Original Research

A single low dose of cadmium exposure induces benign prostate hyperplasia like condition in rat: A novel benign prostate hyperplasia rodent model

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Abstract

Abnormal prostate growth is the most prevalent pathological sign in aged human males, as reflected by high incidence of benign prostate hyperplasia (BPH) and prostate cancer. In spite of the high prevalence, the etiology and pathophysiology of BPH is unclear due to the lack of any established rodent model for study. It has been demonstrated that the cadmium (Cd) mimics the activity of androgen or estrogen by interacting with the steroid hormone receptors in the prostate and elicits BPH, but the specific receptor which binds to Cd is still unknown. Our lab studies with BPH patients highlighted a strong co-relation between smokings with increased Cd content. Changes in the maximum urinary flow rate (Qmax) and prostatic acid phosphatase (PAP) level further supports that Cd can induce BPH like condition. Therefore, the present study was aimed to induce BPH like condition in rats by Cd administration. The dose of cadmium was standardized in an age- and time-dependent manner, which was further examined by prostate weight, histology, and PAP levels that elucidated the pathogenesis of BPH. Further to understand the molecular basis, steroid hormone receptor antagonist experiment was performed. Gene expression and immunohistochemistry data suggest that Cd induces hyperplasia like condition by activating the androgen receptor and estrogen receptor-α and suppresses the action of estrogen receptor-β. The experimental model used here is a cost effective, less time consuming and potentially valuable tool for investigating the respective functions of epithelial and stromal hormone receptors. The applicability of this model would be helpful in understanding the pathogenesis of BPH and its progression.

Keywords: Prostate, benign prostate hyperplasia, cadmium, steroid hormone receptor, animal model

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Introduction

Benign prostate hyperplasia (BPH) is a common disease of old age. It has a high public health impact and is one of the most common reasons for surgical intervention among elderly men. Anatomic or microscopic evidence of BPH is present at autopsy in approximately 55% of men aged between 60 and 70 years.[1] Many attempts have been made during the last decade to obtain a thorough understanding of the BPH pathogenesis. In spite of this, the etiology and pathophysiology of the disease remains unclear because of the lack of suitable animal models. Transurethral resection has been the treatment of choice from the last decade. Recently, less invasive therapies such as laser prostatectomy,[2] thermotherapy,[3,4] and complementary medications[5] have been introduced. Nonsurgical methods and laser treatments have been satisfactory and cost-effective therapeutic options for patients. As a consequence, human BPH tissue would be unavailable for future studies. Thus, it is inevitable to develop an animal model in order to unravel the disease pathogenesis.

Spontaneous BPH is rare in species other than human. It has only been described in dogs and chimpanzees.[6] Gene knockdown, xenograft, and hormone-induced *in vitro* models are the alternatives for BPH induction in other species.[7–9] Variety of growth factors, steroid hormones, and proteases are involved in normal prostatic morphogenesis and function; however, their role in BPH and prostate cancer (PCa) are poorly understood. The development of BPH in men is commonly attributed to testicular hormones and aging. The principal androgen responsible for prostate development is dihydrotestosterone or DHT (a derivative of testosterone). Testosterone gets converted into DHT by prostate specific enzyme 5α-reductase, which occurs in three isoenzyme forms. Type-1 isoenzyme is predominantly expressed in the liver and skin whereas type-2 and 3 are

expressed in the prostate.[10,11] DHT has a very high binding affinity to androgen receptors. Studies showed that hyperplasic areas usually have higher concentrations of androgen receptors as compared to the normal areas[12] with altered testosterone to DHT ratio, one of the major factor for the cause of BPH.[13]

Occupational and environmental studies suggest a potential role of cadmium (Cd) in the prostate enlargement.[14,15] Cd is responsible for increased incidence of the prostate and other cancers in men exposed to high levels of this metal or its compounds[16] due to its potent androgenic and estrogenic activity.[17] The metal binds with high affinity to the hormone-binding domain of both androgen and estrogen receptors and activates them.[18] Rat and mice prostates have been documented for their response to hormone and chemical carcinogen treatment. However, only the dorso-lateral lobe of the rodent prostate is ontogenetically comparable to the human prostate.

Increased expression of Androgen Receptor (AR) in the dorsal and lateral lobes of the prostate is directly associated with the continuous growth of the gland in age-dependent spontaneous hyperplasia.[19]

In normal and malignant human prostate, Estrogen Receptor-α (ER-α) is predominantly expressed in the stroma, whereas Estrogen Receptor-β (ER-β) is a dominant estrogen receptor in both normal stroma and epithelium. It has been earlier reported that the loss of ER-β expression is associated with progression from hyperplastic prostate epithelium to PCa.[20]

Histological examination is one of the techniques to identify prostate morphology. The histology of PCa shows irregular growth of glandular epithelium with loss of ductal morphology.[21] Basal cells are absent in adenocarcinoma of the prostate, whereas they are present in the BPH.[22]

Martin *et al.* 2002 reported presence of epithelial proliferation and infolding in animals treated with cadmium.[23] Similarly results from our lab with BPH patients' data also demonstrated possible association of Cd content, smoking, maximum urinary flow rate (Qmax), and prostatic acid phosphatase (PAP) level with severity of BPH.[24]

The purpose of this study was to establish BPH like condition in rats using heavy metal Cd. To further understand the mechanism, how Cd induces BPH by its androgenic mimicking action, an experiment was performed with steroid hormone receptor blockers. *In vivo* models are useful for studying, the mechanisms of disease progression and regulation, as well as in understanding the pathophysiology of the organ. Hence, in the present study an attempt was made to develop an *in vivo* model for understanding the pathogenesis of BPH.

Materials and methods

Chemicals

All primary mouse monoclonal antibodies and antagonists were purchased from Sigma-Aldrich, USA (Anti-Androgen Receptor cat no A9853, Anti-Vimentin cat no. C9080, Anti Ki-67 cat no. P6834, Nilutamide cat no. N8534, MPP cat no. M7068 and 4-Hydroxytamoxifen cat no. H7904) and Anti-E-Cadherin cat no.610181 from BD Biosciences, USA. Reverse-Transcriptase PCR (RT-PCR) reagents from Fermentas, Germany. Cadmium acetate and sodium acetate were obtained from SISCO Pvt Ltd Research Laboratories, India. All the chemicals were extra pure and of analytical grade.

Animals

Healthy adult male Charles foster rats, weighing about 250–350 g of age 5 months and 1 year were used. The animals were housed in clean polypropylene cages and kept in an air-conditioned animal house with constant 12-h light/dark cycle. Rats were allowed free access to drinking water throughout the experimental period. The animals were fed with standard rat pellet diet (Lipton India Ltd, Mumbai, India). The experiments were approved by the Institute Animal Ethical Committee (CEPSC Reg. No. 938/a/06/CPCSEA).

Time-dependent study

To determine the optimum time of BPH development, a single intraperitoneal (i.p.) dose of 20 μg/kg body weight Cd is administered (the dose used here was equivalent to the daily exposure of metal from food and drinking water as per the literature).[25,26] Animals were divided into six groups, each group contained six rats. Animals were administered with a single cadmium acetate dose by i.p. injection with their respective controls. Rats were sacrificed after 10th, 20th, and 30th days of experimental regime and prostate glands were surgically removed.

