LIST OF PUBLICATIONS

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

Akhilesh Prajapati, Akshay Rao, Jhanvi Patel, Sharad Gupta and Sarita Gupta

Exp Biol Med (Maywood) 2014 239: 829 originally published online 28 May 2014
DOI: 10.1177/1535370214536118

Original Research

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

Akhilesh Prajapati[1], Akshay Rao[1], Jhanvi Patel[1], Sharad Gupta[2] and Sarita Gupta[1]

[1]Department of Biochemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat 390002, India; [2]Gupta Pathology laboratory, Vadodara, Gujarat 390001, India
Corresponding author: Sarita Gupta. Email: sglmescrl@gmail.com

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

***Experimental Biology and Medicine* 2014; 239: 829–841. DOI: 10.1177/1535370214536118**

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).

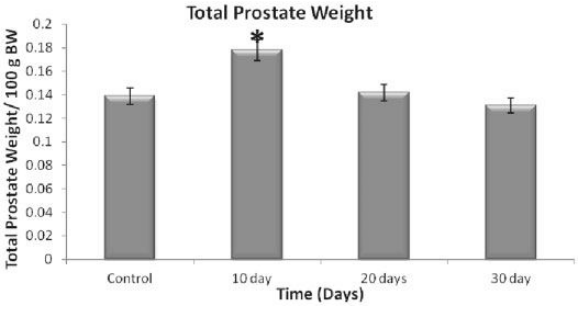


Figure 1 Time-dependent effect of a single dose of cadmium on rat prostate. The results represent the mean of six animals ± SEM, *p < 0.05, 20 μg/kg Bw Cd versus control

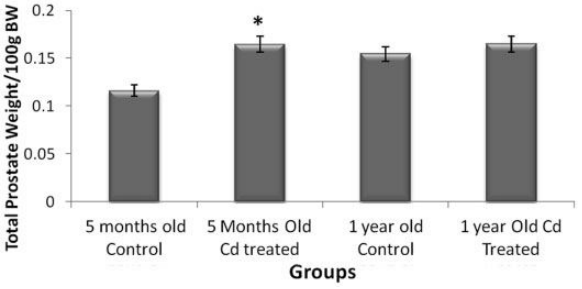


Figure 2 Age-dependent effect of a single dose of cadmium on rat prostate. The results represent the mean of six animals ± SEM, *p < 0.05, 20 μg/kg Bw Cd versus control

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).

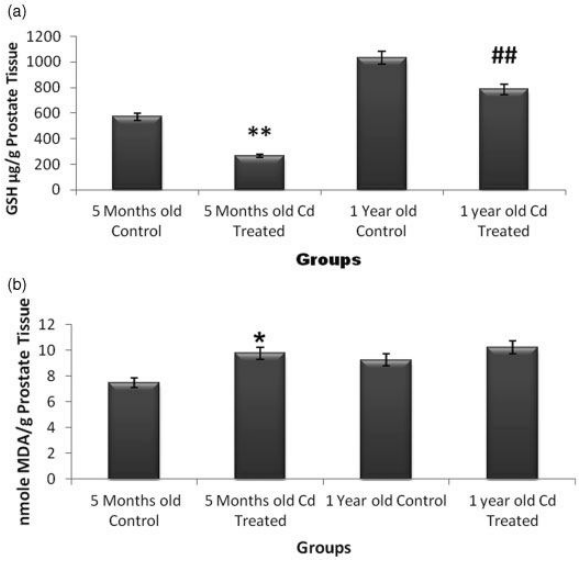


Figure 3 Age-dependent effect of a single dose of cadmium at GSH and LPO level in rat prostate. The results represent the mean of six animals ± SEM, (a) **$p < 0.01$, 5-month-old Cd treated versus 5-month-old control, ##$p < 0.01$, 1-year-old Cd treated versus 1-year-old control, (b) *$p < 0.05$, 5-month-old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

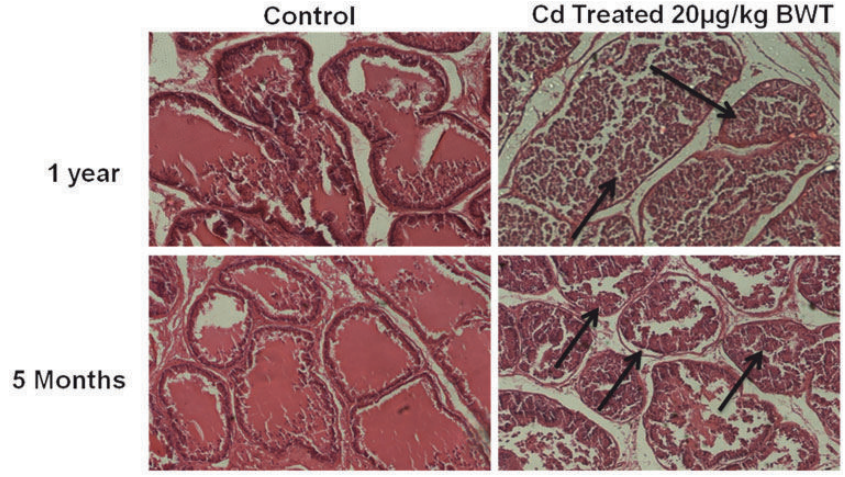


Figure 4 Age-dependent histological changes of a single dose of cadmium in rat prostate. Sections were stained by hematoxylin/eosin staining. Images were captured by light microscope depicting epithelial infolding and acinar growth pattern using 20× objective. (A color version of this figure is available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

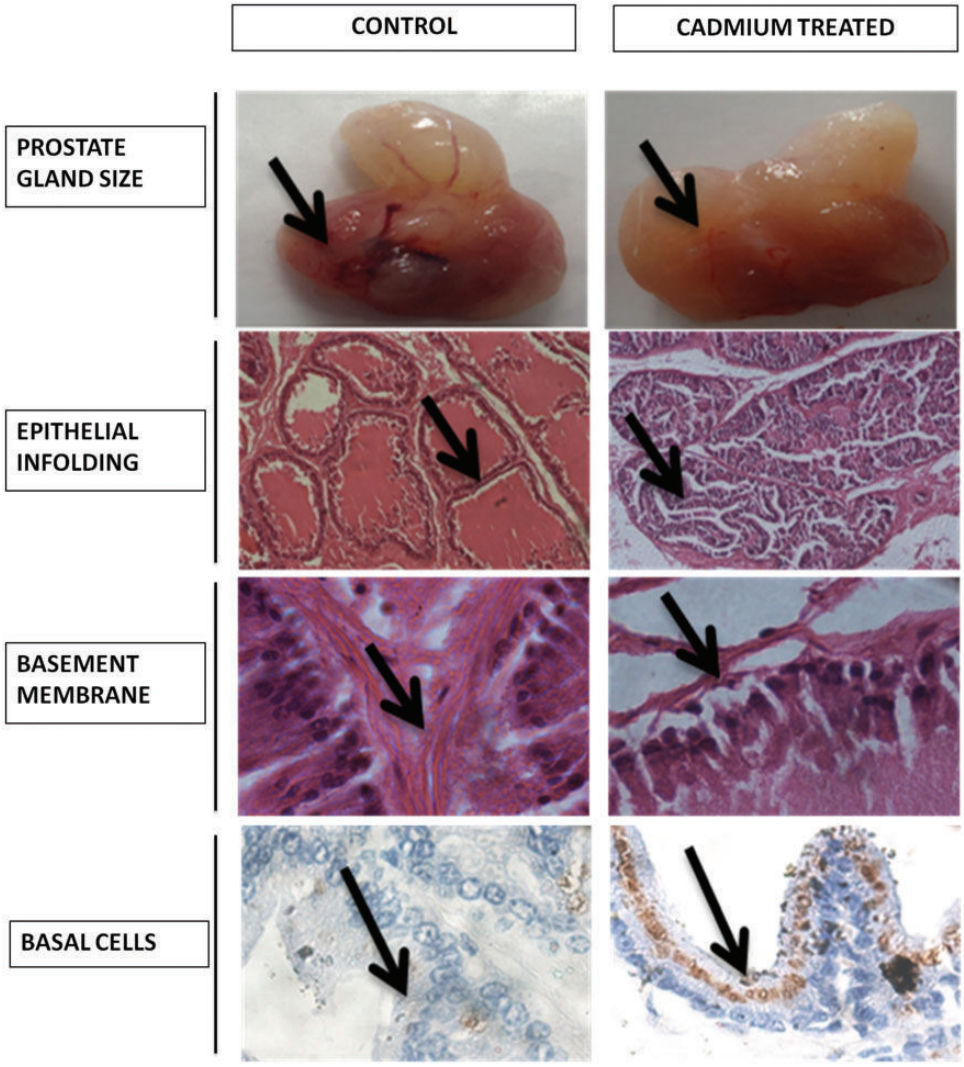


Figure 5 Histological evaluation of a single dose of cadmium in 5-month-old rat prostate. Sections were stained by hematoxylin/eosin staining. Images were captured by light microscope using 20× objective. (A color version of this figure is available in the online journal.)

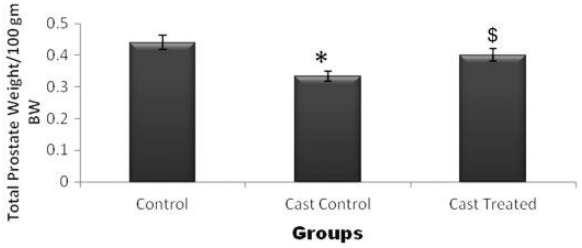


Figure 6 Effect of a single dose of cadmium on 5-month-old castrated rat prostate. The results represent the mean of six animals ± SEM, *$p < 0.05$, castration control versus control, \$ $p < 0.05$, castration treated versus castration control

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,

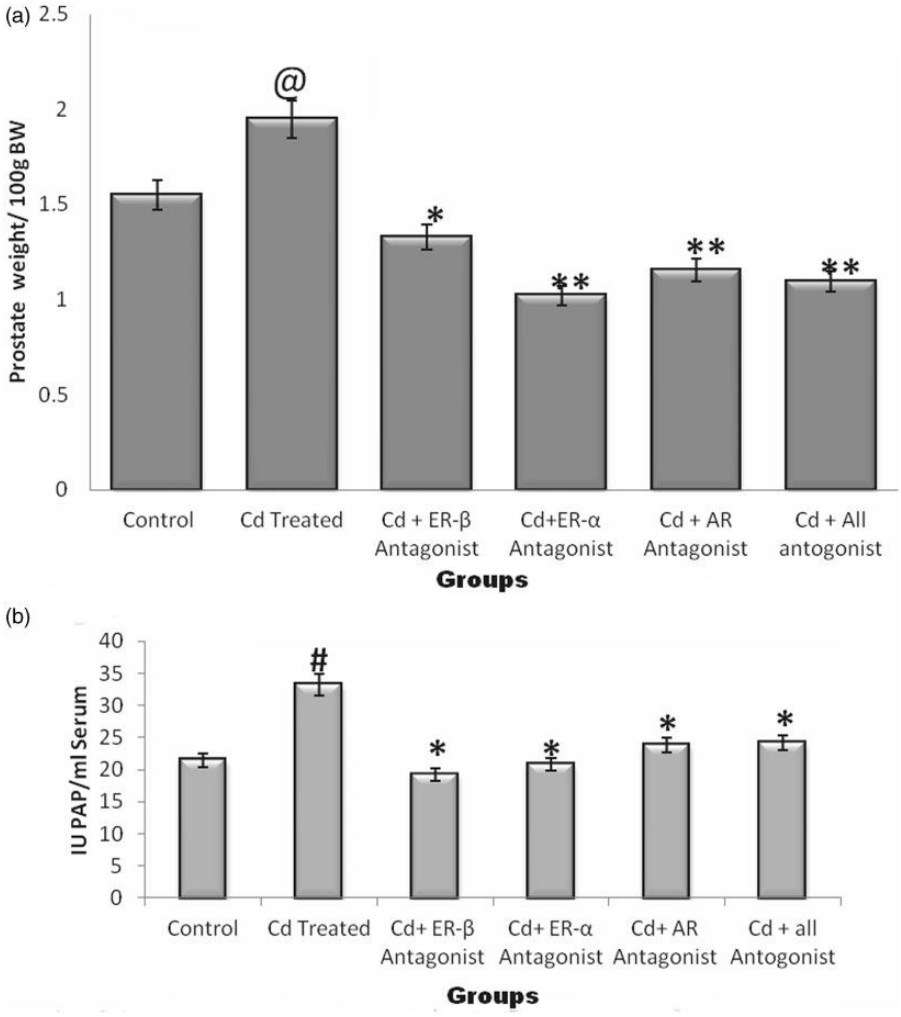


Figure 7 Effect of a single dose of cadmium along with steroid hormone receptor antagonist on prostate weight and prostatic acid phosphatase activity. The results represent the mean of six animals ± SEM, (a) @$p < 0.05$, Cd treated versus control. @ $p < 0.05$, Cd treated versus control, *$p < 0.05$,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

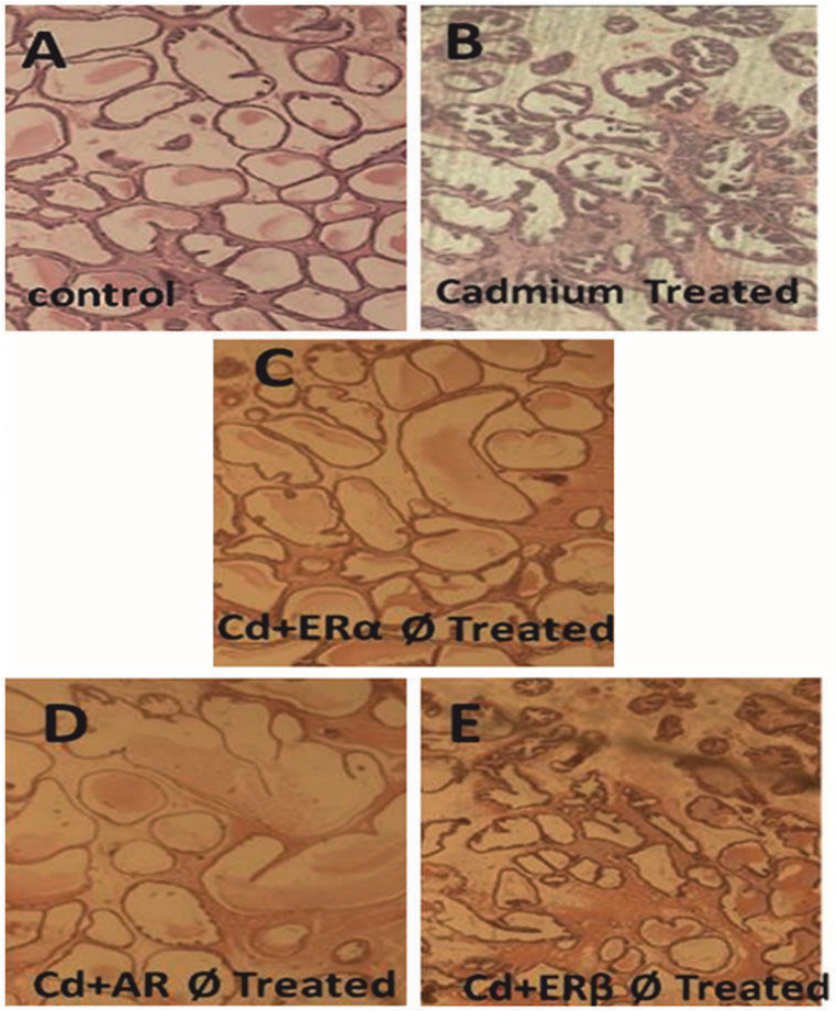


Figure 8 Histological changes in rat prostate of a single dose of cadmium treated group along with antagonists. Sections were stained by hematoxylin/eosin staining. Images were captured by light microscope showing epithelial infolding and acinar growth pattern using 20× objective. φ=antagonist. (A color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

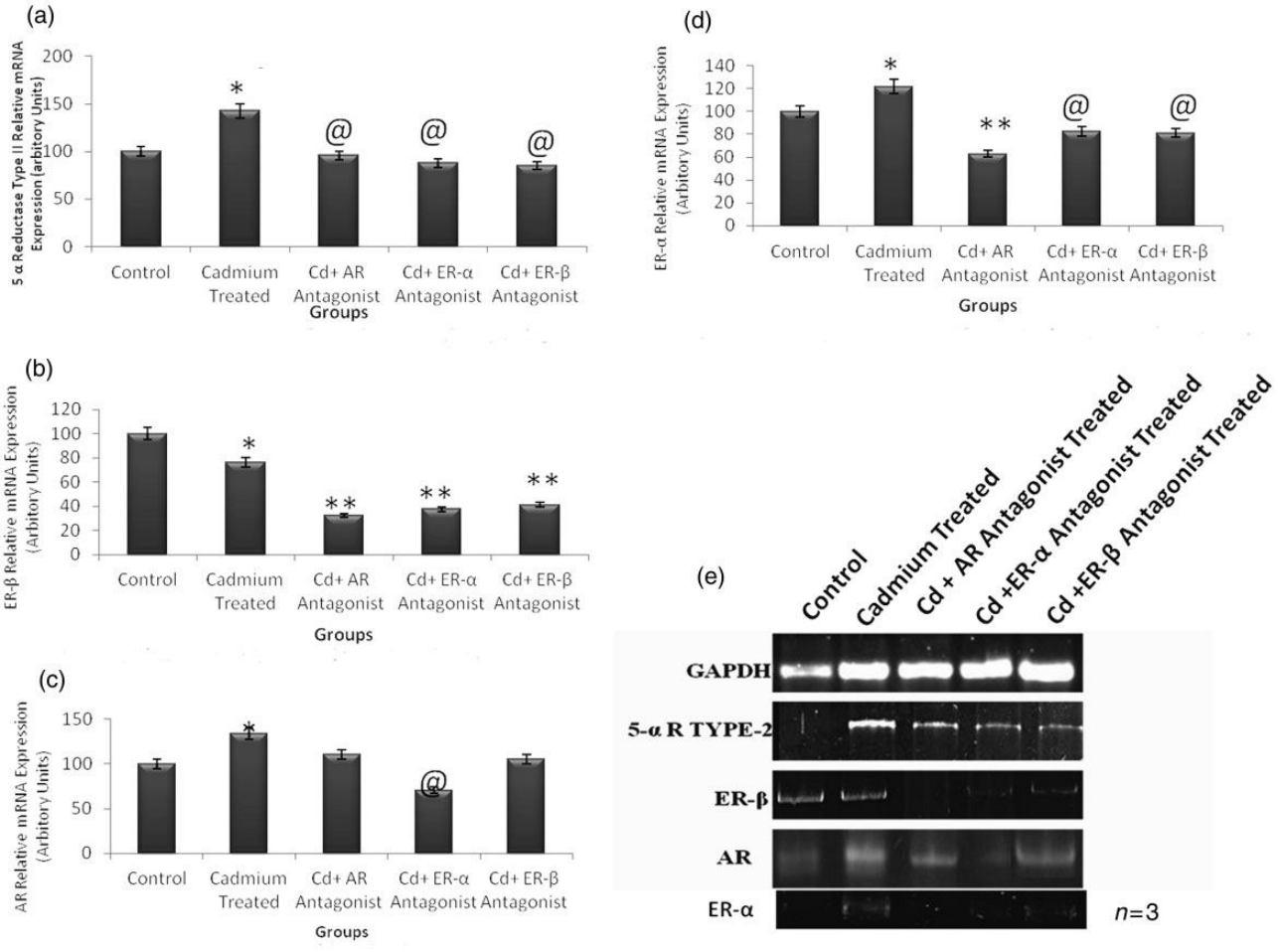


Figure 9 Effect of a single dose of cadmium on prostatic genes expression profile in presence of antagonists. The results represent the mean of three animals ± SEM, (a) *$p < 0.05$, Cd versus control, @$p < 0.05$, Cd versus AR, ER-α, β-antagonist. (b) *$p < 0.05$, Cd versus control, **$p < 0.01$, Cd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

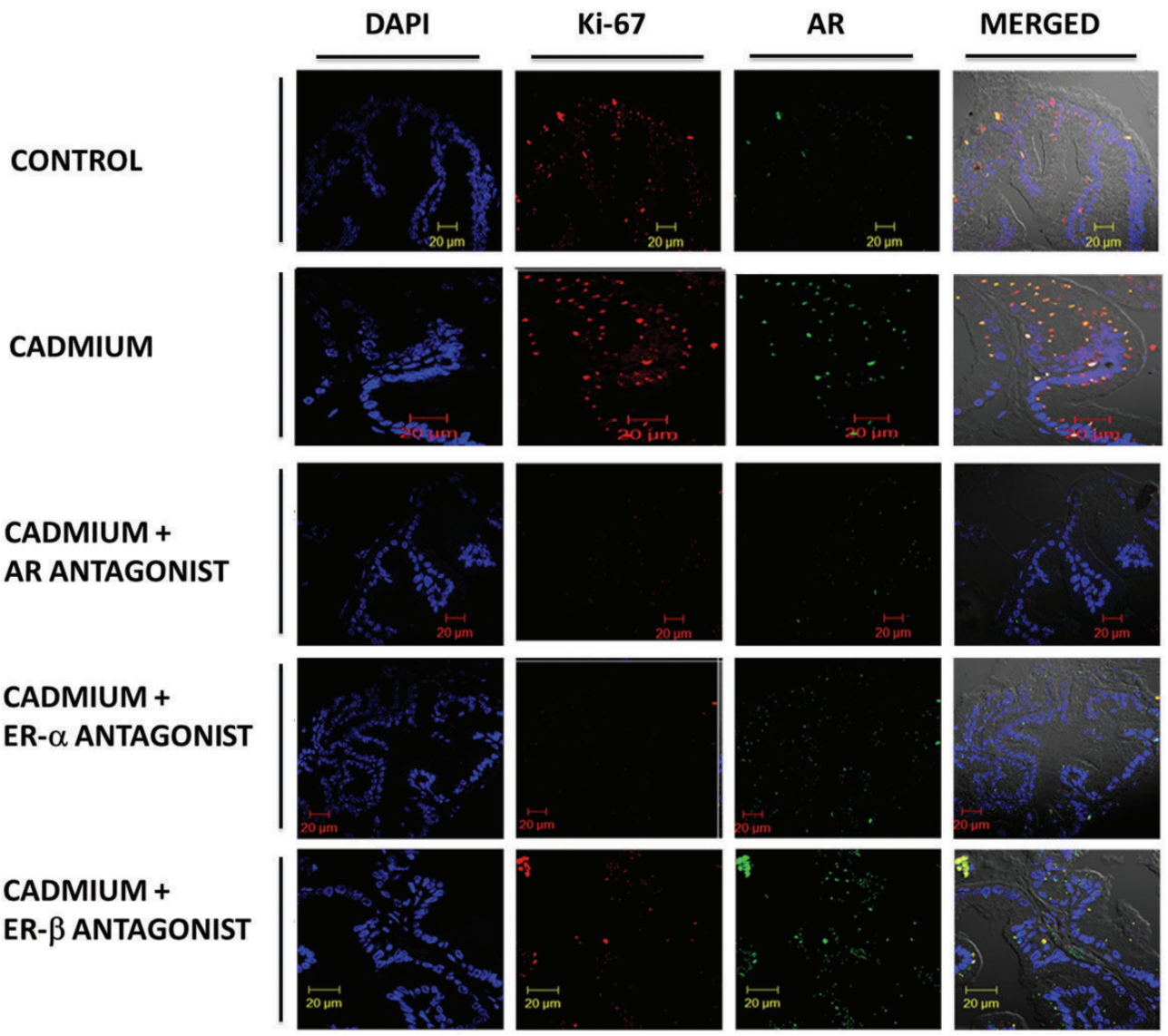


Figure 10 Effect of a single dose of cadmium on expression profile of ki-67 and AR on rat prostate in the presence of antagonists by immunofluorescence method. Tissue sections were stained with secondary antibodies conjugated to FITC (AR) and TRITC (ki-67) fluorophores along with DAPI for nuclear staining. 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.

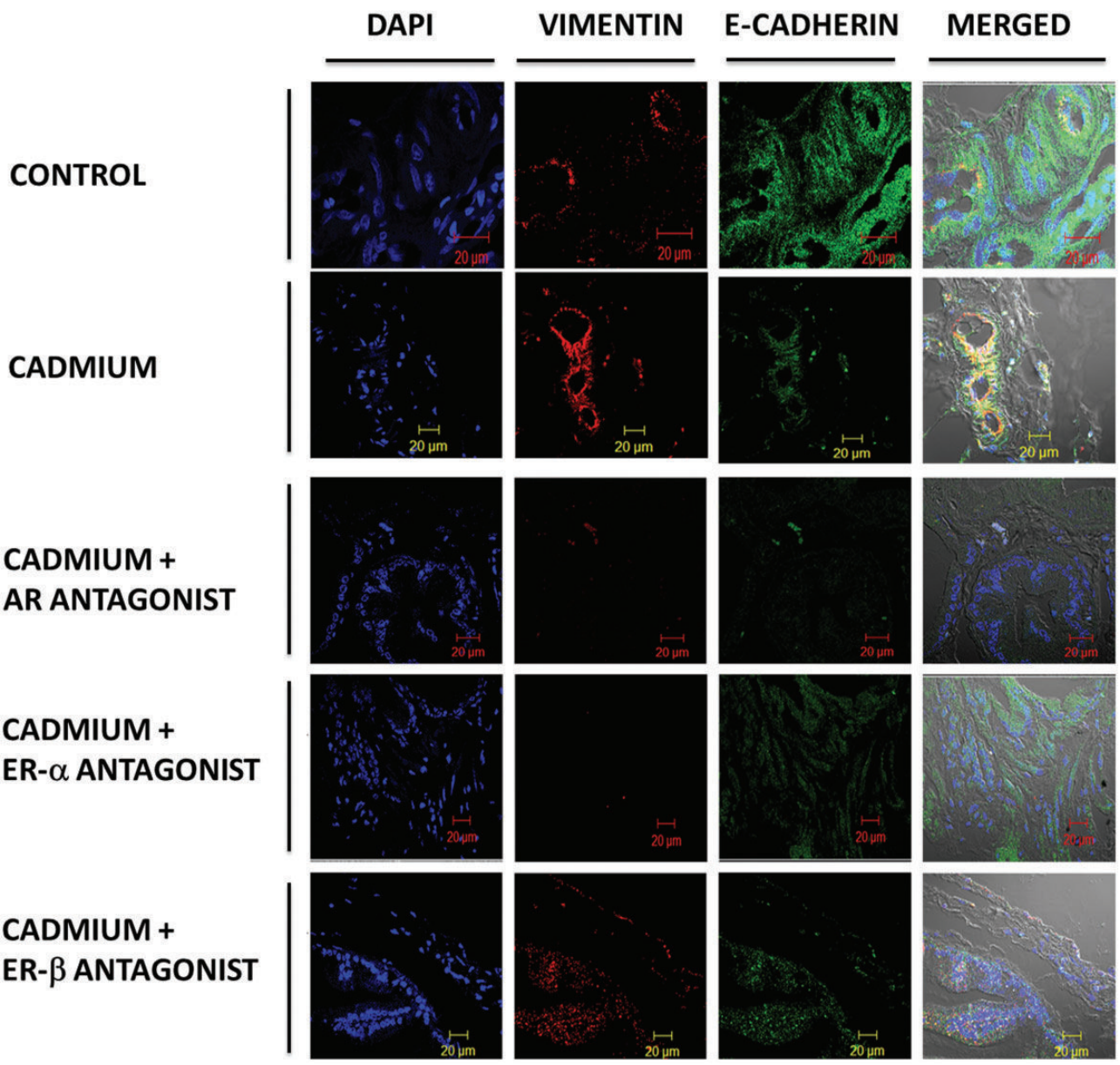


Figure 11 Effect of a single dose of cadmium on expression profile of vimentin and E-cadherin on rat prostate in the presence of antagonists by immunofluorescence method. Tissue sections were stained with secondary antibodies conjugated to CY5 (vimentin) and FITC (e-cadherin) fluorophores along with 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].

ACKNOWLEDGEMENTS

We would like to thank Dr Gitika kharkwal for discussion of the manuscript. This work was funded by a grant from DBT, India under DBT-MSUB-ILSPARE Project.

REFERENCES

1. Ziada A, Rosenblum M, Crawford ED. Benign prostatic hyperplasia: An overview. *Urology* 1999;**53**:1–6
2. Chertin B, Moriel EZ, Hadas-Halperin I, Abu-Arafeh W, Lupa S, Zilberman M, Farkas A. Laser prostatectomy. Long-term follow-up of 303 patients. *Eur Urol* 1999;**35**:285–8
3. Djavan B, Seitz C, Ghawidel K, Basharkhah A, Bursa B, Hruby S, Marberger M. High-energy transurethral microwave thermotherapy in

patients with acute urinary retention due to benign prostatic hyperplasia. *Urology* 1999;**54**:18–22
4. Thalmann GN, Graber SF, Bitton A, Burkhard FC, Gruenig O, Studer UE. Transurethral thermotherapy for benign prostatic hyperplasia significantly decreases infravesical obstruction: Results in 134 patients after 1 year. *J Urol* 1999;**162**:387–93
5. Cooper KL, McKiernan JM, Kaplan SA. Alpha-adrenoceptor antagonists in the treatment of benign prostatic hyperplasia. *Drugs* 1999;**57**:9–17
6. Steiner MS, Couch RC, Raghow S, Stauffer D. The chimpanzee as a model of human benign prostatic hyperplasia. *J Urol* 1999;**162**:1454–61
7. Mahapokai W, Van Sluijs FJ, Schalken JA. Models for studying benign prostatic hyperplasia. *Prostate Cancer Prostatic Dis* 2000;**3**:28–33
8. Oudot A, Oger S, Behr-Roussel D, Caisey S, Bernabe J, Alexandre L, Giuliano F. A new experimental rat model of erectile dysfunction and lower urinary tract symptoms associated with benign prostatic hyperplasia: The testosterone-supplemented spontaneously hypertensive rat. *BJU Int* 2012;**110**:1352–8
9. Rick FG, Abi-Chaker A, Szalontay L, Perez R, Jaszberenyi M, Jayakumar AR, Shamaladevi N, Szepeshazi K, Vidaurre I, Halmos G, Krishan A, Block NL, Schally AV. Shrinkage of experimental benign prostatic hyperplasia and reduction of prostatic cell volume by a gastrin-releasing peptide antagonist. *Proc Natl Acad Sci USA* 2013;**110**:2617–22
10. Steers WD. 5alpha-reductase activity in the prostate. *Urology* 2001;**58**:17–24. (discussion)
11. Azzouni F, Godoy A, Li Y, Mohler J. The 5 alpha-reductase isozyme family: A review of basic biology and their role in human diseases. *Adv Urol* 2012;**2012**:18
12. Barrack ER, Bujnovszky P, Walsh PC. Subcellular distribution of androgen receptors in human normal, benign hyperplastic, and malignant prostatic tissues: Characterization of nuclear salt-resistant receptors. *Cancer Res* 1983;**43**:1107–16
13. Carson C 3rd, Rittmaster R. The role of dihydrotestosterone in benign prostatic hyperplasia. *Urology* 2003;**61**:2–7
14. van der Gulden JW, Kolk JJ, Verbeek AL. Prostate cancer and work environment. *J Occup Med* 1992;**34**:402–9
15. Vinceti M, Venturelli M, Sighinolfi C, Trerotoli P, Bonvicini F, Ferrari A, Bianchi G, Serio G, Bergomi M, Vivoli G. Case-control study of toenail cadmium and prostate cancer risk in Italy. *Sci Total Environ* 2007;**373**:77–81
16. Anetor JI, Ajose F, Anetor GO, Iyanda AA, Babalola OO, Adeniyi FA. High cadmium/zinc ratio in cigarette smokers: potential implications as a biomarker of risk of prostate cancer. *Niger J Physiol Sci* 2008;**23**:41–9
17. Johnson MD, Kenney N, Stoica A, Hilakivi-Clarke L, Singh B, Chepko G, Clarke R, Sholler PF, Lirio AA, Foss C, Reiter R, Trock B, Paik S, Martin MB. Cadmium mimics the in vivo effects of estrogen in the uterus and mammary gland. *Nat Med* 2003;**9**:1081–4
18. Stoica A, Katzenellenbogen BS, Martin MB. Activation of estrogen receptor-alpha by the heavy metal cadmium. *Mol Endocrinol* 2000;**14**:545–53
19. Banerjee PP, Banerjee S, Brown TR. Increased androgen receptor expression correlates with development of age-dependent, lobe-specific spontaneous hyperplasia of the brown Norway rat prostate. *Endocrinology* 2001;**142**:4066–75
20. Gabal SM, Habib FM, Helmy DO, Ibrahim MF. Expression of estrogen receptor-B (ER-B) in benign and malignant prostatic epithelial cells and its correlation with the clinico-pathological features. *J Egypt Natl Canc Inst* 2007;**19**:239–48
21. Gleason DF. Classification of prostatic carcinomas. *Cancer Chemother Rep* 1966;**50**:125–8
22. Humphrey PA. Diagnosis of adenocarcinoma in prostate needle biopsy tissue. *J Clin Pathol* 2007;**60**:35–42
23. Martin MB, Voeller HJ, Gelmann EP, Lu J, Stoica EG, Hebert EJ, Reiter R, Singh B, Danielsen M, Pentecost E, Stoica A. Role of cadmium in the regulation of AR gene expression and activity. *Endocrinology* 2002;**143**:263–75
24. Pandya C, Gupta S, Pillai P, Bhandarkar A, Khan A, Bhan A, Prajapati A, Gupta S. Association of cadmium and lead with antioxidant status and incidence of benign prostatic hyperplasia in patients of Western India. *Biol Trace Elem Res* 2013;**152**:316–26
25. Gartrell MJ, Craun JC, Podrebarac DS, Gunderson EL. Pesticides, selected elements, and other chemicals in adult total diet samples, October 1980–March 1982. *J Assoc Off Anal Chem* 1986;**69**:146–59
26. Gartrell MJ, Craun JC, Podrebarac DS, Gunderson EL. Pesticides, selected elements, and other chemicals in infant and toddler total diet samples, October 1980–March 1982. *J Assoc Off Anal Chem* 1986;**69**:123–45
27. Bowers GN Jr, McComb RB. Measurement of total alkaline phosphatase activity in human serum. *Clin Chem* 1975;**21**:1988–95
28. Beutler E, Gelbart T. Plasma glutathione in health and in patients with malignant disease. *J Lab Clin Med* 1985;**105**:581–4
29. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979;**95**:351–8
30. Horsmans Y, Lannes D, Larrey D, Tinel M, Letteron P, Loeper J, Pessayre D. Nilutamide inhibits mephenytoin 4-hydroxylation in untreated male rats and in human liver microsomes. *Xenobiotica* 1991;**21**:1559–70
31. Huang D, Zhang Y, Qi Y, Chen C, Ji W. Global DNA hypomethylation, rather than reactive oxygen species (ROS), a potential facilitator of cadmium-stimulated K562 cell proliferation. *Toxicol Lett* 2008;**179**:43–7
32. Davis AM, Mao J, Naz B, Kohl JA, Rosenfeld CS. Comparative effects of estradiol, methyl-piperidino-pyrazole, raloxifene, and ICI 182 780 on gene expression in the murine uterus. *J Mol Endocrinol* 2008;**41**:205–17
33. Reed CA, Berndtson AK, Nephew KP. Dose-dependent effects of 4-hydroxytamoxifen, the active metabolite of tamoxifen, on estrogen receptor-alpha expression in the rat uterus. *Anticancer Drugs* 2005;**16**:559–67
34. Lee JD, Wu SM, Lu LY, Yang YT, Jeng SY. Cadmium concentration and metallothionein expression in prostate cancer and benign prostatic hyperplasia of humans. *J Formos Med Assoc* 2009;**108**:554–9
35. Aryal M, Pandeya A, Gautam N, Baral N, Lamsal M, Majhi S, Chandra L, Pandit R, Das BK. Oxidative stress in benign prostate hyperplasia. *Nepal Med Coll J* 2007;**9**:222–4
36. Harris MG, Coleman SG, Faulds D, Chrisp P. Nilutamide. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in prostate cancer. *Drugs Aging* 1993;**3**:9–25
37. Kim CS, Choi SJ, Park CY, Li C, Choi JS. Effects of silybinin on the pharmacokinetics of tamoxifen and its active metabolite, 4-hydroxytamoxifen in rats. *Anticancer Res* 2010;**30**:79–85
38. Hieble JP. Animal models for benign prostatic hyperplasia. *Handb Exp Pharmacol* 2011;**202**:69–79
39. Scolnik MD, Servadio C, Abramovici A. Comparative study of experimentally induced benign and atypical hyperplasia in the ventral prostate of different rat strains. *J Androl* 1994;**15**:287–97
40. Constantinou CE, Omata S. Analysis of the relative biomechanical effects of alpha 1 and alpha 2 antagonists in modifying the compliance of the prostate and micturition parameters of the hormonally manipulated male rat. *Neurourol Urodyn* 1996;**15**:85–101
41. Lee JZ, Omata S, Tillig B, Perkash I, Constantinou CE. Chronology and urodynamic characterization of micturition in neurohormonally induced experimental prostate growth in the rat. *Neurourol Urodyn* 1998;**17**:55–69
42. Kim HJ, Andersson LC, Bouton D, Warner M, Gustafsson JA. Stromal growth and epithelial cell proliferation in ventral prostates of liver X receptor knockout mice. *Proc Natl Acad Sci USA* 2009;**106**:558–63
43. Schauer IG, Ressler SJ, Rowley DR. Keratinocyte-derived chemokine induces prostate epithelial hyperplasia and reactive stroma in a novel transgenic mouse model. *Prostate* 2009;**69**:373–84
44. Wennbo H, Kindblom J, Isaksson OG, Tornell J. Transgenic mice overexpressing the prolactin gene develop dramatic enlargement of the prostate gland. *Endocrinology* 1997;**138**:4410–5
45. Shappell S, Masumori N, Thomas T, Case T, Pau M, Kasper S, Matusik R. Transgenic mouse models of prostate carcinoma: anatomic, histopathologic, and molecular considerations. In: Lalani EN, Abel PD (eds). *Prostate cancer: scientific and clinical aspects: bridging the gap*, 1st ed. Hackensack, NJ: Imperial College Press, 2003, pp. 245–319

