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Androgenic to oestrogenic switch in the human adult prostate gland is regulated by epigenetic silencing of steroid 5α-reductase 2

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Abstract

Benign prostatic hyperplasia is the most common proliferative abnormality of the prostate. All men experience some prostatic growth as they age, but the rate of growth varies among individuals. Steroid 5α-reductase 2 (SRD5A2) is a critical enzyme for prostatic development and growth. Previous work indicates that one-third of adult prostatic samples do not express SRD5A2, secondary to epigenetic modifications. Here we show that the level of oestradiol is dramatically elevated, concomitant with significant upregulation of oestrogen response genes, in prostatic samples with methylation at the *SRD5A2* promoter. The phosphorylation of oestrogen receptor-α in prostatic stroma is upregulated when SRD5A2 expression is absent. We show that tumour necrosis factor (TNF)-α suppresses SRD5A2 mRNA and protein expression, and simultaneously promotes expression of aromatase, the enzyme responsible for conversion of testosterone to oestradiol. Concomitant suppression of SRD5A2 and treatment with TNF-α synergistically upregulate the aromatase levels. The data suggest that, in the absence of prostatic SRD5A2, there

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Author contributions statement

The authors contributed in the following way: ZW, LH, AFO: conceived experiments; ZW, LH, RG, CR, JP: performed experiments; ZW, LH, KS, SB, SW, CW, ST, DS: analysed data; ZW, KS, DS, AFO: wrote the paper; ZW, LH, KS, SW, JP: generated figures. All authors approved the submitted and published versions.

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SUPPLEMENTARY MATERIAL ONLINE

Supplementary materials and methods

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is an androgenic to oestrogenic switch. These findings have broad implications for choosing appropriate classes of medications for the management of benign and malignant prostatic diseases.

Keywords

androgenic to oestrogenic switch; prostate; epigenetic silencing; methylation; steroid 5α-reductase 2

Introduction

The prostate is the only solid organ that grows continuously throughout adulthood [1]. All men experience some prostatic growth as they age, but the rate of growth varies significantly among different men. Despite the widespread use of medical therapy, there is no completely effective medical treatment for benign prostatic hyperplasia (BPH). The current management of BPH with lower urinary tract symptoms includes watchful waiting, medical therapy, or surgery [2,3]. The medication regimen includes daily treatment with an α-adrenergic blocker (e.g. doxazosin, terazosin, tamsulosin, or alfuzosin) and/or a 5α-reductase inhibitor (5ARI) (i.e. finasteride or dutasteride). Steroid 5α-reductase 2 (SRD5A2) is necessary for prostatic development [4,5] and growth [6–8]. As a result, 5ARIs have played a major role in treatment of bladder outlet obstruction secondary to BPH. However, one-third of men are resistant to 5ARI therapies [2,3,9].

It has been generally accepted that *SRD5A2* is expressed in all human adult prostatic tissues. However, we have demonstrated that 30% of adult human prostatic tissues do not express SRD5A2 mRNA and protein [10]. We have shown that the somatic suppression of SRD5A2 during adulthood depends on epigenetic changes in the promoter region of the *SRD5A2* gene [11]. As with the neoplastic initiation and progression of many cancers [12], epigenetic changes and variable expression of *SRD5A2* in benign prostatic tissue constitute a plausible molecular mechanism of variable prostatic growth [11,13]. Epigenetic modifications of *SRD5A2* are dependent on the DNA methyltransferase (DNMT) family, and methylation of the *SRD5A2* promoter region is regulated by the upstream inflammatory mediators tumour necrosis factor (TNF)-α, nuclear factor-κB (NF-κB), and interleukin (IL)-6 [11].

Although the prostate is commonly thought of as an androgen target tissue, it is also an important target of oestrogens. Androgens and oestrogens exert similar, but distinct, effects on the prostate, and it is becoming clear that a finely tuned balance between the effects mediated by androgen receptor, by oestrogen receptor-α (ERα) and by oestrogen receptor-β (ERβ) is required for the maintenance of prostatic health [14]. Oestrogens directly and indirectly affect the growth and differentiation of the prostate [15–17]. Testosterone can be metabolized via CYP19/aromatase into the potent oestrogen, i.e. oestradiol-17β. In humans, the correlations of oestrogen levels with prostatic volume and other features of BPH remain unclear [18,19].

We have previously shown that clinical conditions associated with increased levels of inflammatory mediators (obesity and age) affect the methylation and expression of *SRD5A2*. In the present study, we examined the regulation of oestrogenic pathways when

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the prostatic conversion from testosterone to dihydrotestosterone (DHT) was blocked by *SRD5A2* downregulation.

