positivity, representing earlier-stage PCa, compared to other commonly used PCa cell lines, such as PC-3, DU145 and CWR22R-v1 (59). By contrast, C4-2 cells, derived from LNCaP cells, exhibit higher invasiveness and metastatic potential, representing a more advanced stage of PCa (60,61). While C4-2 cells indeed exhibit higher invasiveness and metastatic potential compared with LNCaP cells, they still inherently express AR, representing relatively early stages of PCa. Conversely, other commonly used PCa cell lines, such as PC-3, DU145 and CWR22R-v1, demonstrate higher malignancy, representing advanced stages of PCa. In addition, PC-3 and DU145 cells lack AR expression, with DU145 further classified as a CRPC cell line (62-64). Similarly, although CWR22R-v1 is AR-positive, it is also classified as a CRPC cell line (65). Therefore, to mimic the T2 tumor stage of the clinical specimens, C4-2 cells were chosen as another PCa cell line for investigation. This selection allows for the study of differential responses between less and more aggressive PCa cells, and may improve understanding of the impact of the adipose tissue secretome on tumors at different stages of malignancy. Additionally, the present study incorporated experiments using the prostate normal epithelial cell line PZ-HPV-7 to provide a comprehensive comparison and to observe the effects on normal prostate cells. The proliferation rate of prostate cells was significantly lower in the PPAT-CM group compared with that in the control- and PVAT-CM groups. However, when comparing the cell proliferation rates between the PVAT- and PPAT-CM groups, the cells in the PPAT-CM

group showed a trend of slower growth, but the difference was not statistically significant, possibly due to the insufficient number of groups used in the experiment. The un-paired Student's t-test did not show statistical significance, likely due to individual variations among the samples. Collecting more cases could reduce these individual differences and achieve statistical significance. Furthermore, the cells cultured in PPAT-CM exhibited more pronounced apoptosis than those cultured in control- and PVAT-CM did. Compared with the PCa cell lines, although the CM had a slight effect on the prostate normal epithelial cell line, the impact on PCa cells appeared to be more pronounced. RNA-seq and analysis revealed that immune responses, and the cell death and apoptosis pathways were more activated in PPAT-CM-cultured cells than in PVAT-CM-cultured cells. These findings indicated that the cytokines and other factors secreted from PPAT-CM may have induced PCa cell apoptosis.

Evidence has indicated that adipose tissue can act as an energy reservoir, and it is a metabolically active organ (66) that produces growth factors, hormones and adipokines (67). The secretions of adipose tissue affect both physiological cellular responses, and the paracrine and autocrine signaling networks, especially in tumor microenvironments, where hormonal dependence mediates cancer progression (32,57,68-71). Several cytokines and adipokines, such as IL-6, leptin and vascular endothelial growth factor, have been associated with tumor progression (72,73); however, several others, including adiponectin (74), suppress tumor growth, whereas the effects of factors such as TNF- α remain unclear (75).

The secretome of adipose tissue may vary across different stages of PCa. Sacca *et al* (76) analyzed PPAT-CM from patients either with PCa (including stage T2 and T3 tumors) or from those with benign diseases using liquid chromatography-mass