46. Price D. Comparative aspects of development and structure in the prostate. In: Vollmer EP, Kauffmann G (eds). *Biology of the prostate and related tissues*. Washington, DC: US Government Printing Office, 1963, pp. 1–28
47. Coogan TP, Shiraishi N, Waalkes MP. Minimal basal activity and lack of metal-induced activation of the metallothionein gene correlates with lobe-specific sensitivity to the carcinogenic effects of cadmium in the rat prostate. *Toxicol Appl Pharmacol* 1995;**132**:164–73
48. Yamano T, DeCicco LA, Rikans LE. Attenuation of cadmium-induced liver injury in senescent male Fischer 344 rats: Role of Kupffer cells and inflammatory cytokines. *Toxicol Appl Pharmacol* 2000;**162**:68–75
49. Waalkes MP, Rehm S, Perantoni AO, Coogan TP. Cadmium exposure in rats and tumours of the prostate. *IARC Sci Publ* 1992;**118**:391–400
50. Benbrahim-Tallaa L, Liu J, Webber MM, Waalkes MP. Estrogen signaling and disruption of androgen metabolism in acquired androgen-independence during cadmium carcinogenesis in human prostate epithelial cells. *Prostate* 2007;**67**:135–45
51. Totten RS, Heinemann MW, Hudson PB, Sproul EE, Stout AP. Microscopic differential diagnosis of latent carcinoma of prostate. *AMA Arch Pathol* 1953;**55**:131–41
52. Coffey DS, Isaacs JT. Control of prostate growth. *Urology* 1981;**17**:17–24
53. Thomas LN, Douglas RC, Lazier CB, Too CK, Rittmaster RS, Tindall DJ. Type 1 and type 2 5alpha-reductase expression in the development and progression of prostate cancer. *Eur Urol* 2008;**53**:244–52
54. Gelmann EP. Molecular biology of the androgen receptor. *J Clin Oncol* 2002;**20**:3001–15
55. Prins GS, Birch L, Couse JF, Choi I, Katzenellenbogen B, Korach KS. Estrogen imprinting of the developing prostate gland is mediated through stromal estrogen receptor alpha: studies with alphaERKO and betaERKO mice. *Cancer Res* 2001;**61**:6089–97
56. Barton MC, Shapiro DJ. Transient administration of estradiol-17 beta establishes an autoregulatory loop permanently inducing estrogen receptor mRNA. *Proc Natl Acad Sci USA* 1988;**85**:7119–23
57. Pelletier G, Labrie C, Labrie F. Localization of oestrogen receptor alpha, oestrogen receptor beta and androgen receptors in the rat reproductive organs. *J Endocrinol* 2000;**165**:359–70
58. Cohen I, Beyth Y, Altaras MM, Shapira J, Tepper R, Cardoba M, Yigael D, Figer A, Fishman A, Berenhein J. Estrogen and progesterone receptor expression in postmenopausal tamoxifen-exposed endometrial pathologies. *Gynecol Oncol* 1997;**67**:8–15
59. Alonso-Magdalena P, Brossner C, Reiner A, Cheng G, Sugiyama N, Warner M, Gustafsson JA. A role for epithelial-mesenchymal transition in the etiology of benign prostatic hyperplasia. *Proc Natl Acad Sci USA* 2009;**106**:2859–63

(Received November 16, 2013, Accepted March 27, 2014)

Stem Cell
Research & Therapy

Prajapati et al., J Stem Cell Res Ther 2014, 4:1
http://dx.doi.org/10.4172/2157-7633.1000164

Research Article Open Access

Pluripotent Stem Cell within the Prostate could be Responsible for Benign Prostate Hyperplasia in Human

Akhilesh Prajapati[1], Sharad Gupta[2], Ramesh Bhonde[3] and Sarita Gupta[1]*

[1]Department of Biochemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda,Vadodara, Gujarat 390002, India
[3]Gupta Pathological laboratory, Vadodara, Gujarat, India
[3]Manipal Institute of Regenerative Medicine, Manipal University Campus, Bangalore, Karnataka, India

Abstract

Aim: Abnormal prostate growth is the most prevalent pathological sign in aged human males, reflected by high incidence of Benign Prostatic Hyperplasia (BPH) and Prostate Cancer (PCa). The successful isolation and cultivation of prostate cells, is a prerequisite need for establishing a model cell line for understanding the pathogenesis, unique biological properties and also various evidences suggest the role of stem cells in the pathogenesis of these conditions for a therapeutic point of view.

Methods and results: Here we isolated a candidate pluripotent stem cell population from BPH patients underwent TURP which include the isolation of an enriched population of prostate stem cells through cell culture techniques and the cultivation of prostate stem cells *in vitro* and characterization of these cells and their stem potential, including *in-vivo* teratoma generation. Cytogenetic analysis by G-banding assay demonstrated an aneuploid karyotype with a model chromosome number of 60 and normal Y chromosome. Characterization of isolated cells showed the presence of ONS pluripotency stem cell markers. Beside this these cells were also found positive for stem cell surface markers such as CD49b, CD44, CD117, CD34 and prostatic tissue specific markers like p63 and Androgen Receptor. *In-vitro* differentiation of the cells demonstrated formation of a tri-germinal layer into ectodermal, endodermal and mesodermal cell lineages with defined medium conditions and *In-vivo* teratoma formation in excised tumor in Balb/*c* mouse.

Conclusion: we report here isolation, establishment and characterization of human prostate-derived pluripotent stem cell line. The cell line eventually serves as a potential tool for studies in prostate adult stem cell research, understanding etiopathophysiology and the regulation of BPH and PCa.

Keywords: Benign prostate hyperplasia; Prostate stem cells; Pluripotent stem cell marker; Teratoma; Karyotype; Multi-lineage differentiation

Introduction

The prostate is a hormonally regulated organ whose growth accelerates at sexual maturity due to androgen actions on both stromal and epithelial cells. In men over the age of 40-50 years, prostate gland represents a major medical problem in the form of benign prostate hyperplasia (BPH) and prostate cancer (Pca). Epidemiological data from several studies indicated that both diseases are becoming increasingly prevalent worldwide [1,2]. Unavailability of normal/ benign prostate cell lines and suitable animal model made these attributes difficult to study in vitro and in vivo. At histological level, human prostate contains mainly two types of cells, epithelial and stromal cells. The stromal to epithelial ratio in normal prostate of human is 2:1 [3,4]. The epithelial cell layer is composed of four differentiated cell types known as basal, secretory luminal, neuroendocrine (NE), and transit-amplifying (TA) cells that are identified by their morphology, location, and distinct marker expression. The basal cells form a layer of flattened to cuboidal shaped cells above the basement membrane and express p63 (a homolog of the tumor suppressor gene p53), Bc1-2 (an anti-apoptotic factor), Cluster designation (CD) 44, hepatocyte growth factor (HGF), and the high molecular weight cytokeratins (CK) 5 and 14.The expression of androgen receptor (AR) is low or undetectable in the basal cells, which makes the basal cells independent of androgens for their survival [5-7].

Both human and animal studies have shown that stromal cells are essential for functional and morphological differentiation of prostatic epithelium. It has been hypothesized that the basal layer is the proliferative compartment of the prostate, containing a stem cell population, which can differentiate into secretory epithelium and TA cells. Prostatic stem cells are present within the epithelium and are capable of regenerating the adult organ [8]. Several investigations based on stem cell models have elegantly defined role of stem cells in cellular turnover and morphology in normal human prostate [9]. To further support the role of basal stem cells in prostate development, an experiment on p63 null mice was performed and the resultant progeny of these animals were born devoid of prostate gland [10-12]. As the stem cells are key target for mutagenic changes and tumurogenesis in human prostate, an urge arises to understand more about their status in normal and disease prostate tissue and the cellular and molecular mechanism of BPH pathogenesis.

The concept of stem and progenitor cells with the capacity for self-renewal and multilineage differentiation has been important to understand the molecular mechanisms of normal development and functional homeostasis [6,13]. This is also very important to

***Corresponding author:** Sarita Gupta, Department of Biochemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda,Vadodara, Gujarat 390002, India, Tel: +91-9426319494; +91-265-2795594; E-mail: sglmescrl@gmail.com; akhileshbiotech06@gmail.com

Received November 15, 2013; **Accepted** January 24, 2014; **Published** January 27, 2014

Citation: Prajapati A, Gupta S, Bhonde R, Gupta S (2014) Pluripotent Stem Cell within the Prostate could be Responsible for Benign Prostate Hyperplasia in Human. J Stem Cell Res Ther 4: 164. doi:10.4172/2157-7633.1000164

understand how tissues are remodeled during inflammatory repair, or in carcinogenesis resulting from oxidative stress, inflammation, genomic and metabolic insults [14,15]. However, at a practical level there have been few human cell lines available that accurately recapitulate prostatic development and that can be used to examine these concepts. To pursue studies relevant to normal human prostate biology with associated disorders as a starting point for studies on human disease, there is an urgent need for human prostate cell lines that show phenotypes that match human tissue samples. There are several non-tumorigenic immortalized human prostate epithelial (HPrE) cell lines have been established using viral SV-40Tag or E6/E7 infection including BPH-1 [16], and RWPE-1 [17], none of these accurately recapitulate normal human prostatic growth and function.

In light of this we made an attempt to isolate and establish a candidate population of Human prostatic stem/progenitor cells from BPH patients (underwent TURP) that expresses pluripotency markers. Characterization of isolated cells showed the presence of pluripotency stem cell markers like Oct 3/4, Nanog and Sox-2 by mRNA expression, western blotting and flowcytometery. We further assessed the expression level of stem cell surface markers to identify normal Prostate stem cells (PSCs) interestingly, these cells were found positive for prostate stem cell markers such as CD49b, CD44, CD117, CD34, p63 and prostatic tissue specific marker like Androgen Receptor. Upon the introduction to specific culture condition isolated prostate cells can differentiate into adipocyte, osteocyte and chondrocyte (mesodermal origin), islet formation (endodermal origin) and neuronal differentiation (ectodermal origin) cell lineages in *In-vitro* and *In-vivo* teratoma formation in *Balb/c* mouse with three germ layers. Isolated prostate cells could provide an ideal source of pluripotent-like stem cells with the potential to have a critical impact on regenerative medicine.

Materials and Methods

Prostate samples

Prostatic tissue was obtained from patients (average age of 70 years; range 55-75 years) who underwent TURP, patient detailed demographic and anthropometric data collected in structured Questionnaire with consent and ethical approval. Benign prostatic hyperplasia (BPH) histology was confirmed by a surgical pathologist and contained no adenocarcinoma of the prostate.

Chemicals

All primary antibodies and cell culture media (Anti-Androgen Receptor cat no A9853, Anti-Vimentin cat no. C9080, Anti Ki-67 cat no. P6834) and FACS antibodies (Anti-Oct3/4, Sox-2 and Nanog) purchased from Sigma-Aldrich, USA and BD Biosciences,USA respectively. RT-PCR reagents from fermentas, Germany. All the reagents were extra pure and of cell culture and molecular biology grade.

Isolation of prostate cells from TURP samples

Fresh TURP tissue obtained from human prostate surgical specimens. Prostate tissue samples were minced into small pieces and digested with Collagenase type I enzyme for 1 hr at 37°C in a shaking incubator at 110 rpm followed by grown in DMEM medium with 10% FBS as previously described [18].

Cell growth kinetics

Fully confluent prostatic cells were trypsinized with 0.1% Trypsin EDTA solution and counted under an inverted phase contrast microscope (Nikon TE2000, Japan). 5 x 10^4 cells were seeded into each well of 24-well plates for growth curve studies. Cells were eventually trypsinized and counted at different time points (0 h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h and 168 h). Cell counts were then plotted versus time to demonstrate the growth curve of the cells. Doubling time of the isolated prostate cells were determined using the algorithm Ln (Nt-N0) Ln (t), where Nt and N0 were numbered of cells at the final time point and at the initial seeding point respectively, and t was a time period in hours for which cell counts were recorded.

Karyotyping

To study the chromosomal stability isolated human BPH cells (passage7 and 14) were treated with Colcemid (Gibco 15 210-057), trypsinized, resuspended in 75-mM KCl hypotonic solution, fixed in MeOH/acetic Acid (3:1) and stained for metaphase spreads using a standard G-banding protocol technique [19]. For each sample, at least 20 metaphase spreads were examined, in which there was minimal chromosome overlaps, and long chromosome length, little or no cytoplasm, and high banding resolution were selected for detailed analysis.

RNA extraction and semi quantitative Reverse Transcriptase PCR (RT-PCR)

Total RNA was isolated from cells using TRizol Reagent (Sigma Aldrich, USA) extraction following the manufacturer's instructions and immediately treated with DNase l (fermentas). 5 µg of total RNA was reverse transcribed into first strand cDNA using random primers and subjected to PCR amplification of various stem cell genes. One µl of cDNA products was used to amplify genes using a 2X master mix [Sigma Aldrich, USA], containing 1.5 µl Taq Polymerase, 2 mM dNTP, 10X Tris, glycerol reaction Buffer, 25 mM MgCl2, and 20 pM appropriate forward and reverse primers for each gene. GAPDH served as an internal control (Table 1 for primers sequence and annealing temperature). PCR products were separated on a 10% polyacrylamide gels [Sigma Aldrich, USA], visualized and images were captured by Cambridge UV tech, Chemi-doc instrument.

Western blotting

Western blotting of isolated prostate cells performed as previously described [3]. Isolated prostate cells were lysed with urea containing lysis buffer (1 mM EDTA, 50 mM Tris-HCl pH 7.5, 70 mM NaCl, 1% Triton, 50 mM NaF) supplemented with protease inhibitor cocktail (Fermentas INC.). Total Protein estimation was carried out using

Gene	Primer sequence	Annealing Temp.	Product size
Oct ¾ (NM_002701)	Forward-5'AGCTGGAGAAGGAGAAGCTGG-3' Reverse-5'-TCGGACCACATCCTTCTCGAG-3'	63.5°C	458 bp
Sox-2 (NM003106)	Forward-5'-CACCTACAGCATGTCCTACTC-3' Reverse-5'-CATGCTGTTTCTTACTCTCCTC-3'	60°C	384 bp
Nanog (NM_024865)	Forward-5'GCAAACAACCCACTTCTGC-3' Reverse- 5'AGGCCTTCTGCGTCACAC-3'	55.5°C	287 bp

Table 1: Primers Sequence and annealing temperature.

Bradford reagent according to manufacturer's suggestions (BIO-RAD). Cell lysates (40 µg) were separated on Polyacrylamide gel using the Mini-tetra-cell electrophoresis system (BIO-RAD) and transferred onto a nitrocellulose blotting membrane (Millipore) Blots were then incubated with blocking milk buffer (5% fat free skimmed milk with 0.1% Tween-20 in PBS)s. Primary antibodies were added to blots and incubated overnight at 4°C. Anti-rabbit and Anti-mouse IgG conjugated with HRP were used to develop the blots using Ultra-sensitive enhanced chemiluminiscence reagent (Millipore, USA). And imaged by Chemi-doc instrument Cambridge UV tech, UK.

FACS analysis

Cells were trypsinized, centrifuged, and one million cells were resuspended in 100 µl of wash buffer [PBS containing 10% serum], washed twice with Phosphate buffered saline [PBS] containing 1% bovine serum albumin, and then incubated with primary antibody at 4°C for 1 h. Cells were than labelled with 100 µl of secondary antibody for counter staining, and incubated for an additional 40 min at 4°C [20]. Data were recorded and observations analysed using BD FACS Aria III(BD, USA) and flowjo software respectively.

Immunocytochemistry

Adherent cells were washed with PBS with 2% FBS and then with PBS, the cells were fixed with 2% PFA solution followed by PBS washing and triton-X100 treatment using standard protocol and incubated with primary monoclonal antibodies overnight at 4°C followed by 1 hr incubation with fluorochrome tagged secondary antibodies at room temperature. For negative controls, the primary antibodies were omitted. The expressions of antigens in cells were assessed by immunofluoresense method. Images from Carl Zeiss LSM-710 confocal microscope (Carl Zeiss, Germany) were recorded.

In-vitro Differentiation

Ectodermal lineage

Neuronal differentiation: Isolated prostate cells were plated in six-well plat as described above in the presence of neuro-basal medium (Invitrogen) with N2 supplements and 2 mM glutamate for 10 days. The culture medium was replaced every 4th day. Parallel control cells were cultured without neuronal differentiation medium.

Mesodermal lineage

Osteocyte differentiation: Isolated prostate cells were trypsinized, washed with 10 mM PBS, pH 7.2, and resuspended in DMEM high glucose with 10% FBS medium. Cells were plated into six- well plate at 10^5 cells/well in the presence of osteocyte reagents (20 mM B-glycerol phosphate, 50 ug/ml ascorbic acid and 10 mM dexamethasone) for 10 days. The culture medium was replaced every 3rd day. Parallel control cells were cultured without osteocyte reagents.

Adipocyte differentiation: Isolated prostate cells were plated in six-well plate as described above. Adipogenesis was induced by treatment with IBMax (10 mg/ml),10 mM dexamethasone and 10 mg/l insulin for 8 days. The culture medium was replaced every 3rd day. Parallel control cells cultured without adipocyte differentiation medium.

Chondrocyte differentiation: Isolated prostate cells cells were plated in six- well plate as described above. Chondrocyte differentiation was induced by treatment with 10 mM dexamethasone and 10 mg/l insulin for 20 days. The culture medium was replaced every 5th day. Parallel control cells were cultured without chondrocyte differentiation medium.

Endodermal lineage

Islet differentiation: Isolated prostate cells were plated in six-well plated as described above in the presence of islet differentiation serum free RPMI1640 medium with 10 ng/l activin-A and 10 mg/l insulin for 10 days. The culture medium was replenished every alternate day. Parallel control cells cultured without islet differentiation medium.

In-vivo Differentiation

Teratoma formation

For each graft, approximately 0.2 million isolated prostate cells, washed and resuspended in 300 µl DMEM complete medium, and transplanted subcutaneously (intraperitoneal body cavity) on left side of six Balb/c mice (maintained in MSU in-house animal house facility) with 1.5% melted agarose using 23G needle [21,22]. Right side of the same animal was used for control or placebo i.e. only agarose plugs were injected in that site. The experiment was approved by the Institute Animal Ethical Committee (CEPSC Reg. No. 938/a/06/CPCSEA). After 3 weeks of transplantation, mice were sacrificed. Visible tumours, were dissected out and fixed overnight with 4% PFA solution. The tissues were then paraffin embedded, sectioned, stained with H&E, and were examined for the presence of cells representatives of all three germ layers produced by transplanted cells [23].

Results

Prostate cells isolation and characterization

Surgically removed TURP prostate tissue samples were digested with collagenase type I enzyme for 1 hr at 37°C. After enzymatic digestion microscopic observation of cultured prostatic cell population showed characteristic fibroblastic and epitheliod shaped cells (Figures 1B and 1C). To isolate epithelial cells from the mix population, epithelial cell patches were picked up using sterile filter paper discs soaked in trypsin and transferred the cells into DMEM medium with 10% FBS for enrichment (Figure 1A). To further confirm the nature of isolated cells, immunocytochemistry was performed with different cell markers (Figure 2A).

Growth curve and kinetics

Cells were plated in 24-well plates and used for determining the population doubling potential, progression and proliferative activity. Cumulative population doublings were calculated by considering initial number of cells seeded at 0 hrs and number of cells harvested at each destined time point respectively without passaging. These observations provide a theoretical growth curve that is directly proportional to the cell number. With the help of the curve generated, doubling time was found to be 26 ± 1.3 hrs.

Cytogenetic analysis

Cytogenetic analysis by G-banding assay demonstrated an aneuploid karyotype with a model chromosome number of 60 (range 58 to 62, n=20) with 4 to 5 marker chromosomes, which were structurally rearranged and the Y chromosome was found to be normal (Figure 1D).

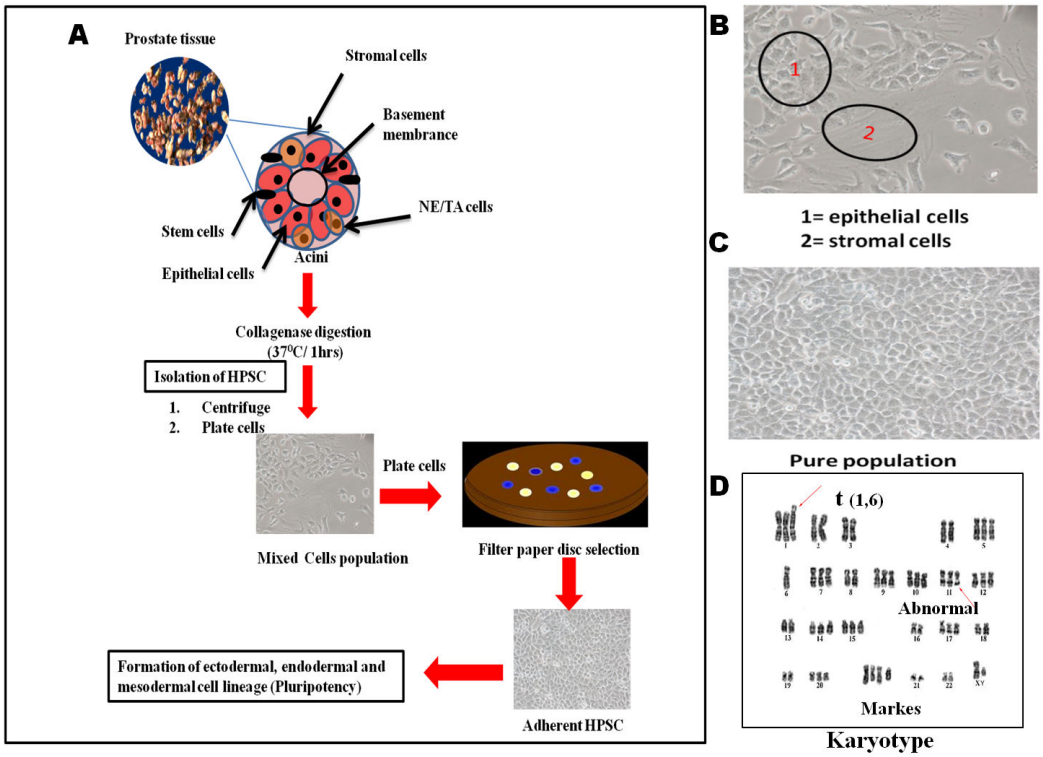


Figure 1: Isolation and cytogenetic characterization of isolated prostate cells from BPH patient. (A) Schematic of prostatic cells isolation from TURP tissue. Cells were obtained after 1 hrs, incubation with collagenase type-I enzyme in DMEM medium without FBS at 37°C. (B and C) Morphological view of isolated cells from mixed population using sterile filter paper discs soaked in trypsin and cultured in DMEM medium with FBS at 37°C under 5% Co_2 (D) karyotype analysis of isolated prostate cells. Cells showed aneuploidy with 4 to 5 marker chromosomes and translocation of 6^{th} chromosome to 1^{st}.

Stem cell characterization

To identify the stem cell properties of isolated cell population, immunocytochemistry for Nestin, E-cadherin, CK19, AR, Vimentin and Ki-67 and FACS analysis for stem cells specific markers such as CD49b, CD44, CD117, CD34 and p63 were performed. Fluorescent microscopic analysis showed cells were positive for stem cell markers (Figure 2A). FACS analysis showed that 3.04% cells were positive for CD49b, 95.3% cells were positive for CD44, 20% cells were positive for CD117, 16.5% cells were positive for CD34 and 96.3% cells were positive for p63 (Figure 2B).

Pluripotency features

To elucidate whether the cells possess pluripotent characteristics, cells were further analyzed for the ONS markers by flow cytometry using BD FACS antibodies. Flowcytometry results showed that1.11% cells were positive for Oct3/4 a protein involved in the self-renewal of human ES cells; Nanog, another transcription factor involved in self-renewal of human ES were 18% in cells and 13.1 % cells were positive for Sox-2,a transcription factor that control genes involved in embryonic development (Figure 3C). RT PCR analysis also showed higher expression of all the three ONS genes, GAPDH served as an internal control (Figure 3A). Further western blot was performed to confirm the expression of ONS at protein level. Western blot analysis showed clear bands for ONS proteins at 34, 117 and 40 kD, respectively, beta actin served as an endogenous control (Figure 3B).

In vitro differentiation

Ectodermal lineage differentiation: To investigate the potential differentiation into ectodermal lineage, isolated prostate cells were cultured for 10 days in neuronal differentiating medium. Cells were positive for MAP-2, a marker for mature neurons (Figure 4).

Mesodermal lineage differentiation: To determine the potential of isolated prostate cells to differentiate into cells of mesodermal lineages: adipocytes, chondrocytes and osteocytes where cells were grown as adherent cells in the respective differentiation culture medium. Differentiation of the prostate cells into a mesodermal lineage was determined by immunocytochemistry and specific staining. Mesodermal markers included PPAR-Y, a marker for adipocyte, CD44, marker for osteocytes and CD 90 and CD44 combined marker for chondrocytes. Futher these cells also stained with Oil Red O, Alizarin red S and Alcian blue stain for Adipocytes, osteocytes and chondrocytes respectively (Figure 5(i-iii)).

Endodermal lineage differentiation: Differentiation of isolated prostate cells to an endodermal lineage (islet differentiation) was detected in prostate cells cultured in defined medium for 10 days. Cells were positive for glucagon and C-Peptide by immunocytochemistry (Figure 6).

In vivo differentiation:

Teratoma formation: To further validate the phenotypic properties of isolated prostate cells in terms of pluripotency, in-vivo experiment for teratoma formation in balb/c mice was carried out, since teratomas formation considered as gold standard technique to prove pluripotency. All of the 6 mice developed evident teratomas (left side) wherein skin bulges were bigger in size than that of agarose plugs

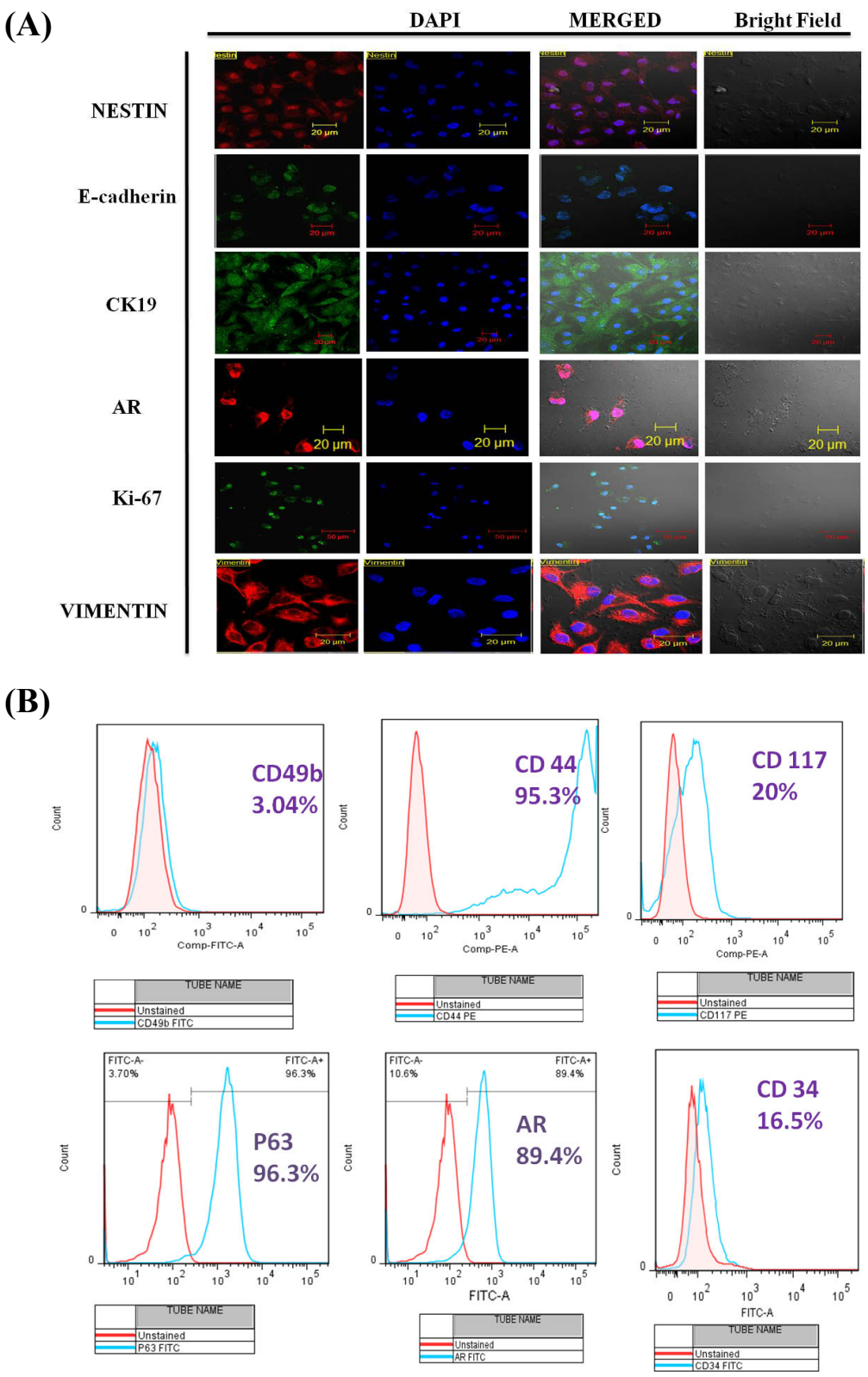


Figure 2: Characterization of isolated prostate cells. (A) Immunofluorescence staining of BPH prostate cells for Nestin, E-cadherin, Ck19, Androgen Receptor, Ki-67 and Vimentin (first panel from left side). The second panel from left side shows DAPI staining and the second panel form right side shows the merged images. Bars represent 20 µm (B) FACS analysis of cell surface markers demonstrate 3.04% CD49b, 95.3% CD44, 20%CD117, 96.3% p63, 89.4%AR and 16.5% CD34 positive cells in isolated prostate cell

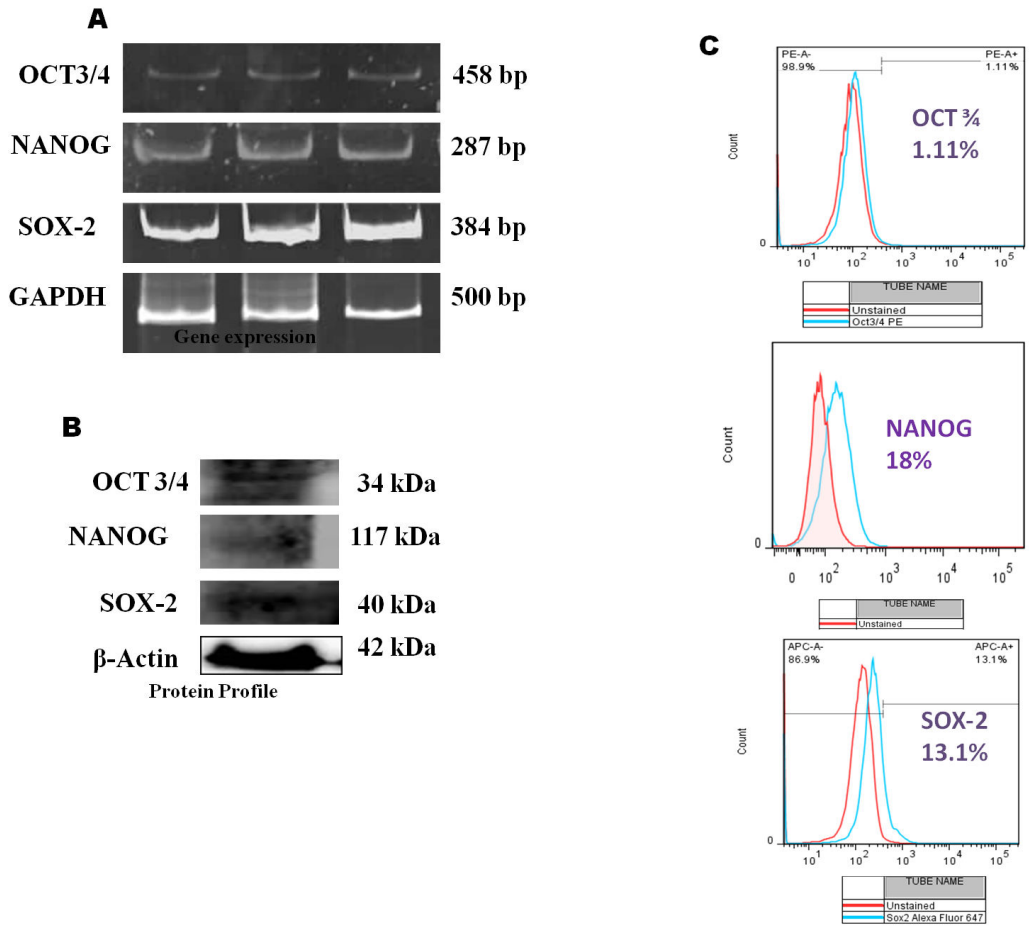


Figure 3: Isolated prostate cells express pluripotent stem cell markers. (A and B) At the genomic and protein level. Gel-electrophoresis of RT-PCR products and protein profile of pluripotency specific genes (Oct 3/4., Sox-2 and Nanog) showed expression. (C) Flow cytometry data showed presence of ONS markers in isolated cells.

