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Research article

The up-stream regulation of polymerase-1 and transcript release factor (PTRF/Cavin-1) in prostate cancer: an epigenetic analysis

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Abstract: The expression of *PTRF* is down-regulated in prostate cell lines and tissues. Restoration of *PTRF* expression leads to a reduction in aggressive phenotypes of prostate cancer cells both *in vitro* and *in vivo*. Epigenetics examines the changes in gene expression that occur without changing DNA sequences. Two main epigenetic mechanisms include hypermethylation of the gene's promoter region and changes to the chromatin structure through histone modification. We investigated the involvement of possible epigenetic up-stream regulatory mechanisms that may down-regulate *PTRF* in prostate cancer cells. Normal (RWPE-1) and prostate cancer (LNCaP and PC3) cell lines were treated with DNA methylation inhibitor, 5-aza-2'-deoxycytidine (5AZA) and histone deacetylase inhibitor, Trichostatin-A (TSA) either independently or in combination. A bioinformatics approach was also used to investigate the changes of epigenetic driver genes *in silico*. In normal prostate cells (RWPE-1), and androgen independent prostate cancer cells (PC3), treatment with 5AZA and/or TSA did not affect *PTRF* expression. However, TSA and TSA + 5AZA treatments, but not 5AZA alone, up-regulated the expression of *PTRF* in LNCaP cells. Bioinformatic analysis of the potential histone deacetylase (HDAC) genes involved showed that *HDAC2*, *HDAC6* and *HDAC10* may be potential candidate genes for the regulation of *PTRF*. This corroborative study describes the possible role of an epigenetic mechanism on *PTRF*, further studies are required to allow a better understanding of the up-stream mechanisms that regulate *PTRF* expression.

Keywords: DNA methylation; histone deacetylase; histone modification

Abbreviations: 5AZA, 5-aza-2'-deoxycytidine; CpG, cytosine and guanine; CpGi, CpG rich islands; HAT, histone acetyltransferase; HDAC, histone deacetylase; miRNA, micro-RNA; PTRF, polymerase-1 and transcript release factor; TSA, Trichostatin-A

1. Introduction

Prostate cancer remains a significant health issue for men world-wide [1]. PTRF is a key molecule involved in transcription termination and caveolae formation [2,3]. The role of PTRF in several health and disease states such as lipodystrophy, cellular senescence, cancer and pulmonary related disorder has recently been discussed [4]. The expression of PTRF is reported to be down-regulated in prostate cancer cell lines and tissues [5]. Restoration of *PTRF* in PC3 prostate cancer cells leads to a reduction in aggressive phenotypes both *in vitro* and *in vivo* [6-8]. Recently, findings from our laboratory suggest that knocking down *PTRF* in normal prostate cells promotes increased proliferation, migration and invasion and is accompanied by up-regulation of phosphorylated STAT3 signaling (Low and Nicholson, unpublished data). However, what causes the down-regulation in *PTRF* expression in prostate cancer remains unknown.

In addition to PTRF, three other homologs have been discovered and described. These include the serum deprivation protein response (SDPR/Cavin-2), sdr related gene product that binds to c-kinase (SRBC/Cavin-3) and muscle restricted coiled-coiled protein (MURC/Cavin-4) [9,10]. These homologs are described to play a role in caveola dynamics and cellular physiology, including caveola morphology, intracellular microtubule transportation of caveola, muscle formation and cardiac dysfunction through the Rho/ ROCK signaling pathway [11-13].

Recently, it was reported that the expression of *PTRF* is down-regulated in breast cancer cell lines and tissues and that this is due to promoter hypermethylation. The expression of *PTRF* was successfully restored through 5-aza-2'-deoxycytidine treatment in breast cancer cell lines [14]. Additionally, the promoter region of *SRBC/Cavin-3* is reported to be methylated in lung and gastric cancer [15,16]. Treatment with 5AZA in lung and gastric cancer cell lines restored the expression of *SRBC/Cavin-3* [15,16]. Taken together, epigenetics may play an important role in suppressing the expression of cavin proteins during tumorigenesis. We hypothesized that the loss of expression of *PTRF* in prostate cancer may be due to epigenetic modification.