Materials and methods

Patient specimens

With institutional review board approval, 59 prostatic specimens were collected from patients who underwent transurethral resection of the prostate (TURP) at Massachusetts General Hospital ($n = 57$) or radical prostatectomy of the prostate for symptomatic BPH at the University of Texas Southwestern Medical Center between November 2011 and December 2016. Retrospective clinical and pathological data for each patient were collected at both institutions. All prostatic samples were from the transition zone and were collected post-surgically in cold saline after pathological examination to exclude malignancy. One portion was formalin-fixed for paraffin embedding, one portion was frozen and stored at −80 °C for protein, DNA and RNA extraction, and another portion was processed for cell digestion and isolation [20–22]. Thirty-five of 57 TURP tissues were processed for immunohistochemistry, or homogenized for steroid sex hormone determination and aromatase evaluation. Clinical characteristics of the subjects are summarized in supplementary material, Tables S1 and S2.

High-performance liquid chromatography-tandem mass spectrometry (HPLC-MS)

The levels of testosterone, DHT and oestradiol were determined by HPLC-MS. Prostatic transition zone samples were homogenized, total protein concentrations were measured, and testosterone, DHT and oestradiol were then extracted by solid-phase extraction, followed by elution with high-performance liquid chromatography. The determination was performed by mass spectrometry with an electrospray ionization source. A deuterated stable isotope was utilized for the assay calibration. Testosterone test precision: intra-assay variation <2% relative standard deviation (RSD); interassay variation <8% RSD. DHT test precision: intra-assay variation <7% RSD; interassay variation <8% RSD. Oestradiol test precision: intra-assay variation <5% RSD; interassay variation <12% RSD.

Microarray analysis

Prostatic specimens obtained after TURP from 22 patients were characterized by *SRD5A2* promoter methylation status (12 methylated; 10 unmethylated). Total RNAs were extracted from each specimen with the RNeasy mini-kit (Qiagen, Valencia, CA). RNAs were then hybridized to Illumina (San Diego, CA) Human HT-12 v4 microarrays for performance of gene expression profiling according to the manufacturer's protocol. The microarray data were normalized by computing the mean background-subtracted signal for each probe and then performing $\log_2$ transformation with the Illumina GenomeStudio (version 2011) and the GenePattern software suite [23]. The microarrays were run in two batches, and batch effects were corrected for by ComBat with the non-parametric method [24]. The processed dataset was then analysed for differential expression between *SRD5A2*-methylated and *SRD5A2*-unmethylated samples by the use of Gene Set Enrichment Analysis (GSEA) [25]. A collection of expert curated 'hallmark' gene sets from the Molecular Signatures Database were tested for enrichment among *SRD5A2*-methylated or *SRD5A2*-unmethylated samples

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by GSEA [25]. Probe sets in the expression dataset were collapsed by gene symbol and ranked by weighted signal-to-noise ratios, and enrichment scores were compared with a null distribution generated by randomly permuting phenotype labels 1000 times. All microarray data were deposited in the NCBI GEO database (Accession Number: GSE101486).

Results

Molecular subtypes exist among BPH patients

To determine whether epigenetic silencing of *SRD5A2* leads to alternative molecular pathways driving growth in human prostatic tissues, we chose 22 patients from our cohort to explore gene expression signatures. Using GSEA to compare 12 *SRD5A2*-methylated with 10 *SRD5A2*-unmethylated samples [25], we identified three gene sets that were most significantly upregulated in *SRD5A2*-methylated samples: oestrogen response, sonic hedgehog signalling, and Myc transcription factor target genes (Figure 1). As oestrogens, like androgens, are known to regulate the development and growth of prostatic tissue [26], we wished to mechanistically assess whether oestrogen family members are differentially regulated in the absence of SRD5A2.

Androgen and oestrogen levels are modified in human prostatic tissue in the absence of SRD5A2