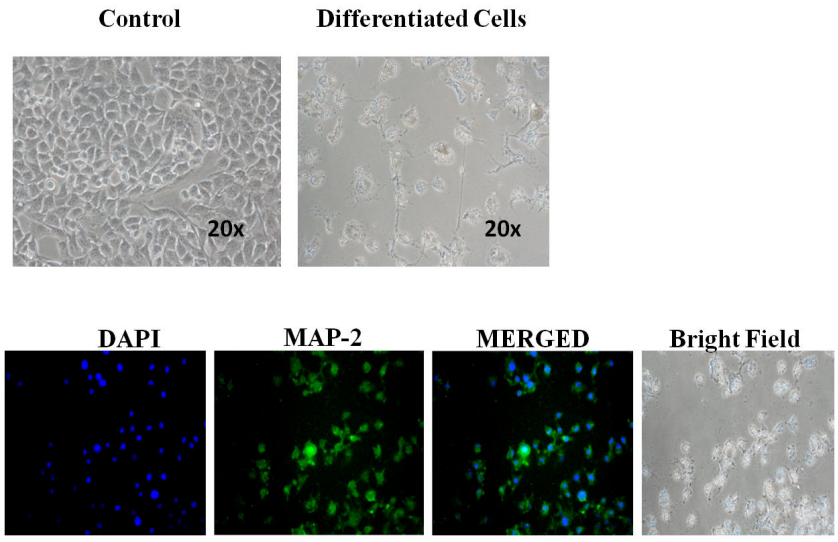


Figure 4: Isolated prostate cells can differentiate to ectodermal cell lineages. (A) Without and (B) with neural differentiation reagents (neuro-basal medium with N2 supplements and 2 mM glutamate) for 10 days. (A) Control. (B) Neural like cells. (C) Neural-like cells were detected by Immunofluorescence staining using MAP-2 antibody. Nuclei were stained with DAPI.

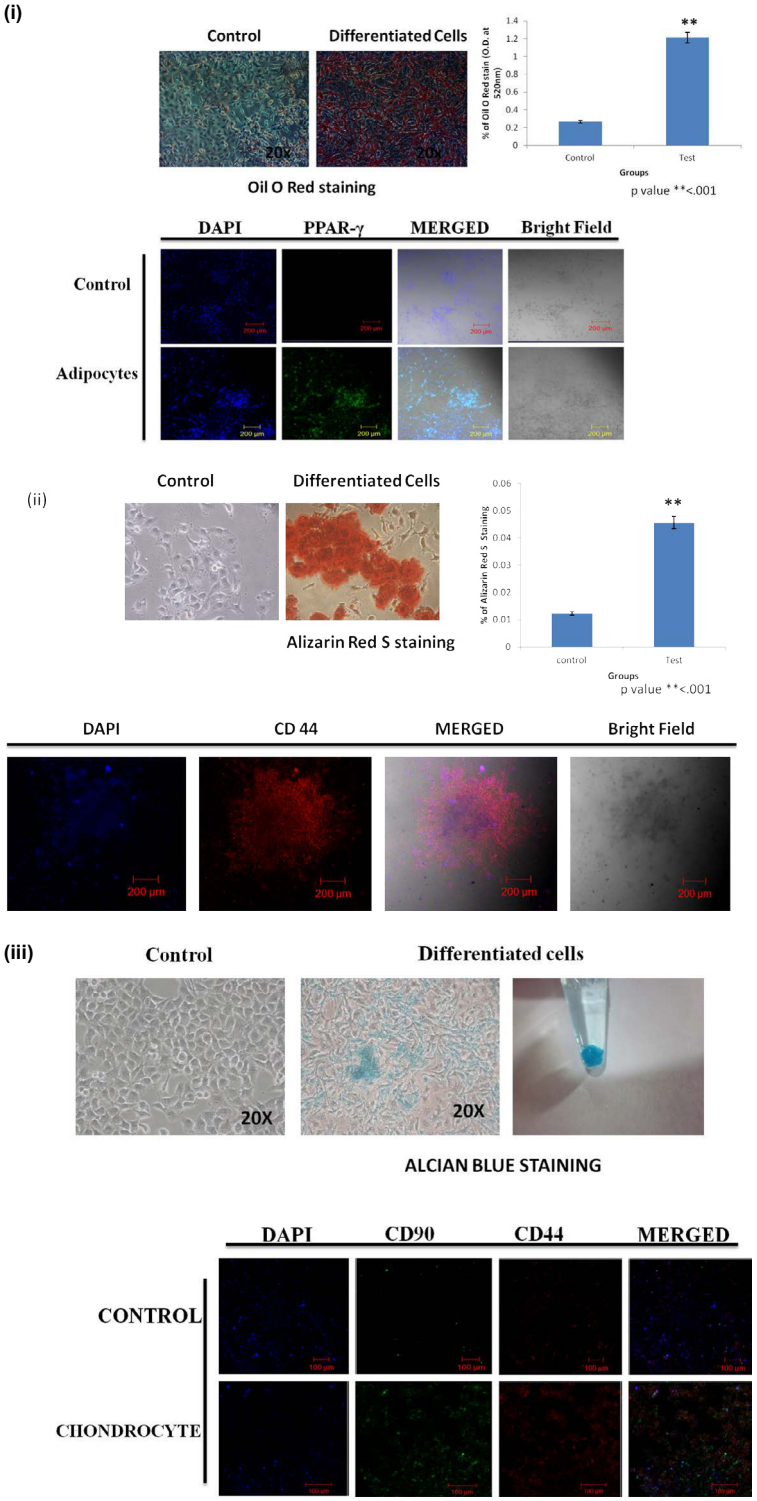


Figure 5: Isolated prostate cells can differentiate to mesodermal cell lineages. (i) Oil red O staining of isolated prostate cells after culture (A) without and (B) with adipocytic differentiation reagents (IBMax 10 mg/ml, 10 mM dexamethasone and 10 mg/l insulin) for 8 days. (A) Control cultures showed Oil Red O negative cells; (B) Positively staining adipocytes. (C) histogram showing the significant oil red O staining in differentiated cells. (D) Immunofluorescence staining of PPAR-γ for differentiated adipocytes. Nuclei were stained with DAPI. (ii) Alizrain red S staining of isolated prostate cells after culture (2A) without and (2B) with osteocyte differentiation reagents (20 mM glycerol phosphate, 50 µg/ml ascorbic acid, 10 mM dexamethasone and 10 mg/l insulin) for 10 days. (2A) Control cultures showed Alizarin red S negative cells; (2B) Positively staining osteocytes. (2C) histogram showing the significant Alizarin red S staining in differentiated cells. (2D) Immunofluorescence staining of CD44 for differentiated osteocytes. Nuclei were stained with DAPI. (iii) Alcian blue staining of isolated prostate cells after culture (A) without and (B) with chondrocyte differentiation reagents (10 mM dexamethasone and 10 mg/l insulin) for 20 days. (A) Control cultures showed Alcian blue negative cells; (B) positively staining chondrocytes. (C) Immunofluorescence staining of CD90 and CD44 for differentiated chondrocytes. Nuclei were stained with DAPI.

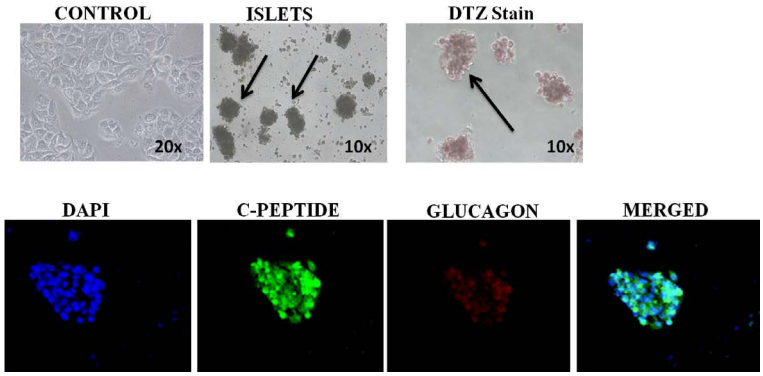


Figure 6: Isolated prostate cells can differentiate to endodermal cell lineages. (A) Without and (B) with islet differentiation reagents (serum free RPMI 1640 medium with 10 ng/l activin-A and 10 mg/l insulin) for 10 days. (A) Control. (B) Islet like cell cluster. (C) DTZ staining. (D) islet-like cell cluster were detected by immunofluorescence staining using C-peptide and glucagon antibodies. Nuclei were stained with DAPI.

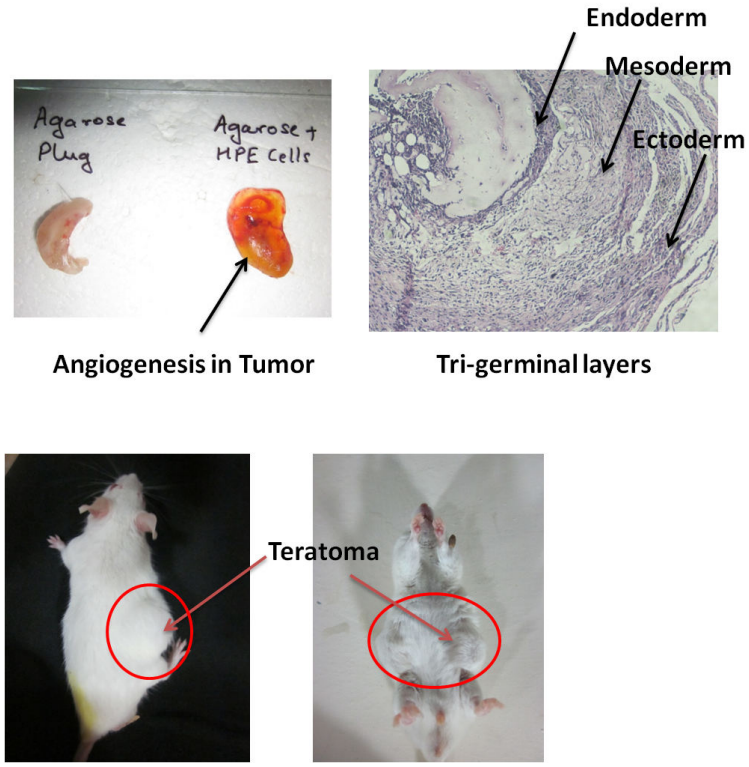


Figure 7: Teratomas formation capability of isolated prostate cells with tri-germinal layer. 0.2 million cells with agarose plug were transplanted in left side body cavity of Balb/c mice. Right side only agarose plug transplanted. (A) Angiogenesis in transplanted tumor. (B) H&E staining showing tri-germinal layer formation in excised tumor transplanted with prostate cells. (D) Visible teratomas in animals.

alone (right side). Also we noted that teratomas formed by isolated prostate cells transplanted with agarose plugs were significantly larger than those formed by agarose plugs alone. Imaging of teratoma sections demonstrated that major mass of tumor plugs were mainly derived from isolated prostate cells (Figure7A).

Histological studies confirmed *in-vivo* functionality and representation of tri-germinal layers in developed teratomas (Figure 7B). Since the cells were encapsulated in agarose and transplanted subcutaneously beneath the forearms and not directly in vasculature; the chances of spreading were minimal to begin with.

The isolation and characterization of human prostate stem cells from BPH patient (underwent TURP) have yielded many interesting findings that these prostate cells possess:

1. Pluripotency stem cell markers.
2. Strong proliferative potential with the ability to differentiate into ectodermal, mesodermal and endodermal lineages and teratoma formation with three germ layers. These cell preparations may serve as a potential tool for studies in prostate adult stem cell research and the regulation of Benign Prostatic Hyperplasia.

Discussion

BPH is a slow progressive enlargement of the prostate gland which can lead to lower urinary tract symptoms (LUTS) in elderly men. It is characterized by hyperplasia of epithelial and stromal cells in the transition zone of the prostate gland, which can be observed histopathologically [24]. Stem cells in the human prostate have been identified and isolated using the cell surface markers such as CD44 [25], integrin α2β1 [26], CD133 (Prominin-1) [27] which, are believed to be responsible for the development and progression of proliferative disorders of the prostate such as prostate cancer and benign prostate hyperplasia [9,28-30]. Based on high expression of α2β1 integrin, Collins and colleagues identified PSCs in the basal layer and showed that the $\alpha2\beta1^{high}$ integrin cells represent ~1% of basal cell population in the human prostate [26].

A very recent finding has demonstrated a relatively high expression of stemness-associated genes, including Oct4A, Sox2, c-Myc, Nanog, and Klf4, in BPH as compared to normal prostate tissue [31]. However, role of ONS and other stem cell markers in hyperplastic prostatic epithelium remain to be established. In the present study, cells were isolated from human TURP (Trans Urethral Resection of Prostate) tissue excised from BPH patients. Stemness nature of isolated cells can be proved by 1. expression of stem cell marker genes, 2. *In vivo* teratoma formation, 3. and *In vitro* multiple-cell lineage differentiation. The expression levels of ONS markers of isolated prostate cells clearly prove pluripotent nature. These cells do possess high level of prostate stem cell markers like CD44 (95%),CD117(c-kit) (20%), p63 (96%), CD49b (3%) and Nestin. The expression levels of CD49b in the present study is high when compared to previous report that showed presence of just 1% of this marker [32]. The p63 a homolog of p53, is present in the basal epithelium of the prostate and in primary cell cultures from normal tissues and its expression is absent in prostate cancer [12,33]. Previous investigation revealed a role of p63 in stem cell functions [34]. In our study isolated cells showed approximately 96.3% p63 positive cells by flow cytometry, further supporting stemness characteristics of isolated prostate cells.

Leong et al. [35] identified CD117 (c-Kit, stem cell factor receptor) as a new marker of a rare adult mouse PSC population which showed self-renewal and full differentiation potential characteristics of stem cells. The CD117(+) with CD44(+) phenotype regenerated functional prostate after transplantation *in vivo*. Moreover, CD117(+) PSCs showed long-term self renewal capacity after serial isolation and transplantation *in vivo*. CD117 expression was predominantly localized to the proximal region of the mouse prostate and was upregulated after castration-induced prostate involution, consistent with prostate stem cell identity and function [35]. CD44 was used as the marker to identify basal stem cells with tissue-regeneration abilities [36]. Interestingly, isolated cells showed both CD117 and CD44 i.e 20% and 95% of these marker respectively, which eventually supports the above fact.

The presence of all the three germ layers (Ecto, Meso and Endoderm) in *in vivo* study clearly demarcates the ability of these cells to form teratomas similar to that of embryonic stem cells. Moreover, results from *in vitro* study suggest that the isolated cells are capable of differentiating into multiple-cell lineages ie. Osteocyte, adipocyte and chondrocyte (Meso-dermal origin), Neural differentiation (Ectodermal origin) and Islet like cell formation (Endo-dermal origin) further providing evidence of pluripotent nature.

It has been reported that stromal to epithelial ratio is altered in BPH, where the ratio increases from 2:1 in normal glands to 5:1 in BPH [15], because the existence of adult stem cells in the prostate stromal compartment is speculated to expand the stroma in response to stimuli during the pathogenesis of BPH [37]. However, involvement of epithelial cells in the prostate development has been less well-understood. It has long been hypothesized and further supported by experiments that a stem/progenitor cell hierarchy exists within the prostate epithelium as well [6,15,38].

The presence of these high proliferative and plastic stem cells isolated from BPH patient in our investigation suggests that BPH could occur as a result of amelioration of stem cell properties that could ultimately give rise to a clonal expansion of specific cell population.

Further cytogenetic study of the isolated prostate cells has demonstrated an aneuploid DNA content and translocation of chromosome 6 to chromosome 1 in prostate. Earlier reports also showed deletion, translocation, inversion and mosaics on chromosomes 1, 7, 16 and Y in south Indian BPH patients and Chromosome 1 showed deletion and translocation in both PC and BPH Patients [39]. Chromosome 1 has a breakage-prone site, which has been reported to be sensitive to environmental clastogens and responsible for tumor development and progression [40-42].

Most interesting finding of our study is that the isolated pluripotent stem cells from BPH patients are expressing both basal(CD44, CD49b, p63 etc.) and secretory (AR,CD117,CK19 etc.) epithelial cell markers and capable to form teratomas when transplanted into balb/c mice along with three germ layers formation. Evidence has been shown that basal and secretory cells have the ability to self-renew [43]. Molecular mechanism and pathways involved in hyperplastic prostate differentiation, especially stem cell differentiation, are poorly understood due to the lack of suitable models. Hence, we made an attempt to develop cell line as referred in present study and cost effective animal model in our earlier study which can be used for understanding the prostate pathology. (Prajapati et al. [30] communicated).

Many methods have been used to establish cell lines using viral oncogenes, overexpression of human TERT or knockdown of specific proteins to inactivate regulatory key pathways, making the cells susceptible to genomic instability and malignant transformation [44-46] which were used to understand pathogenesis and effective therapy for the disease. In light of this, aim of the present investigation was to develop *in vitro* model system to study the pathogenesis of BPH and its potential for assessing therapeutics. In this study, we used method of serial passaging to establish immortalize cell line. The advantages of this approach include limiting genetic damage to key cell cycle checkpoints and allowing for the derivation of cells that are able to recapitulate key aspects of physiology.

This BPH stem/progenitor cell line with pluripotent stem cell characteristics provide the first *in vitro* model which can be used to enhance our understanding of human benign tumor development and provide a tool for testing diagnostic, treatment, and prevention strategies for BPH and cancer patients. Furthermore, this established cell line provides in-depth knowledge to study the role of stem cells, cancer stem cells and epithelial cell differentiation mechanism in disease progression. Because many epithelial cancers and benign tumors seem to arise from cancer stem cells and often exhibit similar characteristics, knowledge generated by the BPH epithelial stem/progenitor cell line will likely be applicable to other epithelial tumurogenesis.

Conclusion

One of the major strength of our study is that the pluripotent cells we obtained from human BPH form a connecting link between embryonic pluripotent stem cells and mesenchymal stem cells. This is evidenced by the expression of beautiful admixture of pluripotent (ONS expression, trilineage *in vitro* differentiation, teratoma formation) and mesenchymal markers (adipo, chondro, osteogenic differentiation and CD marker expression) exhibited by this pluripotent stem cell line we established. Multi-lineage differentiation characteristics of prostate cells can be exploited for stem cell treatment in patients suffering with other disease. While there are chromosomal alterations associated with with crisis and subsequent immortalization, these cells are behaviorally benign as assessed with histopathological and immunocytochemistry criteria. As such, this cell line represent potentially useful model to investigate mechanisms associated with both benign and malignant prostatic disorders and may open avenues for developing intervention therapies for prevention of BPH and cancer progression.

Acknowledgements

We would like to thank Dr. Frenny Sheth (FRIGE House, Ahmedabad) for technical assistance in karyotyping. This work was funded by grant from DBT, India under DBT-MSUB-ILSPARE Project.

References

1. SUZUKI K (2009) Epidemiology of Prostate Cancer and Benign Prostatic Hyperplasia. JMAJ 52: 478-483.
2. Orsted DD, Bojesen SE (2013) The link between benign prostatic hyperplasia and prostate cancer. Nat Rev Urol 10: 49-54.
3. Timms BG (2008) Prostate development: a historical perspective. Differentiation 76: 565-577.
4. Bartsch G, Rohr HP (1980) Comparative light and electron microscopic study of the human, dog and rat prostate. An approach to an experimental model for human benign prostatic hyperplasia (light and electron microscopic analysis)--a review. Urol Int 35: 91-104.
5. Bonkhoff H, Remberger K (1993) Widespread distribution of nuclear androgen receptors in the basal cell layer of the normal and hyperplastic human prostate. Virchows Arch A Pathol Anat Histopathol 422: 35-38.
6. Wang Y, Hayward S, Cao M, Thayer K, Cunha G (2001) Cell differentiation lineage in the prostate. Differentiation 68: 270-279.
7. Long RM, Morrissey C, Fitzpatrick JM, Watson RW (2005) Prostate epithelial cell differentiation and its relevance to the understanding of prostate cancer therapies. Clin Sci (Lond) 108: 1-11.
8. Litvinov IV, Vander Griend DJ, Xu Y, Antony L, Dalrymple SL, et al. (2006) Low-calcium serum-free defined medium selects for growth of normal prostatic epithelial stem cells. Cancer Res 66: 8598-8607.
9. Isaacs JT (2008) Prostate stem cells and benign prostatic hyperplasia. Prostate 68: 1025-1034.
10. Mills AA, Zheng B, Wang XJ, Vogel H, Roop DR, et al. (1999) p63 is a p53 homologue required for limb and epidermal morphogenesis. Nature 398: 708-713.
11. Mills AA, Qi Y, Bradley A (2002) Conditional inactivation of p63 by Cre-mediated excision. Genesis 32: 138-141.
12. Signoretti S, Waltregny D, Dilks J, Isaac B, Lin D, et al. (2000) p63 is a prostate basal cell marker and is required for prostate development. Am J Pathol 157: 1769-1775.
13. Li H, Jiang M, Honorio S, Patrawala L, Jeter CR, et al. (2009) Methodologies in assaying prostate cancer stem cells. Methods Mol Biol 568: 85-138.
14. Isaacs JT (1987) Control of cell proliferation and cell death in the normal and neoplastic prostate: a stem cell model. In: Rodgers CH, Coffey DS, Cunha G, Grayhack JT, Hinman F Jr, Horton R, editors. Benign Prostatic Hyperplasia. Washington, DC: US Department of Health and Human Services 85-94. Rodgers CH, Coffey DS, Cunha G, Grayhack JT, Hinman F Jr, Horton R NIH Publication No. 87-2881. US Department of Health and Human Services.
15. Tsujimura A, Koikawa Y, Salm S, Takao T, Coetzee S, et al. (2002) Proximal location of mouse prostate epithelial stem cells: a model of prostatic homeostasis. J Cell Biol 157: 1257-1265.
16. Hayward SW, Dahiya R, Cunha GR, Bartek J, Deshpande N, et al. (1995) Establishment and characterization of an immortalized but non-transformed human prostate epithelial cell line: BPH-1. In Vitro Cell Dev Biol Anim 31: 14-24.
17. Bello D, Webber MM, Kleinman HK, Wartinger DD, Rhim JS (1997) Androgen responsive adult human prostatic epithelial cell lines immortalized by human papillomavirus 18. Carcinogenesis 18: 1215-1223.
18. Chaproniere DM, McKeehan WL (1986) Serial culture of single adult human prostatic epithelial cells in serum-free medium containing low calcium and a new growth factor from bovine brain. Cancer Res 46: 819-824.
19. Barch MJ, Knutsen T, Spurbeck JL (1997) The AGT cytogenetics laboratory manual. (3rdedn), Lippincott-Raven, Philadelphia, USA.
20. Yoon BS, Moon JH, Jun EK, Kim J, Maeng I, et al. (2010) Secretory profiles and wound healing effects of human amniotic fluid-derived mesenchymal stem cells. Stem Cells Dev 19: 887-902.
21. Awad HA, Wickham MQ, Leddy HA, Gimble JM, Guilak F (2004) Chondrogenic differentiation of adipose-derived adult stem cells in agarose, alginate, and gelatin scaffolds. Biomaterials 25: 3211-3222.
22. Chen SS, Fitzgerald W, Zimmerberg J, Kleinman HK, Margolis L (2007) Cell-cell and cell-extracellular matrix interactions regulate embryonic stem cell differentiation. Stem Cells 25: 553-561.
23. Byrne JA, Nguyen HN, Reijo Pera RA (2009) Enhanced generation of induced pluripotent stem cells from a subpopulation of human fibroblasts. PLoS One 4: e7118.
24. Schuster GA, Schuster TG (1999) The relative amount of epithelium, muscle, connective tissue and lumen in prostatic hyperplasia as a function of the mass of tissue resected. J Urol 161: 1168-1173.
25. Liu AY, True LD, LaTray L, Nelson PS, Ellis WJ, et al. (1997) Cell-cell interaction in prostate gene regulation and cytodifferentiation. Proc Natl Acad Sci U S A 94: 10705-10710.
26. Collins AT, Habib FK, Maitland NJ, Neal DE (2001) Identification and isolation of human prostate epithelial stem cells based on alpha(2)beta(1)-integrin expression. J Cell Sci 114: 3865-3872.
27. Richardson GD, Robson CN, Lang SH, Neal DE, Maitland NJ, et al. (2004) CD133, a novel marker for human prostatic epithelial stem cells. J Cell Sci 117: 3539-3545.
28. Reya T, Morrison SJ, Clarke MF, Weissman IL (2001) Stem cells, cancer, and cancer stem cells. Nature 414: 105-111.
29. Lawson DA, Witte ON (2007) Stem cells in prostate cancer initiation and progression. J Clin Invest 117: 2044-2050.
30. Prajapati A, Gupta S, Mistry B, Gupta S (2013) Prostate Stem Cells in the Development of Benign Prostate Hyperplasia and Prostate Cancer: Emerging Role and Concepts. BioMed Res Int 2013: 10.
31. Le Magnen C, Bubendorf L, Ruiz C, Zlobec I, Bachmann A, et al. (2013) Klf4 transcription factor is expressed in the cytoplasm of prostate cancer cells. Eur J Cancer 49: 955-963.
32. Bhatt RI, Brown MD, Hart CA, Gilmore P, Ramani VA, et al. (2003) Novel method for the isolation and characterisation of the putative prostatic stem cell. Cytometry A 54: 89-99.
33. Davis LD, Zhang W, Merseburger A, Young D, Xu L, et al. (2002) p63 expression profile in normal and malignant prostate epithelial cells. Anticancer Res 22: 3819-3825.
34. McKeon F (2004) p63 and the epithelial stem cell: more than status quo? Genes Dev 18: 465-469.
35. Leong KG, Wang BE, Johnson L, Gao WQ (2008) Generation of a prostate from a single adult stem cell. Nature 456: 804-808.
36. Garraway IP, Sun W, Tran CP, Perner S, Zhang B, et al. (2010) Human prostate sphere-forming cells represent a subset of basal epithelial cells capable of glandular regeneration in vivo. Prostate 70: 491-501.
37. Lin VK, Wang SY, Vazquez DV, C CX, Zhang S, et al. (2007) Prostatic stromal

cells derived from benign prostatic hyperplasia specimens possess stem cell like property. Prostate 67: 1265-1276.

38. Isaacs JT, Coffey DS (1989) Etiology and disease process of benign prostatic hyperplasia. Prostate Suppl 2: 33-50.

39. Balachandar V, Mohana Devi S, Lakshman Kumar B, Sangeetha R, Manikantan P, et al. (2008) Cytogenetic analysis of benign prostate hyperplasia (BPH) and prostate cancer (PC) patients from Tamil Nadu, South India. Scientific Research and Essay 3: 212-214.

40. Conforti-Froes N, el-Zein R, Abdel-Rahman SZ, Zwischenberger JB, Au WW (1997) Predisposing genes and increased chromosome aberrations in lung cancer cigarette smokers. Mutat Res 379: 53-59.

41. Grosovsky AJ, Parks KK, Giver CR, Nelson SL (1996) Clonal analysis of delayed karyotypic abnormalities and gene mutations in radiation-induced genetic instability. Mol Cell Biol 16: 6252-6262.

42. Paraskeva C, Finerty S, Powell S (1988) Immortalization of a human colorectal adenoma cell line by continuous in vitro passage: possible involvement of chromosome 1 in tumour progression. Int J Cancer 41: 908-912.

43. Evans GS, Chandler JA (1987) Cell proliferation studies in rat prostate. I. The proliferative role of basal and secretory epithelial cells during normal growth. Prostate 10: 163-178.

44. Wieser M, Stadler G, Jennings P, Streubel B, Pfaller W, et al. (2008) hTERT alone immortalizes epithelial cells of renal proximal tubules without changing their functional characteristics. Am J Physiol Renal Physiol 295: F1365-1375.

45. Gudjonsson T, Villadsen R, Ronnov-Jessen L, Petersen OW (2004) Immortalization protocols used in cell culture models of human breast morphogenesis. Cell Mol Life Sci 61: 2523-2534.

46. Bhatia B, Jiang M, Suraneni M, Patrawala L, Badeaux M, et al. (2008) Critical and distinct roles of p16 and telomerase in regulating the proliferative life span of normal human prostate epithelial progenitor cells. J Biol Chem 283: 27957-27972.

Citation: Prajapati A, Gupta S, Bhonde R, Gupta S (2014) Pluripotent Stem Cell within the Prostate could be Responsible for Benign Prostate Hyperplasia in Human. J Stem Cell Res Ther 4: 164. doi:10.4172/2157-7633.1000164

Hindawi Publishing Corporation
BioMed Research International
Volume 2013, Article ID 107954, 10 pages
http://dx.doi.org/10.1155/2013/107954

Review Article

Prostate Stem Cells in the Development of Benign Prostate Hyperplasia and Prostate Cancer: Emerging Role and Concepts

Akhilesh Prajapati,[1] Sharad Gupta,[2] Bhavesh Mistry,[1] and Sarita Gupta[1]

[1] *Department of Biochemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat 390005, India*
[2] *Ex-assistant Professor karamsad medical college and Gupta Pathological laboratory, Vadodara, Gujarat 390001, India*

Correspondence should be addressed to Sarita Gupta; sglmescrl@gmail.com

Received 23 April 2013; Revised 14 June 2013; Accepted 14 June 2013

Academic Editor: Mauro S. G. Pavao

Benign Prostate hyperplasia (BPH) and prostate cancer (PCa) are the most common prostatic disorders affecting elderly men. Multiple factors including hormonal imbalance, disruption of cell proliferation, apoptosis, chronic inflammation, and aging are thought to be responsible for the pathophysiology of these diseases. Both BPH and PCa are considered to be arisen from aberrant proliferation of prostate stem cells. Recent studies on BPH and PCa have provided significant evidence for the origin of these diseases from stem cells that share characteristics with normal prostate stem cells. Aberrant changes in prostate stem cell regulatory factors may contribute to the development of BPH or PCa. Understanding these regulatory factors may provide insight into the mechanisms that convert quiescent adult prostate cells into proliferating compartments and lead to BPH or carcinoma. Ultimately, the knowledge of the unique prostate stem or stem-like cells in the pathogenesis and development of hyperplasia will facilitate the development of new therapeutic targets for BPH and PCa. In this review, we address recent progress towards understanding the putative role and complexities of stem cells in the development of BPH and PCa.