Epigenetics is the study of changes in gene expression that occur without a change in the DNA sequence. Two main epigenetic mechanisms include hypermethylation of the promoter region and changes to the chromatin structure through histone modification. Unlike gene mutation that involves a change to the DNA sequence and is irreversible, epigenetic changes are reversible through development and life and can be modified with the use of chemical agents [17-19]. Promoter hypermethylation results from the addition of a methyl group to cytosine residues in the cytosine and guanine (CpG) dinucleotides to form 5-methylcytosine. CpG dinucleotides are unevenly distributed in the mammalian genome to form short sequences which have high densities of CpG dinucleotides, known as CpG rich islands (CpGi). Methylation occurs in CpGi, and gene promoters that are highly methylated are transcriptionally silenced as heavily methylated promoters are not accessible by transcriptional elements. Conversely, gene promoters that are hypomethylated are critical for active gene transcription [19]. Histone acetylation is also able to regulate gene expression. To achieve gene expression, the ε-amino group in the lysine residue of the histone cores must be acetylated by histone acetyltransferases (HATs). In the histone core, a class of enzymes called histone deacetylases (HDACs) oppose the effect of HATs by removing acetyl groups from lysine residues. The removal of acetyl group by HDACs restores the positive charges on lysine residues. As a result, this will cause the histone tails to tightly coil to the DNA leading to transcriptional inactivation [20].

This study investigates the involvement of epigenetic modifications on the expression of *PTRF* in prostate cancer cell lines using *in vitro* and *in silico* approaches.

2. Materials and methods

2.1. Cell culture

The human normal prostate epithelial cell line, RWPE-1 (ATCC, Manassas, VA) was cultured and maintained in keratinocyte serum free medium supplemented with 5 ng/mL of recombinant epidermal growth factor, 0.05 mg/mL of bovine pituitary extract (Invitrogen, Carlsbad, CA), 10 nM/L of dihydrotestosterone (Sigma-Aldrich, Co., St. Louis, MO). The androgen dependent prostate cancer cell line, LNCaP (ATCC, Manassas, VA) and androgen independent prostate cancer cell line, PC3 (ATCC, Manassas, VA) were grown in RPMI-1640 medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS) (Thermo Fisher Scientific, Waltham, MS) and 10 nM/L of dihydrotestosterone. All cell lines were grown in a humidified incubator at 37 °C with 5% CO_2.

2.2. Treatment with epigenetic reversing drugs

5-aza-2'-deoxycytidine (5AZA) and Trichostatin-A (TSA) were purchased from Sigma-Aldrich (Sigma-Aldrich, Co., St. Louis, MO). Cells were harvested and seeded at a density of 8.0×10^4 cells (for TSA treatment) or 1.5×10^4 (for 5AZA and combination treatment) into 6 well plates. The following day, culture medium was removed and cells were treated with 2 μM of 5AZA for 6 days [21]. Culture medium was refreshed every 2 days and supplemented with a similar dose of 5AZA. For TSA treatment, cells were treated with 400 nM of TSA for 48 h [22]. For combination treatment, cells were initially treated with 2 μM of 5AZA and medium was refreshed every 2 days. At the fourth day, both 5AZA and TSA were added and cells were then cultured for another 48 h. At the end of each treatment, cells were harvested.

2.3. Isolation of total RNA

Total RNA was harvested from the cells at the end of each treatment with RNeasy mini kit (Qiagen, Hilden, Germany) according to manufacturer's protocol. Briefly, culture medium was aspirated and cells were washed with ice-cold PBS. Cells were lysed with RLT lysis buffer and the lysate was passed through the provided spin columns to trap the RNA. RNA was then eluted, quantified and stored at −80 °C for further use.

2.4. Synthesis of first strand complimentary DNA

Following RNA isolation, 2 μg of RNA was used to synthesize first strand complementary DNA (cDNA) by using SuperScript® III First-Strand Synthesis kit (Invitrogen, Carlsbad, CA) according to manufacturer's protocol. Briefly, 2 μg of RNA was mixed with 1 μL of random hexamer primer (50 ng/μL), 1 μL of dNTP mix (10 mM stock) and was topped up with UltraPure™ DNase/RNase-Free Distilled Water (Invitrogen, Carlsbad, CA) to 10 μL. The mixture was heated at 65 °C for 5 min and then cooled on ice for at least 3 min. Then, it was mixed with 2 μL of 10× RT buffer, 4 μL of $MgCl_2$, 1 μL of RNase inhibitor, 1 μL of SuperScript III reverse transcriptase and 2 μL of Dithiothreitol (DTT) to make a final volume of 20 μL and heated at 20 °C for 10 min. Next, the mixture was heated at 50 °C for 50 min, followed by 85 °C for 5 min to inactivate SuperScript® III. The product was chilled on ice and 1 μL of RNase H was added and incubated for 20 min at 37 °C.