Although the importance of androgens in the induction and progression of prostate cancer is well established, the effect of metabolized byproducts of androgens and oestrogens on benign prostatic growth remains unclear [27]. As we found that lack of SRD5A2 transcription and translation is associated with upregulation of oestrogen response genes (Figure 1), we next wished to validate our findings. After immunohistochemical analysis of 35 subjects' prostatic samples, we divided the cohort into two groups: *SRD5A2*-methylated with no SRD5A2 protein expression [designated the SRD5A2(−) group], and *SRD5A2*-unmethylated with SRD5A2 protein expression [designated the SRD5A2(+) group] (supplementary material, Figure S1). We first confirmed by enzyme-linked immunosorbent assay (ELISA) that the SRD5A2 level was higher in the SRD5A2(+) group than in the SRD5A2(−) group ($P = 0.001$; Figure 2A). We then investigated whether oestrogenic pathways are upregulated when androgenic pathways are blocked in the absence of prostatic SRD5A2. We found that the level of testosterone was higher in the SRD5A2(−) group than in the SRD5A2(+) group ($P = 0.002$; Figure 2B). As expected, DHT levels were lower in the SRD5A2(−) group ($P = 0.0003$; Figure 2C). The level of oestradiol in the SRD5A2(−) group was elevated as compared with the SRD5A2(+) group, which suggests preferential conversion of testosterone to oestradiol ($P = 0.0002$; Figure 2D). As conversion of testosterone to oestradiol is facilitated by CYP19/aromatase, we evaluated aromatase levels, which were elevated in prostatic samples lacking SRD5A2 ($P = 0.003$, Figure 2E). Furthermore, prostatic DHT levels were inversely correlated with testosterone levels (rho = −0.614, $P = 0.005$, Spearman rank correlation test; Figure 2F). Aromatase levels were also negatively correlated with DHT levels (rho = −0.519, $P = 0.023$; Figure 2G). Although we found a positive trend between oestradiol and aromatase, and a negative trend between oestradiol and DHT, these were not statistically significant (supplementary material, Figure S2).

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The ratio of androgen to oestrogen [testosterone/oestradiol (T/E) ratio] in the serum and prostatic tissue decreases with age [28]. We have found that ageing is associated with reduced SRD5A2 levels and increased methylation of the *SRD5A2* promoter region [11]. To determine whether absence of SRD5A2 expression is associated with prostatic hormonal changes, we calculated T/E ratios, which were lower in the SRD5A2(−) samples ($P = 0.04$; Figure 2H). Collectively, these data suggest that, in the absence of SRD5A2 resulting from promoter methylation [11], there is an androgenic to oestrogenic switch in adult human prostatic tissue.

Because of its role in the conversion of androgens to oestrogens, we evaluated the level of aromatase in prostatic cellular compartments. Consistent with previous studies [29], aromatase was exclusively expressed in the stromal compartment, as assessed in primary cultured prostatic epithelial and stromal cells at the mRNA level, and in prostatic stromal tissue at the protein level (supplementary material, Figure S3). We next wished to evaluate the oestrogen response molecules that respond to an absence of SRD5A2. Phosphorylated ERα (pERα) was upregulated in human prostatic samples that lacked SRD5A2 protein expression, whereas protein levels of ERα, ERβ and phosphorylated ERβ (pERβ) were not affected (Figure 3A). These findings were confirmed by immunohistochemical analysis of prostatic tissue, which showed selective upregulation of aromatase and pERα in samples lacking SRD5A2 expression (Figure 3B).

TNF-α selectively activates aromatase in prostatic stromal cells but not in epithelial cells

Inflammatory changes have long been associated with the histological changes seen in BPH, and autonomous regulation of prostatic epithelial cell proliferation has been shown to include upregulation of specific cytokines [22]. We have shown that methylation of the *SRD5A2* promoter region is regulated by the inflammatory mediators TNF-α, NF-κB and IL-6 via DNMT1 [11]. Here, we investigated whether cytokines influence the activity of aromatase. We isolated primary prostatic stromal cells from fresh tissues that were harvested after debulking surgery for the management of bladder outlet obstruction secondary to BPH. Primary prostatic stromal cells were treated with TNF-α at 5, 10, 20 and 40ng/ml for 24 h. Administration of TNF-α resulted in reductions in SRD5A2 mRNA (Figure 4A) and protein levels [11]. More importantly, TNF-α upregulated the mRNA expression of aromatase (*CYP19A1*) and ERα (*ESR1*) in a dose-dependent manner (Figure 4B, C). In contrast, we found no change in SRD5A1 (Figure 4D) or ERβ (data not shown) expression when stromal cells were treated with TNF-α.

As interactions between stromal and epithelial cells play an important role in maintaining prostatic homeostasis [30,31], we tested whether the effect of TNF-α on epithelial cells is associated with SRD5A2 expression. In benign prostatic epithelial (BPE) BPH-1 cells, increasing doses of TNF-α did not affect the levels of SRD5A2, aromatase, or ER-α (supplementary material, Figure S4). Our data suggest that TNF-α affects the prostatic androgen and oestrogen balance by targeting the stromal cells, with little effect on epithelial cells.

To further evaluate the role of TNF-α in oestrogen biosynthesis in the stroma at the post-transcriptional level, we treated primary prostatic stromal cells with different concentrations

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of TNF-α for 24 h, and then immunostained for SRD5A2 and aromatase (Figure 4E). Administration of TNF-α to stromal cells inhibited the expression of SRD5A2 (green), but simultaneously promoted the expression of aromatase (red), in a dose-dependent manner. Quantification of the immunofluorescent images suggested that aromatase levels were elevated with increasing concentrations of TNF-α, and there was a statistically significant difference from controls at 40 ng/ml (Figure 4F, G). Together, these data suggest that TNF-α not only suppresses SRD5A2 expression, but also promotes upregulation of aromatase.