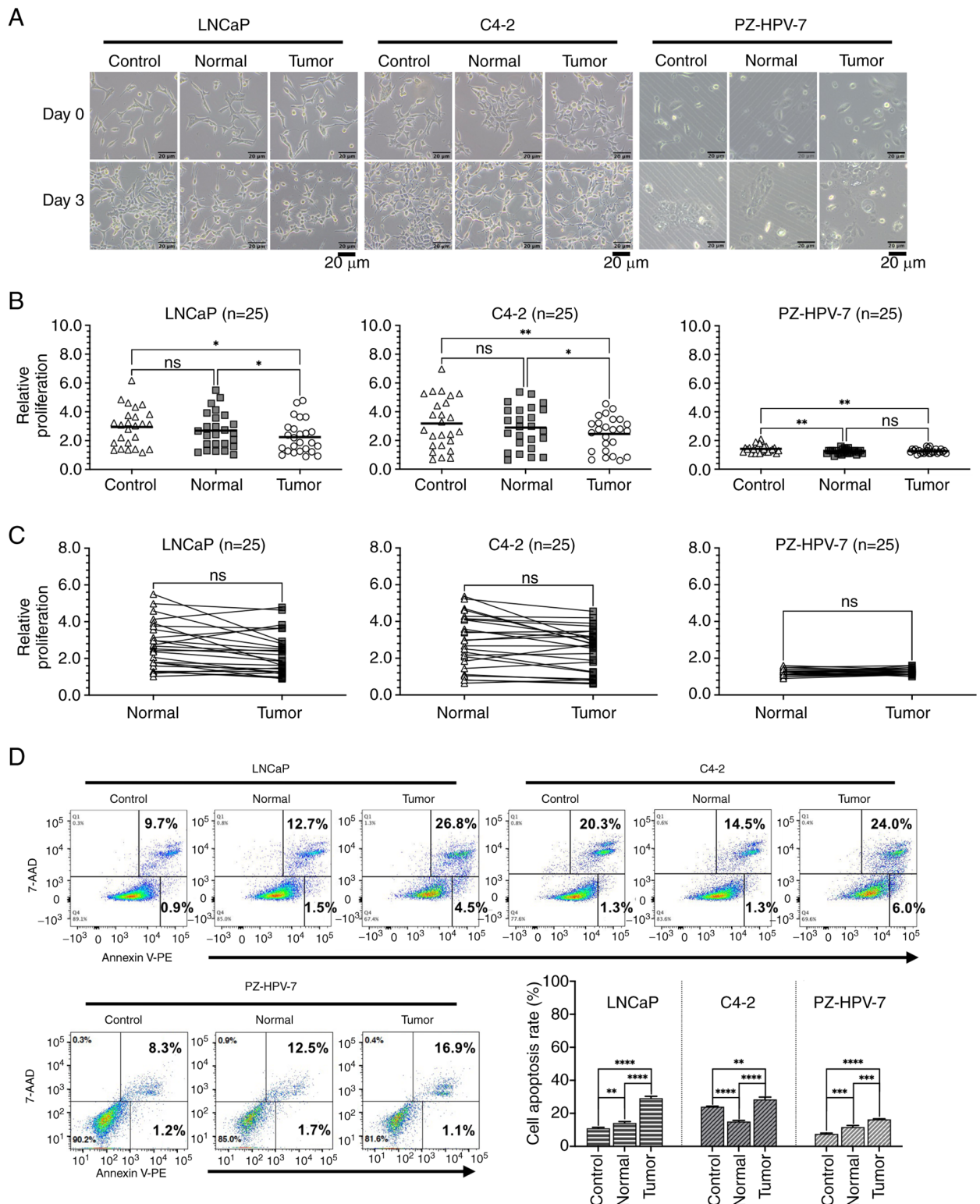


Figure 2. Effect of different CM on the proliferation and apoptosis of LNCaP and C4-2 prostate cancer cell lines, and the PZ-HPV-7 prostate epithelial cell line. Cells were seeded in 6-well culture plates. After 24 h, the medium was changed to serum-free RPMI 1640 medium containing 50% M199 medium (Control), periprostatic adipose tissue-CM (Tumor) or perivesicle adipose tissue-CM (Normal) with a final concentration of 2% FBS. After 72 h of incubation, cell morphology, cell proliferation and cell apoptosis were assessed. (A) Representative cell morphology (original magnification, $\times 100$; scale bar, 20 μ m). (B) Scatter plots and (C) paired scatter plots representing the relative fold-change in cell proliferation, calculated by dividing the total cell count at day 3 by the total cell count at day 0 (n=25). (D) Apoptosis was analyzed by Annexin V-PE/7-AAD staining using the BD LSRFortessa System. Apoptotic cells were compared between various groups, with three independent replicates. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$. CM, conditioned medium.