Age-dependent study

BPH is a progressive disorder of old age males. To further explore the effect of cadmium, 1-year and 5-month-old animals were treated with a single i.p. dose of Cd 20 μg/kg body weight along with control animals. All lobes of the prostate gland were dissected out from each group on the 10th day after Cd administration for analysis and evaluation of disease conditions.

Castration study

Prostate growth is primarily dependent on androgens. The heavy metal cadmium has an androgen mimicking activity. In the present study, 5-month-old animals were surgically castrated. After a 7-day recovery, the animals received a single i.p. injection of Cd (20 μg/kg body weight) whereas control animals were treated with sodium acetate. After 10 days prostate gland was surgically removed to examine the androgenic activity of Cd.

Prostatic acid phosphatase analysis

Measurement of serum PAP level indicates prostatic cell growth. Hence, blood was collected and PAP activity was estimated in serum by hydrolyzed phenol method. PAP converts p-nitrophenyl phosphate into p-nitrophenol which can be measured at 405 nm.[27] The addition of tartrate in the sample will lead to inhibition of PAP and by subtracting it from the total activity (without tartrate) will give the PAP activity.

Reactive oxygen species (ROS) parameters

ROS are known to be the mediators of phenotypic and genotypic changes that lead to neoplasia. Hence, it is important to investigate the role of Cd in the production of ROS and its action as a potent carcinogen. For determination of ROS in cadmium induced BPH like condition, prostate tissue was evaluated for reduced glutathione (GSH) content measured by the method of Beutler and Gelbart.[28] Reduced GSH reacts with 5-5′ Dithiobis (2-nitrobenzoic) acid to yield a yellow color which can be measured at 412 nm. Lipid peroxidation (LPO) is estimated by the method of Ohkawa *et al*. LPO leads to the formation of an endoperoxide and gives Thiobarbituric acid reactive substances (TBARS), which can be measured at 532 nm.[29]

Histological examination

Prostate glands were fixed in 10% buffered formalin solution, 3 μm thick tissue sections were cut and stained with hematoxylin/eosin stain. Histological observations such as number of acini and mitotic figures were quantified per 40× objective microscopic field (Table 1), and epithelial cell invaginations and basement membrane integrity were examined by Nikon TES2000 microscope (Nikon, Japan) using 20× objective. Histology of BPH samples was evaluated by a surgical pathologist.

Antagonist studies

The aim of this study was to determine the molecular mechanism of BPH progression due to cadmium, using the steroid hormone receptor antagonist in Cd induced BPH rats. Animals were divided into nine groups and administered with a different steroid hormone receptor antagonist along with Cd (20 μg/kg body weight). AR antagonist nilutamide: 10 mg/kg/day i.p.,[30,31] ER-α antagonist methyl piperidine pyrazole: 50 μg/kg body weight/day i.p.,[32] and ER-β antagonist 4-hydroxytamoxifen: 1 mg/kg/day administered subcutaneously[33] everyday till 10 days (required time period for BPH development) as per available literatures. Animals were sacrificed after 10th day. The weight of the dissected prostate was noted and the tissues were subjected to histological examination, biochemical analysis, gene expression, and immunohistochemistry studies.

Relative gene expression studies

Total RNA was isolated from freshly removed complete prostate gland and resuspended in RNA stabilizing solution procured from Amrisco laboratories. RNA samples (n = 3) were quantified by spectrophotometer at 260/280 nm.

Complementary DNA (cDNA) was synthesized by reverse transcriptase (RT) using 1 μg RNA (Fermentas First stand cDNA synthesis kit). After reverse transcription cDNA samples were amplified by RT-PCR using gene-specific primers for AR, ER-α, ER-β, and 5 α reductase (type 2) genes. GAPDH was used as an endogenous control (Table 2). Reactions were carried out in an Eppendorf Gradient PCR. The PCR products were electrophoresed on an ethidium bromide stained 2% agarose gel in Tris-acetate-EDTA (TAE) buffer. Gels were photographed by gel documentation unit from UVITEC Cambridge alliance

Table 1 Histological analysis of the prostate gland

Number of mitotic figures/microscopic field	Age of animal	Control	Cd treated (20 μg/KBW)
	1-year-old animals	6.1 ± 0.12	10.05 ± 0.29**
	5-month-old animals	5.6 ± 0.5	10.70 ± 0.62***
Number of acini/microscopic field	1-year-old animals	18.9 ± 0.54	23.2 ± 1**
	5-month-old animals	19.1 ± 1.15	25.6 ± 0.97***

All values are presented as mean of six animals ± SEM, **$p < 0.01$, ***$p < 0.001$.

Table 2 RT-PCR primers sequence and annealing temperature

Gene name	Primer sequence	Annealing temperature	Product size (bp)
Estrogen receptor-α (ER-α) NM_012689.1	Fw: 5′CCTTCTAGACCCTTCAGTGAAGCC-3′ Rv: 5′ACATGTCAAAGATCTCCACCATGCC-3′	59.3	287
Estrogen receptor-β (ER-β) NM_012754.1	Fw: 5′AAAGCCAAGAGAAACGGTGGGCAT-3′ Rv: 5′GCCAATCATGTGCACCAGTTCCT-3′	57.7	204
Androgen receptor (AR) NM_012502.1	Fw: 5′ATCGAGGAGCGTTCCAGAATCTG-3′ Rv: 5′ATATGGTCGAATTGCCCCCTAGG-3′	58	630
5α-reductase type-2 NM_022711.4	Fw: 5′ ATCCTGTGCTTAGGGAAAC 3′ Rv: 5′ CATACGTAAACAAGCCACC 3′	54.5	496
GAPDH NM_017008.4	FW: 5′CAAGGTCATCCATGACAACTTTG3 ′ RW: 5′GTCCACCACCCTGTTGCTGTAG 3′	58	496

4.7 and densitometrical analysis was carried out using Image J software.

Immunohistochemistry

Tissue sections (3 μm) were deparaffinized and rehydrated using standard protocols and incubated overnight with primary mouse monoclonal antibodies at 4°C. Sections were then rinsed twice with washing buffer (1:10 dilution of blocking buffer in PBS) followed by 1 h incubation with secondary antibodies conjugated with Fluorescein isothiocunate (FITC) and Tetramethyrhodamine isothiocynate fluorophores (Sigma Aldrich, USA) in the dark at room temperature. For negative controls, the primary antibodies were omitted. Tissue sections were mounted with mounting medium containing 4′6′-diamidino-2-phenylindole dihydrochloride (Sigma Aldrich, USA). The expression of antigens in tissue sections was assessed by immunofluorescence method. Images were captured by confocal microscope LSM710 (Carl Zeiss, Germany) using 63× objective.

Statistical analysis

The values were represented as mean ± SEM at n = 6 animals. The values were accepted as significant at $P \leq 0.05$ Newman–Keuls *post hoc* one-way analysis of variance and t-test by using Prism software version 5.0.