2.5. Quantitative real time polymerase chain reaction

QuantiTect primer assays for *PTRF* (QT00059430) and *GAPDH* (QT00079247) for quantitative real time polymerase chain reaction (qRT-PCR) were purchased from Qiagen (Qiagen, Hilden, Germany). The reaction mixtures for qRT-PCR were prepared using QuantiTect SYBR Green PCR kit (Qiagen, Hilden, Germany). Briefly, 4 μL (50 ng) of cDNA that had been diluted previously was mixed with 5 μL of SYBR Green Master Mix and 1 μL of Quantitect primer assay. qRT-PCR was then carried out using a Stratagene MX3000p qRT-PCR Instrument (Agilent Technologies, Santa Clara, CA) and cycle parameters of real time PCR consisted of three steps: (I) one cycle of 95 °C for 15 min, (II) 40 cycles of 94 °C for 15 s, 55 °C for 30 s and 72 °C for 30 s each cycle and (III) one cycle of 95 °C for 1 min, 55 °C for 30 s and 95 °C for 30 s. Analysis was run using the built in software and the CT values were recorded. To obtain the fold change of gene expression, results were calculated using a relative quantification method, $2^{-\Delta\Delta}$CT.

2.6. Western blot

Cells (5.0×10^4 cells) were resuspended in Complete® Protease Cocktail Inhibitor (Roche Diagnostics, Basel, Switzerland). Samples were then separated with a 10% (w/v) resolving gel 100 V for 90 min and transferred onto nitrocellulose membrane (GE Healthcare, Buckinghamshire, UK) in ice-cold buffer at 200 mA for 90 min. The resulting membrane was blocked with Odyssey Blocking Solution (LI-COR Biosciences, Lincoln, NE) for 1 h at room temperature. Primary PTRF antibody (1:5000) (Abcam ab48824; Abcam, Cambridge, UK) diluted in Odyssey Blocking Solution was added and left incubated at 4 °C overnight on a shaker. β-actin (1:10,000) (Cell Signaling 4970; Cell Signaling, Danvers, MA) was used as a protein loading control. The following day, the membrane was washed with Tris-Buffered Saline (TBS) for 5 min, followed by TBS with Tween for 5 min and finally with TBS again for 5 min. Fluorescent conjugated secondary antibody, IRdye 800CW donkey anti rabbit IgG or IRdye 680RD donkey anti mouse (LI-COR Biosciences, Lincoln, NE) diluted with Odyssey Blocking Solution was added to the membrane which was incubated at room temperature for 2 h on a shaker protected from light. The membrane was then washed with TBS-Tween followed by TBS for 5 min each, before being dried and scanned on an Odyssey Infrared Imager (LI-COR Biosciences, Lincoln, NE). Quantification of protein was performed using Odyssey Application Software Version 3.0 (LI-COR Biosciences, Lincoln, NE).

2.7. Bioinformatic analysis on HDAC genes

Data mining was performed through a PubMed search of microarray data sets that have been deposited in the National Centre for Biotechnology Information. Data set full SOFT files (GEO ascension number; GDS3761, GDS4159 and GDS3973) were downloaded (http://www.ncbi.nlm.nih.gov/gds). Data set GDS3761 represents transcriptomes of RWPE-1 cells (n = 12). Data set GDS4159 represents transcriptomes of LNCaP (n = 4) and data set GDS 3973 represents the transcriptomes of PC3 (n = 3) cells. Microarray analysis of the 3 data sets was performed on a common platform, Affymetrix Human Genome U133 Plus 2.0 Array. Histone deacetylase (HDAC) gene expression profiles for Class I (*HDAC1*, *2*, *3*, *8*), Class IIA (*HDAC4*, *7*, *9*), Class IIB (*HDAC6*, *10*) and housekeeping genes (*RPS18*, *RPL11*, *RPS27A*) were extracted and normalized with a housekeeping gene, glyceraldehyde

3-phosphate dehydrogenase (*GAPDH*) (Table 1). This was to ensure that the changes observed were not due to variation between the samples in the data set. Upon normalisation, the gene expression profiles were analysed. One-way ANOVA was performed with Tukey multiple post hoc comparison. A Bonferonni adjustment was then performed to ensure that across all genes tested there was only a 5% probability that any of the sets of pairwise tests will result in a type I error. To limit the *P*-value to a maximum of one, the following formula was applied after the second adjustment of the *P*-values;

$$= \text{MIN}(1,\text{RC}[-1]*12)$$

A *P*-value <0.05 after the final adjustments was considered statistically significant. Expression profiles of 3 housekeeping genes (*RPL11*, *RPS18*, *RPS27A*) (Table 1) were also extracted and underwent the same analysis as described above to act as the control group of this study. This was to ensure that the changes in gene expression were solely due to the different cell lines studied rather than caused by differences between three data sets.