1. Introduction

Prostate gland is a male accessory reproductive endocrine organ, which expels proteolytic solution in the urethra during ejaculation. In humans, the prostate is located immediately below the base of the bladder surrounding the neck region of the urethra. It is mainly associated with three types of disorders, namely, benign prostate hyperplasia (BPH), prostate cancer (PCa), and prostatitis. BPH and PCa are the most common pathophysiological conditions of prostate gland in elderly men. These diseases already represent significant challenges for health-care systems in most parts of the world. Epidemiologically, BPH is more prevalent in Asian population [1, 2]. Whereas, PCa is more common in the western world [3, 4]. Both the diseases are complex and multifactorial. Factors predisposing to the development of BPH or PCa include hormonal imbalance, oxidative stress, environmental pollutants, inflammation, hereditary, aging, and, more particularly, stromal to epithelial cells crosstalk [5–7]. So far, variety of growth factors and hormonal factors, including androgens and estrogens, has been described in the hyperplastic development of the prostate gland [8–10]. However, the cellular and molecular processes underlying the pathogenesis and development of BPH or PCa are poorly understood.

Stem cells have an extensive capacity to propagate themselves by self-renewal and to differentiate into tissue-specific progeny. It is well know that stem cells are required to maintain and repair tissues throughout the lifetime. The requirement to understand the biology of stem cells derived from the prostate is increasing, as new evidence suggests that BPH and PCa may arise from the stem or stem-like cell compartments [11–13]. This review summarises the biology of prostate stem or stem-like cells and their contribution in pathogenesis and development of BPH and PCa.

2. Prostatic Cellular Compartments

The prostate is a hormonally regulated glandular organ whose growth accelerates at sexual maturity due to androgen action on both stromal and epithelial cells [14, 15]. The human

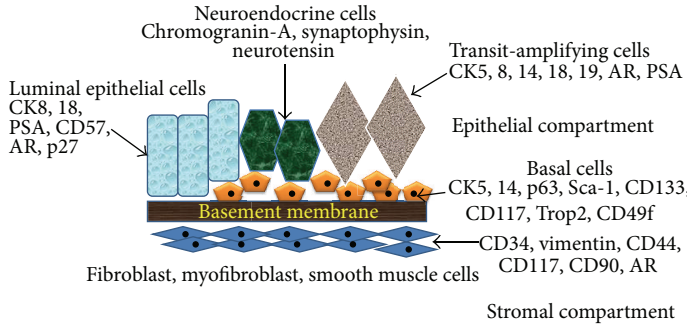


Figure 1: Prostatic cellular compartments and stem cell identity markers. Pictorial representation of different prostatic cells and their respective cellular markers.

prostate is a complex ductal-acinar gland that is divided into three anatomically distinct zones: peripheral, transitional, and central zones, which are surrounded by a dense and continuous fibromuscular stroma [16–18]. BPH, a nonmalignant overgrowth found in older men, mainly, develops in the transitional zone, while PCa arises primarily in the peripheral zone [19].

At histological level, human prostate contains mainly two types of cells that are called epithelial and stromal cells. The stromal to epithelial ratio in normal prostate of human is 2 : 1 [18, 20]. The epithelial cell layer is composed of four differentiated cell types known as basal, secretory luminal, neuroendocrine (NE), and transit-amplifying (TA) cells that are identified by their morphology, location, and distinct marker expression (Figure 1). The basal cells form a layer of flattened to cuboidal shaped cells above the basement membrane and express p63 (a homolog of the tumor suppressor gene *p53*), Bc1-2 (an anti-apoptotic factor), Cluster designation (CD) 44, hepatocyte growth factor (HGF), and the high molecular weight cytokeratins (CK) 5 and 14. The expression of androgen receptor (AR) is low or undetectable in the basal cells, which makes the basal cells independent of androgens for their survival [21–23]. The luminal cells are the major cell type of the prostate that form a layer of columnar-shaped cells above the basal layer and constitute the exocrine compartment of the prostate, secreting prostate-specific antigen (PSA) and prostatic acid phosphatase (PAP) into the lumen. They are terminally differentiated, androgen dependent, and nonproliferating cells, expressing low molecular weight CK8 and 18, CD57 and $p27^{Kip1}$ (a cell cycle inhibitor) [22–24] along with high levels of AR. NE cells are rare cells scattered in the basal and luminal layers of the prostate. They are terminally differentiated and androgen-insensitive cells, expressing chromogranin A, synaptophysin, and neuron-specific enolase (NSF) [23, 25, 26]. The NE cells also produce and secrete neuropeptides such as bombesin, calcitonin, and neurotensin that are believed to support epithelial cell growth and differentiation [19, 27, 28]. Additionally, there is a small group of intermediate cells referred to as TA cells that express both basal as well as luminal cell markers (CK5, CK8, CK14, CK18, AR, and PSA) [29–32]. The epithelial layer is surrounded by a stromal layer, which forms a peripheral boundary of the prostate gland. The stromal cell layer consists of several types of cells that include smooth muscle cells (the most abundant cell type in stroma), fibroblasts, and myofibroblasts. Stromal cells express mesenchymal markers like CD34, vimentin, CD44, CD117, and CD90 [33].

3. Stem Cell in Normal Prostate

Prostatic epithelium is, structurally and functionally, a highly complex tissue composed of multiple differentiated cell types, including basal, luminal, and neuroendocrine cells, along with small population of relatively undifferentiated cells generally known as "stem cells" that are endowed with self-renewal and differentiation capacities [26]. If the stem cells are key target for mutagenic changes and tumourigenesis in human prostate, we need to understand more about stem cell status in normal prostate tissue.

As the adult prostate is relatively slow-growing organ with limited cycles of cell proliferation and apoptosis, the possible existence of adult prostate stem cells (PSCs) was controversial for many years. Several investigations based on stem cell models have elegantly defined role of stem cells in cellular turnover and morphogenesis of normal prostate [30, 34]. Evidence for the existence of the stem cells in normal prostate came from the studies which demonstrated that adult rodent prostate can undergo multiple rounds of castration-induced regression and testosterone-induced regrowth [35–37]. Adult PSCs were believed to reside within the basal cell layer because of the ability of the basal cells to survive and undergo regression and regeneration following repeated castration and androgen replacement [38–40]. Adult mouse prostate epithelial cells, when transplanted along with the urogenital sinus mesenchymal cells under the renal capsule, generated normal murine prostate like structures [41]. Prostate glands were also regenerated when dissociated cells were implanted in Matrigel subcutaneously into immunodeficient mice [42]. Studies, including 5-bromo-2-deoxyuridine (BrdU) retention analysis, showed that the enriched population of BrdU-labelled cells possessing stem cell features (quiescent, high proliferation potential) are localized at the proximal region of mouse prostate duct [43] and are programmed to regenerate proximal-distal ductal axis [44]. The proximal region of the prostatic duct is surrounded by a thick band of smooth muscle cells [45] that are known to produce high level of transforming growth factor-beta (TGF-β) [46], which is known to play a critical role in maintaining the relative

dormancy of the PSCs [47]. Independent study by Burger et al. also identified a candidate population of PSCs in the proximal region of mouse prostatic ducts, using stem cell surface marker known as stem cell antigen 1 (Sca-1, also known as Ly6a) [48]. In addition to high expression of Sca-1, these cells were shown to coexpress integrin α6 (CD49f) and Bcl-2. The cells with these properties showed a higher efficiency to generate prostatic tissue in an *in vivo* reconstitution assay [48]. Lawson et al. showed that sorting prostatic cells for CD45(−)CD31(−)Ter119(−)Sca-1(+)CD49f(+) antigenic profile results in a 60-fold enrichment for colony and sphere-forming cells that can self-renew and expand to form spheres for many generations [49]. Leong and colleagues identified CD117 (c-Kit, stem cell factor receptor) as a new marker of a rare adult mouse PSC population that showed all the functional characteristics of stem cells including self-renewal and full differentiation potential. The CD117(+) single stem cell defined by the phenotype Lin(−)Sca-1(+)CD133(+)CD44(+)CD117(+) regenerated functional, secretion-producing prostate after transplantation *in vivo*. Moreover, CD117(+) PSCs showed long-term self renewal capacity after serial isolation and transplantation *in vivo*. CD117 expression was predominantly localized to the proximal region of the mouse prostate and was upregulated after castration-induced prostate involution, consistent with prostate stem cell identity and function [50].

Stem cells in the human prostate have been identified and isolated using the cell surface markers such as integrin $\alpha2\beta1$ [51], CD133 (Prominin-1) [52], and CK6a (cytokeratin 6a) [53]. Based on high expression of $\alpha2\beta1$ integrin, Collins and colleagues identified PSCs in the basal layer and showed that the $\alpha2\beta1^{high}$ integrin cells represent ~1% of basal cell population in the human prostate [51]. This selected PSC population was enriched through rapid adherence to the type I collagen and showed higher colony-forming efficiency *in vitro*. Furthermore, when the $\alpha2\beta1^{high}$ integrin cells were grafted subcutaneously together with stromal cells in Matrigel into nude mice, they formed prostatic gland structures *in vivo*. Nevertheless, these glandular-like structures, although containing basal cytokeratin positive as well as AR, PAP, and PSA positive cells, lack well-defined basal and luminal organizations [51]. However, recent studies by Missol-Kolka et al. have reported that the overall expression of CD133 in human prostate is not strictly limited to the rare basal stem and progenitor cells, but it is also expressed in some of the secretory luminal cells [54]. Furthermore, it has been shown that CD133 is downregulated in prostate cancer tissues and upregulated in the luminal cells in the vicinity of cancer area. In contrast to the human CD133, the mouse CD133 has been shown to express widely in prostate [54]. Several other surface markers, such as aldehyde dehydrogenase (ALDH), tumor-associated calcium signal transducer 2 (Trop-2), ATP-binding cassette transporter family membrane efflux pump (ABCG2), p63, and CD44, have also been reported for identification and isolation of the PSCs from the prostate tissues of human and mouse [49, 55–60]. Moreover, Trop2(+)CD44(+)CD49f(+) were used as the markers to identify basal stem cells with enhanced prostasphere-forming and tissue-regenerating abilities [61]. Unlike the murine PSCs, the human PSCs are randomly distributed within the basal epithelial layer throughout the acini and ductal regions of the prostate [51, 52]. In addition to the expression of stem-cell-specific markers, different studies have also shown that PSCs express both basal and luminal cell-specific markers in fetal and adult stages of prostate development [13, 22, 31, 62, 63]. Several studies have proposed the existence of different cell compartments based on stem-cell-driven differentiation hierarchical arrangements within the prostate epithelium [24, 29, 30, 64].

In addition to prostate epithelial stem cells, stromal stem cells (SSCs) have also been reported to exist in the prostate, where they are postulated to carry out function of replacing and regenerating local cells that are lost to normal tissue turnover, injury, or aging [65–67]. These subpopulation of SSCs expressed mesenchymal stem cell (MSC) markers such as CD34 and Sca-1, showed a high proliferative activity and ability to differentiate into fibroblastic, myogenic, adipogenic, and osteogenic lineages [68]. Of all these potential lineages, the most characteristic cell type derived from prostate stromal stem cell is fibroblast or smooth muscle cells [68, 69]. Growth factors that have regulatory effects on SSCs include members of TGF-β superfamily, the insulin-like growth factors, the fibroblast growth factors, the platelet-derived growth factor, and Wnts [70]. It is believed that the differentiation of stromal stem cells to smooth muscle cells is due to paracrine effects of prostrate epithelial cells, which permanently commit the stromal stem cells to mature into androgen receptor (AR) expressing smooth muscle cells [68].

4. Stem Cell in Benign Prostate Hyperplasia (BPH)

BPH is a slow progressive enlargement of the prostate gland which can lead to lower urinary tract symptoms (LUTS) in elderly men. It is characterized by hyperproliferation of epithelial and stromal cells in the transition zone of the prostate gland, which can be observed histopathologically [71]. Despite of its obvious importance as a major health problem, little is known in terms of biological processes that contribute to the development of BPH. To explain the etiology behind the pathogenesis of BPH, several theories, including stem cell, hormonal imbalance, apoptosis, epithelial-mesenchymal transition, embryonic awakening, and inflammation, have been proposed in recent years, and all of them seem to contribute together to some extent in the pathogenesis of BPH [12, 72]. According to stem cell theory, the stem cell population residing in the prostate gland is increased due to abnormal proliferation and apoptosis of stem cells, which may eventually contribute to BPH pathogenesis. Earlier, it was reported by Berry et al. that stem cell population is responsible for prostate gland maintenance [73]. Changes in tissue consistency and cellular hyperplasia are accompanied by downregulation of apoptotic factors and increased level of antiapoptotic factors that decrease the rate of prostatic cell death and, thus, contributing to hyperproliferation of prostatic tissue [74]. It has been reported that stromal to epithelial

ratio is altered in BPH, where the ratio increases from 2 : 1 in normal glands to 5 : 1 in BPH [75]. Because stromal hyperproliferative activity is thought to promote the development of BPH, the existence of adult stem cells in the prostate stromal compartment is speculated to expand the stroma in response to stimuli during the pathogenesis of BPH [68]. Lin et al. showed that primary culture of prostate cells from BPH patients possessed many common stem cell markers, including CD30, CD44, CD54, neuronspecific enolase (NSE), CD34, vascular endothelial growth factor receptor-1 (Flt-1), and stem cell factor (SCF, also known as KIT ligand or steel factor) [68]. Compared to CD30, CD44, CD54, and NSE, the CD34, Flt-1, and SCF markers were expressed at low level. These stem cells were negative for CD11b, stem cell antigen-1 (SCA-1), SH2, AA4.1, and c-Kit. Furthermore, among this stem cell population only a fraction (5%) of the stem cells was positive for CD133 [68]. Although the origin of these stem cells is not known, the CD49(+)CD54(+)NSE(+)SCF(+) cell marker profile of these cells suggests that they are in a lineage closely related to MSCs. The stem cell population with the above profile possessed ability to differentiate or transdifferentiate into myogenic, adipogenic, and osteogenic lineages [68, 76]. Ceder et al. reported the possible existence of prostate stromal stem/progenitor cells in the adult human prostate [76]. This stromal population expressed vimentin (a mesenchymal marker), CD133, c-Kit, and SCF, with expression profiles similar to those observed in the Cajal cells of gastrointestinal tract, which represent a subset of stem cell-like cells. Several studies have identified c-Kit-expressing interstitial cells in the stromal compartment of human prostate [77–79]. Altered patterns of c-Kit expression have been reported in benign lesions of prostate and breast tissues [80, 81]. It has been shown that the c-Kit expression and number of c-Kit(+) interstitial cells were significantly higher in BPH than those of the normal prostate. Furthermore, it has been suggested that c-Kit regulates cell proliferation in prostate and plays a crucial role in the pathophysiology of BPH via altering the expression of JAK2 and STAT1 [77].

Stem cells from the BPH samples expressing CD49f, CD44, or CD133 markers have been shown to possess monolayer- and spheroid-colony-forming ability, where the highest (98%) recovery of colony-forming cells (CFCs) was achieved by CD49f(+) cells as compared to CD44(+) (17%) or CD133(+) (3%) cells [82]. These CFCs showed the capacity to undergo clonal proliferation, generates branching ductal structures, and they expressed both basal and luminal lineage markers. Further characterization of CD49f(+) cells revealed that they are comprised of two cell types: CK5(+) basal epithelial cells and CD31(+) endothelial cells [82]. Sca-1- and CD34-expressing cells isolated from BPH tissue showed a high proliferative capacity and increased plasticity, as these cells were able to differentiate into fibroblastic, myogenic, adipogenic and osteogenic lineages, similar to that of MSCs [68, 83]. Furthermore, Burger and colleagues found that cells with high Sca-1 expression had considerably more growth potential, and proliferative capabilities than cells expressing low or no Sca-1 antigen [48]. Expression of pluripotency markers such as *Oct4A*, *Sox2*, *c-Myc*, and *Klf4* might represent a stemness-specific gene signature. A very recent study has demonstrated a relatively high expression of stemness-associated genes, including *Oct4A*, *Sox2*, *c-Myc*, *Nanog*, and *Klf4*, in BPH as compared to normal prostate tissue [84]. Thus, several studies have revealed the presence of stem cells that express pluripotency-associated markers and are hyperproliferative and capable of differentiation into different cell lineages within the hyperplastic prostate tissue. The presence of these high proliferative and plastic stem cells in the BPH tissue samples suggests that BPH could occur as a result of changes in the stem cell properties that could ultimately give rise to a clonal expansion of cell populations.

5. Stem Cell in Prostate Cancer (PCa)

PCa is the most prevalent and is the second most frequently diagnosed cancer and sixth leading cause of cancer-related deaths among men in the world [85]. Its etiology, although not clear, is partly attributed to multigenic and epigenetic mechanisms and the heterogeneous nature of this disease [4, 86–88]. Gleason and others described that when the transition of normal gland into adenocarcinoma of prostate takes place, its normal histological structure is disrupted and results in abnormal proliferation of the glandular structure, destruction of basement membrane, and progressive loss of basal cells (<1%) [87, 89]. In addition, AR(+) luminal cells increase and contribute in bulk of prostate mass (>99%) in PCa [90]. It is hypothesised that prostate cancer arises from AR(+) luminal cells and dramatic loss of basal cells. To support this hypothesis several investigations have been conducted [4, 91–93]. In addition, mouse basal population expressing Lin(−)Sca-1(+)CD49f^{high} cells can differentiate into luminal cells in xenograft [49]. Lin(−)Sca-1(+)CD49f^{high} cells from a Pten−/− mouse model display cancer stem cells phenotypes, which gave rise to adenocarcinoma after transplantation [94]. It has been reported that basal cells are the possible cells of prostate cancer origin [95]. When Goldstein et al., especially injected the mixture of urogenital sinus mesenchyme (UGSM) with human prostate basal (expressing CD49f^{high} and Trop2^{high}) or luminal cells (expressing CD49f^{low} and Trop2^{high}) into the subcutaneous space of immunodeficient NOD(−)SCID(−)IL(−)2Rg−/− mice, only basal cells formed prostatic duct after 16 week, whereas no prostatic duct or adenocarcinoma developed when using luminal cells [91, 95]. Luminal derived grafts lack epithelial structures and mimicked transplantation of UGSM cell alone [95]. Collins et al. reported basal cancer stem cells isolated from human prostate cancer biopsies expressing Cd44(+), $\alpha 2\beta 1^{high}$, and Cd133(+) and cell surface markers were of self renewal *in vitro* [96]. ALDH^{high} is another marker used for cancer stem cells in human prostate cancer cell lines. Cells expressing ALDH$^{high}\alpha 2(+)/\alpha 6(+)/\alpha v(+)$-integrin CD44(+) showed increased tumourigenicity and metastasis *in vivo* and enhanced invasiveness *in vitro* [97]. Prostate cancer stem cells isolated from LNCaP and DU145 cell lines also showed expression of CD44(+), $\alpha 2\beta 1^{high}$, and CD133(+) markers [98, 99]. In addition, CD44(+) population isolated from xenograft human tumour and cell lines displayed high tumour initiating

ability and metastasis *in vitro* [100]. Recently, Rajasekhar and his group isolated a small cell population expressing TRA-1-60(+)CD151(+)CD166(+) markers that displayed stem cell like features with increased NF-kB signalling along with basal cell markers, and this recapitulates the cellular hierarchy of the tumour origin from basal cells [101].

Over all data from several investigators indicated that origin of prostate cancer can be from basal stem cell population, which expresses CD44(+), $\alpha 2\beta 1^{high}$, CD133(+), $ALDH^{high}$, and other normal basal stem cell markers.

6. Stem Cell Niche and Plasticity

Stem cells are localized in a defined microenvironment, which is known as their "niche." The main function of a niche probably is to provide specific factors necessary for the maintenance of the stem cell properties via a combination of intracellular and intercellular signalling. These factors include a complex array of growth factors, cytokines, chemokines, and adhesive molecules known to be capable of altering the balance between proliferation, differentiation, and quiescence in stem cell populations [102, 103]. One can probably assume that this is equally true for prostatic stem cells as it is for other stem cell populations.

PSCs reside in niche areas within the basal layer of the epithelial compartment at a low percentage of approximately 0.5–1% [34]. PSCs population in the prostate undergoes a series of phenotype changes. Specifically, the basal SCs do not express the AR or the p63 protein. They have extended proliferative potential by slow cycling. According to these studies, it is postulated that, in addition to the reserve stem-cell population, there is a "TA" cell type, which is characterized by the expression of p63, as well as other basal markers such as CK5 and 14, Jagged-1, and Notch-1 [64, 104, 105]. A TA cell does not express AR protein and it is dependent, for proliferation, but not for survival, on andromedans secreted by stromal cells [105]. Under normal conditions a PSC is slow cycling in that it divides occasionally, undergoing asymmetric division to give rise to a new PSC along with a more differentiated TA daughter cell. TA cell undergoes a limited number of rapidly amplifying cell division cycles to increase the cell population derived from a single PSC before leaving the proliferative compartment to produce intermediate cell [106]. This intermediate cell expresses both epithelial specific (CK5 and 14) and luminal specific (CK8 and 18) cytokines, AR mRNA (but not protein), and prostate stem cell antigen (PSCA) [105, 107]. As an intermediate cell migrates through the basal layer, it differentiates into various terminally differentiated cell lineages of prostate epithelium.

7. Is BPH/PCa a Stem Cells Disease?

Numerous investigators demonstrated presence of stem cell in prostate tissue by using various high-end techniques that may contribute to local invasive to metastatic disease in human and research animals. In normal tissue-development, homeostasis is maintained by differentiation of stem cells and

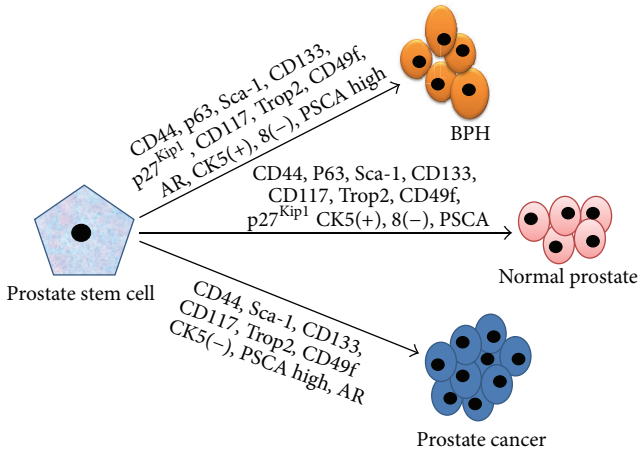


Figure 2: Cellular identity of stem cells in prostate. Stem cell model of normal tissue renewal, BPH and PCa.

programmed cells death in regular cell cycle. This mechanism is established through interactions with tissue specific environmental factors such as growth factors and steroid hormones. Many signalling molecules and factors involvement have been reported in stem cell self-renewal and implication in cancer stem cells (CSCs) regulation (Figure 2).

Although the precise role of stem cells in tumourigenesis is still in debate, it is widely accepted that cancers can arise from normal stem cells which may accumulate mutation, genetic changes, and molecular pathway alterations that disrupt self-renewal control capacity (Table 1). It has been reported that, in prostate, putative stem/progenitor cells can reside in CK5(+) 8(−) basal cells. A diagnostic feature of human prostate cancer is the loss of basal cells [108], indicating cancer origin cells as basal cells. In BPH, CD133(+) cells expressed genes related to undifferentiated cells such as TDGF1 (teratocarcinoma-derived growth factor 1) and targets of the Wnt and Hedgehog developmental pathways, whereas CD133(−) cells showed upregulation of genes related to proliferation and metabolism. In cancer, CD133(+) cells specifically displayed more TA population phenotype with increased metabolic activity and proliferation, possibly explaining the transition from a relatively quiescent state to an active growing tumour phenotype. This reflects that CD133 isolates from benign and malignant tissues show biologically distinct characteristics [109]. CSCs exploit many of the signal pathways such as notch, hedgehog- and TGF-β, which play, important role in proliferation and differentiation in prostate stem cell [110, 111]. The sonic hedgehog signalling element receptor PTCH1 and glioma-associated oncogene homolog-1 (GL1) transcription factor were especially reported to be colocalized with p63 basal marker in BPH and PCa cells, expressing CD44/CK8/14. This suggests that hedgehog pathway may induce differentiation of prostate stem/progenitor cells into CD44(+)/P63(+/−) hyperplasia basal cells [112]. Other studies on DNA damage and proliferation markers $p27^{Kip1}$, cyclin D3, and Ki-67, revealed interesting findings. It has been shown that $p27^{Kip1}$ is significantly upregulated in BPH,

Table 1: Molecular alterations in BPH and PCa.

Factors	Normal prostate	BPH	PCa
Prostate-specific factors			
5 α reductase	Normal	Upregulated	Upregulated
Androgen receptor (AR)	Normal	Upregulated	Upregulated
AR coactivator	Normal	Upregulated	Upregulated
Androgen corepressor	Normal	Upregulated	Upregulated
PSA level in serum	(0–4 ng/mL)	(2–8 ng/mL)	(4–10 ng/mL)
Growth factors	FGF-2,7,9 IGF 1,2 IGFBP-2	FGF 1,2,9 IGF-2 high IGFBP-3	FGF-1,2,6,8 IGF-1 high IGFBP-2 high IGFBP-3 high
NE cells	Normal	Number decrease	Number increase
Luminal cell factors	Vimentin Intracellular space normal PMSA normal	Vimentin increase Intracellular space increase PMSA decrease	Vimentin over exp Intracellular space decrease PMSA increase
Basal cells	Present	Present	Absent
Stromal cell factor	Fibroblast content normal NMMHC Elastin SMMHC	Fibroblast content increase NMMHC increase Elastin decrease SMMHC decrease	Fibroblast content increase NMMHC Elastin increase SMMHC decrease
Stem cell markers	CD44, P63, Sca-1, CD133, CD117, Trop2, CD49f, $p27^{Kip1}$, CK5(+), 8(−), PSCA	CD44, p63, Sca-1, CD133, $p27^{Kip1}$, CD117, Trop2, CD49f, AR, CK5(+), 8(−), PSCA high	CD44, Sca-1, CD133, CD117, Trop2, CD49f, CK5(−), PSCA high, AR

whereas it is downregulated in PCa. In addition to downregulation of $p27^{Kip1}$, there is also up regulation of Ki-67 and cyclin D3 in PCa [113].

Several lines of evidence have been indicated that CSCs exhibit both stem cells and cancer cells characteristics. CSCs have the ability to form tumors when transplanted into an animal host. CSCs can be distinguished from other cells within the tumor by cell division and alterations in their gene expression profile [114].

Advanced prostate cancer is androgen independent and basal cells can be phenotypically identified in the majority of metastases [115]. Studies from several investigators revealed that tumor-initiating cells are negative for AR and p63 and expressed the stem cell markers Oct-4, Nanog, Sox-2, Nestin, CD44, CD133, and CD117. Moreover, Sca-1-positive cells having the ability with prostate-regeneration activity, showed evidence of a basal and luminal lineage [96, 100, 116, 117]. Gu et al. demonstrated human telomerase reverse transcriptase-(hTERT-) positive epithelial cells could regenerate tumor in mice that resembled the original tumor in patients [118]. These finding may be indicative of CSC role in prostate cancer.

The growing understanding of the prostate stem cell biology provides the rationale for acute approaches. But without a clear definition of stem cells in normal prostate and BPH/PCa, it is difficult to determine whether the cancer cell of origin in prostate is a stem cell, multipotent progenitor/TA cells, or a more differentiated progeny. Nonetheless, evidence exists that the cellular origin can include both basal and luminal cells.

8. Conclusion

The prostate stem cells are a key role player in prostate tumourigenesis and enlargement disorders. But their precise role in disease pathogenesis remains unknown. The prostate stromal and epithelial compartments and their reciprocal paracrine and autocrine interactions are crucial regulators of prostatic tissue homeostasis. The combination of the prostatic cell surface markers, such as Sca-1, CD133, p63, and CD49f, can aid in the identification of prostate stem cell populations. However, a prostate-specific stem cell marker has yet to be identified. The study of CSCs is still in its early stages. No standard treatments have yet been developed as a result of research on CSCs. The isolation and characterization of epithelial, stromal stem cells and cancer stem cells in the prostate will lead to understanding normal stem cells and CSCs activity to identify new strategies for the control of prostate diseases without harming normal cells milieu.

References

[1] M. L. Gaynor, "Isoflavones and the prevention and treatment of prostate disease: is there a role?" *Cleveland Clinic Journal of Medicine*, vol. 70, no. 3, pp. 203–204, 2003.

[2] L. Denis, M. S. Morton, and K. Griffiths, "Diet and its preventive role in prostatic disease," *European Urology*, vol. 35, no. 5-6, pp. 377–387, 1999.

[3] A. Jemal, R. Siegel, E. Ward, Y. Hao, J. Xu, and M. J. Thun, "Cancer statistics, 2009," *CA Cancer Journal for Clinicians*, vol. 59, no. 4, pp. 225–249, 2009.

[4] M. M. Shen and C. Abate-Shen, "Molecular genetics of prostate cancer: new prospects for old challenges," *Genes and Development*, vol. 24, no. 18, pp. 1967–2000, 2010.

[5] W. W. Barclay, R. D. Woodruff, M. C. Hall, and S. D. Cramer, "A system for studying epithelial-stromal interactions reveals distinct inductive abilities of stromal cells from benign prostatic hyperplasia and prostate cancer," *Endocrinology*, vol. 146, no. 1, pp. 13–18, 2005.

[6] S. M. Harman, E. J. Metter, J. D. Tobin, J. Pearson, and M. R. Blackman, "Longitudinal effects of aging on serum total and free testosterone levels in healthy men," *Journal of Clinical Endocrinology and Metabolism*, vol. 86, no. 2, pp. 724–731, 2001.

[7] J. C. Nickel, J. Downey, I. Young, and S. Boag, "Asymptomatic inflammation and/or infection in benign prostatic hyperplasia," *BJU International*, vol. 84, no. 9, pp. 976–981, 1999.

[8] J. D. McConnell, "The pathophysiology of benign prostatic hyperplasia," *Journal of Andrology*, vol. 12, no. 6, pp. 356–363, 1991.

[9] M. Mimeault and S. K. Batra, "Recent advances on multiple tumorigenic cascades involved in prostatic cancer progression and targeting therapies," *Carcinogenesis*, vol. 27, no. 1, pp. 1–22, 2006.

[10] M. Marcelli and G. R. Cunningham, "Hormonal signaling in prostatic hyperplasia and neoplasia," *Journal of Clinical Endocrinology and Metabolism*, vol. 84, no. 10, pp. 3463–3468, 1999.

[11] A. Y. Nikitin, A. Matoso, and P. Roy-Burman, "Prostate stem cells and cancer," *Histology and histopathology*, vol. 22, no. 9, pp. 1043–1049, 2007.

[12] M. Notara and A. Ahmed, "Benign prostate hyperplasia and stem cells: a new therapeutic opportunity," *Cell Biology and Toxicology*, vol. 28, no. 6, pp. 435–442, 2012.

[13] G. L. Powers and P. C. Marker, "Recent advances in prostate development and links to prostatic diseases," *Wiley Interdisciplinary Reviews*, vol. 5, no. 2, pp. 243–256, 2013.

[14] G. S. Prins and O. Putz, "Molecular signaling pathways that regulate prostate gland development," *Differentiation*, vol. 76, no. 6, pp. 641–659, 2008.

[15] Y. Sugimura, G. R. Cunha, and A. A. Donjacour, "Morphogenesis of ductal networks in the mouse prostate," *Biology of Reproduction*, vol. 34, no. 5, pp. 961–971, 1986.

[16] J. E. McNeal, "Anatomy of the prostate and morphogenesis of BPH," *Progress in Clinical and Biological Research*, vol. 145, pp. 27–53, 1984.

[17] J. E. McNeal and D. G. Bostwick, "Anatomy of the prostatic urethra," *Journal of the American Medical Association*, vol. 251, no. 7, pp. 890–891, 1984.

[18] B. G. Timms, "Prostate development: a historical perspective," *Differentiation*, vol. 76, no. 6, pp. 565–577, 2008.

[19] C. Abate-Shen and M. M. Shen, "Molecular genetics of prostate cancer," *Genes and Development*, vol. 14, no. 19, pp. 2410–2434, 2000.

[20] G. Bartsch and H. P. Rohr, "Comparative light and electron microscopic study of the human, dog and rat prostate: an approach to an experimental model for human benign prostatic hyperplasia (light and electron microscopic analysis): a review," *Urologia Internationalis*, vol. 35, no. 2, pp. 91–104, 1980.

[21] H. Bonkhoff and K. Remberger, "Widespread distribution of nuclear androgen receptors in the basal cell layer of the normal and hyperplastic human prostate," *Virchows Archiv*, vol. 422, no. 1, pp. 35–38, 1993.

[22] Y. Wang, S. W. Hayward, M. Cao, K. A. Thayer, and G. R. Cunha, "Cell differentiation lineage in the prostate," *Differentiation*, vol. 68, no. 4-5, pp. 270–279, 2001.

[23] R. M. Long, C. Morrissey, J. M. Fitzpatrick, and R. W. G. Watson, "Prostate epithelial cell differentiation and its relevance to the understanding of prostate cancer therapies," *Clinical Science*, vol. 108, no. 1, pp. 1–11, 2005.

[24] A. M. De Marzo, A. K. Meeker, J. I. Epstein, and D. S. Coffey, "Prostate stem cell compartments: expression of the cell cycle inhibitor p27(Kip1) in normal, hyperplastic, and neoplastic cells," *The American Journal of Pathology*, vol. 153, no. 3, pp. 911–919, 1998.

[25] H. Bonkhoff, U. Stein, and K. Remberger, "Endocrine-paracrine cell types in the prostate and prostatic adenocarcinoma are postmitotic cells," *Human Pathology*, vol. 26, no. 2, pp. 167–170, 1995.

[26] J. A. Schalken and G. van Leenders, "Cellular and molecular biology of the prostate: stem cell biology," *Urology*, vol. 62, supplement 1, no. 5, pp. 11–20, 2003.

[27] G. P. Amorino and S. J. Parsons, "Neuroendocrine cells in prostate cancer," *Critical Reviews in Eukaryotic Gene Expression*, vol. 14, no. 4, pp. 287–300, 2004.

[28] P. A. Abrahamsson, "Neuroendocrine differentiation in prostatic carcinoma," *Prostate*, vol. 39, no. 2, pp. 135–148, 1999.