TNF-α promotes upregulation of aromatase while suppressing SRD5A2 levels

To further examine the effect of TNF-α on aromatase in the presence and absence of SRD5A2, primary cultured prostatic stromal cells were treated with TNF-α (20ng/ml, 24 h). The aromatase level was increased with TNF-α treatment, and also when SRD5A2 expression was suppressed by small interfering RNA transfection (Figure 5A). Concomitant suppression of SRD5A2 and treatment with TNF-α synergistically increased aromatase levels (Figure 5A). On the other hand, ectopic expression of SRD5A2 in prostatic stromal cells did not affect intracellular aromatase levels (data not shown).

In addition to assessing intracellular aromatase levels, we determined whether TNF-α affects paracrine secretion of aromatase. Unlike intracellular levels, aromatase levels in culture medium were not significantly affected after treatment with TNF-α or suppression of SRD5A2 (Figure 5B). However, after ectopic expression of SRD5A2 in prostatic stromal cells, the level of secreted aromatase in culture medium was reduced (Figure 5C).

Paracrine signalling and mesenchymal–epithelial cell interactions are essential components of androgenic control of the prostate gland [30]. We have previously shown that stromal cells can regulate the proliferation of epithelial cells [31,32]. Here, we examined whether aromatase levels were modified in epithelial cells with modifications in SRD5A2, and whether this change in epithelial cells affects stromal cells. We chose prostatic primary BPE cells, which express SRD5A2 and are not methylated at the *SRD5A2* promoter [10]. As aromatase is predominantly expressed in stromal cells (supplementary material, Figure S3), we detected only trace levels of aromatase (20 ng/g tissue) in prostatic epithelial cells at baseline when SRD5A2 was silenced (Figure 5D). Addition of TNF-α dramatically stimulated aromatase production (Figure 5D). Our findings are in line with previous reports that increased production of prostatic and circulating aromatase is associated with inflammatory change [33].

Finally, to test whether epithelial cell secretion affects aromatase levels in stromal cells, we cultured prostatic stromal cells with BPE cell-conditioned medium. Aromatase levels were unchanged in stromal cells after treatment with the conditioned medium of control cells, of cells subjected to SRD5A2 suppression, or cells subjected to SRD5A2 suppression plus TNF-α treatment (Figure 5E). However, when we used conditioned medium from BPH-1 cells, which do not express SRD5A2 [10], treatment with TNF-α alone increased the stromal cell aromatase expression (Figure 5F). However, conditioned medium from BPH-1 cells with restored SRD5A2 expression plus TNF-α treatment suppressed stromal cell aromatase expression (Figure 5F).

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Discussion

Prostatic development and growth depend largely on androgens. Despite advances in our understanding of prostatic development during gestation [34] and the pathogenesis of prostatic neoplasia [35–37], very little is known about prostatic growth during adulthood. We have shown that SRD5A2 is absent in 30% of adult human prostatic tissues [11], owing to methylation of the *SRD5A2* promoter, regulated by the DNMT1 family of genes (a process that is enhanced by conditions associated with increased inflammatory mediators), ageing, and obesity. Here, we demonstrate that absence of SRD5A2 expression is associated with an androgenic to oestrogenic switch, which may explain the variable growth pattern of human adult prostates, and provides a rationale for alternative pathways for the management of BPH in patients who are resistant to 5ARIs.

Two distinct oestrogen receptors, ERα and ERβ, act as hormone-inducible transcription factors. In the normal prostate and BPH, ERα is localized to stromal cells, and oestrogen mediates its effects on the prostatic epithelium through paracrine pathways [14,38], although epithelial cells in prostatic periurethral ducts also express ERα [39]. The action of oestrogens can be complex, and they can have both proliferative and inhibitory effects via ERα and ERβ. The role of ERα has been associated with increased proliferation and inflammatory changes in the prostate [27]. Here, we found that pERα was activated in human prostatic samples that lack SRD5A2 protein expression, although protein levels of ERα, ERβ and pERβ were not affected (Figure 3A). As the balance between ERα and ERβ plays a major role in modulating prostatic growth [15,17,40], our findings suggest that, in the absence of SRD5A2, alternative oestrogenic pathways are activated that may affect prostatic proliferative capacity [27], and that may serve as alternative targets for the treatment of BPH in selected patients who lack SRD5A2 expression [41].