Table 1. Information on genes extracted from microarray data sets for bioinformatic analysis.

Class Type	Gene name	Official gene symbol	Affymetrix probe set ID
I	Histone deacetylase-1	*HDAC1*	201209_at
I	Histone deacetylase-2	*HDAC2*	201833_at
I	Histone deacetylase-3	*HDAC3*	216326_s_at
I	Histone deacetylase-8	*HDAC8*	223909_s_at
IIA	Histone deacetylase-4	*HDAC4*	204225_at
IIA	Histone deacetylase-7	*HDAC7*	217937_s_at
IIA	Histone deacetylase-9	*HDAC9*	205659_at
IIB	Histone deacetylase-6	*HDAC6*	206846_s_at
IIB	Histone deacetylase-10	*HDAC10*	232870_at
	Glyceraldehyde 3-phosphate	*GAPDH*	217398_x_at
	Ribosomal protein L11	*RPL11*	200010_at
	Ribosomal protein S18	*RPS18*	201049_s_at
	Ribosomal protein S27A	*RPS27A*	200017_at

2.8. Statistical analysis

Student's *t*-test was used to analyze the differences between the treatment and control group. Statistical significance was achieved with a $P < 0.05$. Data are presented as mean ± standard error of 3 observations, unless otherwise stated. GraphPad Prism 5.0 software (GraphPad Software, Inc., San Diego, CA) was used for statistical analysis.

3. Results

3.1. Endogenous mRNA and protein expression of PTRF

Initially, the expression of PTRF in the three cell lines used in this study was confirmed at both mRNA and protein level. Results from qRT-PCR show that mRNA expression of *PTRF* is reduced in

LNCaP and PC3 cells compared to RWPE-1 cells (Figure 1A). Similarly, RWPE-1 cells showed a greater expression of PTRF at the protein level compared to the other two cell lines (Figure 1B).

3.2. TSA but not 5AZA up-regulated the expression of PTRF in vitro in LNCaP cells

Treatment with 5AZA did not result in any changes in the expression of *PTRF* in RWPE-1, LNCaP or PC3 cells (Figure 2A). However, TSA treatment significantly up-regulated the expression of *PTRF* in LNCaP cells ($P < 0.05$) (Figure 2B) but not in RWPE-1 or PC3 cells (Figure 2B). In the combination treatment (5AZA and TSA), the expression of *PTRF* was up-regulated in LNCaP cells ($P < 0.05$) (Figure 2C) but no changes in *PTRF* expression in RWPE-1 or PC3 cells were seen (Figure 2C).

3.3. Bioinformatic analysis of possible candidate histone deacetylase genes that may be up-regulated in androgen dependent prostate cancer

Therefore, the histone deacetylase (HDAC) genes that might be up-regulated to cause the down-regulation of *PTRF* were investigated. HDAC enzymes are classified into 4 families and only those from Class I, IIA and IIB are sensitive to TSA [23]. Therefore, it was postulated that some members of the HDAC Class I, IIA and IIB enzymes may be up-regulated in LNCaP cells. In order to provide an initial view on the changes on these genes in prostate cancer, a bioinformatic analysis was carried out. To show that the significant changes observed were due to the differences between the transcriptome of the cell lines and not due to the differences in information between the data sets, expression of three housekeeping genes (*RPS18, RPL11* and *RPS27A*) were used as a control group for this study. No significant differences in the expression of *RPS18* (one-way ANOVA, $P = 1$) (Figure 3A), *RPL11* (one-way ANOVA, $P = 0.7521$) (Figure 3B) and *RPS27A* (one-way ANOVA, $P = 0.2093$) (Figure 3C) across the three cell lines were observed.

Analysis of Class I HDAC genes revealed significant differences between the normal and prostate cancer cell lines. Up-regulation of *HDAC1* was noted in LNCaP cells compared to RWPE-1 and PC3 cells (one-way ANOVA, $P < 0.001$) (Figure 3D). The expression of *HDAC2* was significantly up-regulated in cancer cell lines compared to RWPE-1, but expression was greater in LNCaP than PC3 (one-way ANOVA, $P < 0.001$) (Figure 3E). A small decrease in the expression of *HDAC3* was noted in PC3 cells compared to RWPE-1 and LNCaP cells (one-way ANOVA, $P < 0.001$) (Figure 3F). Lastly, the expression of *HDAC8* was significantly up-regulated in both LNCaP and PC3 cells when compared to RWPE-1 cells (one-way ANOVA, $P < 0.001$) (Figure 3G).