[29] H. Bonkhoff, U. Stein, and K. Remberger, "Multidirectional differentiation in the normal, hyperplastic, and neoplastic human prostate: simultaneous demonstration of cell-specific epithelial markers," *Human Pathology*, vol. 25, no. 1, pp. 42–46, 1994.

[30] H. Bonkhoff and K. Remberger, "Differentiation pathways and histogenetic aspects of normal and abnormal prostatic growth: a stem cell model," *Prostate*, vol. 28, no. 2, pp. 98–106, 1996.

[31] Y. Xue, F. Smedts, F. M. Debruyne, J. J. de la Rosette, and J. A. Schalken, "Identification of intermediate cell types by keratin expression in the developing human prostate," *Prostate*, vol. 34, no. 4, pp. 292–301, 1998.

[32] D. L. Hudson, M. O'Hare, F. M. Watt, and J. R. W. Masters, "Proliferative heterogeneity in the human prostate: evidence for epithelial stem cells," *Laboratory Investigation*, vol. 80, no. 8, pp. 1243–1250, 2000.

[33] T. Takao and A. Tsujimura, "Prostate stem cells: the niche and cell markers," *International Journal of Urology*, vol. 15, no. 4, pp. 289–294, 2008.

[34] J. T. Isaacs and D. S. Coffey, "Etiology and disease process of benign prostatic hyperplasia," *Prostate*, vol. 2, pp. 33–50, 1989.

[35] H. F. English, R. J. Santen, and J. T. Isaacs, "Response of glandular versus basal rat ventral prostatic epithelial cells to androgen withdrawal and replacement," *Prostate*, vol. 11, no. 3, pp. 229–242, 1987.

[36] G. S. Evans and J. A. Chandler, "Cell proliferation studies in the rat prostate: II. The effects of castration and androgen-induced regeneration upon basal and secretory cell proliferation," *Prostate*, vol. 11, no. 4, pp. 339–351, 1987.

[37] A. P. M. Verhagen, T. W. Aalders, F. C. S. Ramaekers, F. M. J. Debruyne, and J. A. Schalken, "Differential expression of keratins in the basal and luminal compartments of rat prostatic epithelium during degeneration and regeneration," *Prostate*, vol. 13, no. 1, pp. 25–38, 1988.

[38] D. P. DeKlerk and D. S. Coffey, "Quantitative determination of prostatic epithelial and stromal hyperplasia by a new technique. Biomorphometrics," *Investigative Urology*, vol. 16, no. 3, pp. 240–245, 1978.

[39] N. Kyprianou and J. T. Isaacs, "Identification of a cellular receptor for transforming growth factor-β in rat ventral prostate and its negative regulation by androgens," *Endocrinology*, vol. 123, no. 4, pp. 2124–2131, 1988.

[40] M. Montpetit, P. Abrahams, A. F. Clark, and M. Tenniswood, "Androgen-independent epithelial cells of the rat ventral prostate," *Prostate*, vol. 12, no. 1, pp. 13–28, 1988.

[41] L. Xin, H. Ide, Y. Kim, P. Dubey, and O. N. Witte, "In vivo regeneration of murine prostate from dissociated cell populations of postnatal epithelia and urogenital sinus mesenchyme," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, supplement 1, pp. 11896–11903, 2003.

[42] M. Azuma, A. Hirao, K. Takubo, I. Hamaguchi, T. Kitamura, and T. Suda, "A quantitative matrigel assay for assessing repopulating capacity of prostate stem cells," *Biochemical and Biophysical Research Communications*, vol. 338, no. 2, pp. 1164–1170, 2005.

[43] A. Tsujimura, Y. Koikawa, S. Salm et al., "Proximal location of mouse prostate epithelial stem cells: a model of prostatic homeostasis," *Journal of Cell Biology*, vol. 157, no. 7, pp. 1257–1265, 2002.

[44] K. Goto, S. N. Salm, S. Coetzee et al., "Proximal prostatic stem cells are programmed to regenerate a proximal-distal ductal axis," *Stem Cells*, vol. 24, no. 8, pp. 1859–1868, 2006.

[45] J. A. Nemeth and C. Lee, "Prostatic ductal system in rats: regional variation in stromal organization," *Prostate*, vol. 28, no. 2, pp. 124–128, 1996.

[46] J. A. Nemeth, J. A. Sensibar, R. R. White, D. J. Zelner, I. Y. Kim, and C. Lee, "Prostatic ductal system in rats: tissue-specific expression and regional variation in stromal distribution of transforming growth factor-beta 1," *Prostate*, vol. 33, no. 1, pp. 64–71, 1997.

[47] S. N. Salm, P. E. Burger, S. Coetzee, K. Goto, D. Moscatelli, and E. L. Wilson, "TGF-β maintains dormancy of prostatic stem cells in the proximal region of ducts," *Journal of Cell Biology*, vol. 170, no. 1, pp. 81–90, 2005.

[48] P. E. Burger, X. Xiong, S. Coetzee et al., "Sca-1 expression identifies stem cells in the proximal region of prostatic ducts with high capacity to reconstitute prostatic tissue," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 20, pp. 7180–7185, 2005.

[49] D. A. Lawson, L. Xin, R. U. Lukacs, D. Cheng, and O. N. Witte, "Isolation and functional characterization of murine prostate stem cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 1, pp. 181–186, 2007.

[50] K. G. Leong, B.-E. Wang, L. Johnson, and W.-Q. Gao, "Generation of a prostate from a single adult stem cell," *Nature*, vol. 456, no. 7223, pp. 804–810, 2008.

[51] A. T. Collins, F. K. Habib, N. J. Maitland, and D. E. Neal, "Identification and isolation of human prostate epithelial stem cells based on $\alpha 2\beta 1$-integrin expression," *Journal of Cell Science*, vol. 114, no. 21, pp. 3865–3872, 2001.

[52] G. D. Richardson, C. N. Robson, S. H. Lang, D. E. Neal, N. J. Maitland, and A. T. Collins, "CD133, a novel marker for human prostatic epithelial stem cells," *Journal of Cell Science*, vol. 117, no. 16, pp. 3539–3545, 2004.

[53] M. Schmelz, R. Moll, U. Hesse et al., "Identification of a stem cell candidate in the normal human prostate gland," *European Journal of Cell Biology*, vol. 84, no. 2-3, pp. 341–354, 2005.

[54] E. Missol-Kolka, J. Karbanová, P. Janich et al., "Prominin-1 (CD133) is not restricted to stem cells located in the basal compartment of murine and human prostate," *Prostate*, vol. 71, no. 3, pp. 254–267, 2011.

[55] R. I. Bhatt, M. D. Brown, C. A. Hart et al., "Novel method for the isolation and characterisation of the putative prostatic stem cell," *Cytometry A*, vol. 54, no. 2, pp. 89–99, 2003.

[56] P. E. Burger, R. Gupta, X. Xiong et al., "High aldehyde dehydrogenase activity: a novel functional marker of murine prostate stem/progenitor cells," *Stem Cells*, vol. 27, no. 9, pp. 2220–2228, 2009.

[57] A. S. Goldstein, D. A. Lawson, D. Cheng, W. Sun, I. P. Garraway, and O. N. Witte, "Trop2 identifies a subpopulation of murine and human prostate basal cells with stem cell characteristics," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 52, pp. 20882–20887, 2008.

[58] A. Y. Liu, L. D. True, L. Latray et al., "Cell-cell interaction in prostate gene regulation and cytodifferentiation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 20, pp. 10705–10710, 1997.

[59] J. C. Pignon, C. Grisanzio, Y. Geng, J. Song, R. A. Shivdasani, and S. Signoretti, "p63-expressing cells are the stem cells of developing prostate, bladder, and colorectal epithelia," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 20, pp. 8105–8110, 2013.

[60] M. Yao, R. A. Taylor, M. G. Richards et al., "Prostate-regenerating capacity of cultured human adult prostate epithelial cells," *Cells Tissues Organs*, vol. 191, no. 3, pp. 203–212, 2010.

[61] I. P. Garraway, W. Sun, C. P. Tran et al., "Human prostate sphere-forming cells represent a subset of basal epithelial cells capable of glandular regeneration in vivo," *Prostate*, vol. 70, no. 5, pp. 491–501, 2010.

[62] G. van Leenders, H. Dijkman, C. Hulsbergen-van de Kaa, D. Ruiter, and J. Schalken, "Demonstration of intermediate cells during human prostate epithelial differentiation in situ and in vitro using triple-staining confocal scanning microscopy," *Laboratory Investigation*, vol. 80, no. 8, pp. 1251–1258, 2000.

[63] W. W. Barclay, L. S. Axanova, W. Chen et al., "Characterization of adult prostatic progenitor/stem cells exhibiting self-renewal and multilineage differentiation," *Stem Cells*, vol. 26, no. 3, pp. 600–610, 2008.

[64] R. A. Taylor, R. Toivanen, and G. P. Risbridger, "Stem cells in prostate cancer: treating the root of the problem," *Endocrine-Related Cancer*, vol. 17, no. 4, pp. R273–R285, 2010.

[65] C. G. Roehrborn, J. D. McConnell, M. Lieber et al., "Serum prostate-specific antigen concentration is a powerful predictor of acute urinary retention and need for surgery in men with clinical benign prostatic hyperplasia," *Urology*, vol. 53, no. 3, pp. 473–480, 1999.

[66] M. J. Naslund and M. Miner, "A review of the clinical efficacy and safety of 5α-reductase inhibitors for the enlarged prostate," *Clinical Therapeutics*, vol. 29, no. 1, pp. 17–25, 2007.

[67] P. Boyle, C. Roehrborn, R. Harkaway, J. Logie, J. de La Rosette, and M. Emberton, "5-alpha reductase inhibition provides superior benefits to alpha blockade by preventing AUR and BPH-related surgery," *European Urology*, vol. 45, no. 5, pp. 620–627, 2004.

[68] V. K. Lin, S.-Y. Wang, D. V. Vazquez, C. C. Xu, S. Zhang, and L. Tang, "Prostatic stromal cells derived from benign prostatic hyperplasia specimens possess stem cell like property," *Prostate*, vol. 67, no. 12, pp. 1265–1276, 2007.

[69] G. R. Cunha, S. W. Hayward, R. Dahiya, and B. A. Foster, "Smooth muscle-epithelial interactions in normal and neoplastic prostatic development," *Acta Anatomica*, vol. 155, no. 1, pp. 63–72, 1996.

[70] C. Richard, G. Kim, Y. Koikawa et al., "Androgens modulate the balance between VEGF and angiopoietin expression in prostate epithelial and smooth muscle cells," *Prostate*, vol. 50, no. 2, pp. 83–91, 2002.

[71] G. A. Schuster and T. G. Schuster, "The relative amount of epithelium, muscle, connective tissue and lumen in prostatic hyperplasia as a function of the mass of tissue resected," *Journal of Urology*, vol. 161, no. 4, pp. 1168–1173, 1999.

[72] J. Tang and J. Yang, "Etiopathogenesis of benign prostatic hypeprlasia," *Indian Journal of Urology*, vol. 25, no. 3, pp. 312–317, 2009.

[73] S. J. Berry, D. S. Coffey, J. D. Strandberg, and L. L. Ewing, "Effect of age, castration, and testosterone replacement on the development and restoration of canine benign prostatic hyperplasia," *Prostate*, vol. 9, no. 3, pp. 295–302, 1986.

[74] N. Kyprianou, H. Tu, and S. C. Jacobs, "Apoptotic versus proliferative activities in human benign prostatic hyperplasia," *Human Pathology*, vol. 27, no. 7, pp. 668–675, 1996.

[75] E. Shapiro, M. J. Becich, V. Hartanto, and H. Lepor, "The relative proportion of stromal and epithelial hyperplasia is related to the development of symptomatic benign prostate hyperplasia," *Journal of Urology*, vol. 147, no. 5, pp. 1293–1297, 1992.

[76] J. A. Ceder, L. Jansson, R. A. Ehrnström, L. Rönnstrand, and P.-A. Abrahamsson, "The characterization of epithelial and stromal subsets of candidate stem/progenitor cells in the human adult prostate," *European Urology*, vol. 53, no. 3, pp. 524–532, 2008.

[77] M. Imura, Y. Kojima, Y. Kubota et al., "Regulation of cell proliferation through a KIT-mediated mechanism in benign prostatic hyperplasia," *Prostate*, vol. 72, no. 14, pp. 1506–1513, 2012.

[78] A. Lammie, M. Drobnjak, W. Gerald, A. Saad, R. Cote, and C. Cordon-Cardo, "Expression of c-kit and kit ligand proteins in normal human tissues," *Journal of Histochemistry and Cytochemistry*, vol. 42, no. 11, pp. 1417–1425, 1994.

[79] A. Shafik, I. Shafik, and O. El-Sibai, "Identification of c-kit-positive cells in the human prostate: the interstitial cells of Cajal," *Archives of Andrology*, vol. 51, no. 5, pp. 345–351, 2005.

[80] A. Kondi-Pafiti, N. Arkadopoulos, C. Gennatas, V. Michalaki, M. Frangou-Plegmenou, and P. Chatzipantelis, "Expression of c-kit in common benign and malignant breast lesions," *Tumori*, vol. 96, no. 6, pp. 978–984, 2010.

[81] R. Simak, P. Capodieci, D. W. Cohen et al., "Expression of c-kit and kit-ligand in benign and malignant prostatic tissues," *Histology and Histopathology*, vol. 15, no. 2, pp. 365–374, 2000.

[82] H. Yamamoto, J. R. Masters, P. Dasgupta et al., "CD49f is an efficient marker of monolayer- and spheroid colony-forming cells of the benign and malignant human prostate," *PLoS ONE*, vol. 7, no. 10, Article ID e46979, 2012.

[83] M. F. Pittenger, A. M. Mackay, S. C. Beck et al., "Multilineage potential of adult human mesenchymal stem cells," *Science*, vol. 284, no. 5411, pp. 143–147, 1999.

[84] C. Le Magnen, L. Bubendorf, C. Ruiz et al., "Klf4 transcription factor is expressed in the cytoplasm of prostate cancer cells," *European Journal of Cancer*, vol. 49, no. 4, pp. 955–963, 2013.

[85] A. Jemal, F. Bray, M. M. Center, J. Ferlay, E. Ward, and D. Forman, "Global cancer statistics," *CA Cancer Journal for Clinicians*, vol. 61, no. 2, pp. 69–90, 2011.

[86] N. J. Maitland and A. Collins, "A tumour stem cell hypothesis for the origins of prostate cancer," *BJU International*, vol. 96, no. 9, pp. 1219–1223, 2005.

[87] N. J. Maitland, F. M. Frame, E. S. Polson, J. L. Lewis, and A. T. Collins, "Prostate cancer stem cells: do they have a basal or luminal phenotype?" *Hormones and Cancer*, vol. 2, no. 1, pp. 47–61, 2011.

[88] E. E. Oldridge, D. Pellacani, A. T. Collins, and N. J. Maitland, "Prostate cancer stem cells: are they androgen-responsive?" *Molecular and Cellular Endocrinology*, vol. 360, no. 1-2, pp. 14–24, 2011.

[89] D. F. Gleason, "Classification of prostatic carcinomas," *Cancer Chemotherapy Reports*, vol. 50, no. 3, pp. 125–128, 1966.

[90] C. Grisanzio and S. Signoretti, "p63 in prostate biology and pathology," *Journal of Cellular Biochemistry*, vol. 103, no. 5, pp. 1354–1368, 2008.

[91] Z. A. Wang and M. M. Shen, "Revisiting the concept of cancer stem cells in prostate cancer," *Oncogene*, vol. 30, no. 11, pp. 1261–1271, 2011.

[92] H. Korsten, A. Ziel-van der Made, X. Ma, T. van der Kwast, and J. Trapman, "Accumulating progenitor cells in the luminal epithelial cell layer are candidate tumor initiating cells in a Pten knockout mouse prostate cancer model," *PLoS ONE*, vol. 4, no. 5, Article ID e5662, 2009.

[93] X. Ma, A. C. Ziel-van der Made, B. Autar et al., "Targeted biallelic inactivation of Pten in the mouse prostate leads to prostate cancer accompanied by increased epithelial cell proliferation but not by reduced apoptosis," *Cancer Research*, vol. 65, no. 13, pp. 5730–5739, 2005.

[94] D. J. Mulholland, L. Xin, A. Morim, D. Lawson, O. Witte, and H. Wu, "Lin-Sca-1+CD49fhigh stem/progenitors are tumor-initiating cells in the Pten-null prostate cancer model," *Cancer Research*, vol. 69, no. 22, pp. 8555–8562, 2009.

[95] A. S. Goldstein, J. Huang, C. Guo, I. P. Garraway, and O. N. Witte, "Identification of a cell of origin for human prostate cancer," *Science*, vol. 329, no. 5991, pp. 568–571, 2010.

[96] A. T. Collins, P. A. Berry, C. Hyde, M. J. Stower, and N. J. Maitland, "Prospective identification of tumorigenic prostate cancer stem cells," *Cancer Research*, vol. 65, no. 23, pp. 10946–10951, 2005.

[97] C. van den Hoogen, G. van der Horst, H. Cheung et al., "High aldehyde dehydrogenase activity identifies tumor-initiating and metastasis-initiating cells in human prostate cancer," *Cancer Research*, vol. 70, no. 12, pp. 5163–5173, 2010.

[98] E. M. Hurt, B. T. Kawasaki, G. J. Klarmann, S. B. Thomas, and W. L. Farrar, "CD44+CD24- prostate cells are early cancer progenitor/stem cells that provide a model for patients with poor prognosis," *British Journal of Cancer*, vol. 98, no. 4, pp. 756–765, 2008.

[99] C. Wei, W. Guomin, L. Yujun, and Q. Ruizhe, "Cancer stem-like cells in human prostate carcinoma cells DU145: the seeds of the cell line?" *Cancer Biology and Therapy*, vol. 6, no. 5, pp. 763–768, 2007.

[100] L. Patrawala, T. Calhoun, R. Schneider-Broussard et al., "Highly purified CD44+ prostate cancer cells from xenograft human tumors are enriched in tumorigenic and metastatic progenitor cells," *Oncogene*, vol. 25, no. 12, pp. 1696–1708, 2006.

[101] V. K. Rajasekhar, L. Studer, W. Gerald, N. D. Socci, and H. I. Scher, "Tumour-initiating stem-like cells in human prostate cancer exhibit increased NF-κB signalling," *Nature Communications*, vol. 2, article 162, 2011.

[102] A. D. Whetton and G. J. Graham, "Homing and mobilization in the stem cell niche," *Trends in Cell Biology*, vol. 9, no. 6, pp. 233–238, 1999.

[103] A. Spradling, D. Drummond-Barbosa, and T. Kai, "Stem cells find their niche," *Nature*, vol. 414, no. 6859, pp. 98–104, 2001.

[104] I. V. Litvinov, D. J. Vander Griend, Y. Xu, L. Antony, S. L. Dalrymple, and J. T. Isaacs, "Low-calcium serum-free defined medium selects for growth of normal prostatic epithelial stem cells," *Cancer Research*, vol. 66, no. 17, pp. 8598–8607, 2006.

[105] J. T. Isaacs, "Prostate stem cells and benign prostatic hyperplasia," *Prostate*, vol. 68, no. 9, pp. 1025–1034, 2008.

[106] D. L. Hudson, "Epithelial stem cells in human prostate growth and disease," *Prostate Cancer and Prostatic Diseases*, vol. 7, no. 3, pp. 188–194, 2004.

[107] C. P. Tran, C. Lin, J. Yamashiro, and R. E. Reiter, "Prostate stem cell antigen is a marker of late intermediate prostate epithelial cells," *Molecular Cancer Research*, vol. 1, no. 2, pp. 113–121, 2002.

[108] P. A. Humphrey, "Diagnosis of adenocarcinoma in prostate needle biopsy tissue," *Journal of Clinical Pathology*, vol. 60, no. 1, pp. 35–42, 2007.

[109] C. J. Shepherd, S. Rizzo, I. Ledaki et al., "Expression profiling of CD133+ and CD133- epithelial cells from human prostate," *Prostate*, vol. 68, no. 9, pp. 1007–1024, 2008.

[110] A. T. Collins and N. J. Maitland, "Prostate cancer stem cells," *European Journal of Cancer*, vol. 42, no. 9, pp. 1213–1218, 2006.

[111] R. Blum, R. Gupta, P. E. Burger et al., "Molecular signatures of prostate stem cells reveal novel signaling pathways and provide insights into prostate cancer," *PLoS ONE*, vol. 4, no. 5, Article ID e5722, 2009.

[112] B.-Y. Chen, J.-Y. Liu, H.-H. Chang et al., "Hedgehog is involved in prostate basal cell hyperplasia formation and its progressing towards tumorigenesis," *Biochemical and Biophysical Research Communications*, vol. 357, no. 4, pp. 1084–1089, 2007.

[113] D. Nikoleishvili, A. Pertia, O. Trsintsadze, N. Gogokhia, L. Managadze, and A. Chkhotua, "Expression of p27(Kip1), cyclin D3 and Ki67 in BPH, prostate cancer and hormone-treated prostate cancer cells," *International Urology and Nephrology*, vol. 40, no. 4, pp. 953–959, 2008.

[114] J. M. Rosen and C. T. Jordan, "The increasing complexity of the cancer stem cell paradigm," *Science*, vol. 324, no. 5935, pp. 1670–1673, 2009.

[115] A. Y. Liu, P. S. Nelson, G. D. van Engh, and L. Hood, "Human prostate epithelial cell-type cDNA libraries and prostate expression patterns," *Prostate*, vol. 50, no. 2, pp. 92–103, 2002.

[116] G. J. L. H. van Leenders and J. A. Schalken, "Stem cell differentiation within the human prostate epithelium: implications for prostate carcinogenesis," *BJU International*, vol. 88, Supplement, no. 2, pp. 35–42, 2001.

[117] N. Craft, C. Chhor, C. Tran et al., "Evidence for clonal outgrowth of androgen-independent prostate cancer cells from androgen-dependent tumors through a two-step process," *Cancer Research*, vol. 59, no. 19, pp. 5030–5036, 1999.

[118] A. Gu, J. Yuan, M. Wills, and S. Kasper, "Prostate cancer cells with stem cell characteristics reconstitute the original human tumor in vivo," *Cancer Research*, vol. 67, no. 10, pp. 4807–4815, 2007.

Biol Trace Elem Res (2013) 152:316–326
DOI 10.1007/s12011-013-9630-y

Association of Cadmium and Lead with Antioxidant Status and Incidence of Benign Prostatic Hyperplasia in Patients of Western India

Chirayu Pandya · Sharad Gupta · Prakash Pillai · Ajay Bhandarkar · Arif Khan · Arunodhay Bhan · Akhilesh Prajapati · Sarita Gupta

Received: 3 December 2012 / Accepted: 7 February 2013 / Published online: 13 March 2013

Abstract The association of cadmium (Cd) and lead (Pb) in the pathophysiology and progression of benign prostate hyperplasia (BPH) has been evaluated in an epidemiological study with 116 BPH patients of the western part of India. The prostatic acid phosphatase activity, prostate-specific antigen, maximum urinary flow rate (Q_{max}), and redox status of BPH patients were correlated with Cd and Pb contents. Additionally, patients were also separated on the basis of their age, genetic lineage, and additive habits and correlated with the Cd, Pb, and Q_{max} levels. Our results suggest that the accumulation of toxic metals in prostate tissue has a significant positive correlation with the pathogenesis of BPH. Cd and Pb exert their effects through altered antioxidant defense mechanisms, ultimately leading to increased BPH severity. Progression of the pathogenesis also depends on other factors such as additive habits, genetic lineage, and age of the patients.

Keywords Pb · Cd · Antioxidant enzyme · Reactive oxygen species (ROS) · Benign prostate hyperplasia (BPH)

Chirayu Pandya and Sharad Gupta contributed equally.

C. Pandya
Psychiatry and Health Behavior, Georgia Health Sciences University, Augusta, GA, USA

P. Pillai · A. Khan · A. Bhan · A. Prajapati · S. Gupta (✉)
Department of Biochemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat 390002, India
e-mail: saritagupta9@yahoo.com

S. Gupta
Gupta Pathological Laboratory, Vadodara, Gujarat, India

A. Bhandarkar
Sujay Urological Hospital, Vadodara, Gujarat, India

Introduction

Constantly increasing environmental pollutants due to rapid industrialization, urbanization, use of diesel generators/diesel exhaust, and through scientific and technical advancement have stimulated interest in studying toxic substances and their effects on the biological system [1]. Cd and Pb are well-known endocrine disrupters that act as estrogens or androgens that disturb the normal reproductive system [2, 3]. The International Agency for Research on Cancer considered Cd and Pb as potent human carcinogens which are of great interest to study their role in the pathogenesis of benign prostate hyperplasia (BPH) and cancer of the prostate (CaP) [4, 5]. BPH represents microscopic evidence of prostatic stromal and epithelial hyperplasia. In man, this proliferative process occurs predominantly in the transitional zone and peri-urethral glands, which is associated with age. However, CaP is a malignant condition of the prostate, either androgen-dependent or androgen-independent. Several studies have been undertaken to understand the etiopathogenesis of BPH; however, till date, no clear evidence exists to delineate their mechanisms. Increased risk of BPH induction in migrants from low- risk to high-risk areas suggests the involvement of environmental factors in the etiology of the disease [6, 7]. It has been reported that both BPH and CaP develop due to DNA damage and mutations, which could arise through various reasons, including environmental

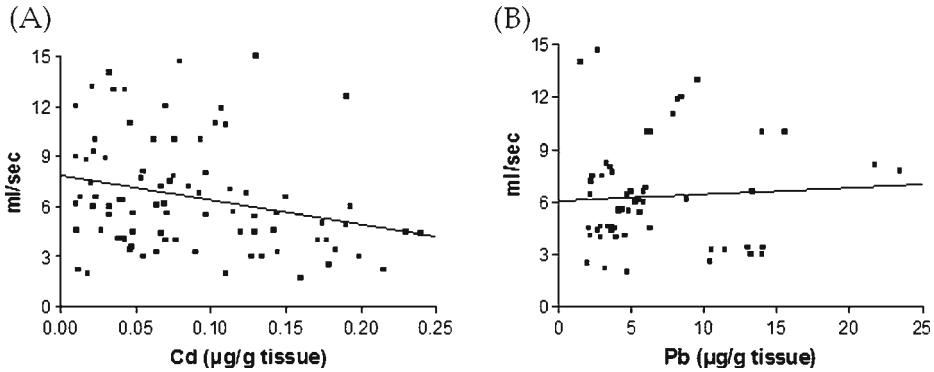


Fig. 1 Correlation trend of Cd (r^2=0.07, p<0.05, N=85) (**a**) and Pb (r^2=0.004, ns, N=58) (**b**) contents with Q_{max} level in the prostate of BPH patients. Cd showed a significant association with BPH pathogenesis compared to Pb. Data are represented as basis of correlation/trends

contaminants such as Cd and Pb exposure [8–10]. Recently, Guzel et al. [11] showed the association of Cd and Pb to BPH development and progression through the altered pro-oxidant–antioxidant balance in blood and/or tissue of 25 patients with BPH. Similarly, studies carried out with trace metals like Cr, Mn, Fe, Ni, Zn, and Cu also suggest the importance of metal ions in either promoting or inhibiting prostatic disorders [12]. Altogether, the studies showed that Cd and Pb play a critical role in the pathogenesis of BPH.

Apart from environmental influence, certain other risk factors are also responsible for the manifestation of BPH. These factors include age, genetic lineage, or additive habits such as cigarette smoking [13]. Epidemiological studies reported that the prevalence of BPH rises greatly with older age. It has been reported that 70 % of US men between the ages of 60 and 69 years are diagnosed with BPH; however, this increased to 80 % in those 70 years or older [14, 15]. Population-based studies of other research teams have also demonstrated similar trends [16–18]. The literature suggests that there are strong genetic components to BPH pathogenesis [19]. In a case–control analysis, it was shown that lineage individuals ≤64 years old were found to have four- to sixfold higher age-specific risks of BPH surgery among all primary male relatives. A similar group of researchers has demonstrated that 50 % of men ≤60 years of age undergoing surgery for BPH had a genetic lineage [20]. Several other findings have also reported higher risk of BPH to genetically lineage individuals at a younger age [21–24]. Although age and genetics play important roles in the etiology of BPH, cigarette smoking is also recognized as one of the strongest epidemiologic risk factors [13]. Surprisingly, several studies show the protective effect of smoking to the risk of BPH; however, others have reported no risk or increased risk [25]. Thus, discrepancy in the available literature suggests that further study needs to be carried out for definitive conclusions.

In view of this, the aim of the present study was to understand the association of environmental pollutants (Cd and Pb) with the incidence of BPH in patients from the western part of India. The importance of our study is reflected by the fact that this demographic study has been carried out directly in the target tissue, i.e., the prostate tissue from patients undergoing transurethral resection of the prostate (TURP) for BPH. Additionally, the present study will also help us understand the mechanisms behind the association of environmental contaminants and other risk factors to the progression and pathophysiology of BPH.

Materials and Methods

Chemicals

Tert-butyl hydroperoxide (*t*-BOOH) was obtained from Sigma, USA. Bovine serum albumin fraction V (BSA), nicotinamide adenine dinucleotide, and 5,5′-dithiobis(2-

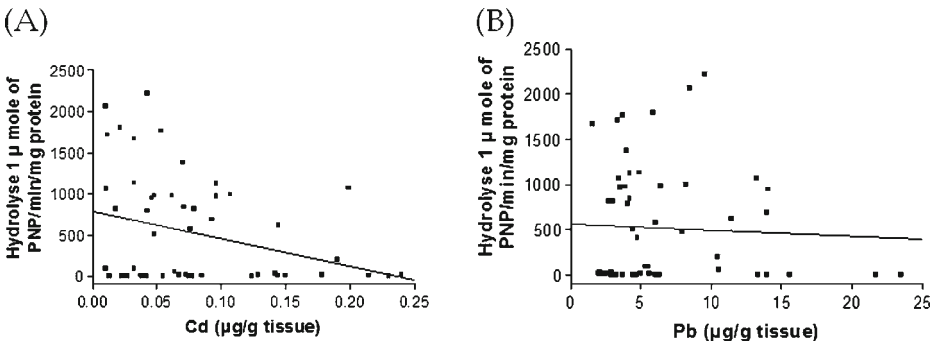


Fig. 2 Correlation of Cd (r^2= 0.095, p<0.05, N=54) (**a**) and Pb (r^2=0.003, ns, N=54) (**b**) contents with PAP level in BPH patients. Similar observations as in Fig. 1 further correlate Cd with BPH severity. Data are represented as basis of correlation/trends

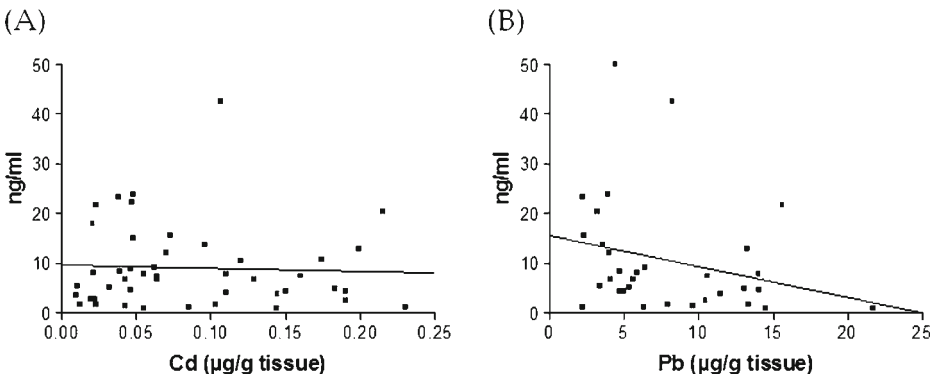


Fig. 3 Correlation of Cd (r^2= 0.002, ns, N=45) (**a**) and Pb (r^2=0.07, ns, N=34) (**b**) contents with PSA level in serum of BPH patients. Data are representes as basis of correlation/trends

nitrobenzoic acid) were obtained from SRL, India. Reduced glutathione (GSH) was a product of Hi Media, India. All other chemicals were of the highest purity grade and were purchased locally.

Subjects

A total of 116 prostate tissue samples from BPH patients who underwent TURP (~1 g tissue weight) were collected from the Sujay Hospital, Baroda, in ice-cold conditions (4 °C) in normal buffer saline and brought immediately to the lab for further processing. Sample collection was approved by the institutional ethical committee since the study was performed in the diseased tissue sample. Control prostate tissue was not possible for comparison.

Patients' detailed demographic and anthropometric data were collected using a structured questionnaire. Patients were asked several questions about their dietary habits, addictive habits, and environmental pollutant exposure status at their place of residence and work and whether patients had any genetic lineage of BPH from their family background.

Blood was collected for prostate-specific antigen (PSA) measurement [26]. Five hundred microliter serum was used for the serum PSA-based on enzyme immunoassay using monoclonal antibodies (PSA Monobind ELISA kits), with 0.05 ng sensitivity and maximum urinary flow rate (Q_{max}) [27] using uroflowmetry for voided volume (V). Peak flow rate analysis was performed on each patient to monitor clinical status and the severity of the disease condition.

Histology

Prostate tissue samples were submitted for histopathological diagnosis by standard histological techniques. Samples were fixed in 10 % buffered formalin fixative. Sections of 5-µm thickness were cut and stained with hematoxylin/eosin stain. Histological observations were made for every sample using light microscopy. BPH samples histologically confirmed by the pathologist were used for analysis.

Metal Analysis

Around 0.5 g of prostate sample was used for Pb and Cd estimation using an atomic absorption spectrophotometer [28]. The samples were digested in analytic grade nitric acid–perchloric acid (2:1) mixture. Digestion was continued until the samples became colorless. Then, the acid mixture was evaporated and the precipitate thus obtained was dissolved in a few drops of concentrated HCl. Pb and Cd levels were determined using a thermo-atomic absorption spectrophotometer by acetylene–air flame. Sensitivities of the assays were 0.06 and 0.009 µg/ml for Pb and Cd, respectively.