With advancing age, oestrogen levels remain constant in the epithelium, but increase in the stroma [42]. In elderly men, the ratio of free testosterone to free oestradiol in plasma declines by up to 40% [43], reflecting an androgenic to oestrogenic switch. Here, we found that human prostatic tissues lacking SRD5A2 expression have a lower T/E ratio than prostatic tissues that express SRD5A2 (Figure 2H). Our data suggest that, in the absence of SRD5A2, there is an androgenic to oestrogenic switch in the human adult prostatic tissue, owing to methylation of the *SRD5A2* promoter region [11].

Aromatase encoded by the *CYP19A1* gene catalyses the conversion of androgens to oestrogen [44]. Associations between aromatase gene polymorphisms and the risks of prostatic hyperplasia and malignancy have been identified [45–48]. Aberrantly expressed aromatase in prostatic epithelial cells and infiltrating inflammatory cells is also associated with the progression of prostate cancer [27]. However, the molecular mechanism and actions of aromatase in human benign prostatic disease remain largely unknown. Aromatase plays a key role in maintaining prostatic homeostasis. Loss of aromatase expression causes decreased oestrogen-induced prostatic proliferation [29,49], although, in clinical trials, the selective aromatase inhibitor atamestane had no effect on established BPH [50,51], which may have been a result of inadequate selection of patients who have elevated aromatase levels in the setting of absent SRD5A2. The proinflammatory cytokines IL-1, IL-6 and TNF-

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α stimulate aromatase activity and induce the conversion of androgens to oestrogens. In the breast tissue of overweight and obese women, clinical data suggest that inflammation associated with obesity promotes aromatase activity [52]. These findings suggest that low androgen levels and disease states associated with increased levels of inflammatory mediators promote aromatase activity. In concert with these studies, we found that a significant increase in aromatase level is associated with absence of SRD5A2 expression and methylation of the *SRD5A2* promoter. More importantly, increased aromatase levels are negatively correlated with DHT levels (Figure 2G), suggesting that increased oestrogenic levels in the absence of SRD5A2 may serve as an alternative pathway to promote prostatic growth.

We have shown that TNF-α regulates aromatase activity in an SRD5A2-dependent manner. TNF-α increases aromatase levels in primary cultured prostatic stromal cells from BPH patients. Furthermore, suppression of SRD5A2 in stromal cells increased aromatase activity, and TNF-α and SRD5A2 suppression synergistically promoted aromatase activity (Figure 5A). Interestingly, in ectopic SRD5A2-overexpressing stromal and epithelial BPH-1 cells, TNF-α did not increase aromatase activity as expected, but reduced aromatase production both in cells and in culture media (Figure 5B, D, G).

Our findings have broad implications for the chronic use of 5AR2 inhibitors for the management of BPH [9]. With activated oestrogenic pathways that modulate prostatic growth, alternative treatment strategies for the management of BPH in carefully selected patients who lack SRD5A2 expression may be more scientifically sound [41].

Beyond the management of benign prostatic diseases, variable expression of SRD5A2 has implications for the use of 5ARIs for prostate cancer chemoprevention [53,54], and even the treatment of patients with castration-resistant prostate cancer (CRPC). In CRPC managed with the CYP17A1 inhibitor abiraterone, addition of 5ARI has been shown to block the production of tumour-promoting metabolites and permit accumulation of Δ^4-abiraterone, which has stronger antitumour activity [55]. Therefore, the presence of SRD5A2 may be crucial for efficacy when combinations of CYP17A1 and 5ARIs are considered for patients with advanced prostate cancer.

In summary, lack of SRD5A2 expression in the prostate induces an androgenic to oestrogenic switch in human benign prostatic tissues, via increased stromal levels of aromatase and pERα. The proinflammatory mediator TNF-α suppresses SRD5A2, while simultaneously promoting the expression of aromatase. We conclude that, in the absence of prostatic SRD5A2 when androgenic pathways are blocked, the alternative oestrogenic pathways are upregulated to drive prostatic growth. Whereas anti-androgen pathways have been major targets for the treatment of patients with BPH [56], our data suggest that, in carefully selected patients who lack SRD5A2 expression, oestrogenic pathways may serve as effective treatment targets. We believe that the knowledge gained from this study will enable us to appropriately offer 5ARI therapies to those who express SRD5A2.