The expression of Class IIA HDAC genes (*HDAC4*, *7*, *9*) was significantly up-regulated in prostate cancer cell lines compared to RWPE-1 (one-way ANOVA, $P < 0.001$) (Figure 3H to J). Among the three candidates, *HDAC9* showed (Figure 3J) the most increase in magnitude of change in PC3 cells, followed by LNCaP cells when compared to RWPE-1 cells. The expressions of *HDAC4* and *HDAC7* (Figure 3H) were shown to be higher in PC3 and LNCaP cells when compared to RWPE-1. Finally, results showed that there was a significant increase in Class IIB HDAC genes, *HDAC*6 (one-way ANOVA, $P < 0.001$) (Figure 3K) and *HDAC10* (one-way ANOVA, $P < 0.001$) (Figure 3L) in prostate cancer cell lines compared to RWPE-1 cells. The increase of magnitude of *HDAC10* was more in LNCaP cells than PC3 cells when compared to RWPE-1 cells (Figure 3L). Although the expression of *HDAC6* (Figure 3K) demonstrated similar changes in direction to

HDAC10 (≈ 4 fold increase), the change of magnitude was less in *HDAC6* (≈ 0.2 fold increase). The changes in gene expression (compared to RWPE-1 cells) of the examined *HDAC* genes are summarized in Table 2.

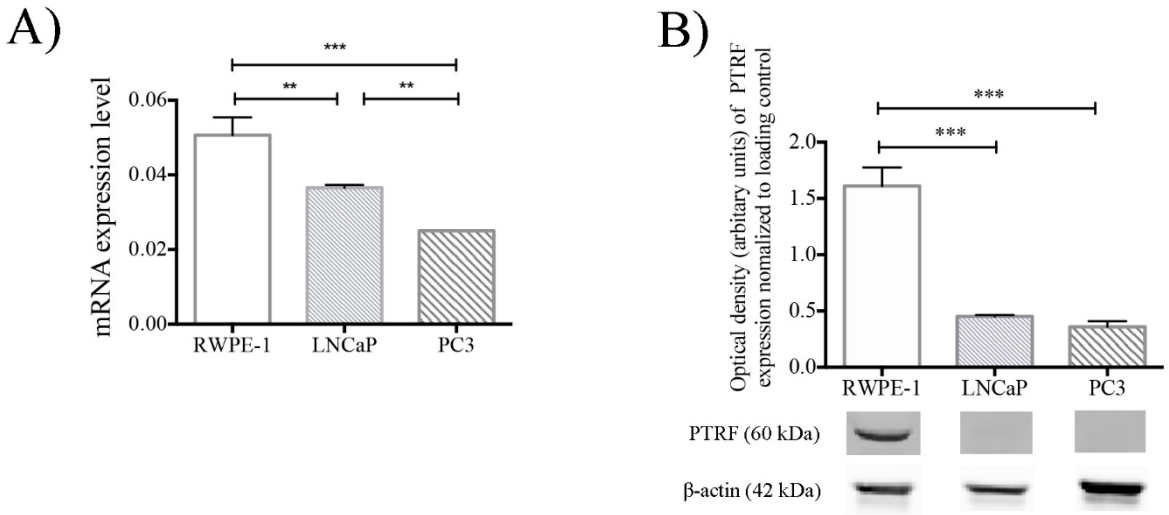

Figure 1. Endogenous mRNA and protein expression of PTRF in RWPE-1, LNCaP and PC3 cells. (A) Shows decreased expression of mRNA in LNCaP and PC3 cells compared with RWPE-1 cells through qRT-PCR. Data represent mean ± SE. One-way ANOVA with Tukey post hoc test, $^{**}P < 0.01$, $^{***}P < 0.001$, $n = 3$. (B) Western blot analysis showed a loss of PTRF protein expression in LNCaP and PC3 cells compared to RWPE-1 cells. Data represent mean ± SE. One-way ANOVA with Tukey post hoc test, $^{***}P < 0.001$, $n = 3$.