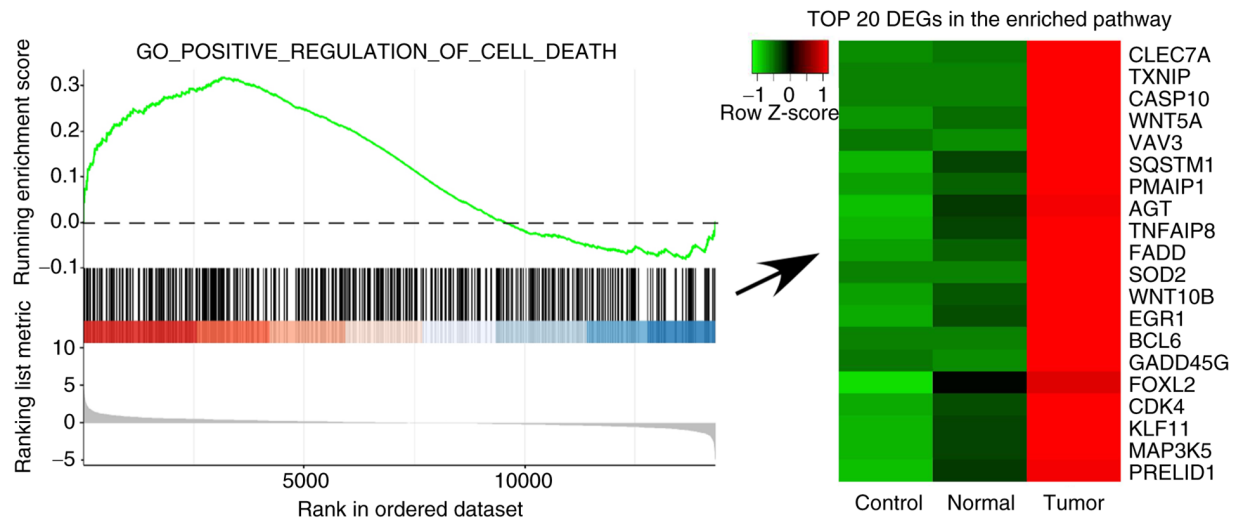
spectrometry-based proteomics. The results observed that the PPAT from patients with cancer exhibited stronger immune responses. Moreover, the PPAT from patients with stage T3

PCa was rich in catalytic proteins, whereas that from patients with stage T2 PCa was rich in defense and immune response proteins. These findings indicated that, in the early stages of

A

ID	*ES	#NES	P-value
GO_RESPONSE_TO_LIPID	0.344	1.591	0.00149
GO_POSITIVE_REGULATION_OF_CELL_DEATH	0.318	1.472	0.00149
GO_POSITIVE_REGULATION_OF_CELL_POPULATION_PROLIFERATION	0.346	1.598	0.00149
GO_NEGATIVE_REGULATION_OF_CATALYTIC_ACTIVITY	0.373	1.718	0.00149
HP_UNUSUAL_INFECTION	0.350	1.617	0.00149
GO_REGULATION_OF_IMMUNE_RESPONSE	0.435	2.000	0.00149
GO_REGULATION_OF_PROTEOLYSIS	0.369	1.694	0.00150
GO_LEUKOCYTE_MEDIATED_IMMUNITY	0.399	1.826	0.00150
GO_CELL_CELL_ADHESION	0.347	1.587	0.00150
GO_ESTABLISHMENT_OF_PROTEIN_LOCALIZATION_TO_ORGANELLE	0.334	1.539	0.00150
GO_INNATE_IMMUNE_RESPONSE	0.482	2.198	0.00151
GO_REGULATION_OF_DEFENSE_RESPONSE	0.445	2.010	0.00151
GO_APOPTOTIC_SIGNALING_PATHWAY	0.379	1.727	0.00151
GO_MYELOID_LEUKOCYTE_ACTIVATION	0.355	1.608	0.00152
GO_CELLULAR_RESPONSE_TO_LIPID	0.353	1.578	0.00153
GO_REGULATION_OF_VESICLE_MEDIATED_TRANSPORT	0.359	1.614	0.00153
GO_ENDOSOME_MEMBRANE	0.436	1.972	0.00153
GO_DNA_BINDING_TRANSCRIPTION_ACTIVATOR_ACTIVITY	0.357	1.594	0.00153
GO_PROTEIN_HOMODIMERIZATION_ACTIVITY	0.332	1.509	0.00153
GO_CYTOKINE_MEDIATED_SIGNALING_PATHWAY	0.478	2.170	0.00153