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Supplementary Material

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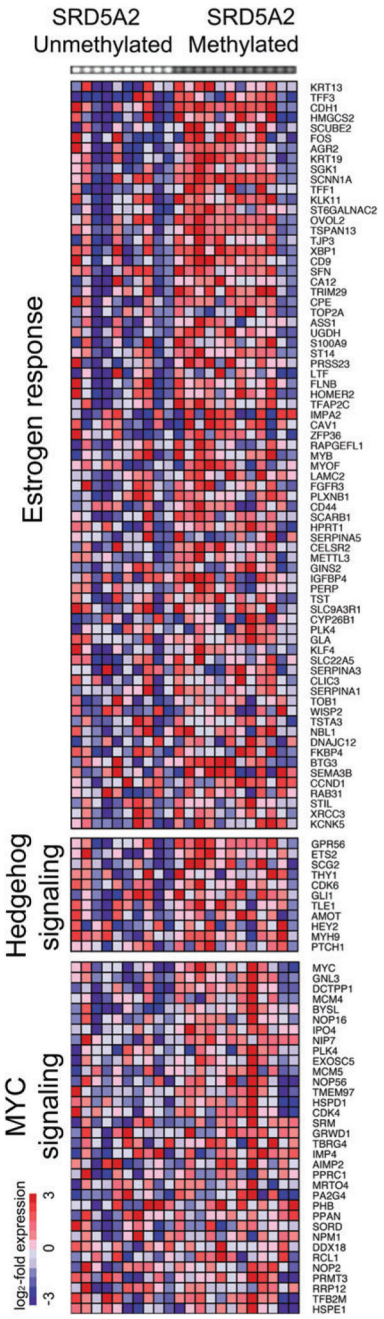

Figure 1.

Microarray gene expression analysis of human prostatic tissue, comparing samples with or without methylation at the *SRD5A2* promoter. Oestrogen response, hedgehog signalling and MYC signalling are among the most significantly upregulated pathways in *SRD5A2*-methylated samples as compared with unmethylated samples.

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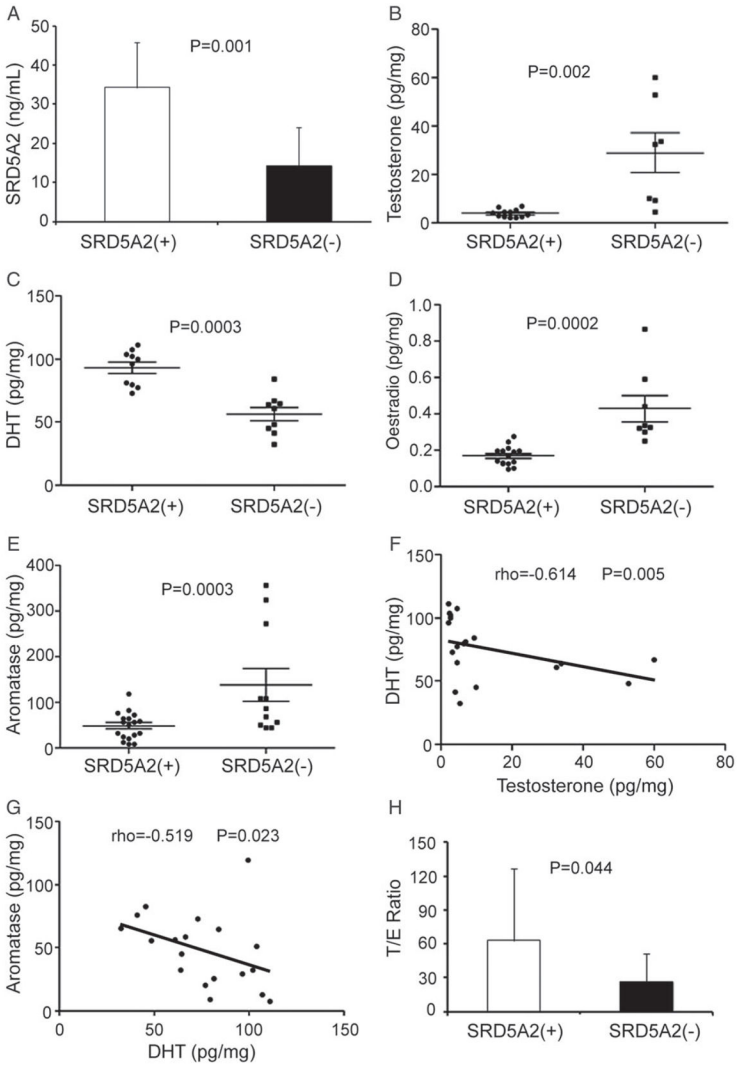

Figure 2.

Androgen, oestrogen and aromatase levels in BPH prostatic samples. (A) SRD5A2 level, measured with ELISA. (B–D) Levels of testosterone (B), DHT (C) and oestradiol (D), measured by HPLC-MS. (E) Aromatase level, measured with ELISA. (F) DHT levels are negatively correlated with testosterone levels. (G) Aromatase levels are negatively correlated with DHT levels. (H) T/E ratio. Continuous variables were assessed with the Wilcoxon rank sum test. The association between two different parameters was assessed with Spearman rank correlation. The data in (A) and (H) represent means of average determinants ± standard error of the mean.