Results

A positive co-relation between cadmium concentration and severity of BPH disease in Indian human population has been depicted in our previous lab studies[24] supported by other reports.[34] It has also been reported that cadmium has a potent androgen and estrogen like activity in the prostate gland.[23] Thus, the goal of the present study was to ascertain whether the metal binds with steroid hormone receptors in the rat prostate, inducing hyperplasia like condition.

Time-dependent study

To establish BPH like condition, a single dose of cadmium 20 μg/kg body weight (~108 nmol/kg) was administered (intraperitoneal) to the 5-month-old animals. The dose used was 1/500 of LD50 of the metal, equivalent to the daily exposure from food and drinking water.[25,26] To determine the optimal time of BPH development, a time-dependent exposure of Cd (20 μg/kg body weight) was performed for 10, 20, and 30 days (Figure 1). Significant increase in prostate weight of animals was observed within 10 days after a single dose of Cd exposure, indicating the development of BPH like condition.

Age-dependent study

As BPH is an age-dependent pathology, we further explored the effect of 20 μg/kg body weight Cd dose in 1 year aged animals and compared with 5-month-old animals. Though 20 μg/kg body weight dose of Cd significantly induced prostate weight in both the age groups, the increase was significantly higher in 5-month-old Cd treated as compared to the 1-year-old animals with their respective controls (Figure 2).

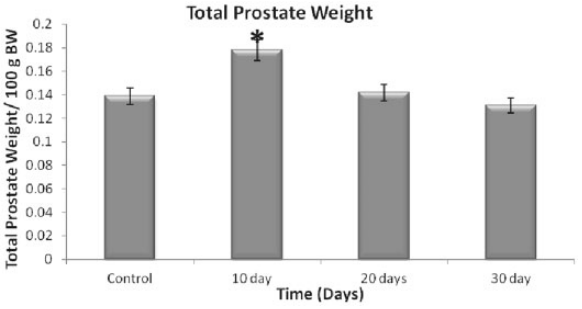

Figure 1 Time-dependent effect of a single dose of cadmium on rat prostate. The results represent the mean of six animals ± SEM, *p < 0.05, 20 μg/kg Bw Cd versus control

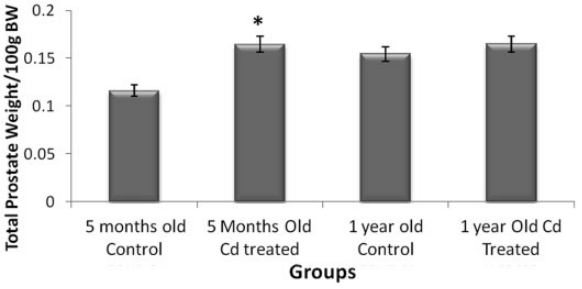

Figure 2 Age-dependent effect of a single dose of cadmium on rat prostate. The results represent the mean of six animals ± SEM, *p < 0.05, 20 μg/kg Bw Cd versus control

Oxidative stress has been implicated in pathogenesis of several diseases. Previous studies of our lab and other groups showed Cd as an inducer of oxidative stress and potent clinical and biochemical environmental toxicant for BPH pathogenesis.[24,35] Cd treated animals demonstrated significant decrease in GSH and increase in LPO level (Figure 3(a) and (b)) which further supported our previous results and role of cadmium as an oxidative stress inducer causing BPH like condition.

Confirmation of BPH model by histological studies

A single 20 μg/kg body weight cadmium dose was selected for the development of BPH rat model in 5-month-old animal, which was supported by histological observations. The histology reveals a significant increase in the number of acini and mitotic figures in 5-month-old Cd treated group (Table 1) as compared with 1-year-old animal (Figure 4). As evident from Figure 5 normal prostate is characterized by compound tubular alveolar glands with presence of basement membrane. The cell lining of the duct is columnar to cuboidal with basally located nuclei that are round to oval in shape. The alveolar portions of gland contain primary and secondary infoldings of secretory epithelium that project into the alveolar lumen (Figure 5) and the alveoli separated by a delicate fibrous connective tissue stroma with an increased number and irregular acinar growth pattern. Each of the lobule is larger and has more elaborate

branching than in the normal gland. In addition, the size of the secretory epithelial cells is increased principally due to an increase in the amount of cytoplasm. The amount of stroma is relatively less than normal gland, and the basement membrane appears somewhat attenuated (Figure 5).

Further basal cells proliferation was observed by immunohistochemisty using anti-Ki-67 antibody which is a marker of epithelial proliferation (Figure 5).

Mechanism of cadmium in prostate hyperplasia induction

To determine whether cadmium mimics the androgenic response in animals, the effects of the metal along with the wet weight of the prostate were tested in 5-month-old castrated animals. Results demonstrated statistical difference in an average weight of the prostate (Figure 6). Previous lab data suggest that cadmium mimics the action of steroid hormone; however, its action via binding to steroid hormone receptors was not well defined. To answer this question, an experiment was designed where animals were treated with steroid hormone receptor antagonists.

The animals received antagonists, namely nilutamide, methyl piperidino pyrazole, and 4-hydroxytamoxifen till 10 days. First, rats were treated with steroid hormone receptor blocker and then Cd was administered after 3 h of AR antagonist and 30 min of ER antagonists treatment[36,37] to block the availability of steroid hormone receptors for Cd. Increase in wet weight of the prostate and PAP activity in antagonist treated group were blocked (Figure 7(a) and (b)). Further histological examination of the prostate from antagonists treated group exhibited large and regular acini with no epithelial infolding in AR and ER-α antagonists group (Figure 8), whereas epithelial infoldings were observed in ER-β antagonist group (Figure 8).

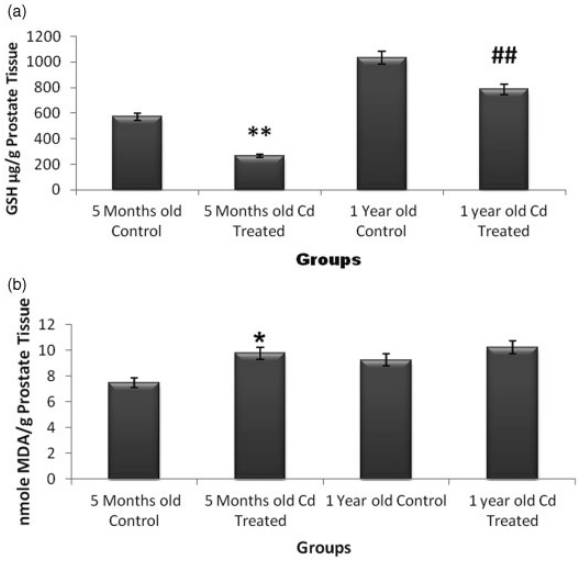

Figure 3 Age-dependent effect of a single dose of cadmium at GSH and LPO level in rat prostate. The results represent the mean of six animals ± SEM, (a) **$p < 0.01$, 5-month-old Cd treated versus 5-month-old control, ##$p < 0.01$, 1-year-old Cd treated versus 1-year-old control, (b) *$p < 0.05$, 5-month-old Cd treated versus 5-month-old control

Relative gene expression and immunohistochemistry

The metal has an ability to bind to steroid hormone receptors and stimulate proliferation. To shed light into the gene expressions underlying the response of cadmium treatment, steroid hormone receptor antagonist groups along with cadmium treated animals were examined for gene expression profile (AR, ER-α, ER-β, and 5α reductase type-2) by RT-PCR method (Figure 9).