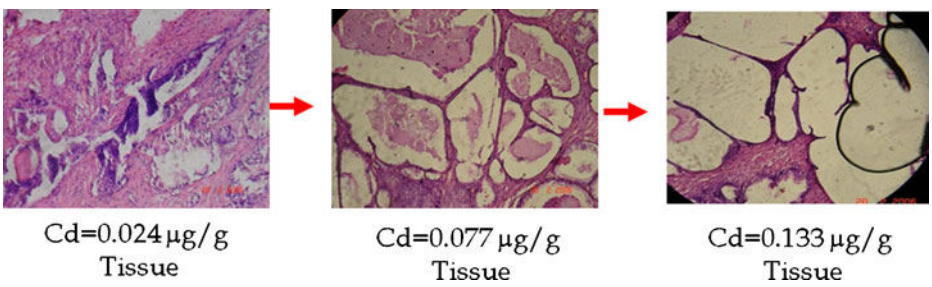


Fig. 4 Histopathological observation showed larger and more numbers of acini with increase Cd content in the prostate of BPH patients

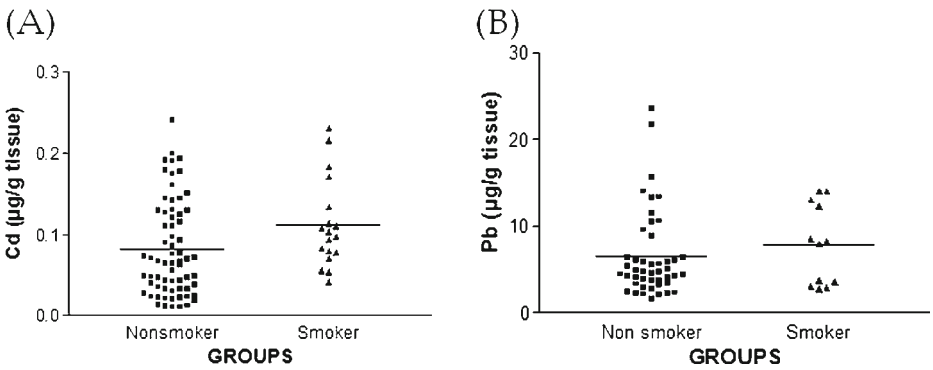


Fig. 5 Cd (non-smoker=67, smoker=18, $p<0.05$) (**a**) and Pb (non-smoker=46, smoker=12, ns) (**b**) levels in non-smokers and smokers: an effect of smoke in BPH patients. *Horizontal bars* indicate the mean. Cd was significantly higher in smokers compared to non-smokers

Biochemical Analyses

Prostate tissue was weighed and homogenized in chilled (4 °C) isolation medium (0.25 M sucrose, 10 mM Tris–HCl buffer, pH 7.4, 1 mM EDTA, and 250 µg BSA/ml). The isolation of the mitochondria and post-mitochondrial fractions was carried out [29]. The mitochondrial and post-mitochondrial fractions obtained were subjected to biochemical estimations/analysis of the reactive oxygen species (ROS)-related parameter. ROS parameters including the GSH content was measured using the method of Beutler and Gelbart [30], lipid peroxidation (LPO) was according to the methods of Ohkawa et al. [31], and superoxide dismutase (SOD) activity was determined according to the methods described by Marklund and Marklund [32]. Catalase activity was assayed by monitoring the decrease of H_2O_2 at 240 nm using the method of Hugo [33], and glutathione peroxidase (GPx) activity was determined using the procedure described by Hafeman et al. [34]. The activity of prostatic acid phosphatase (PAP) [35] was estimated in the post-mitochondrial fraction. Protein estimation has been carried out to express enzyme activity in terms of specific activity [36].

Statistical Analyses

The total number of samples was analyzed on the basis of correlation/trends in relation with various variables using SPSS version 15.0.1 statistical software program. The values were presented as significant at $p \leq 0.05$.

Results

The results of the present study represent the correlation(s) /trend(s) of patients suffering from BPH. To evaluate the pathogenesis of BPH, the known biochemical markers PSA and PAP and the clinical marker Q_{max} were correlated with both Cd and Pb contents. A strong correlation was observed with increasing Cd content to that of Q_{max} (Fig. 1) and PAP

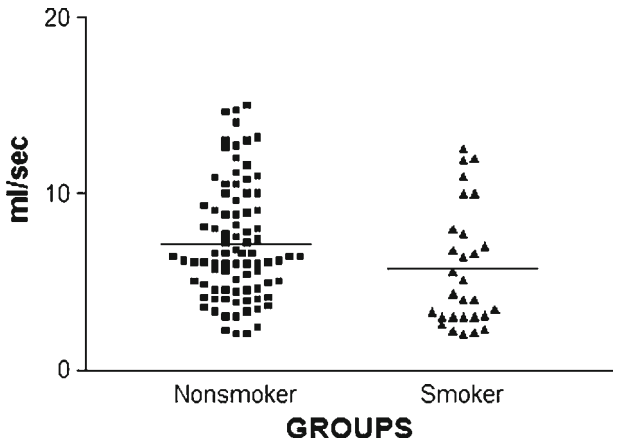


Fig. 6 Q_{max} levels in non-smokers and smokers: effect of smoking in BPH patients (non-smoker=87, smoker=29, $p<0.05$). *Horizontal bars* indicate the mean. Q_{max} was found to be significantly lower in smokers

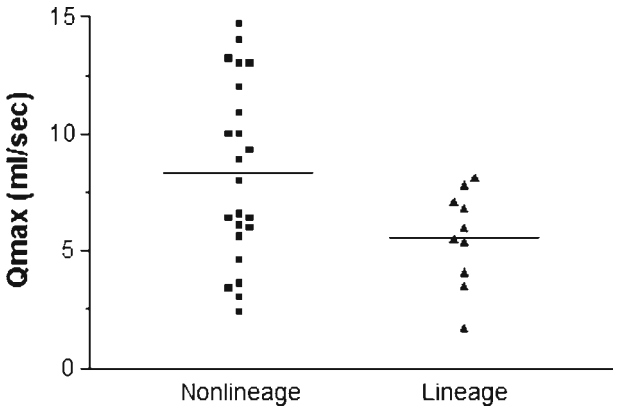


Fig. 7 Q_{max} of genetically non-lineage and lineage BPH patients between 45 and 64 years (non-lineage=23, lineage=10, $p<0.05$). *Horizontal bars* indicate the mean. Q_{max} of genetically lineage patients was significantly lower compared to non-lineage

(Fig. 2) values without any relation with the Pb content. However, the PSA level did not show any significant correlation with Cd and Pb (Fig. 3) contents.

The correlation between the severity of BPH with Cd and Pb contents was analyzed histopathologically. Cd and Pb contents showed a positive correlation with the severity of BPH. The results of few representative slides are shown in Fig. 4.

Record of patient history was also maintained so as to obtain information regarding dietary habits, addictive habits (cigarette smoking, tobacco chewing, alcoholism), and environmental pollutant exposure and genetic lineage of BPH. On the basis of the record, patients were divided into two groups: those having additive habits of smoking or not and those having any first- or second-degree genetic lineage of BPH or not. Parameters such as metal content (Cd and Pb) and Q_{max} were analyzed in smokers and non-smokers. Amongst the metals, cadmium was significantly more in smokers compared to non-smokers, while Pb did not show any significant accumulation (Fig. 5). The mean Q_{max} was found to be significantly lower in smokers than non-smokers (Fig. 6). The Q_{max} of genetically predisposed patients was significantly lower compared to genetically non-predisposed BPH patients (Fig. 7). Also, the incidence of BPH in genetically predisposed patients was found to be higher at an early age (Fig. 8). Patients were also divided into three age groups (55–64, 65–74, and 75–84 years) and compared using Q_{max} (Fig. 9). The Q_{max} value was found to be significantly decreased with older age.

To evaluate the activities of the antioxidant defense system in BPH patients, we assayed lipid peroxidation, glutathione level, and antioxidant enzyme activity (SOD, catalase, and GPx) from the mitochondrial and post-mitochondrial fractions. Oxidative stress parameters are correlated with the levels of Cd and Pb as a representation of environmental pollutants in BPH patients. Both the mitochondrial (Fig. 10a) and post-mitochondrial (Fig. 10b) LPO levels were found to be significantly increased with an increase in Cd levels (positive correlation). Similarly, significant positive trends were obtained in LPO levels with Pb accumulation in both the fractions used (Fig. 15a, b). SOD activity demonstrated a decreasing trend with respect to increased Cd levels in both subcellular fractions (negative correlation; Fig. 11a, b). Catalase activity does not exhibit any changes with both Cd and Pb accumulation. In the post-mitochondrial (Fig. 12a, b) fractions, GPx activity was significantly increased and positively correlated with increasing Cd content (Fig. 13). Similarly, statistical correlation of all antioxidant enzyme activities was carried out with the Pb content in BPH patients (Figs. 14 and 15). SOD (Fig. 16a, b) and enzyme activity exhibited negative correlation with Cd and Pb. Additionally, a significant decrease in GPx (Fig.17a, b) was found with higher Pb-exposed patients. Non-enzymatic antioxidants such as GSH were also correlated with the Cd and Pb levels of BPH patients. However, the GSH levels of the mitochondrial and post-mitochondrial fraction show a decreasing trend, but do not show significant alterations with cadmium (Fig. 14a, b) and lead (Fig. 18a, b).

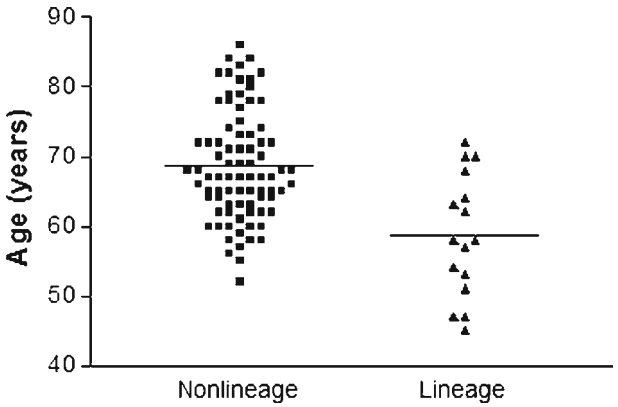


Fig. 8 Age of genetically non-lineage and lineage BPH patients (non-lineage=87, lineage=29, $p<0.001$). *Horizontal bars* indicate the mean. BPH in genetically predisposed patients was found to be higher at an early age

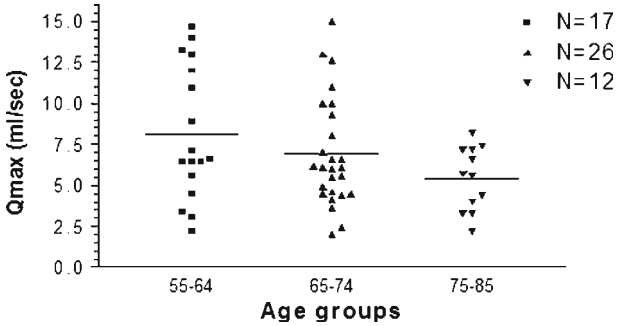


Fig. 9 Age-dependent effect on the Q_{max} level of BPH patients (55–64 vs. 75–85, $p<0.05$). *Horizontal bars* indicate the mean. Q_{max} value was found to be significantly decreased with older age

Discussion

The study was carried out with patients who underwent TURP to ascertain the association of environmental pollutants with prostatic disorders .Clinical and histopathological evaluation classified them as BPH patients. The toxic metals Pb and Cd have been correlated with biochemical markers

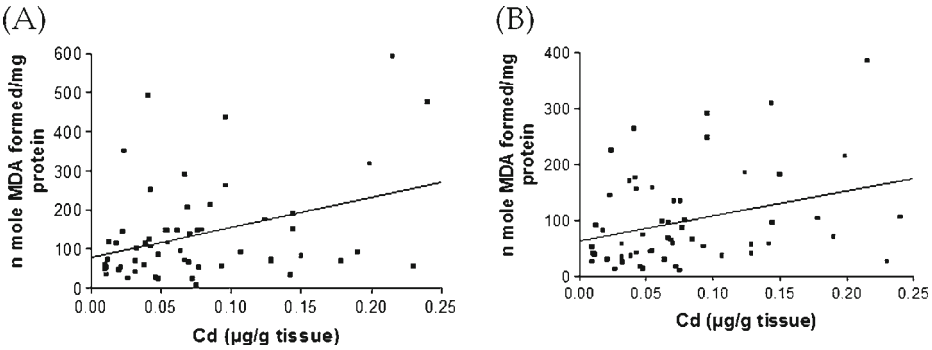


Fig. 10 Correlation of Cd content with mitochondrial (r^2=0.128, p<0.01, N=58) (**a**) and post-mitochondrial (r^2= 0.097, p<0.05, N=56) (**b**) LPO levels in the prostate of BPH patients. LPO levels were found to be significantly increased with an increase in Cd levels in both fractions

of BPH. The increase in the Cd level was significantly correlated with decreased levels of Q_{max} and PAP activity. The negative correlation of Cd with Q_{max} and PAP suggests the severity of BPH due to Cd accumulation. Although the mean level of Pb accumulation was 100 times higher than Cd accumulation in BPH patients, it did not show any significant correlation with Q_{max} and PAP, suggesting it as a weak toxicant. The serum PSA level does not show any significant correlation with Cd and Pb accumulation, suggesting that there is no correlation of metal with tissue damage at this concentration. Cadmium was found to be a potent toxicant associated with the clinical status of BPH pathogenesis.

It is well known that toxic metals act as catalysts in the generation of reactive oxygen species [37].We were interested in investigating whether the association of Cd and Pb with the incidence of BPH pathogenesis is due to oxidative damage. We observed that increased levels of Cd and Pb were significantly correlated with higher levels of LPO in the mitochondrial and post-mitochondrial fractions of prostate tissue. Elevated levels of LPO with higher Cd and Pb may lead to membrane alterations [38, 39]. Our earlier studies have reported changes in LPO levels in liver and membrane fluidity with both Pb and Cd exposures [40]. The enhanced LPO in the present study also indicates failure of antioxidant defense mechanism, which otherwise prevents the formation of excess free radical.

To further understand this mechanism, antioxidant enzymes were monitored in mitochondrial and/or the post-mitochondrial fraction of prostate. The activities of antioxidant enzymes such as SOD, catalase, and GPx were measured in the subcellular compartments of BPH subjects. The activity of antioxidant enzyme SOD was diminished when correlated with metal ions; however, the altered activity was statistically non-significant. SOD generally dismutates the superoxide anion radical (O_2^-) into hydrogen peroxide (H_2O_2), which is degraded by catalase and GPx. The decreased SOD activity could be due to replacement of the essential metal ions such as manganese, copper, and zinc from the active site of the SOD by Pb [41] and Cd [42]. Catalase did not show any alteration with Cd and Pb contents. GPx activity was found to be significantly altered with Cd and Pb levels in BPH subjects. GPx activity was increased with Cd levels, however decreased when correlated with Pb levels in the mitochondrial and post-mitochondrial fractions of prostate tissue. Thus, the major route of H_2O_2 elimination would seem to be via GPx. The increased activity of GPx may indicate a stress response of the prostate to Cd toxicity. The negative correlation of GPx activity to Pd content could be due to the replacement of selenium ion from the active site of the enzyme [43]. However, a non-significant negative

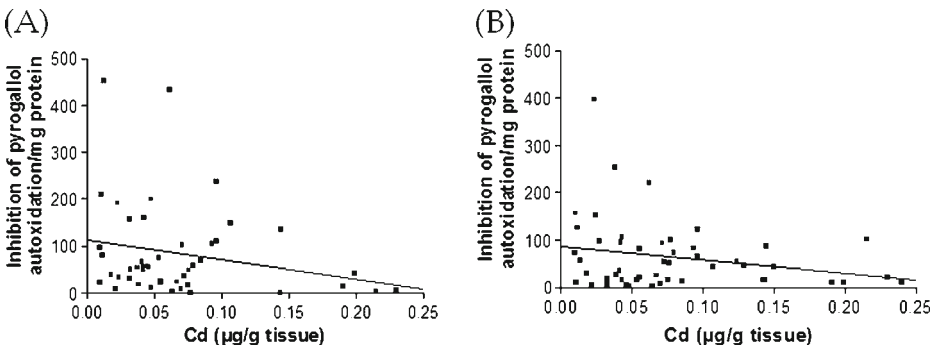


Fig. 11 Correlation of SOD activity with Cd content in the mitochondrial (r^2=0.051, ns, N=47) (**a**) and post-mitochondrial (r^2=0.054, ns, N =50) (**b**) fractions of human BPH prostate. A decreasing trend with respect to increased Cd levels is demonstrated

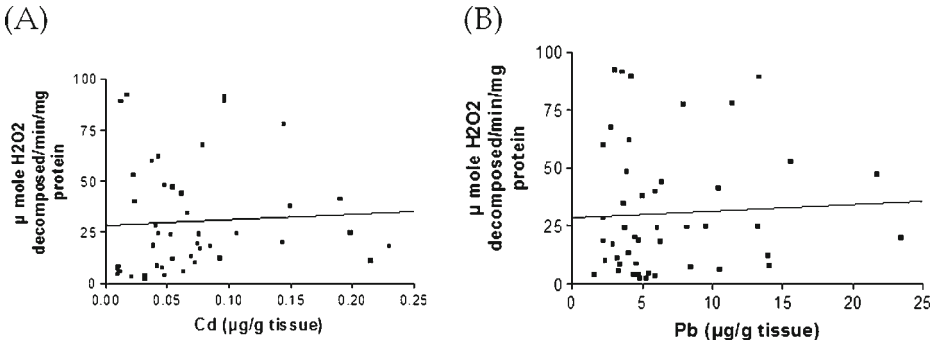


Fig. 12 Catalase activity in BPH patients showed correlation trend of Cd (r^2= 0.003, ns, N=45) (**a**) and Pb (r^2=0.002, ns, N=47) (**b**) contents with the post-mitochondrial fraction

association of Cd and Pb could be established with GSH content. Thus, altered antioxidant enzyme activity, especially GPx, might lead to an increase in oxidative stress, causing ROS-induced damage to macromolecules such as DNA, proteins, and key enzymes involved in prostatic enlargement. Earlier, in a similar kind of metal-exposed study, the altered activities of antioxidant enzyme in pituitary [44], ovary [45], testis [39], and liver [46] were also reported in Cd- and Pb-exposed rats. It is also to be noted that the alteration pattern of oxidative parameters in the mitochondrial and post-mitochondrial fractions were similar, suggesting similar subcellular effects of Cd and Pb.

Since BPH is considered to be an age-related disorder, we divided the patients into three age groups. Q_{max} was used as a measure of severity for the different age groups; an inverse relation with Q_{max} was found, which suggests increased severity with age. It has been reported earlier that an increase in age causes an increase 5α-reductase activity in prostate tissue [47], which will imbalance hormonal milieu and cause BPH. A positive family history also showed a negative association with Q_{max} and onset of the disease at an earlier age. The possible explanation for the early incidence and higher severity of BPH in genetically predisposed people could be attributed to single nucleotide polymorphisms in the genes of various enzymes or certain receptors like androgen receptor-2 and estrogen receptors [48]. In the present study, patients were also divided into genetically lineage and non-lineage based on their background of having first-degree BPH patients in the family. The mean age of lineage patients was significantly lower than non-lineage BPH patients, suggesting age-related disease.

There are many other factors which play an important role in the incidence of BPH pathology and the increase in its severity. Therefore, patients were also divided into two groups, namely, smokers and non-smokers, from their history.

The Q_{max} of smokers is significantly lower than that of non-smokers, suggesting the higher level of severity of BPH in smokers. There are several potential mechanisms whereby cigarette smoking may increase the risk of prostate cancer and hyperplasia. One is the ability of cigarette smoking to increase the bioavailable testosterone and decrease the bioavailable estradiol, which may alter the hormonal milieu favoring higher androgenic exposure to the prostate [49]. Another possible mechanism for an association between smoking and prostatic hyperplasia is exposure to carcinogenic substances found in cigarettes. For example, Cd and Pb are inorganic toxicants also found in cigarettes in higher concentrations [50, 51]. In the present study, Cd and Pb contents were measured in both groups, and the Cd level

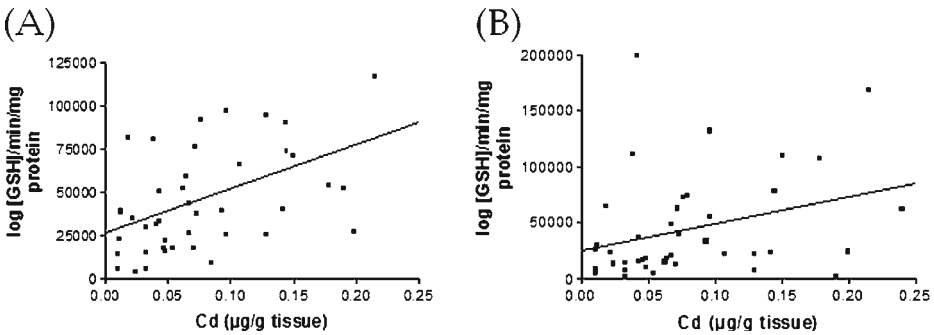


Fig. 13 Correlation of Cd content with the mitochondrial (r^2=0.177, p<0.01, N=42) (**a**) and post-mitochondrial (r^2= 0.1, p<0.05, N=42) (**b**) GPx activity in the prostate of BPH patients

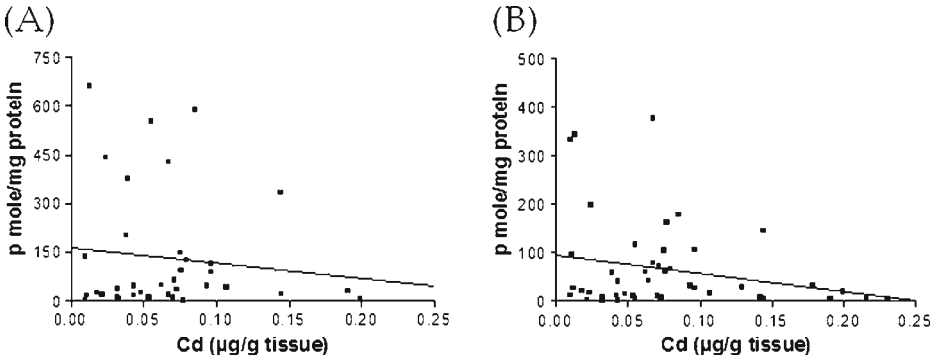


Fig. 14 Correlation of Cd content with the mitochondrial (r^2=0.014, ns, N=37) (**a**) and post-mitochondrial (r^2=0.055, ns, N=45) (**b**) reduced glutathione levels in the prostate of BPH patients

was found to be significantly higher in the prostate of smokers. The higher levels of Cd in smokers were due to the presence of about 0.5–2 μg Cd per cigarette: 10% of the cadmium content is inhaled (WHO 1992) [52]. Similarly, Pb is also present in tobacco at concentrations of approximately 2.5–12.2 μg/cigarette, of which approximately 2–6 % may actually be inhaled by the smoker (WHO 1977). We did not find significantly elevated levels of Pb in smokers, which could be due to the high concentration of Pb in the environment, which the non-smokers will also be exposed to. In 1993, the International Agency for Research on Cancer designated cadmium as a human carcinogen. Although not directly mutagenic in the prostate, cadmium has been shown to indirectly induce prostate carcinogenesis through interaction with the androgen receptor [53]. Studies also reported that cadmium has the property of activating the androgen receptor response in human prostate cancer cell lines. Furthermore, when applied in combination with androgen, cadmium enhances androgen-mediated transcriptional activity in the prostate [53]. Chronic cadmium exposure in rats has been shown to induce prostate tumors in the presence of normal testicular function. Therefore, chronic smoking in men with otherwise normal testicular function and androgen levels may effectively increase their androgen exposure through the interaction of cadmium with the androgen receptor, thus increasing the risk of prostate cancer and prostatic hyperplasia over a period of time. In addition to the role of cadmium in oxidative stress mechanism, cadmium is also known to act as an endocrine disruptor by binding to the estrogen receptor and functioning as an estrogenic mimic. Along with aging and positive family history, our data demonstrated the association of cadmium with the severity of BPH pathogenesis.

Thus, in the present study, a linkage between an increase in reactive oxygen radicals causing lipid peroxidation due to cadmium accumulation as an endocrine disruptor and the pathogenesis of BPH has been well established. Patients with higher accumulation of cadmium content also demonstrated more imbalances in antioxidant enzymes, and positive association with smoking and family history was very well demonstrated.

Conclusion

When the antioxidant control mechanisms are exhausted or overrun, the cellular redox potential shifts toward an oxidative stress, in turn increasing the potential for damage to the cellular component and the severity of BPH. Cadmium as an inducer of oxidative stress and as an endocrine disruptor was

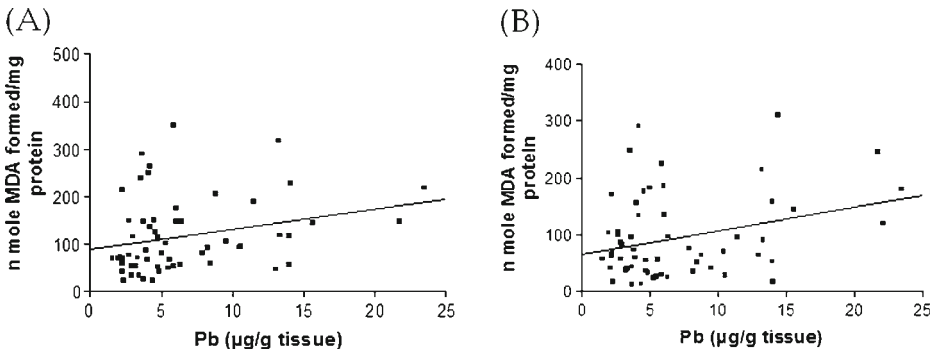


Fig. 15 Correlation of Pb content with the mitochondrial (r^2=0.07, p<0.05, N=58) (**a**) and post-mitochondrial (r^2= 0.091, p<0.05, N=59) (**b**) LPO levels in the prostate of BPH patients

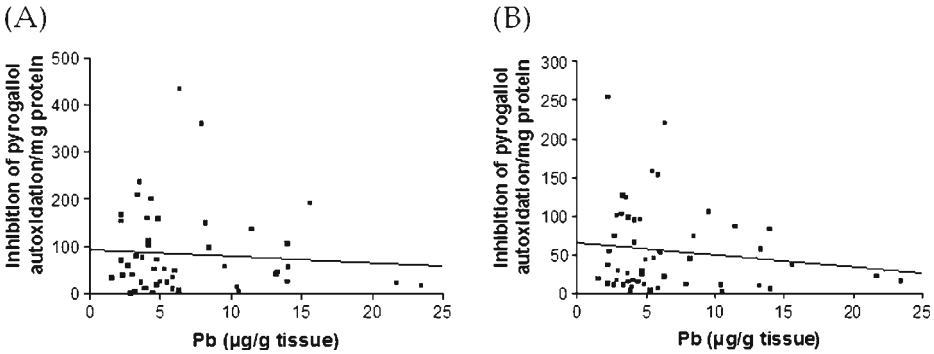


Fig. 16 Correlation of Pb content with the mitochondrial (r^2=0.006, ns, N=48) (**a**) and post-mitochondrial (r^2=0.02, ns, N=52) (**b**) SOD activity in the prostate of BPH patients

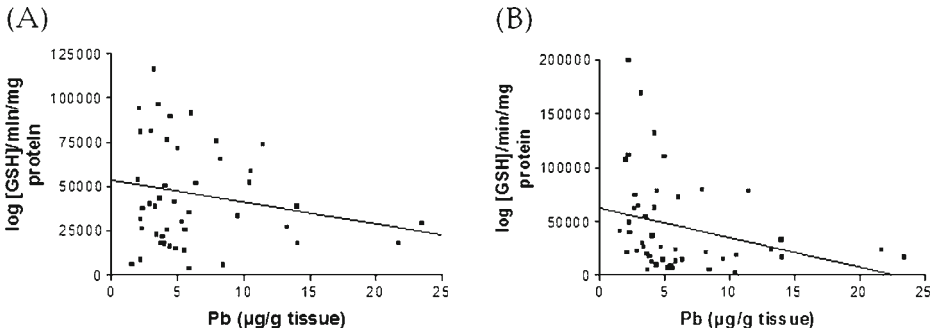


Fig. 17 Correlation of Pb content with the mitochondrial (r^2=0.034, ns, N=46) (**a**) and post-mitochondrial (r^2= 0.08867, p<0.05, N=46) (**b**) GPx activity in the prostate of BPH patients

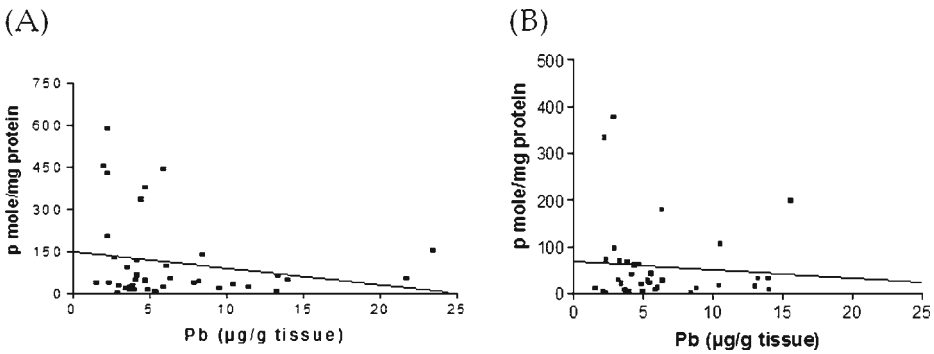


Fig. 18 Correlation of Pb content with the mitochondrial (r^2=0.04124, ns, N=39) (**a**) and post-mitochondrial (r^2=0.006, ns, N=35) (**b**) reduced glutathione levels in the prostate of BPH patients

found to be a potent clinical and biochemical environmental toxicant for BPH pathogenesis.

Acknowledgments We are thankful to Dr. S. Sharma (Geology Department, the M.S. University of Baroda) for his help in metal estimation. The authors thank Mr. Nitin Patel (Laboratory Assistant, Gupta Pathology, Vadodara) for the help in sample collection during surgery.