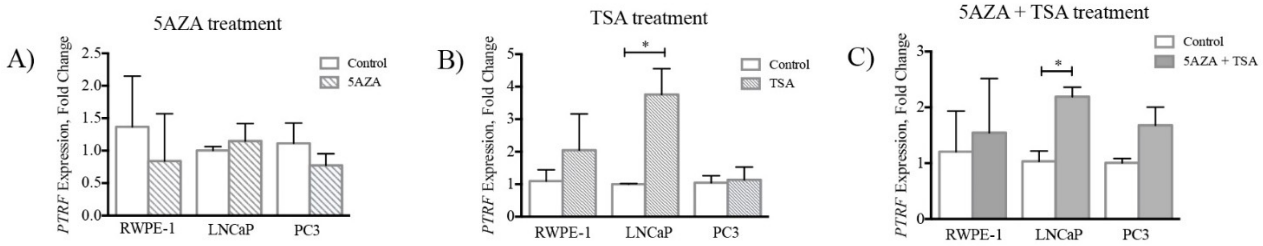

Figure 2. The effects of 5AZA, TSA and combination treatment on *PTRF* expression in normal and prostate cancer cell lines. (A) 5AZA treatment did not affect *PTRF* expression in RWPE-1, LNCaP and PC3 cells. (B) TSA treatment up-regulated the expression of *PTRF* in LNCaP cells. No changes were observed in RWPE-1 and PC3 cells. (C) Combination treatment of 5AZA and TSA up-regulated the expression of *PTRF* in LNCaP cells, but not RWPE-1 and PC3 cells. Data are expressed as fold change relative to the non-treated controls for each group. Data represent mean ± SE, Student's t-test $^{*}P < 0.05$, $n = 3$.