The relative expression of 5α reductase type II, an enzyme responsible for conversion of testosterone into DHT showed significant decreased expressions in all antagonists groups when compared with Cd treated group, indicating decreased conversion of testosterone to DHT and hence less proliferation. Cadmium has an ability to transactivate AR. Increased expression of AR and ER-α along with decreased expression of ER-β was observed in the cadmium treated group compared with control.

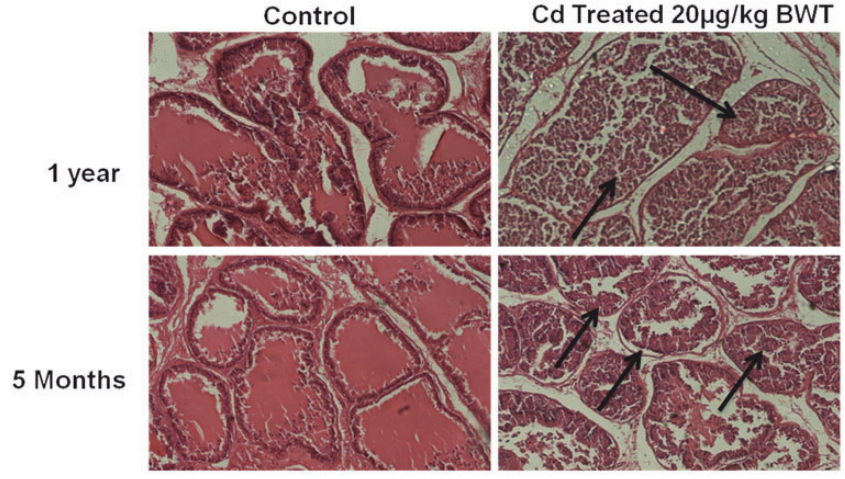

Figure 4 Age-dependent histological changes of a single dose of cadmium in rat prostate. Sections were stained by hematoxylin/eosin staining. Images were captured by light microscope depicting epithelial infolding and acinar growth pattern using 20× objective. (A color version of this figure is available in the online journal.)

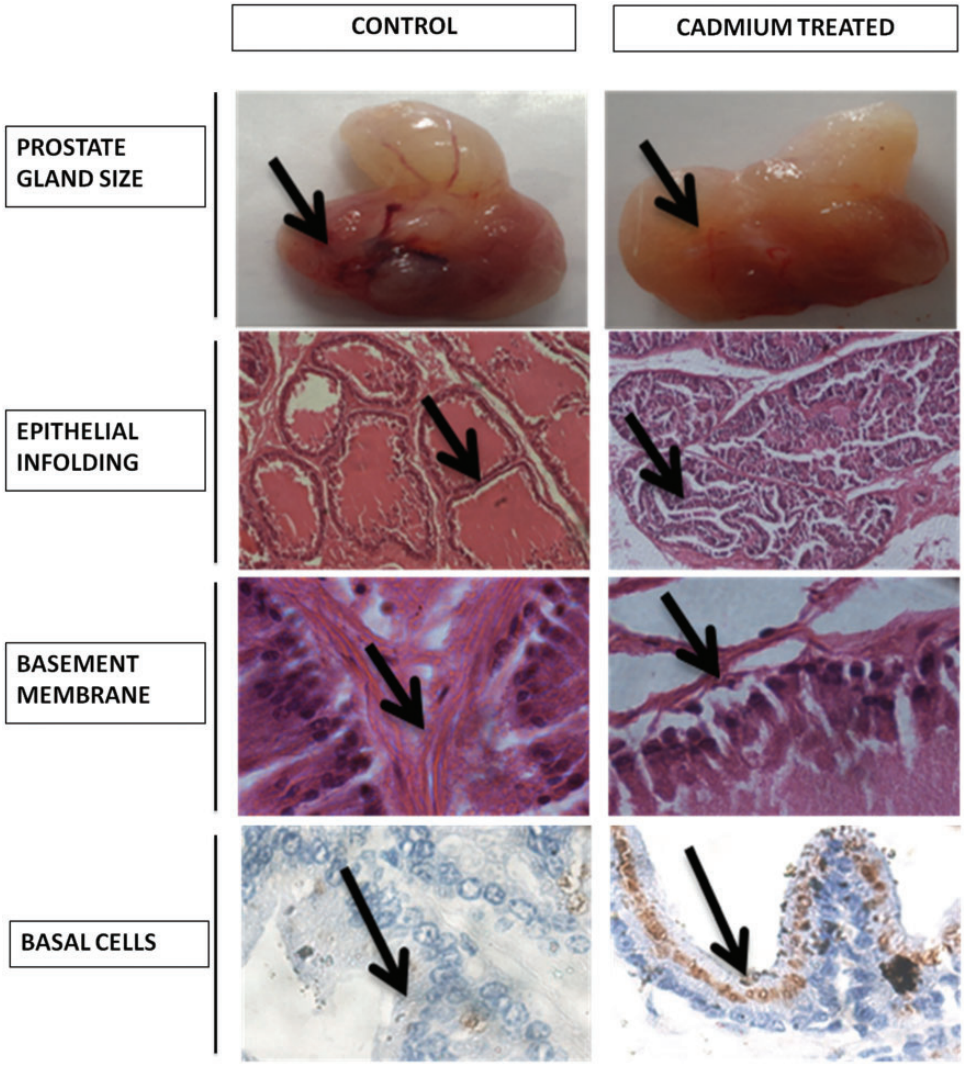

Figure 5 Histological evaluation of a single dose of cadmium in 5-month-old rat prostate. Sections were stained by hematoxylin/eosin staining. Images were captured by light microscope using 20× objective. (A color version of this figure is available in the online journal.)

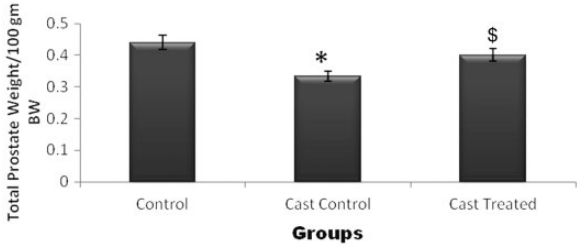

Figure 6 Effect of a single dose of cadmium on 5-month-old castrated rat prostate. The results represent the mean of six animals ± SEM, *$p < 0.05$, castration control versus control, \$ $p < 0.05$, castration treated versus castration control

To further substantiate the action of Cd via steroid receptor, immunohistochemistry was performed. Cadmium treated group exhibited a higher Ki-67 index, which clearly indicated epithelial cell proliferation than in the normal section of the gland, whereas AR and ER-α antagonists group showed no proliferation. Similarly, when the sections were stained with anti-AR antibody, Cd treated group showed a significant increase in expression of AR compared to antagonists group (Figure 10). ER-β treated group showed Ki-67 and AR positive staining. Weak E-cadherin and abundant expression of vimentin in the Cd treated group were observed and compared with control group which further provided information about EMT (epithelial to mesenchymal transformation) in the BPH pathogenesis (Figure 11).