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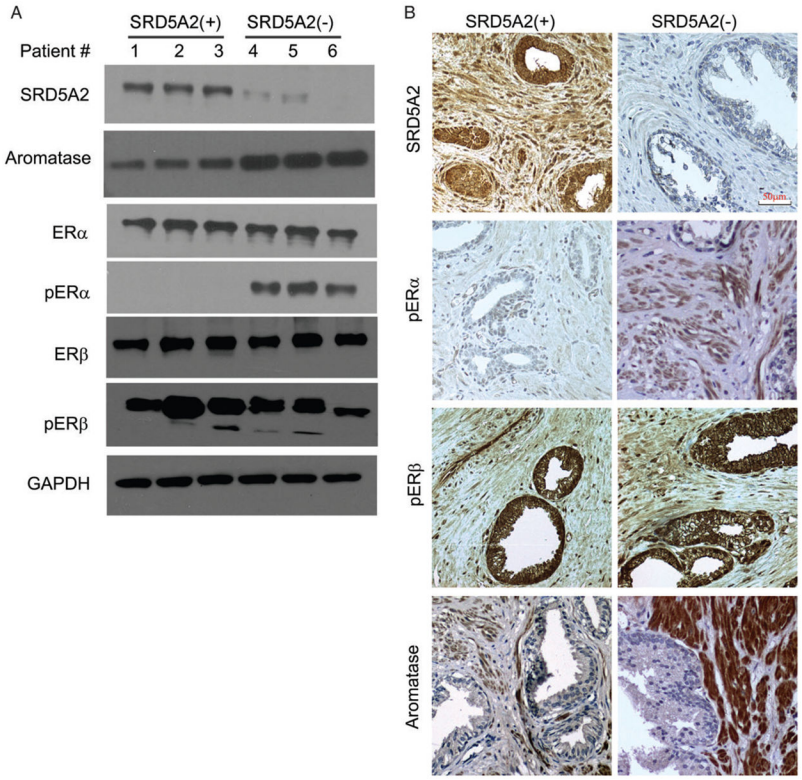

Figure 3.

Aromatase and pERα are upregulated in the absence of SRD5A2. (A) Representative figures for protein expression by immunoblot assay. Patients 1, 2 and 3 represent *SRD5A2*-unmethylated samples; patients 4, 5 and 6 represent *5RD5A2*-methylated samples. (B) Representative pictures of immunohistochemical analyses demonstrating increased levels of pERα and aromatase when SRD5A2 expression was absent. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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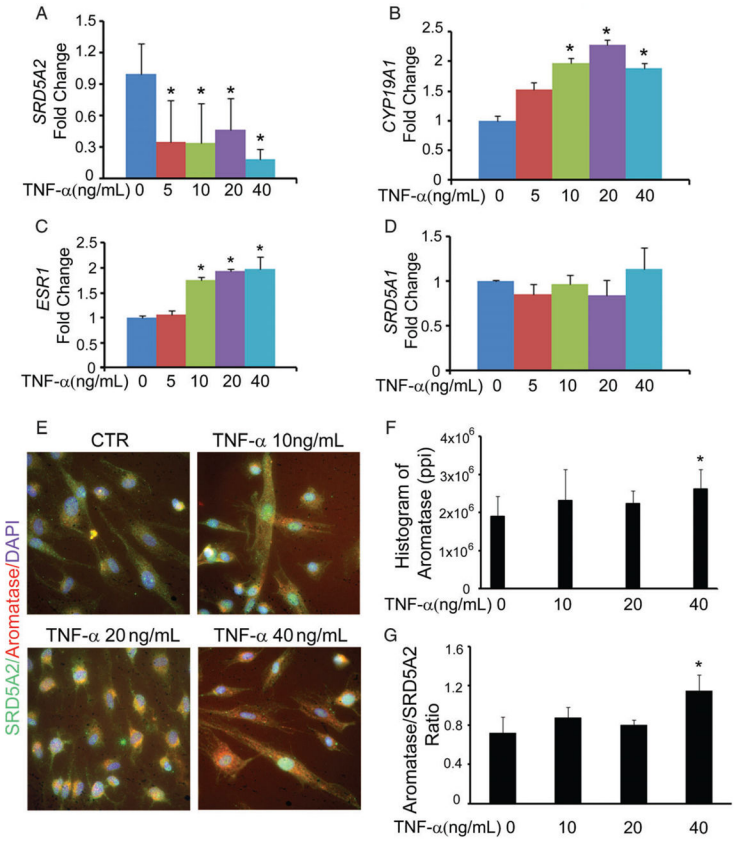

Figure 4.