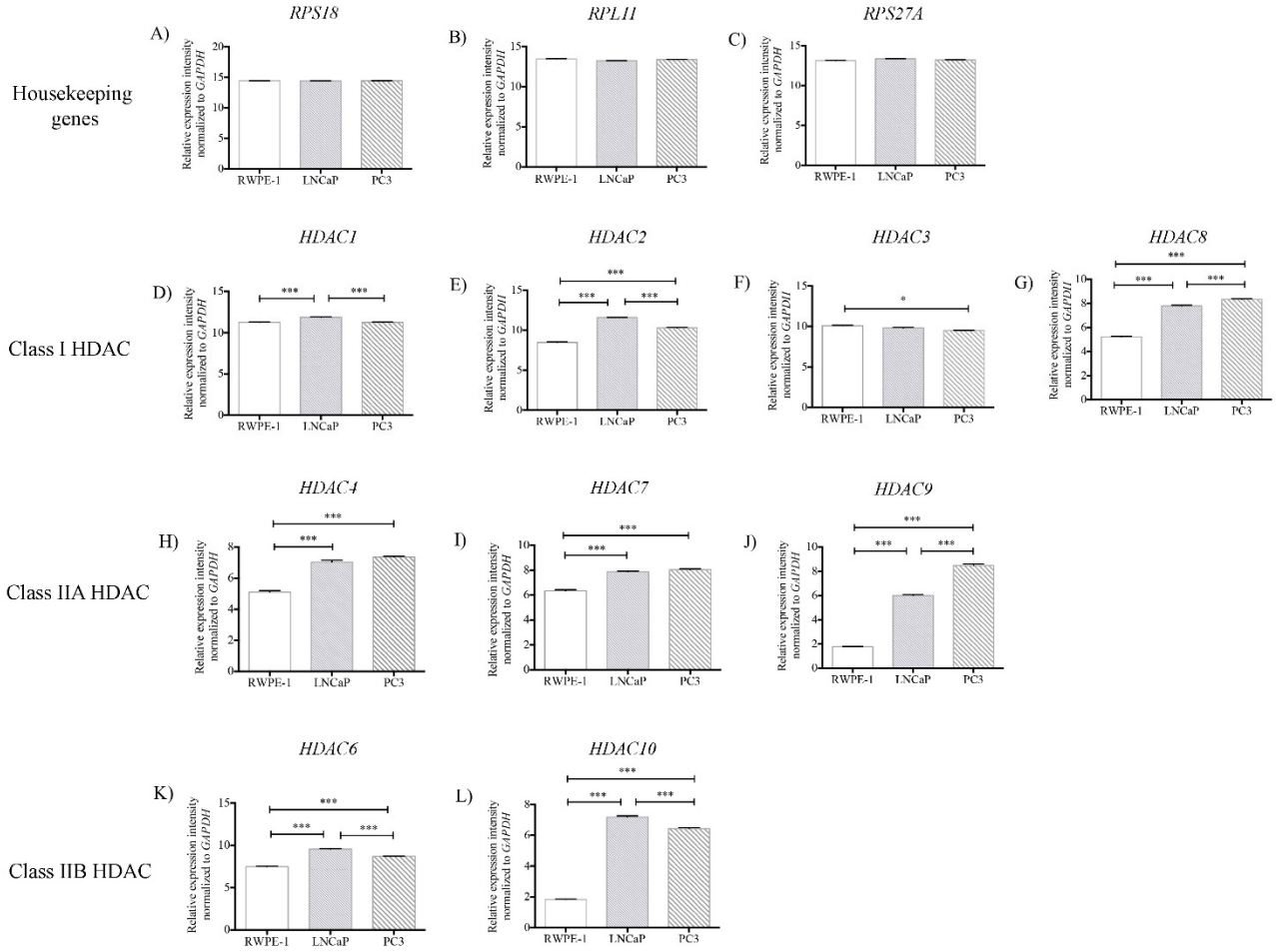

Figure 3. Bioinformatic analysis of possible candidate histone deacetylase genes that may be up-regulated in androgen dependent prostate cancer. A to C, Expression analysis of housekeeping genes in RWPE-1, LNCaP and PC3 cell lines to act as control group in this study. No significant changes were noted in the expression of *RPS18*, *RPL11* and *RPS27A*. D to G, Comparison of the expression of Class I HDAC genes, *HDAC1*, *HDAC2*, *HDAC3* and *HDAC8* in RWPE-1, LNCaP and PC3 cell lines. H to J, Expression analysis of Class IIA HDAC genes *HDAC4*, *HDAC7* and *HDAC9* in RWPE-1, LNCaP and PC3 cell lines. K and L, Expression analysis of Class IIB HDAC genes, *HDAC6* and *HDAC10* in RWPE-1, LNCaP and PC3 cell lines. Data represent mean ± SE. One-way ANOVA with Tukey post hoc test, $*P < 0.05$, $***P < 0.001$. RWPE-1 ($n = 12$), LNCaP ($n = 4$), PC3 ($n = 3$).

4. Discussion

In cancer, regulation of gene expression is controlled by both genetic and epigenetic mechanisms [17,18]. Our qRT-PCR and western blot data suggest that mRNA and protein expression of PTRF is down-regulated in both LNCaP and PC3 cells. This confirms the earlier report on the protein expression of PTRF in normal and prostate cancer cell lines and tissues [5]. With evidence that epigenetic mechanisms may play an important role in modifying the expression of *PTRF* [14], this study

hypothesized that the down-regulation of *PTRF* in prostate cancer might be related to epigenetic mechanisms.

Table 2. Summary of the changes in gene expression of HDAC genes in prostate cancer cell lines compared to RWPE-1 cells.

Class Type	*HDAC* genes	LNCaP	PC3
I	*HDAC1*	↑	No change
I	*HDAC2*	↑↑	↑
I	*HDAC3*	No change	↓
I	*HDAC8*	↑↑	↑↑↑
IIA	*HDAC4*	↑↑	↑↑
IIA	*HDAC7*	↑	↑
IIA	*HDAC9*	↑↑	↑↑↑
IIB	*HDAC6*	↑↑	↑
IIB	*HDAC10*	↑↑↑	↑↑

This study did not show any significant changes in *PTRF* expression after 5AZA or TSA treatment in normal, RWPE-1, prostate cells, or androgen independent prostate cancer cells (PC3). However, the expression of *PTRF* was significantly up-regulated in LNCaP cells after TSA and combination treatment suggesting that the expression of *PTRF* maybe suppressed through deacetylation of histones in the less aggressive and androgen dependent rather than the highly aggressive and androgen independent prostate cancer cell line. Additionally, our finding suggests that PTRF protein expression is almost undetectable in LNCaP cells. The expression of caveolin-1 (CAV1) is dependent on the expression of PTRF [24], and both CAV1 and PTRF proteins are only present at very low levels in LNCaP cells [5]. It is possible that *CAV1* is also down-regulated in LNCaP cells by histone deacetylation. However, more studies are required to validate this assumption.

DNA methylation and histone deacetylation have been reported to have synergistic effects in silencing cancer genes and it has been suggested that in order to maximize the re-expression of genes silenced in cancer, both mechanisms should be blocked [25]. It has been shown that tumor suppressor genes that have dense promoter methylation cannot be transcriptionally activated by histone deacetylase inhibitors unless DNA demethylating agents are first used [25]. This suggests that DNA methylation is usually the more dominant force in transcriptionally inactivating genes in cancer [25]. However, in this study, TSA successfully up-regulated the *PTRF* expression in LNCaP cells without the presence of a DNA methylating agent. Furthermore, addition of 5AZA into the treatment did not cause a further increase in magnitude of re-expression of *PTRF*. Thus, the expression of *PTRF* may be solely regulated through a histone deacetylation mechanism in LNCaP cells and independent of DNA methylation. Another possible explanation is that there is no significant methylation present on the promoter region of *PTRF*, however, more evidence is required to validate this assumption.