Discussion

The pathogenesis of BPH remains very elusive in prostate biology till date. Many attempts have been made during the last decades to understand the pathophysiology of the disease. In this context several *in vitro* and *in vivo* animal models have been developed for studying BPH.[7,8,38] Rat prostate has been documented to respond for hormone and other chemical treatments such as citral, exogenous testosterone, DHT, and estradiol.[39,40] Similarly, Lee *et al.* also developed BPH rat model by combined administration of DHT and adenoreceptor antagonist prazosin,

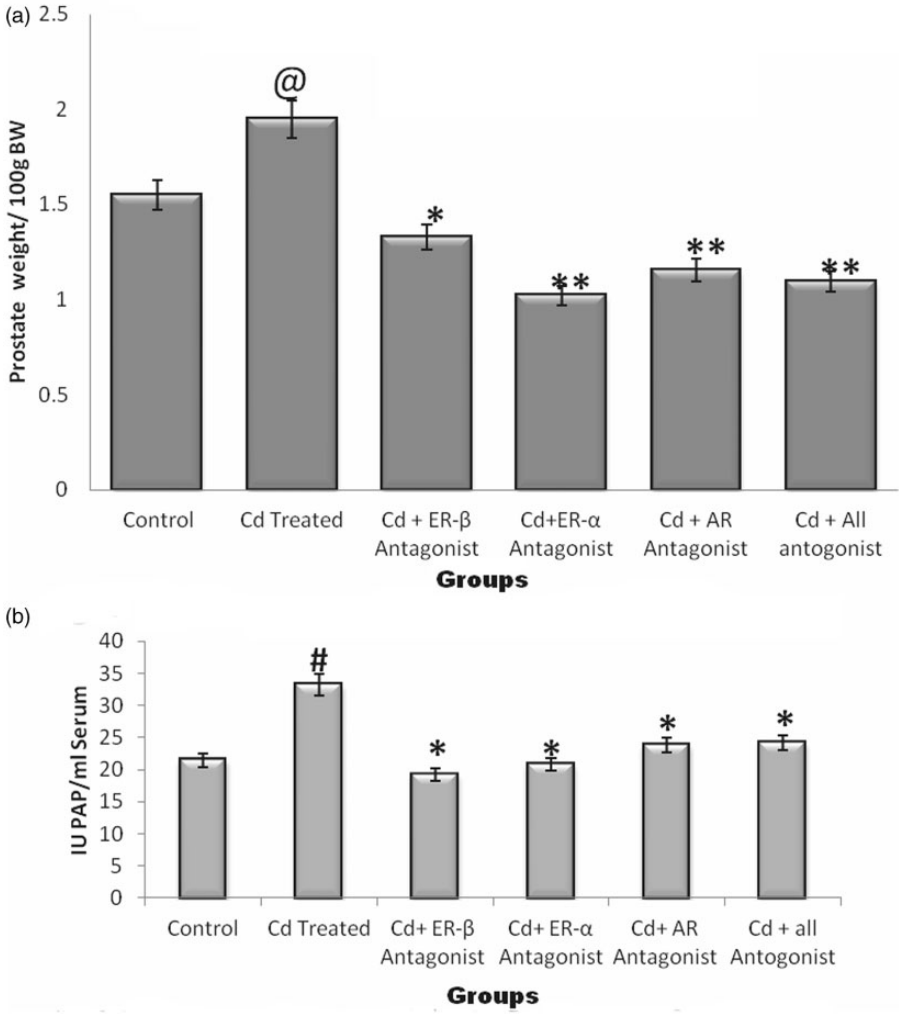

Figure 7 Effect of a single dose of cadmium along with steroid hormone receptor antagonist on prostate weight and prostatic acid phosphatase activity. The results represent the mean of six animals ± SEM, (a) @$p < 0.05$, Cd treated versus control. @ $p < 0.05$, Cd treated versus control, *$p < 0.05$, Cd treated versus ER-β. **$p < 0.01$, Cd treated versus ER-α, AR, and all antagonist. (b) #$p < 0.05$, Cd treated versus control. *$p < 0.05$, cd treated versus ER-α,β, AR, and all antagonist

subcutaneously for 14 days.[41] Besides these steroid hormone and other chemical induced BPH rat model, recently several transgenic and knockout animal models were also developed to study the pathogenesis of BPH such as liver X receptor (is a ligand-activated transcription factor) knockout mouse, overexpression of keratinocyte derived chemokine, the murine analog of the chemokine IL-8, and prolactin overexpressing rodent models.[42–44] However, above-mentioned animal models are costly, time consuming, and required transgenic or knockout species.

To study the pathogenesis of BPH, spontaneous and hormone-induced models are more desirable.[8,9,38] Hormone-induced spontaneous BPH model in the dogs and chimpanzees is more readily available, but ethical and financial matters need to be considered. However, rat and human prostate differ markedly including differences in the gross and microanatomy that have implications for pathological interpretation in clinicopathologic characteristics of human prostatic disorders.[45,46] Yet, the rodents and human prostate have many anatomical similarities such as development of the gland in the form of lobular glands from the Wolffian ducts and the urogenital sinuses. Both species have androgen-sensitive organs and distinctly differentiated epithelial cells with similar functions. The rat dorso-lateral prostate has been documented to be the most homologous to the human peripheral zone. These similarities help to support the rat models for the study of molecular alterations in the development and progression of prostatic enlargement.[46]

To the best of our knowledge, the present work depicts the development of cadmium induced rat model for the first time, which is cost effective, less time consuming, and aids in revealing the mystery of pathogenesis of BPH with great ease, compared to other available models. This model had also showed a broad spectrum of histopathological lesions corresponding from normal to hyperplasia progression and is useful for understanding disease pathogenesis and drug discovery.

The present study suggests that cadmium has significant potential as an inducer of prostate hyperplasia in Charles foster rats. A significant increase in prostate weight with characteristic histological features in 5-month-old animals