TNF-α regulates aromatase activity in prostatic stromal cells. Primary prostatic stromal cells were treated with 5, 10, 20 and 40 ng/ml TNF-α for 24 h, and this was followed by RNA extraction or immunostaining. (A–D) Data of quantitative polymerase chain reaction analysis are presented as the fold change of mRNA expression for *SRD5A2* (A), aromatase (*CYP19A1*) (B), ERα (*ESR1*) (C), and *SRD5A1* (D). (E–G) Stromal cells were immunostained with anti-SRD5A2 and anti-aromatase primary antibodies. (E) Representative pictures of immunostained cells. (F) Histogram of image stained with anti-aromatase antibody. Ppi, pixels per inch. (G) The aromatase-positive/SRD5A2-positive ratio. The data represent means of average determinants ± standard error of the mean. All experiments were repeated independently at least three times, with similar results. *$P < 0.05$ as compared with the phosphate-buffered saline-treated vehicle control group (CTR). DAPI, 4′,6-diamidino-2-phenylindole.

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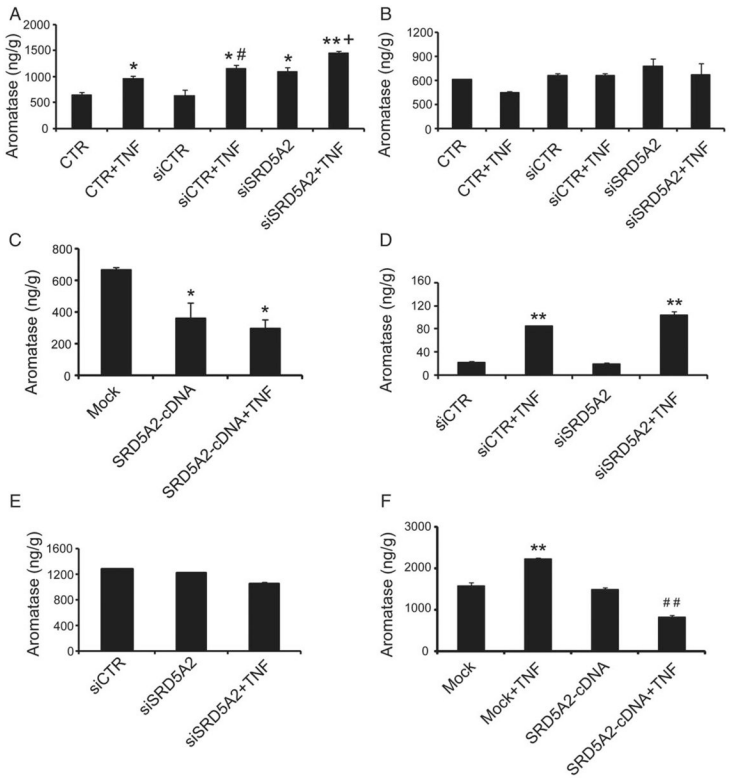

Figure 5.

TNF-α and SRD5A2 synergistically regulate aromatase activity in prostatic stromal cells. (A—C) Primary prostatic stromal cells were transfected with SRD5A2 siRNA (A and B) or SRD5A2-cDNA plasmids (C) for 48 h; this was followed by 20 ng/ml TNF-α treatment for 24 h, and then cell lysate (A) or culture medium (B and C) was analysed to evaluate aromatase with ELISA. (D) Primary prostatic epithelial BPE cells were transfected with SRD5A2 siRNA for 48 h, subjected to 20 ng/ml TNF-α treatment for 24 h, and then lysed for evaluation of aromatase with ELISA; the medium was used for stromal cell culture. (E and F) Stromal cells were harvested for aromatase evaluation after culture with BPE-conditioned medium (E) or BPH-1-conditioned medium (F) for 24 h. Prostatic epithelial BPE/siSRD5A2 cells (E) or BPH-1/SRD5A2 cells (F) were reseeded and treated with 20 ng/ml TNF-α for 24 h, and conditioned medium was collected. Stromal cells were cultured with conditioned medium for 24 h, and cell lysate was analysed. The data represent means of average determinants ± standard error of the mean. All experiments were repeated independently at least three times, with similar results. $^{*}P < 0.05$ and $^{**}P < 0.01$ as compared with mock or phosphate-buffered saline-treated control group (CTR). $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ as compared with siCTR (A) or SRD5A2-cDNA (C and F). $^{+}P < 0.05$ as compared with siSRD5A2 (A). Mock: control for SRD5A2 cDNA.