B



C

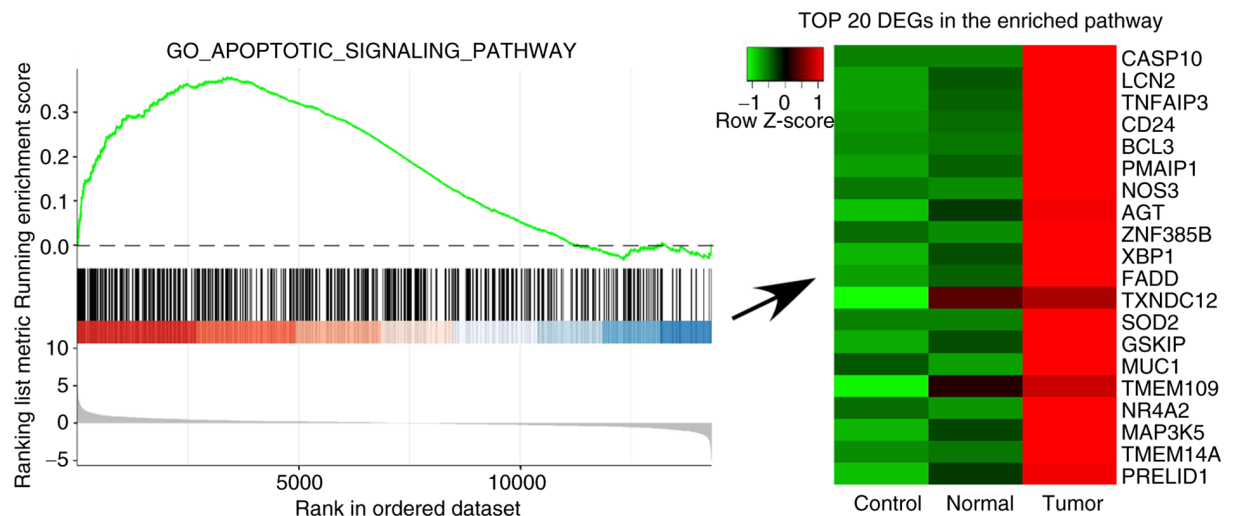


Figure 3. GSEA gene sets and relative expression heatmap for LNCaP cells cultured in control-CM, PVAT-CM, and PPAT-CM. (A) GSEA of RNA sequencing data from LNCaP cells cultured in PPAT-CM, with the C5 ontology gene sets serving as a reference. GSEA enrichment plot and heatmap of the top 20 DEGs among the core enrichment genes for the gene set in LNCaP cells cultured in control-CM, PVAT-CM and PPAT-CM. (B) GO_POSITIVE_REGULATION_OF_CELL_DEATH and (C) GO_APOPTOTIC_SIGNALING_PATHWAY. ES, enrichment score; NES, normalized enrichment score; DEGs differentially expressed genes.

localized PCa, the secretome of PPAT, including cytokines, may activate immune defense responses and induce cell apoptosis. This mechanism potentially acts as a first line of defense in the early stages of PCa. However, when the disease progresses and cancer cells extend to adipose tissue, their crosstalk with the tissue may induce catalytic activity and alter the tumor micro-environment, triggering progression or invasion.

The present study explored the role of PPAT in the early stages of PCa and revealed that the secretome of PPAT could inhibit cell proliferation by inducing apoptosis. However, this study has several limitations. First, PPAT was obtained only from patients harboring early-stage tumors (T2); samples from patients with more advanced PCa are required to further validate that PPAT inhibits cell proliferation. Second, different PCa cell lines cultured with PPAT should be used to thoroughly understand the influence of adipose tissue on cancer cells. Finally, the mechanism underlying the changes in the secretome of PPAT with the progression of PCa and its related signaling pathways remain to be elucidated.

In conclusion, the present study revealed that PPAT was significantly associated with extracapsular PCa extension. Furthermore, *in vitro* experiments revealed that PPAT could inhibit PCa cell proliferation by secreting factors that activated immune responses and could thereby promote cancer cell apoptosis. This mechanism may act as a first line of defense in the early stages of PCa. The mechanisms underlying further crosstalk between PPAT and PCa cells remain to be elucidated.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author. The RNA-seq data generated in the present study may be found in the National Center for Biotechnology Information-Gene Expression Omnibus database under accession number GSE267084 or at the following URL: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE267084>.

Authors' contributions

IHS, CTW, CHH and STP conceived and designed the study. IHS, YiHC, LKH, YCC, HCK, PHL, KJY, MLH, CKC, CTW and STP participated in data acquisition. IHS, THC, YHH, TWS and YuHC participated in the collection and assembly of data. TWS, LJW and YuHC participated in image analysis. IHS, THC, YHH and CHH participated in data analysis and interpretation. IHS, THC, CHH and STP participated in manuscript writing. IHS, CHH and STP confirmed the authenticity of all raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was conducted in accordance with the ethical principles outlined in The Declaration of Helsinki (2013). This study was approved by the Chang Gung Medical Foundation Institutional Review Board (IRB no. 1912230098). Written informed consent was obtained from the patients for tissue collection.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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