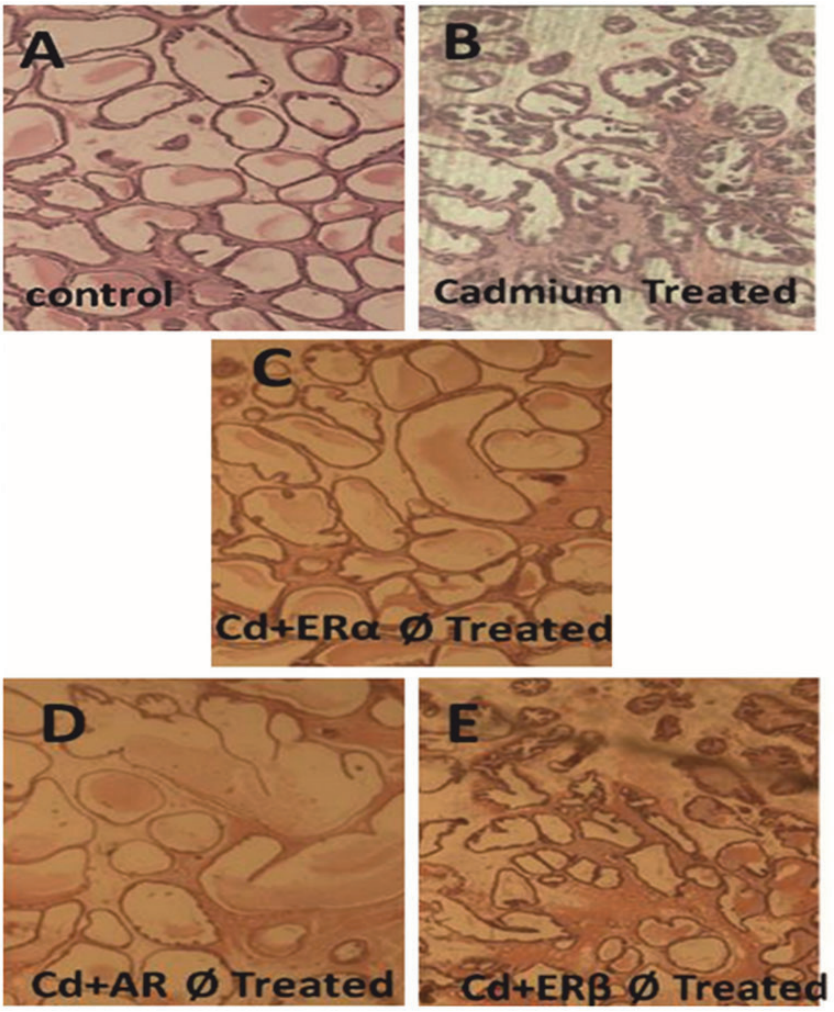

Figure 8 Histological changes in rat prostate of a single dose of cadmium treated group along with antagonists. Sections were stained by hematoxylin/eosin staining. Images were captured by light microscope showing epithelial infolding and acinar growth pattern using 20× objective. φ=antagonist. (A color version of this figure is available in the online journal.)

treated with a single i.p. dose of 20 μg cadmium/kg body weight developed BPH like condition within 10 days' time compared with 1-year-old animals.

The metal-binding protein, metallothionein (MT), is thought to be involved in detoxification of various metal toxicities including, Cd. It has also been reported that MT is poorly expressed in ventral prostate whereas high basal expression has been observed in dorsolateral prostate of rats,[47] suggesting protective role of MT in later time period of cadmium dose (20 and 30 days) as demonstrated in our results.

Cadmium exposure induces cell proliferation, depicted by increased prostate weight. Previous reports suggested that the old age rats were more resistant to cadmium induced toxicity compared with young age rats[48] which supported our results of less weight gain in 1-year-old rats. The current findings suggest that a single dose of Cd causes 1.62-fold increases in the prostate weight compared to control, which is in concordance to earlier reports by Martin *et al.*[23] Several studies reported the induction of prostate carcinoma by administration of Cd; however, the doses of Cd used were much higher than that used in the present study.[49,50] Moreover, histological studies suggest that in BPH, the ductal morphology is maintained, unlike in PCa where unorganized growth is observed. Also the presence of basal cells, a characteristic of BPH further strengthens the cadmium induced BPH condition in the present study. It was reported earlier that epithelial cells originate from basal cells and play important role in prostate development and exhibit higher proliferation in BPH like condition, whereas the basal cells are absent in adenocarcinoma of the prostate.[51]

The overall maintenance of the prostate is dependent on androgens, and the prostate demonstrates regression after withdrawal of androgen, such as castration.[50,52] In the present study also a decrease in prostate weight was observed in castrated group of animals, supporting androgen mimicking activity of cadmium, which was ameliorated with Cd treatment suggesting cadmium induces a hyperplasia like condition.

Further ability of antagonists to block these effects suggests that the effects of cadmium are mediated through the steroid hormone receptor. In antagonist experiment, the effects from prostate weight and PAP activity were more