This study reports the involvement of histone deacetylation in an androgen dependent prostate cancer cell line. Histone deacetylases (HDACs) are the enzymes that facilitate deacetylation. With 18 mammalian HDACs identified to date [23], it is unclear which of the HDACs is playing a role in

regulating the expression of *PTRF* in prostate cancer. The HDACs can be sub-divided into several families. Class I HDAC consists of HDAC 1, 2, 3 and 8 that are ubiquitously expressed in human cell lines and tissues. These enzymes are predominantly localized to the nucleus. Class II HDACs enzymes can be further sub-divided into Class IIa and IIb. The expression of Class IIa enzymes (4, 7, 9) and IIb (6, 10) are tissue specific and these enzymes are able to move between the nucleus and cytoplasm, suggesting the ability of these enzymes to acetylate non-histone proteins. Class III HDACs (also known as sirtuins) consist of 7 members (1 to 7) that are homologous with the yeast Sir2 family of proteins and their sub-cellular localization and tissue distribution remains unknown. Lastly, HDAC 11 has been recently discovered and is the only member of the Class IV HDAC enzyme. HDACs from Class I and II are sensitive to TSA whereas Class III HDAC enzymes are insensitive to TSA [23]. Since data from this study showed that TSA can up-regulate the expression of *PTRF* in LNCaP cells, this narrows down the possibility to the involvement of Class I and Class II HDAC enzymes in modifying the expression of *PTRF* in androgen dependent prostate cancer.

Results from the bioinformatic analysis showed significant changes in the expression of HDAC genes from Class I and II. However, while some of the identified changes were statistically significant, they were minimal (e.g. *HDAC1*), questioning the biological significance of the changes. The HDAC genes (*HDAC2*, *HDAC6* and *HDAC10*) were up-regulated in LNCaP cells compared to RWPE-1 and PC3 cells (Table 2). This suggests that these *HDAC* genes are over-expressed in LNCaP cells and may be potential candidates worthy of further investigation. Collectively, it would be interesting to further examine the functional role of *HDAC2*, *HDAC6* and *HDAC10* in LNCaP cells in the future to further understand the histone deacetylation mechanism in regulating the expression of *PTRF*.

There are several limitations in this study. First, this study has only used 3 cell lines and further experiments using a broader range of cell lines and prostate tissues are required to validate our data. Data on the effect of knocking down the identified HDAC genes that are up-regulated in LNCaP cells is needed to address our hypothesis. Additionally, gene expression data from our bioinformatics study might not directly reflect the changes in the identified HDAC protein expression. A possible discrepancy between mRNA and protein expression might be present due to post-transcriptional mechanisms [26]. Therefore, our future strategy to address the above issues would be to quantify the protein expression of the identified HDAC genes and targeted knock down in LNCaP cells. More specific approaches such as detection of histone modification with antibodies and co-immunoprecipitation would strengthen our findings.

The use of HDAC inhibitors has shown positive results as an anti-cancer agent with promising efficacy in hematological and solid tumors both *in vitro* and *in vivo*. Furthermore, HDAC inhibitors do not possess any significant toxic side effects when introduced *in vivo* [27]. It is also possible to combine HDAC inhibitors with other treatment modalities such as traditional chemotherapy and radiation therapy to maximize the success rate and eradication of tumor cells [28].

Apart from acetylation, there are other modification mechanisms that are able to modify histone residues, including; methylation, phosphorylation, sumolyation, ubiquitination and ADP-ribosylation [29,30]. Notably, only acetylation and methylation of lysine will influence the chromatin structure and ultimately affect the transcriptional status of genes [29,30]. In addition to epigenetic silencing, another possible explanation for the observed down-regulation of endogenous *PTRF* expression in prostate cancer might be micro-RNAs (miRNAs), which are also up-stream regulators of gene expression.

It may be possible that mutations of the *PTRF* gene may take place in prostate cancer, although there are no reports to support this hypothesis. *PTRF* mutations have been identified and are associated congenital generalized lipodystrophy [31]. Hence, it is possible that genetic mutation of *PTRF* might play a role in promoting prostate cancer progression.

5. Conclusion

In this study, histone deacetylation was shown to be involved in regulating the expression of *PTRF* in LNCaP cells, but not PC3 cells. This may suggest that at different stages of prostate cancer different up-stream regulatory mechanisms are involved in modifying the expression of genes. However, as this study only investigated the role of epigenetics in the context of *PTRF* of *in vitro* and *in silico* models, it is important to interpret the data with care. There may be other up-stream regulatory mechanisms that are potentially involved in regulating the expression of *PTRF* in prostate cancer. Further studies are required to allow a better understanding of the up-stream mechanisms that regulate *PTRF* expression, especially histone deacetylation as it may provide a new treatment option for prostate cancer patients.

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Conflict of interests

The authors declare no conflict of interests in this publication.

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