References

1. Gopalkrishnan K (1998) Characteristics of semen parameters in a selected population of Indian men over a period of 10 years. Curr Sci 75(9):939–942
2. Martin MB, Voeller HJ, Gelmann EP, Lu J, Stoica EG, Hebert EJ et al (2002) Role of cadmium in the regulation of AR gene expression and activity. Endocrinology 143(1):263–275
3. Stoica A, Katzenellenbogen BS, Martin MB (2000) Activation of estrogen receptor-alpha by the heavy metal cadmium. Mol Endocrinol 14(4):545–553
4. Waalkes MP (2003) Cadmium carcinogenesis. Mutat Res 533(1–2):107–120
5. Bertin G, Averbeck D (2006) Cadmium: cellular effects, modifications of biomolecules, modulation of DNA repair and genotoxic consequences (a review). Biochimie 88(11):1549–1559
6. Gann PH (2002) Risk factors for prostate cancer. Rev Urol 4(Suppl 5):S3–S10
7. Angwafo FF (1998) Migration and prostate cancer: an international perspective. J Natl Med Assoc 90(11 Suppl):S720–723
8. Fenech M, Ferguson LR (2001) Vitamins/minerals and genomic stability in humans. Mutat Res 475(1–2):1–6
9. De Bont R, van Larebeke N (2004) Endogenous DNA damage in humans: a review of quantitative data. Mutagenesis 19(3):169–185
10. Burcham PC (1999) Internal hazards: baseline DNA damage by endogenous products of normal metabolism. Mutat Res 443(1–2):11–36
11. Guzel S, Kiziler L, Aydemir B, Alici B, Ataus S, Aksu A et al (2012) Association of Pb, Cd, and Se concentrations and oxidative damage-related markers in different grades of prostate carcinoma. Biol Trace Elem Res 145(1):23–32
12. Guntupalli JN, Padala S, Gummuluri AV, Muktineni RK, Byreddy SR, Sreerama L et al (2007) Trace elemental analysis of normal, benign hypertrophic and cancerous tissues of the prostate gland using the particle-induced X-ray emission technique. Eur J Cancer Prev 16(2):108–115
13. Parsons JK (2010) Benign prostatic hyperplasia and male lower urinary tract symptoms: epidemiology and risk factors. Curr Bladder Dysfunct Rep 5(4):212–218
14. Benoff S, Jacob A, Hurley IR (2000) Male infertility and environmental exposure to lead and cadmium. Hum Reprod Update 6(2):107–121
15. Wei JT, Calhoun E, Jacobsen SJ (2005) Urologic diseases in America project: benign prostatic hyperplasia. J Urol 173(4):1256–1261
16. Kupelian V, Wei JT, O'Leary MP, Kusek JW, Litman HJ, Link CL et al (2006) Prevalence of lower urinary tract symptoms and effect on quality of life in a racially and ethnically diverse random sample: the Boston Area Community Health (BACH) Survey. Arch Intern Med 166(21):2381–2387
17. Taylor BC, Wilt TJ, Fink HA, Lambert LC, Marshall LM, Hoffman AR et al (2006) Prevalence, severity, and health correlates of lower urinary tract symptoms among older men: the MrOS Study. Urology 68(4):804–809
18. Parsons JK, Bergstrom J, Silberstein J, Barrett-Connor E (2008) Prevalence and characteristics of lower urinary tract symptoms in men aged > or =80 years. Urology 72(2):318–321
19. Gronberg H (2003) Prostate cancer epidemiology. Lancet 361(9360):859–864
20. Sanda MG, Beaty TH, Stutzman RE, Childs B, Walsh PC (1994) Genetic susceptibility of benign prostatic hyperplasia. J Urol 152(1):115–119
21. Pearson JD, Lei HH, Beaty TH, Wiley KE, Isaacs SD, Isaacs WB et al (2003) Familial aggregation of bothersome benign prostatic hyperplasia symptoms. Urology 61(4):781–785
22. Sanda MG, Doehring CB, Binkowitz B, Beaty TH, Partin AW, Hale E et al (1997) Clinical and biological characteristics of familial benign prostatic hyperplasia. J Urol 157(3):876–879
23. Rohrmann S, Fallin MD, Page WF, Reed T, Partin AW, Walsh PC et al (2006) Concordance rates and modifiable risk factors for lower urinary tract symptoms in twins. Epidemiology 17(4):419–427
24. Partin AW, Page WF, Lee BR, Sanda MG, Miller RN, Walsh PC (1994) Concordance rates for benign prostatic disease among twins suggest hereditary influence. Urology 44(5):646–650
25. Parsons JK (2007) Modifiable risk factors for benign prostatic hyperplasia and lower urinary tract symptoms: new approaches to old problems. J Urol 178(2):395–401
26. Acevedo B, Perera Y, Ruiz M, Rojas G, Benitez J, Ayala M et al (2002) Development and validation of a quantitative ELISA for the measurement of PSA concentration. Clin Chim Acta 317(1–2):55–63
27. Porru D, Jallous H, Cavalli V, Sallusto F, Rovereto B (2002) Prognostic value of a combination of IPSS, flow rate and residual urine volume compared to pressure-flow studies in the preoperative evaluation of symptomatic BPH. Eur Urol 41(3):246–249
28. Muslehiddinoglu J, Uludag Y, Ozbelge HO, Yilmaz L (1998) Determination of heavy metal concentration in feed and permeate streams of polymer enhanced ultrafiltration process. Talanta 46(6):1557–1565
29. Sahoo DK, Roy A, Bhanja S, Chainy GB (2005) Experimental hyperthyroidism-induced oxidative stress and impairment of antioxidant defence system in rat testis. Indian J Exp Biol 43(11):1058–1067
30. Beutler E, Gelbart T (1985) Plasma glutathione in health and in patients with malignant disease. J Lab Clin Med 105(5):581–584
31. Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95(2):351–358
32. Marklund S, Marklund G (1974) Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 47(3):469–474
33. Hugo EA (1987) Catalase. In: Bergmeyer HU, Bergmeyer J, Grabt M (eds) Methods of biochemical analysis, vol. 3. VCH Publishers, New York, pp 277–282
34. Hafeman DG, Sunde RA, Hoekstra WG (1974) Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. J Nutr 104(5):580–587
35. Bowers GN Jr, McComb RB (1975) Measurement of total alkaline phosphatase activity in human serum. Clin Chem 21(13):1988–1995
36. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193(1):265–275
37. El-Maraghy SA, Gad MZ, Fahim AT, Hamdy MA (2001) Effect of cadmium and aluminum intake on the antioxidant status and lipid peroxidation in rat tissues. J Biochem Mol Toxicol 15(4):207–214
38. Casalino E, Sblano C, Landriscina C (1997) Enzyme activity alteration by cadmium administration to rats: the possibility of iron involvement in lipid peroxidation. Arch Biochem Biophys 346(2):171–179
39. Pandya C, Pillai P, Nampoothiri LP, Bhatt N, Gupta S (2012) Effect of lead and cadmium co-exposure on testicular steroid metabolism

and antioxidant system of adult male rats. Andrologia 44(Suppl 1):813–822
40. Pillai A, Laxmi Priya PN, Gupta S (2002) Effects of combined exposure to lead and cadmium on pituitary membrane of female rats. Arch Toxicol 76(12):671–675
41. Gupta A, Shukla GS (1997) Enzymatic antioxidants in erythrocytes following heavy metal exposure: possible role in early diagnosis of poisoning. Bull Environ Contam Toxicol 58(2):198–205
42. Sen Gupta R, Sen Gupta E, Dhakal BK, Thakur AR, Ahnn J (2004) Vitamin C and vitamin E protect the rat testes from cadmium-induced reactive oxygen species. Mol Cells 17(1):132–139
43. Valenzuela A, Lefauconnier JM, Chaudiere J, Bourre JM (1989) Effects of lead acetate on cerebral glutathione peroxidase and catalase in the suckling rat. Neurotoxicology 10(1):63–69
44. Pillai A, Priya L, Gupta S (2003) Effects of combined exposure to lead and cadmium on the hypothalamic–pituitary axis function in proestrous rats. Food Chem Toxicol 41(3):379–384
45. Nampoothiri LP, Agarwal A, Gupta S (2007) Effect of co-exposure to lead and cadmium on antioxidant status in rat ovarian granulose cells. Arch Toxicol 81(3):145–150
46. Pillai A, Gupta S (2005) Antioxidant enzyme activity and lipid peroxidation in liver of female rats co-exposed to lead and cadmium: effects of vitamin E and Mn^{2+}. Free Radic Res 39(7):707–712
47. Berry SJ, Coffey DS, Walsh PC, Ewing LL (1984) The development of human benign prostatic hyperplasia with age. J Urol 132(3):474–479
48. Gupta L, Thakur H, Sobti RC, Seth A, Singh SK (2010) Role of genetic polymorphism of estrogen receptor-alpha gene and risk of prostate cancer in north Indian population. Mol Cell Biochem 335(1–2):255–261
49. Field AE, Colditz GA, Willett WC, Longcope C, McKinlay JB (1994) The relation of smoking, age, relative weight, and dietary intake to serum adrenal steroids, sex hormones, and sex hormone-binding globulin in middle-aged men. J Clin Endocrinol Metab 79(5):1310–1316
50. Kalcher K, Kern W, Pietsch R (1993) Cadmium and lead in the smoke of a filter cigarette. Sci Total Environ 128(1):21–35
51. Ashraf MW (2012) Levels of heavy metals in popular cigarette brands and exposure to these metals via smoking. Scientific World Journal 2010:729430.
52. Elinder CG, Kjellstrom T, Hogstedt C, Andersson K, Spang G (1985) Cancer mortality of cadmium workers. Br J Ind Med 42(10):651–655
53. Ye J, Wang S, Barger M, Castranova V, Shi X (2000) Activation of androgen response element by cadmium: a potential mechanism for a carcinogenic effect of cadmium in the prostate. J Environ Pathol Toxicol Oncol 19(3):275–280

ABSTRACTS, POSTERS & CONFERENCES

Association of Benign Prostate Hyperplasia with respect to Environmental Pollutant Cadmium

Pandya Chirayu D.[1], **Prajapati Akhilesh [1]**, Pillai Prakash[1],Gupta Sharad C.[2], Bhandarker Ajay[3] , Bhatt Niraj[1] , **Gupta Sarita S[1]***

[1]MOLECULAR ENDOCRINOLOGY AND STEM CELL RESEARCH LAB, DEPARTMENT OF BIOCHEMISTRY, THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA, VADODARA, GUJARAT, INDIA.

[2]GUPTA PATHOLOGICAL LABORATORY, VADODARA, [3]SUJAY UROLOGICAL HOSPITAL, VADODARA.

*Corresponding author- Email: saritagupta9@yahoo.com.

ABSTRACT

BPH is characterized by the non-malignant enlargement of the prostate gland which obstructs urethra. Certain risk factors for manifestation of BPH involves advanced age, African-Asian race, environmental influence, cigarette smoking and heavy metal exposure. Amongst the above cadmium which is a known carcinogen is a very potent heavy metal toxicant and is linked to BPH and Prostate cancer in epidemiologic and laboratory animal studies. Cadmium has three major properties, which mechanistically explain how it elicits a majority of their toxic effects. First it is transition metals that promotes hydrogen peroxide, hydroxyl radical and lipid per-oxidation production. The pro-oxidation properties of metals are highlighted by their inhibitory effects on antioxidant processes, in addition cadmium also has been linked to prostatic hyperplasia due to its estrogen mimicking capability, causing onset of tumor formation in Prostate gland. The mechanism of which is still under investigation. Very few reports are available on association of environmental pollutants and prostatic disorders such as prostate cancer and benign prostate hyperplasia.

In view of this a novel epidemiological study has been carried out with 116 benign prostate hyperplasia (BPH) patients, progressive neo-plasic condition using prostate sample to understand the association of environment pollutants (cadmium) with the incidence of BPH of patients of western India. The importance of our study is reflected by the fact that, this demographic study has been carried out directly in the target tissue, i.e. the prostate from patients undergoing TURP (Trans Urethral Resection of Prostate). The objective of the present study is to ascertain the correlation of BPH and its severity to smoking, hereditary factors, maximum urinary outflow rate (Qmax), Cadmium levels and antioxidant status. Strong correlation was observed with increasing cadmium content to that of Qmax and PAP value. PSA Level did not show any significant correlation with cadmium.

INTRODUCTION

Environmental pollution by toxic metals is a global problem, which is aggravated in today's age of increased technological development, which results in an increase in heavy metals like Cadmium above the recommended safety levels causing a deleterious effect to human health. Reports have shown Cadmium's oxidative and sulfhydryl reactive capability leading to the onset of oxidative stress in the prostate gland.

There are many other factors which play a role in causing the incidence of BPH and increasing its severity. It is well known that toxic metal acts as a catalyst in generation of Reactive oxygen species (ROS) (Monteiro *et al.*, 1991; El Maraghy *et al.*, 2001)

ROS are known to be the mediators of phenotypic and genotypic changes that lead from mutation to neo-plasia. ROS generating heavy metals like cadmium (Cd) is of great interest to study their role as a carcinogen. It is transition metal which promotes hydrogen peroxide, hydroxyl radical and lipid per-oxidation production. One modifiable risk factor is cigarette smoking. Effectively, cigarette smoking may establish a hormonal milieu which is favourable for the development or progression of BPH. In addition, cigarette contains significant levels of Cadmium, which has been linked to prostate hyperplasia due to its estrogen mimicking effects (Celia Byrne *et al.,*2009). The present study therefore highlights the association of cadmium as pollutant along with severity of BPH pathogenesis.

MATERIAL AND METHODS

BPH tissue (~2g) were collected in transport medium and patient's detailed demographic and anthropometric data were collected in a questionnaire and informed consents from all the patients were obtained.

(ethical clearance was obtained by ethical committee)

Histological analysis → **Slide preparation**

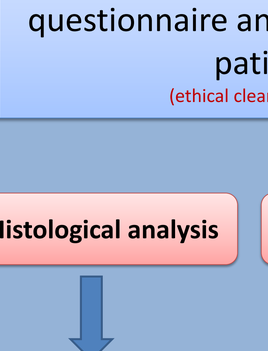
Clinical markers → **Qmax**

Biochemical markers → **Cd content, PSA, PAP, Anti-oxidant status**

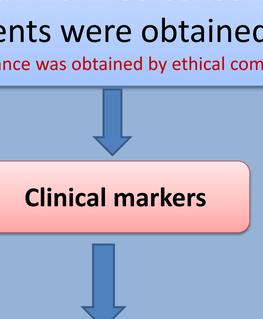
→ **Comparative analysis of Data**

RESULTS

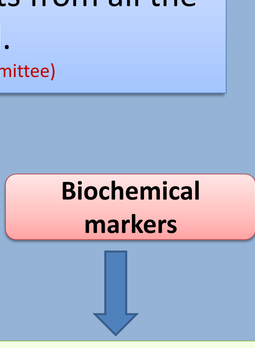
➢Correlation of Cd with Histo-pathological observation of BPH patients.



Cd = 0.024 µg/g tissue

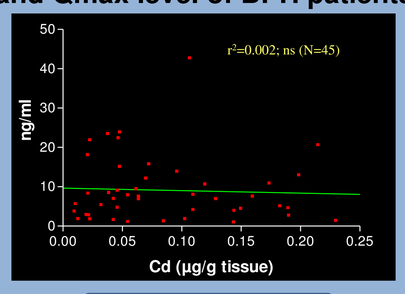


Cd = 0.077 µg/g tissue

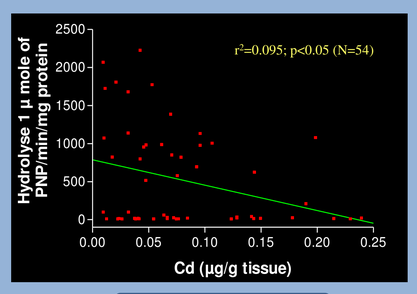


Cd = 0.133 µg/g tissue

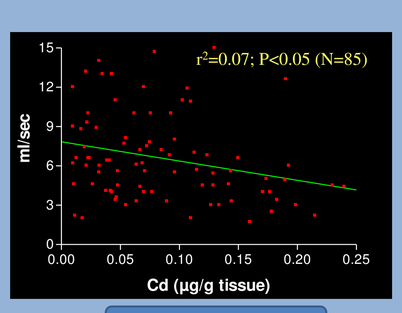
➢correlation of Cd content with serum PSA level ,PAP Activity and Qmax level of BPH patients



Serum PSA Level

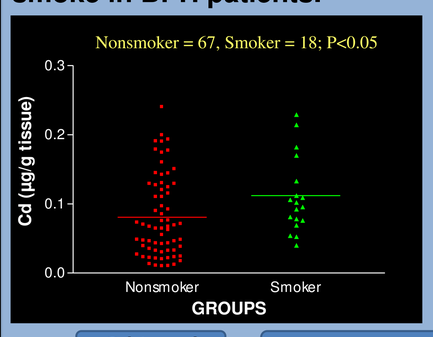


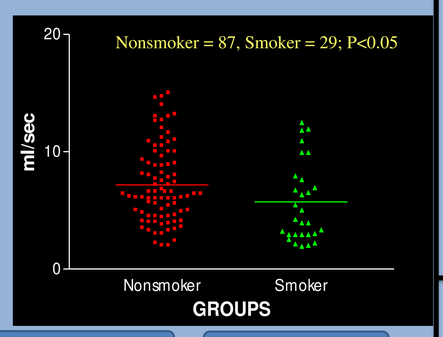
PAP Activity



Qmax Level

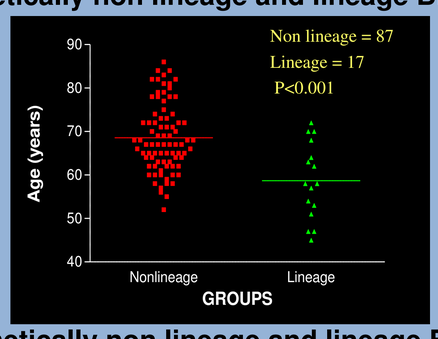
➢Cd and Qmax levels in nonsmokers and smokers: An effect of smoke in BPH patients.



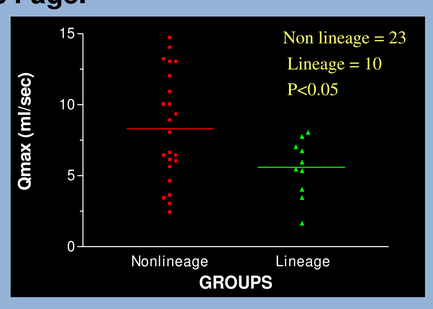


Cd Level | **Horizontal Bars indicate mean** | **Qmax Level**

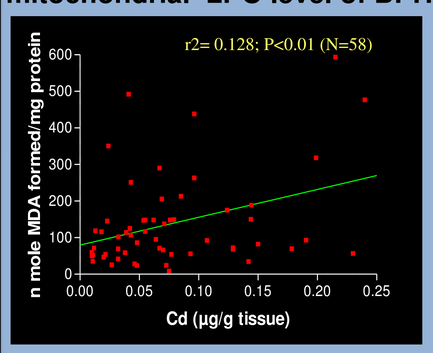
➢ Age of genetically non lineage and lineage BPH patients.

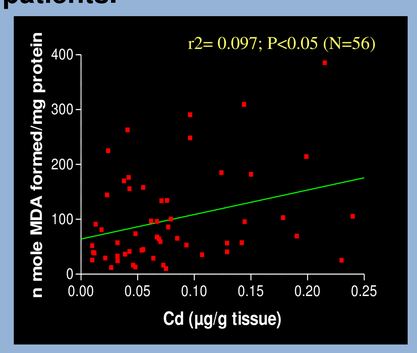


➢ Qmax of genetically non lineage and lineage BPH patients between 45 to 64 age.



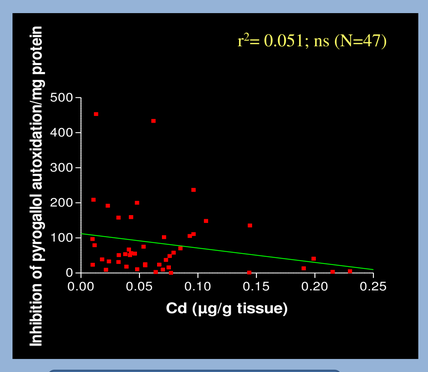
➢Correlation of Cd content with mitochondrial and post mitochondrial LPO level of BPH patients.

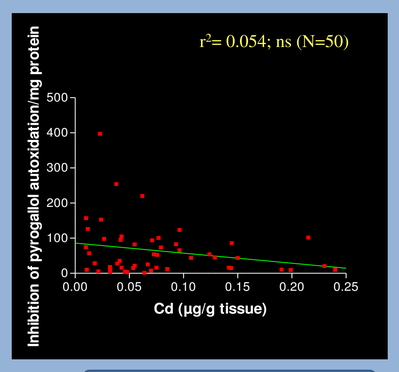




mitochondrial | **Post-mitochondrial**

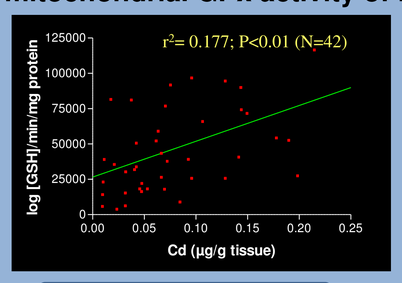
➢Correlation of Cd content with mitochondrial and post mitochondrial SOD activity of BPH patients.

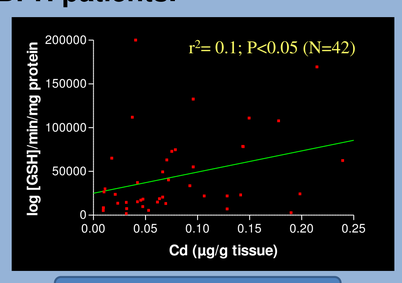




mitochondrial | **Post-mitochondrial**

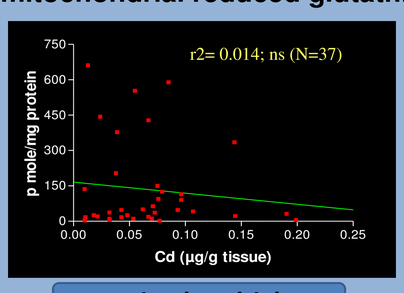
➢Correlation of Cd content with mitochondrial and post mitochondrial GPx activity of BPH patients.

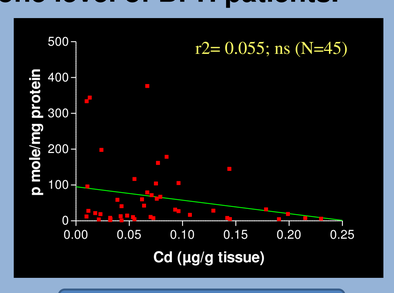




mitochondrial | **Post-mitochondrial**

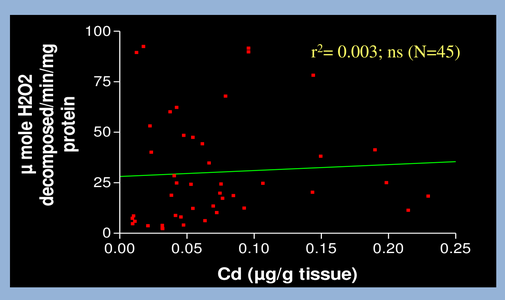
➢Correlation of Cd content with mitochondrial and post mitochondrial reduced glutathione level of BPH patients.





mitochondrial | **Post-mitochondrial**

➢Correlation of Cd content with catalase activity of BPH patients.



DISCUSSION

1. Histological slides demonstrated more severity of BPH condition with respect to increased Cd content.
2. Strong correlation was observed with increasing Cd content to that of Qmax and PAP, Suggesting involvement of Cd in BPH pathogenesis.
3. Decrease in GSH, antioxidant enzyme activities and increase in LPO levels, reflects ROS mediated pathogenesis.
4. Higher severity in old age suggested age dependent effect.
5. Genetically predisposed people showed early incidence and higher severity of BPH.
6. Smokers showed higher Cd content and positively correlated with severity of BPH.

CONCLUSION

Histological analysis, clinical marker (Qmax), biochemical marker (PAP) and anti-oxidant status of tissue demonstrated strong correlation with Cd content which indicates its involvement in the pathogenesis of BPH.

ACKNOWLEDGEMENT

We would like to thanks Mr. Nitin Patel for collecting Prostate tissue samples during surgery.

REFERENCES

➢Bahnson RR,Catalona WJ. Adverse implications of acid phosphatase levels in the upper range of normal. *J urol* 1987, 137,427-430.

➢Berry SW, Coffey DS, Walsh P, *et al*., The development of human benign prostate hyperplasia with age. *J urol.* 1984, 132,474-479.

➢Celia B. *et al.,* Cadmium- a metallohormone. *Toxicol Appl Pharmacol* 2009, 238(3):266-277.

➢El-Maraghy SA, Gad MZ, Fatim AT, Hamdy MA. Effect of cadmium and aluminium intake on the antioxidant status and lipid per-oxidation in rat tissues.*J Biochem Mol Toxicol* 2001,15,207-214.

➢Gunes S, Bagci H, Sarikaya S, Bilen CY, Kara N. Prostate- specific antigen and 17-hydroxylase polymorphic genotypes in patients with prostate cancer and benign prostate hyperplasia. *DNA Cell Biol* 2007,26,873-878.

➢Hickey K,Do KA, Green A. Smoking and prostate cancer review. 2001,23,115-125.

➢Oesterling JE. Benign prostate hyperplasia, a review of its histogenesis and natural history. *Prostate suppl* 1996,6,67-73.

➢Waalkes MP. Cadmium carcinogenesis in review. *J Inorg Biochem*. 2000,79,241-244.

➢Wei JT, Calhoun E,Jacobsen SJ. Urological Diseases in America project, benign prostate hyperplasia. *J urol* 2005,173,1256-1261.

Presented at INTERNATIONAL SYMPOSIUM ON ENDOCRINOLOGY AND REPRODUCTION: MOLECULAR MECHANISMS TO MOLECULAR MEDICINE P-101

AND 28th MEETING OF THE SOCIETY FOR REPRODUCTIVE BIOLOGY AND COMPARATIVE ENDOCRINOLOGY , JNU, New Delhi , INDIA. Feb 4-6, 2010

Cadmium: A Potent BPH Inducer in rat

Akhilesh Prajapati[1], Akshay Rao[1], Sharad Gupta[2], Sarita Gupta[1]*

[1] *Molecular Endocrinology and Stem cell Research lab, Department of Biochemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat -390002, India.*

[2]*Gupta Pathology Laboratory, Vadodara, Gujarat -390002, India.*

* Corresponding Author: saritagupta9@yahoo.com

MOLMED-2011

CHARUSAT — CHAROTAR UNIVERSITY OF SCIENCE & TECHNOLOGY

ABSTRACT

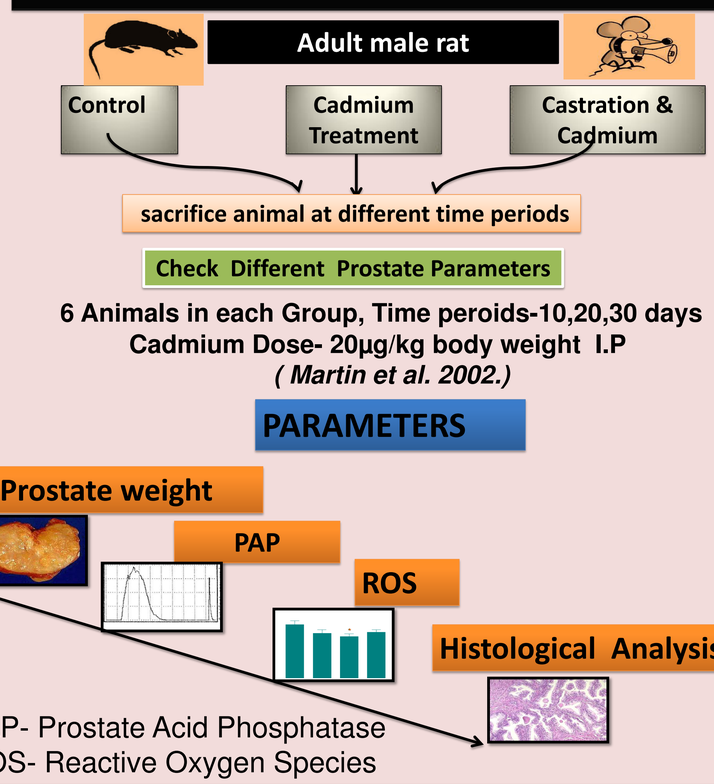
Endocrine disrupters and heavy metals are well known for abnormalities in many organs in human body. A very common pathology in the aging human male is the abnormal growth of the prostate gland, as reflected in the incidence of Benign Prostatic Hyperplasia (BPH) and Prostatic Carcinoma (PCA). Occupational and environmental studies suggest a potential role of cadmium (Cd) in the prostate cancer etiology. The interaction of cadmium with androgen receptors, mimics activity of Androgen or estrogen in the elicitation of benign prostate hyperplasia (BPH), has only recently been recognized. Its mode of action is still uncertain. Cigarette smoking may establish a hormonal milieu that is favorable for the development or progression of prostate cancer and known to contain significant levels of Cd. Our previous lab study has shown co-relation of BPH to smoking and strong co-relation with increasing Cd content affects Qmax and PAP levels.

The objective of the present study was to ascertain Cd induced BPH like condition in rats. It becomes clear that the administration of Cd induces proliferation, which is evident by increase in prostate weight, mitotic figures, ground glass pattern and invaginations of epithelial cells which are comparable with typical BPH histology. Further castration studies showed increase in proliferation of prostatic cells, suggesting its role via AR/ER receptor. Thus, cadmium exposure certainly induces proliferation of prostate cells.

INTRODUCTION

A very common pathology in the aging human male is the abnormal growth of the prostate gland, as reflected in the incidence of benign prostatic hyperplasia (BPH) and prostatic carcinoma (PCA). Prostate cancer is now the second leading cause of cancer-related death in men. Despite the magnitude of morbidity and mortality associated with this disease, very little is known regarding the mechanisms involved in prostate tumorigenesis. A variety of growth factors, steroidal hormones, proteases, and other factors are involved in normal prostatic morphogenesis and function, but their role in BPH and PCA remains poorly understood (1-2). Occupational and environmental studies suggest a potential role of cadmium (Cd) in the prostate cancer& BPH aetiology. Cd seems to be implicated in the increase of the incidence of prostate and other cancers in men exposed to high levels of this metal or its compounds (3). Cadmium has potent androgen/estrogen-like activity. The metal binds with high affinity to the hormone-binding domain of AR/ER and activates the receptors(4).

MATERIAL AND METHODS



PAP- Prostate Acid Phosphatase
ROS- Reactive Oxygen Species

RESULTS

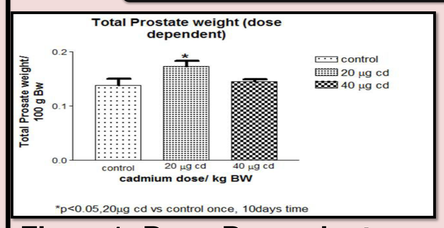


Figure1: Dose Dependent

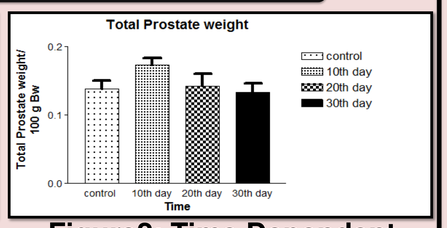


Figure2: Time Dependent

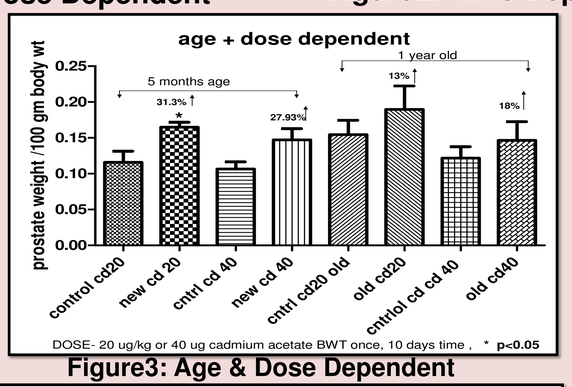


Figure3: Age & Dose Dependent

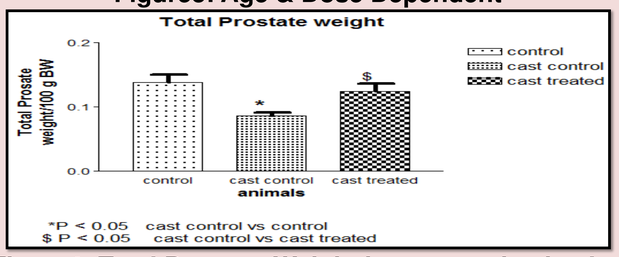


Figure4: Total Prostate Weight in castrated animal

➢ ROS Parameter in Cadmium Treated Rat Prostate

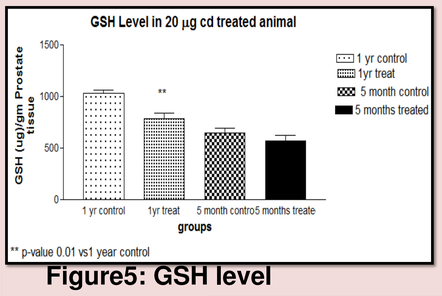


Figure5: GSH level

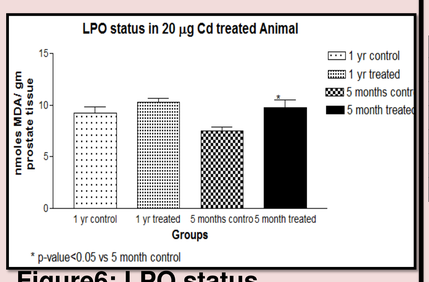


Figure6: LPO status

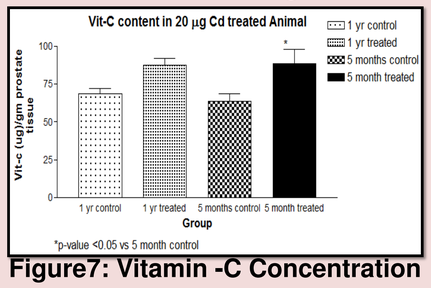


Figure7: Vitamin -C Concentration

➢ Biochemical Parameter

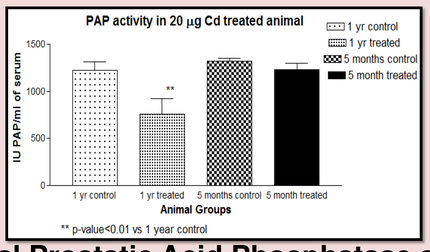
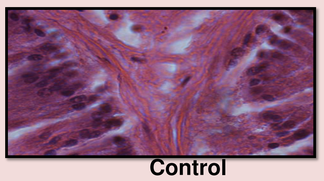


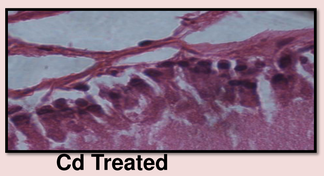
Figure8: Total Prostatic Acid Phosphatase activity

Histological Analysis

➢Figure 9: Basement membrane integrity

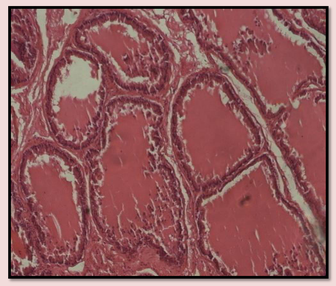


Control

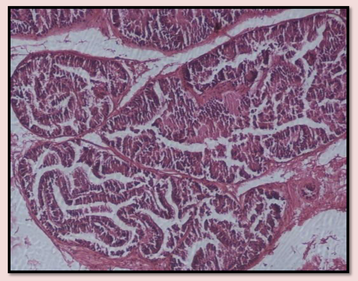


Cd Treated

➢Figure 10: Epithelial cells Invaginations



Control



Cd Treated

➢Table-1: Total Number of Mitotic Figures

AGE+DOSE	No. of mitotic figures/ 50nucleus		%INCREASE
1 year old animals	Control	Treated	
20 µg Cd/Kg BW	6.1±0.12	10.05±0.29 **	47.6 %
40 µg Cd/Kg BW	9.05±0.91	11.75± 0.85 *	23.4 %
5 months old animals			
20 µg Cd/Kg BW	5.6±0.5	10.70±0.62 ***	45.1 %
40 µg Cd/Kg BW	5.5±0.64	9.18±0.9 *	51.1 %

	Mitotic Figures 50 nucleus		% INCREASE
5 months old animals 20 µg Cd/Kg BW	Control	Treated	
Castrated	3.8± 0.26	9.7±1.62 *	60.8 %
Non-Castrated	6.1±0.12	10.05±0.29 ***	47.6 %
Non cast control Vs cast +Cd	6.1±0.12	9.7±1.62*	37.1%

All values are presented as mean ± SEM, p<0.05,**p<0.01,***p<0.001

➢Table-2: Total number of Ground Glass patterns

AGE+DOSE	No. of GROUND GLASS / 50 Nucleus		%INCREASE
1 year old animals	Control	Treated	
20 µg Cd/Kg BW	4.4± 0.29	8.4± 0.27 ***	39.3 %
40 µg Cd/Kg BW	4.2± 0.29	5.8± 0.44 *	27.5 %
5 months old animals			
20 µg Cd/Kg BW	4.7± 0.25	6.4± 0.4 *	26.01 %
40 µg Cd/Kg BW	6.7± 0.16	12.5± 0.85 ***	44.1 %

	no. of ground glass / 50 nucleus		%Increase
5 months old animals 20 µg Cd/Kg BW	Castrated	Castrated + Cd	
	0.007±0.002	0.065±0.01 *	88.9%

All values are presented as mean ± SEM, p<0.05,**p<0.01,***p<0.001

➢Table-3: Total number of Acini

AGE+DOSE	No. of ACINI / FIELD		% INCREASE
1 year old animals	Control	Treated	
20 µg Cd/Kg BW	19.1±1.15	25.6±0.97 ***	19.9%
40µg Cd/ Kg BW	19.1±1.15	24.3±0.98 **	21.4%
5 months old animals			
20 µg Cd/Kg BW	18.9±0.54	23.2±1 **	18.5%
40 µg Cd/Kg BW	19.8±1.4	24.4±1.6 *	18.9%

	Number of ACINI / Field		%INCREASE
5 months old animals 20 µg Cd/Kg BW	Control	Treated	
Castrated	19.1 ± 0.64	26.9±1.35 ***	29.0 %
Non-castrated	19.1± 1.15	25.6±0.97 **	19.1 %
Non cast control Vs cast +Cd	19.1± 1.15	26.9±1.35 ***	28.9%

All values are presented as mean ± SEM, p<0.05,**p<0.01,***p<0.001

All values are presented as mean ± SEM, p<0.05,**p<0.01,***p<0.001

DISCUSSION

1. One time dose of 20 µg cadmium / kg BWT induce Hyperplasia in rat prostate in 10 days time, and it was also supported by histological analysis .
2. 5-months age group animals gave better response to cadmium (both dose) , which was confirmed by histology and prostate weight.
3. Decrease in GSH and increase in LPO level , reflects ROS mediated Pathogenesis.
4. Cadmium has shown its proliferative effect in all cases which is clearly seen in histology and increase in prostate weight .
5. Castration studies show good evidence that cadmium binds to ER/AR and induces proliferation.