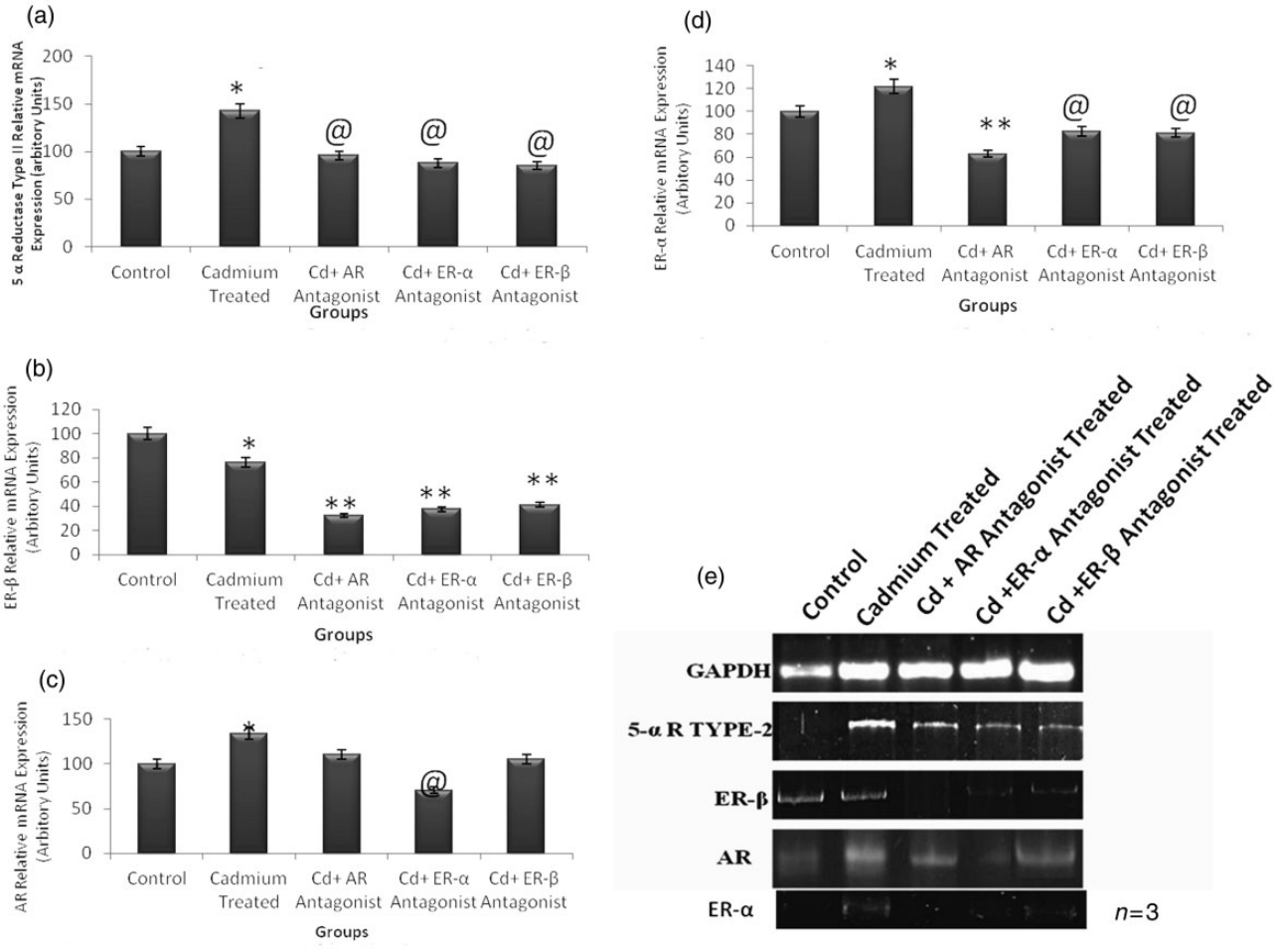

Figure 9 Effect of a single dose of cadmium on prostatic genes expression profile in presence of antagonists. The results represent the mean of three animals ± SEM, (a) *p < 0.05, Cd versus control, @p < 0.05, Cd versus AR, ER-α, β-antagonist. (b) *p < 0.05, Cd versus control, **p < 0.01, Cd versus AR, ER-α, β-antagonist. (c) *p < 0.05, Cd versus control, @p < 0.05, Cd versus ER-α-antagonist. (d) *p < 0.05, Cd versus control, **p < 0.01 Cd versus AR antagonist, @p < 0.05, Cd versus ER-α, β-antagonist. (e) Gel electrophoresis bands

significant in the group treated with AR and ER-α receptor antagonist along with Cd as compared to ER-β receptor antagonist, providing the fact that Cd would probably mediate its effect by binding to the ER-α and AR with more affinity than with ER-β receptor. Previous studies also support that cadmium binds to hormone-binding domain of ER-α and AR with high affinity and activate receptors[18,23] thus, supporting our results. Moreover, histological observations demonstrated larger acini and no epithelial infoldings in AR and ER-α antagonists group compared to Cd treated group. Whereas, ER-β antagonist treated group showed epithelial infoldings, indicating that cadmium treatment blocked antiproliferation activity of ER-β and induced hyperplasia of the gland. Further suggesting that Cd effect is mediated through AR and ER-α receptors, causing hyperplasia like condition.

The gene expression studies were carried out to study the expression levels of the receptors and 5α reductase type II enzyme. 5α Reductase type II enzyme is responsible for conversation of testosterone into DHT. Available literature indicates that the expression of 5α-R2 increases in BPH condition and decreases in PCa.[53] Elevated level of transcriptional activity of the enzyme was noted in Cd treated group and hence more DHT production confirmed androgen mimicking activity of cadmium. Our results showed decreased expression of the same in all antagonists groups compared to cadmium treated group, indicating decreased conversion of testosterone to DHT and hence less proliferation.

AR mRNA levels are regulated by androgens and other steroid hormones.[54] Increased AR mRNA expression in cadmium treaded group and decreased expression in antagonist treated group suggest that Cd mediated its action through AR as reported earlier and modulate the mRNA expression.[23] Moreover, it is also known that ER-α is the dominant ER form mediating the effects of early estrogen exposure on the prostate gland.[55] It has been observed that ER is auto-regulated by estrogen.[56] Stoica *et al.* suggested that Cd interacts with the hormone-binding domain of the receptor and activates ER-α.[18] ER-α m RNA expression was significantly high in Cd treated group compared with control while in other groups it was very less.

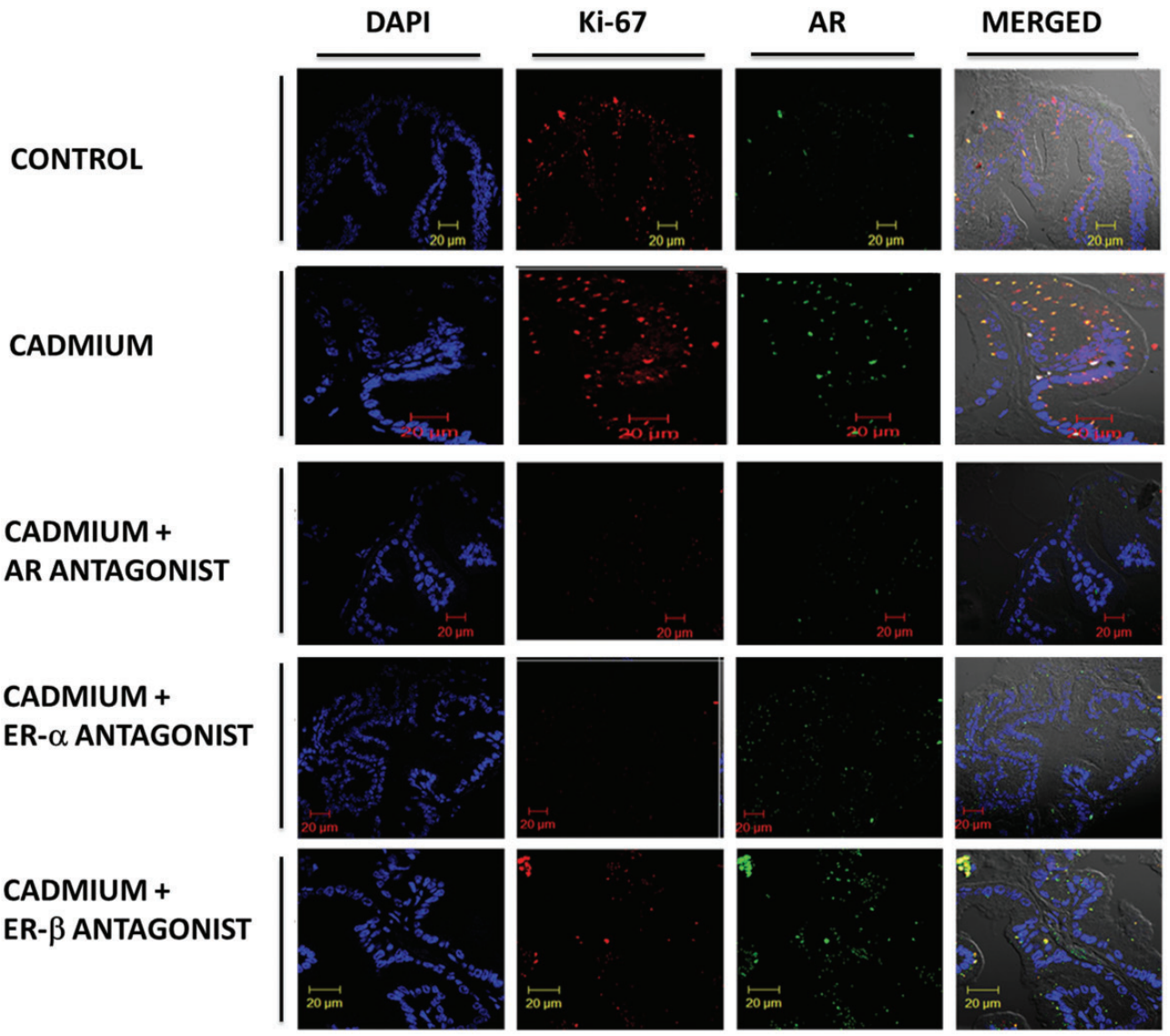

Figure 10 Effect of a single dose of cadmium on expression profile of ki-67 and AR on rat prostate in the presence of antagonists by immunofluorescence method. Tissue sections were stained with secondary antibodies conjugated to FITC (AR) and TRITC (ki-67) fluorophores along with DAPI for nuclear staining. Images were captured by confocal microscope LSM 710 (Carl Zeiss, Germany) using 63× objective. (A color version of this figure is available in the online journal.)

Similarly, other reports also were unable to detect ER-α expression in normal rat prostate tissue.[57] The primary function of ER-β is suppressing proliferation and promoting differentiation of prostatic cells. Decrease in ER-β expression is reported in BPH.[20] We have also noticed a decrease in expression of ER-β in the cadmium treated group which further strengthens the fact that cadmium induces a BPH like condition. Similarly, a study of human breast cancer patients previously treated with estrogen antagonist tamoxifen had reduced ER-β level compared with healthy, age matched controls[58] further supporting our observations.

In the present study, less E-cadherin expression and abundant Ki-67 were observed in Cd treated group. The epithelial characteristics are lost due to high proliferative capability and high vimentin expression indicating possible EMT transition in BPH pathogenesis. During EMT the epithelial cells lose their polarity, stability, and become more fibroblast-like cells. The features with parallel loss of epithelial marker and gaining mesenchymal phenotype which would further alter key signaling pathways responsible for the disease pathogenesis.[59]

The steroid hormone receptor antagonist study suggested Cd induced hyperplasia like condition is by activating the androgen receptor and estrogen receptor alpha action and suppressing estrogen receptor beta action in rats. Therefore, we report for the first time a cost effective and less time consuming rat model of BPH by using low level of Cd which strongly suggests its co-relation to the pathogenesis of human BPH. Therefore, Cd causes BPH like condition upon binding to AR and ER-α receptors which in turn control 5α reductase type 2 enzyme expression, epithelial growth, differentiation, function, and epithelial-stromal cross talk.

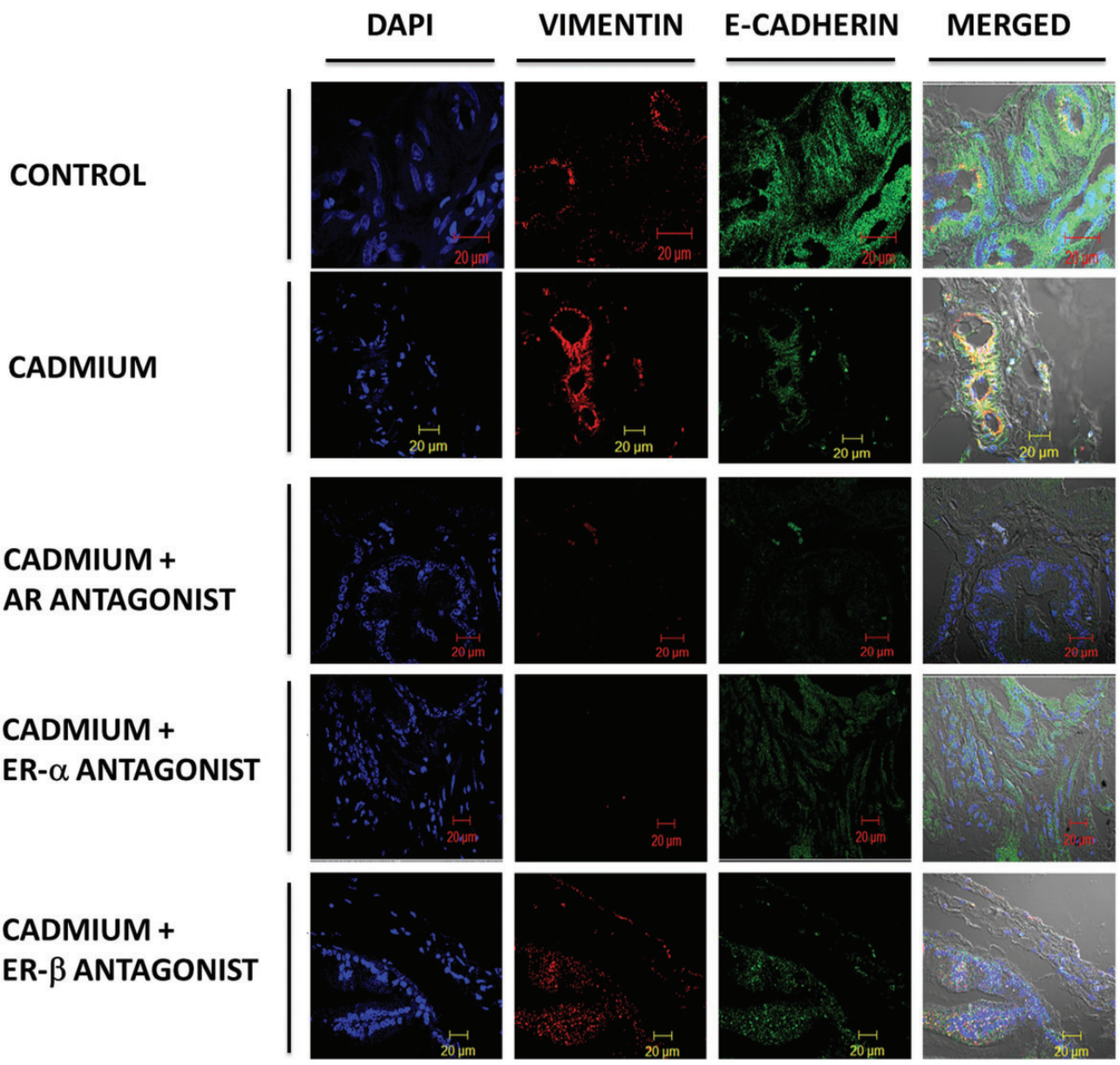

Figure 11 Effect of a single dose of cadmium on expression profile of vimentin and E-cadherin on rat prostate in the presence of antagonists by immunofluorescence method. Tissue sections were stained with secondary antibodies conjugated to CY5 (vimentin) and FITC (e-cadherin) fluorophores along with DAPI for nuclear staining. Images were captured by confocal microscope LSM 710 (Carl Zeiss, Germany) using 63× objective. (A color version of this figure is available in the online journal.)

Conclusion

The experimental model used here is a potentially valuable tool for investigating the respective roles of the epithelial and stromal hormone receptors and for its applicability in the study of the genesis of human BPH, which would be helpful to understand disease pathogenesis and progression and further designing appropriate therapeutics interventions.

Author contributions: Conceived and designed the experiments: AP, SG[1]. Performed the experiments: AP, AR, JP. Analyzed the data: AP, SG[1], SG[2]. Contributed reagents/materials/analysis tools: AP, SG[1], SG[2]. Wrote the paper: AP, SG[1], SG[2].

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