CONCLUSION

The present study suggests that cadmium has significant potency as an inducer of prostate hyperplasia in charles foster rats. Castration studies explain further evidence that cadmium exhibits Androgen/ Estrogen mimicking activity.

REFERENCES

1. Eaton CL. Aetiology and pathogenesis of benign prostatic hyperplasia. *Curr Opin Urol.*13:7-10, 2003.
2. J. W. J. vander Gulden, J. J. Kolk, and A. L. M. Verbeek:Prostate cancer and work environment, *JOM* 34, 402-409 ,1992.
3. Martin Mb, Voeller Hj, Gelmann Ep, Jianming Lu, Stoica Eg, Hebert Ej, Reiter R, Singh B, Danielsen M, Pentecost E, Stoica :, Role of Cadmium in the Regulation of AR Gene Expression and Activity. *Endocrinology* 143(1):263–275, 2002.
4. Michael D J, Nicholas K, Adriana S, Leena H, Baljit S, Gloria C, Rober C, Peter F, Apolonio A, Colby F, Ronald R, Bruce T, Soonmyoung P & Mary Beth M, Cadmium mimics the in vivo effects of estrogen in the uterus and mammary gland. *Nature Medicine* 9, 1081-1084,2003.

TO ASSESS THE POSSIBLE ROLE OF CADMIUM IN BPH PATHOGENESIS VIA STEROID HORMONE RECEPTOR BLOCKER

Akhilesh Prajapati[1], Jhanvi Patel[1], Sharad Gupta[2], Sarita Gupta[1]*

[1]MOLECULAR ENDOCRINOLOGY AND STEM CELL RESEARCH LAB, DEPARTMENT OF BIOCHEMISTRY, THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA, VADODARA, GUJARAT, INDIA.

[2]GUPTA PATHOLOGICAL LABORATORY, VADODARA.

*Corresponding author- Email: saritagupta9@yahoo.com.

ABSTRACT

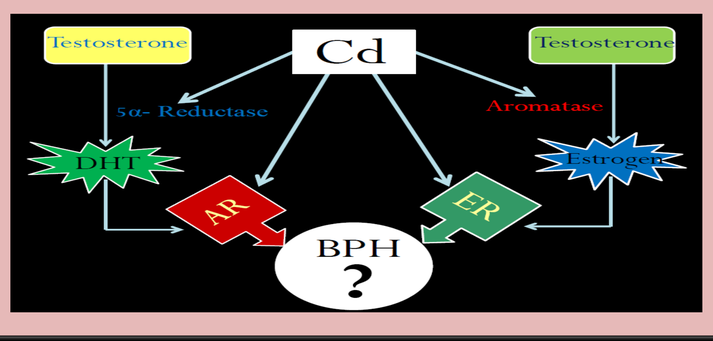
Benign prostatic hyperplasia (BPH) is common disease in old age. It has a high public health impact and is one of the most common reasons for surgical interventions among elderly men. Many attempts have been made during the last decade to obtain a thorough understanding of BPH, in spite of this the etiology and pathophysiology of the disease remains obscure.

Previously in our lab, we developed *in vivo* rat model for study of BPH by cadmium exposure. Cd clearly showed its ability to induce proliferation, which was evident by histological parameters and prostate enlargement. Cd has been reported to have mitogenic and steroid hormone mimicking activities. The present study mainly focuses on the induction of hyperplasia by Cd administration and elucidating the mechanism of cadmium by using androgen and estrogen receptor antagonist. Rat prostate samples were evaluated by histological assessment, immuno-histochemistry and prostate enlargement. The biochemical parameter, PAP was examined along with genes expressions profile of ER, AR, TGF-β and 5α reductase type-2.

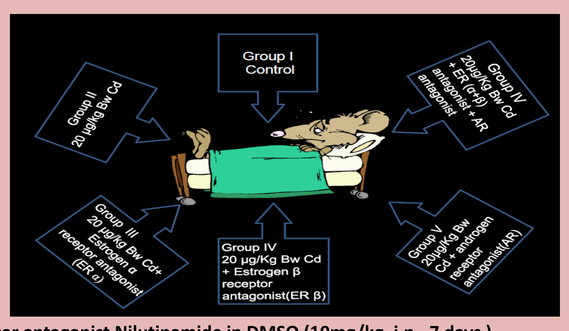
INTRODUCTION

A very common pathology in the aging human male is the abnormal growth of the prostate gland, as reflected in the incidence of benign prostatic hyperplasia (BPH) and prostatic carcinoma (PCA). Prostate cancer is now the second leading cause of cancer-related death in men. Despite the magnitude of morbidity and mortality associated with this disease, very little is known regarding the mechanisms involved in prostate enlargement. A variety of growth factors and steroid hormones are involved in normal prostatic morphogenesis and function, but their role in BPH and PCA remains unclear. Occupational and environmental studies suggest a potential role of cadmium (Cd) in the prostate cancer& BPH etiology (Martin M b et al, 2002). Our previous lab study has shown co-relation of BPH to smoking and strong co-relation with increasing Cd content affects Qmax and PAP levels (chirayu pandya thesis, 2008). Cadmium has potent androgen/estrogen-like activity. The metal binds with high affinity to the hormone-binding domain of AR/ER and activates the receptors(Michael D J et al 2003). Our recent studies showed mitogeneic effect of cadmium via steroid hormone receptor in prostate enlargement.

HYPOTHESIS



MATERIAL AND METHODS



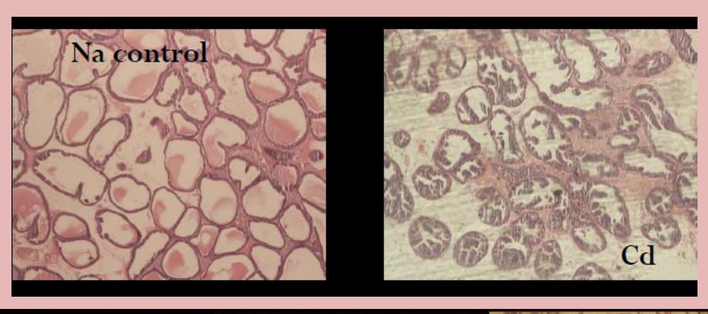
•Androgen receptor antagonist Nilutinamide in DMSO (10mg/kg, i.p., 7 days) (Coleman *et al.,sanofi-aventis Canada Inc.,2006)*

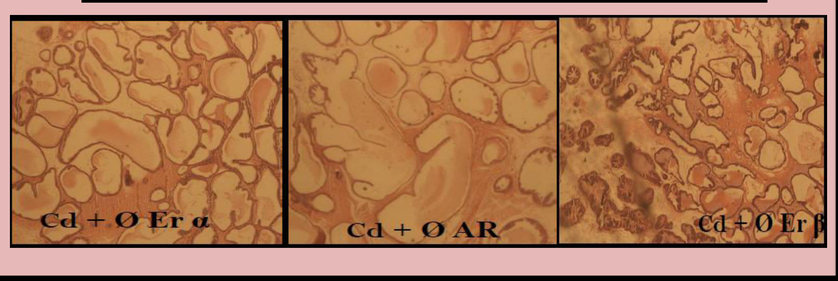
•Estrogen Receptor α antagonist Methyl piperidino pyrazole (in DMSO) 50 µg/kg Bw, 2 doses 24hr apart ,kill on 3rd day (Angela Davis *et. al. 2008, H. C. Shih et. al. 2008)*

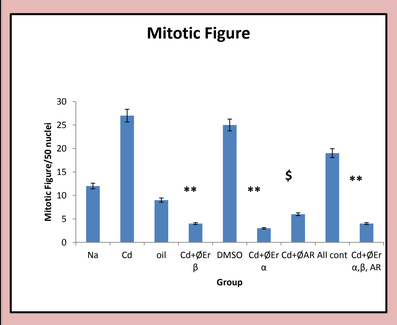
•Estrogen Receptor β antagonist 4- hydroxytamoxifen in seasame oil 1mg/kg 7 days i.p. (Reed & Chad,2005) 0.12 mmol/kg Bw, orally, per day for 7 days (Opsome *et. al. 1999)*

RESULTS

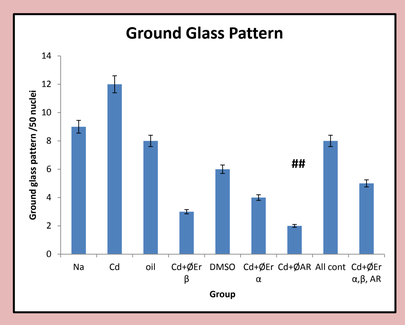
➢**Histological analysis.**



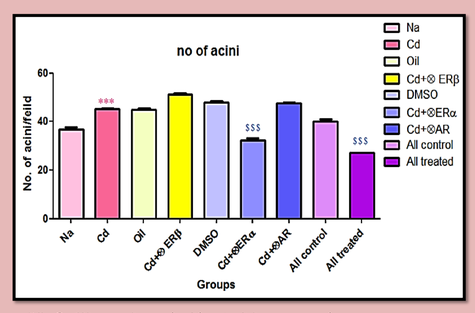




**p<0.001 vs. Cd, $ p<0.01 vs. Cd
*p<0.001 vs. Na

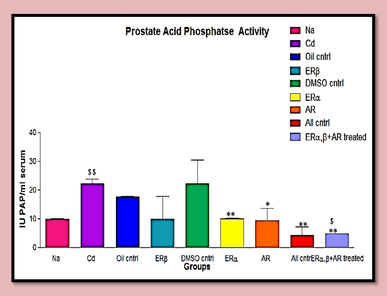


**p<0.01 vs. Cd, * p<0.05 vs. Na, ##p<0.001 vs. Cd, $ p<0.05 vs. Cd



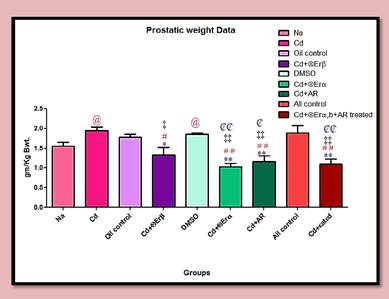
N=3. The values indicated here are in mean±SEM.***p<0.001 vs. Na, $$$p<0.001 vs. Cd

➢BIOCHEMICAL PARAMETERS



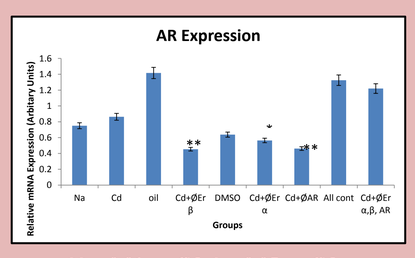
N=4.The values are in mean ±SEM. p=0.090 *p<0.05 vs. Cd, **p<0.01 vs. Cd, $p<0.05 vs. Na, $$p<0.01 vs. Na

➢OTHER PARAMETERS

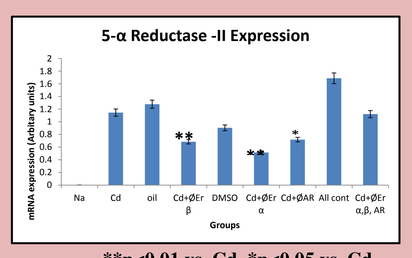


N=4. The values are in Mean ± SEM. @p<0.05 vs. Na, *p<0.05 vs. Cd , **p<0.01 vs. Cd, #p<0.05 vs. all control, ##p<0.001 vs. all control, ‡p<0.05 vs. DMSO, ‡‡p<0.01 vs. DMSO, ₡p<0.05 vs. Oil, ₡₡p<0.01 vs. Oil

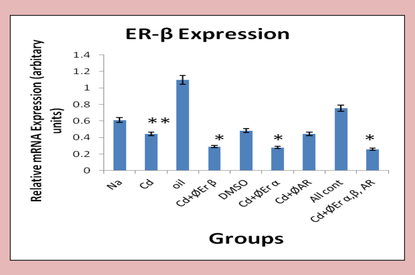
➢GENE EXPRESSION STUDIES



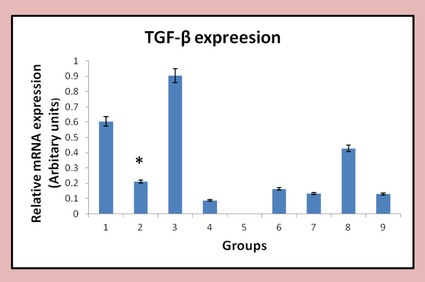
**p<0.01 vs. Cd, *p<0.05 vs. Cd



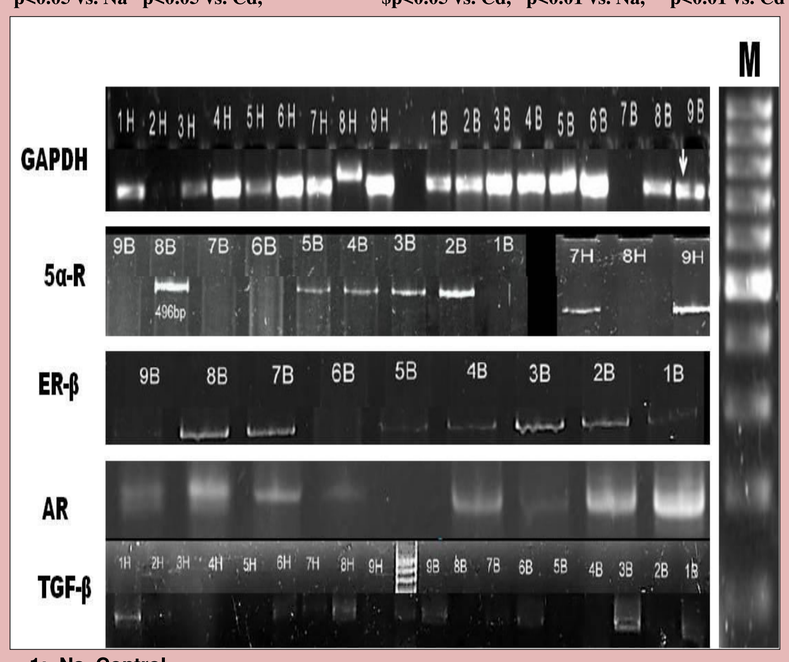
**p<0.01 vs. Cd, *p<0.05 vs. Cd



**p<0.05 vs. Na *p<0.05 vs. Cd,

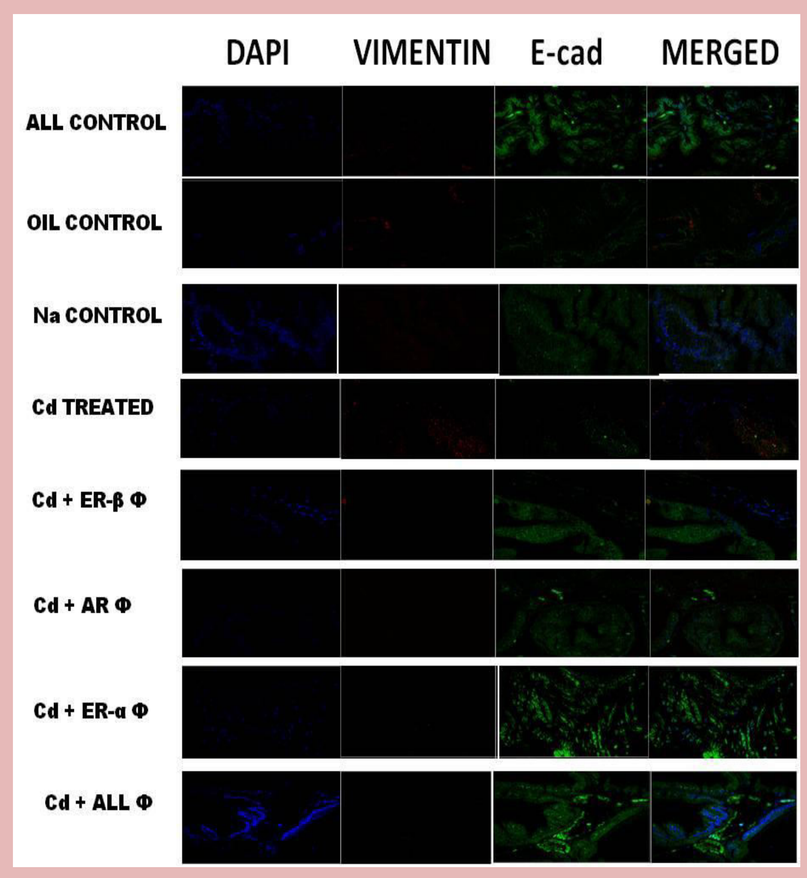


$p<0.05 vs. Cd, *p<0.01 vs. Na, **p<0.01 vs. Cd



1:- Na Control
2:- Cd Treated
3:- Oil control
4:- Cd+ER-β Ø Treated
5:- DMSO control
6:- Cd+ER –α Ø treated
7:- Cd+AR Ø Treated
8:- All control
9:- Cd+Ø (ER-α, β + AR) Treated
Ø=ANTAGONIST, ER=ESTROGEN RECEPTOR,AR=ANDROGEN RECEPTOR

➢IMMUNO- HISTOCHEMISTRY ANALYSIS



DISCUSSION

1. Cd induced prostate enlargement. Cd treatment along with the steroid hormone receptor antagonists (steroid hormone receptor blocker) showed no increase in the prostate weight and PAP activity. Suggesting Cd mediate its effect via steroid hormone receptors.
2. A more significant decrease was seen in case of ER-α and AR genes expression and less significantly in case of ER-β, gene expression.
3. Anti-androgen nilutinamide blocked the effect of Cd suggests the role of Cd through AR.
4. Total number of acini significantly decreases in ER- α antagonist group. Suggesting proliferation of the prostate gland by cadmium via ER- α receptor.
5. A significant decrease was observed in 5α Reductase type II gene relative expression in steroid hormone receptor antagonists group compare with Cd treated group, indicating the decreased conversion of testosterone to DHT and hence less proliferation.
6. Decrease expression of ER-β in cadmium treated group confirmed the Anti proliferative effect of ER-β. and it was also supported by histological analysis in ER-β antagonist treated group.
7. E-cadherin levels in epithelial cells decrease in BPH condition and in contrast, an abundant expression of Vimentin (mesenchymal marker) was observed in hypeplastic glands. (Alonso-Magdalena *et al., 2009 PNAS).* We similarly observed that the expression of Ecad decreased in the Cd treated group compared to the antagonist treated group and vice versa for Vimentin, which further supports our BPH animal model.

CONCLUSION

In presence of the receptor antagonist Cadmium was not able to show its proliferation effect and failed to cause BPH like condition in rats. Thus this study proved that Cadmium mediates proliferation via binding to AR and ER-α receptors. The results from this study can be a stepping stone for translational research in BPH.

REFERENCES

➢Ekman P. The prostate as an endocrine organ: Androgens and estrogens. Prostate Suppl. 2000;10:14-18 .

➢Bosland MC. The role of steroid hormones in prostate carcinogenesis. J Natl Cancer Inst Monogr, 2000 27:39-66.

➢Akduman B, Crawford ED. Terazosin, doxazosin, and prazosin: Current clinical experience. Urology.;2001;58:49-54

➢Gooren LJ, Toorians AW. Significance of oestrogens in male (patho)physiology. Ann Endocrinol (Paris). 2003;64:126-135

➢Suzuki K, Obara K, Kobayashi K, Yamana K, Bilim V, Itoi T, Takahashi K. Role of connective tissue growth factor in fibronectin synthesis in cultured human prostate stromal cells. Urology. 2006;67:647-653

➢Partin AW *et al.* The molecular biology, endocrinology, physiology of the prostate and seminal vesicles. In: Walsh PC, ed. Campbell's urology. Philadelphia:Saunders;2002:1237-1296.

➢**Michael D J, Nicholas K, Adriana S, Leena H, Baljit S, Gloria C, Rober C, Peter F, Apolonio A, Colby F, Ronald R, Bruce T, Soonmyoung P & Mary Beth M, Cadmium mimics the in vivo effects of estrogen in the uterus and mammary gland. *Nature Medicine* 9, 1081-1084,2003**

Presented at INTERNATIONAL CONFERENCE ON REPRODUTIVE HEALTH WITH EMPHASIS ON STRATEGIES FOR FAMILY PLANNING & 22nd ANNUAL MEETING OF THE INDIAN SOCIETY FOR THE STUDY OF REPRODUCTION & FERTILITY (ISSRF) "ICMR CENTINARY CELEBRATION" 1911-2011. 19th -21st FEB,2012,AIIMS, NEW DELHI, INDIA.

HUMAN PROSTATE CELL POPULATION DERIVED FROM BENIGN HYPERPLASIA SPECIMEN DEMONSTRATE PLURIPOTENT STEM CELLS PROPERTIES

Akhilesh Prajapati[1],Nidheesh Dadheech[1], Sharad Gupta[2], Sarita Gupta[1]*

[1]MOLECULAR ENDOCRINOLOGY AND STEM CELL RESEARCH LAB, DEPARTMENT OF BIOCHEMISTRY, THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA, VADODARA, GUJARAT, INDIA. [2]GUPTA PATHOLOGICAL LABORATORY, VADODARA.

*Corresponding author: saritagupta9@yahoo.com. First author: akhileshbiotech06@gmail.com

INTRODUCTION

Abnormal prostate growth is most prevalent pathological sign in aged human males, in the form of Benign Prostate Hyperplasia (BPH) and Prostate Cancer (Pca). Epidemiological data from several studies indicated that both diseases are becoming increasingly prevalent worldwide. At histological level, prostate gland contain mainly two types of cells that are called epithelial and stromal cells. Prostatic stem cells are present within the epithelium and are playing role in prostate development. If the stem cells are key target for mutagenic changes and tumurogenesis in human prostate, we need to understand more about stem cell status in normal prostate tissue. Several investigations based on stem cell models have elegantly defined role of stem cells in cellular turnover and morphology in normal human prostate.

In the present study we made an attempt to characterized stemness property of the tissue. We have isolated a candidate population of prostatic stem cells from BPH patients who underwent TURP that expresses pluripotency markers. Characterization of isolated cells showed presence of embryonic stem cell markers like Oct 3/4, Sox-2 and Nanog by mRNA expression and flowcytometery. Further these cells were also found positive for CD49b, CD44, CD117, CD34 and prostate specific markers like p63 and Androgen Receptor. *In-vitro* differentiation of the cells demonstrated osteocyte, adipocyte, chondrocyte and neural cell lineage differentiation with defined medium and *In-vivo* teratoma formation in *balb/c* mouse with presence of tri-germinal layer.

MATERIALS AND METHODS

BPH tissue (~2g) were collected in transport medium and patient's detailed demographic and anthropometric data were recorded in a questionnaire and informed consents from all the patients were obtained.

Histology

Biochemical & Clinical parameter

Cell Culture

FACS analysis

Immuno-Cytochemistry

Multi-lineage differentiation

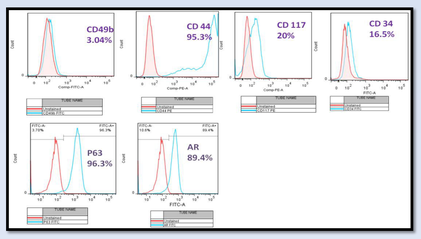
karyotyping

Teratoma formation

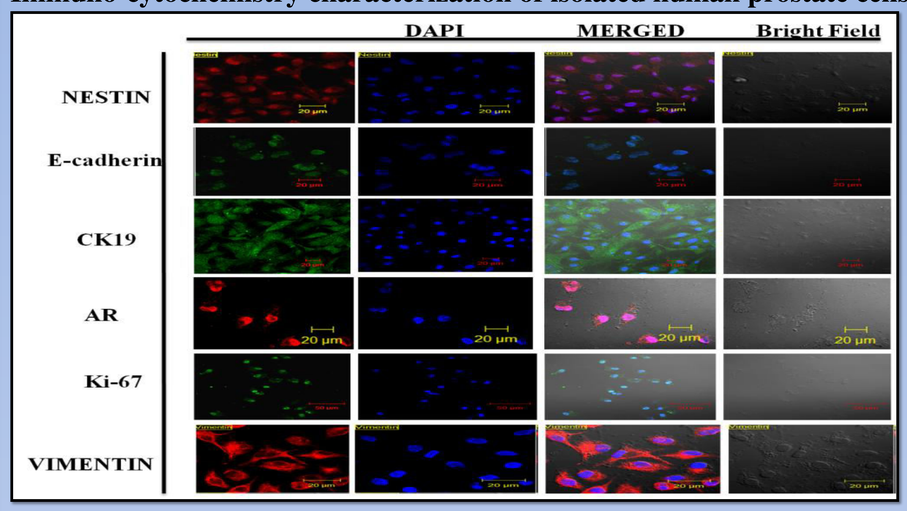
Comparative data analysis

RESULTS

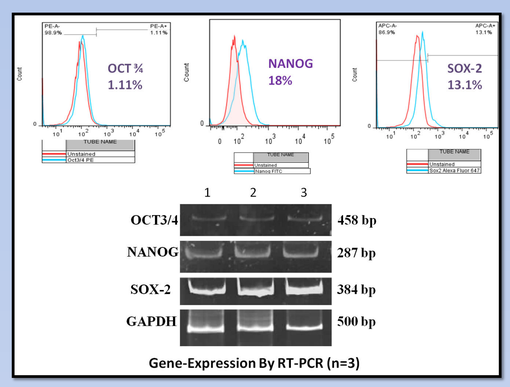
•Stem Cell characterization of Isolated Human Prostate cells from TURP samples by FACS

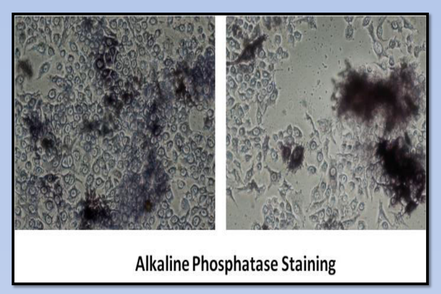


•Immuno-cytochemistry characterization of isolated human prostate cells

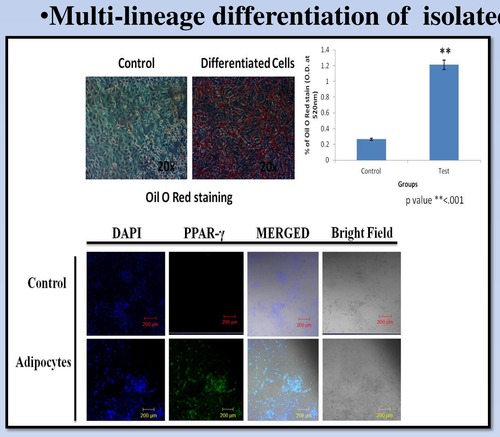


•Pluripotent markers characterization of isolated human prostate cells By FACS and RT-PCR

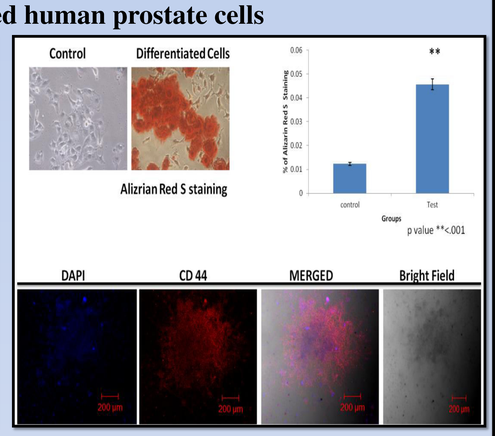




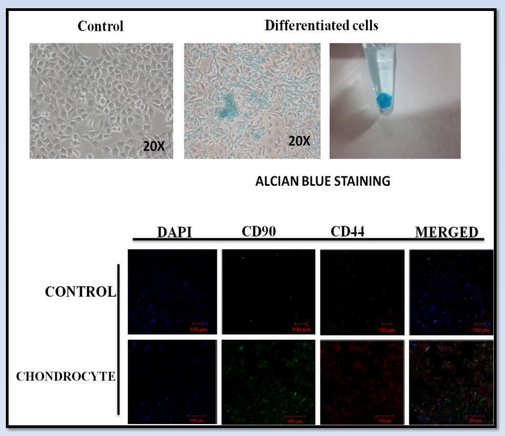
•Multi-lineage differentiation of isolated human prostate cells



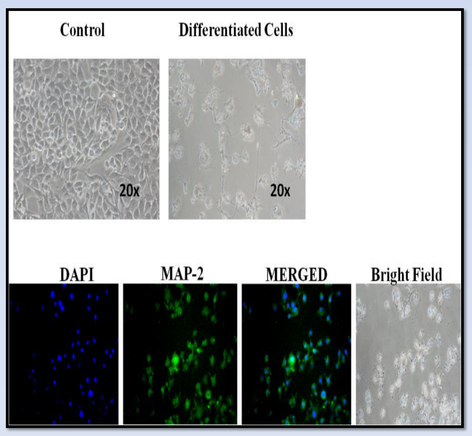
Adipocytes



Osteocytes

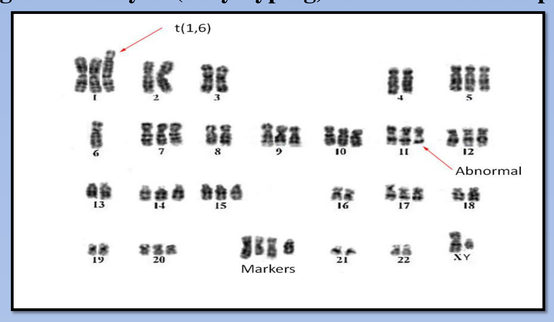


Chondrocytes

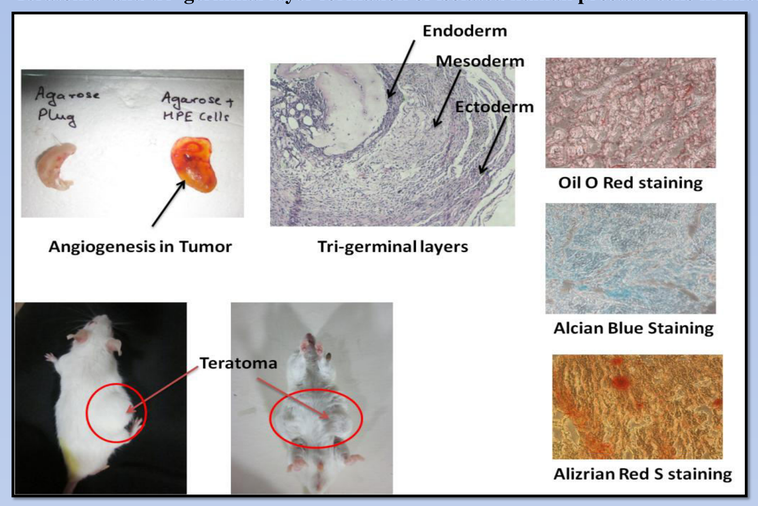


Neuronal

•Cytogenetic analysis (karyotyping) of isolated human prostate cells



•Teratoma and tri- germinal layer formation of isolated human prostate cells in mice



DISCUSSION

1. Flow-cytometry, molecular characterization and immunocytochemistry of isolated Human prostate cells from BPH patient showed embroyonic (Oct3/4, Sox-2, Nanog) and other stem cell markers (CD117,CD44 CD34, p63) indicating multipotency and self-renewal capacity of prostate cells. (Leong KG *et al.,* 2008 Nature)

2. Cytogenetic analysis by G-banding assay demonstrated an aneuploid karyotype with a model chromosome number of 60 (range 58 to 62, n = 20) with 4 to 5 marker chromosomes.

3. In-vitro differentiation of these cells demonstrated osteocyte, adipocyte, chondrocyte and neural cell lineage differentiation and *In-vivo* teratoma formation in *balb/c* mouse demonstrating presence of tri-germinal layer in excised tissue.

CONCLUSION

Our study on primary cells from BPH patients have yielded many interesting findings that these prostate cells possess:

1. Pluripotency markers.
2. Mesenchymal stem cell (MSC) marker.
3. Strong proliferative potential.
4. Ability to differentiate or transdifferentiate to chondrogenic, adipogenic, osteogenic and neurogenic lineages. These cells (stem cells) serve as a potential tool for prostate adult stem cell research and it's role in pathogenesis of Benign Prostate Hyperplasia

REFERENCES

1. Leong KG, Wang BE, Johnson L, Gao WQ. Generation of a prostate from a single adult stem cell. Nature 2008;456(7223):804-808.
2. Monsef N, Soller M, Isaksson M, Abrahamsson PA, Panagopoulos I. The expression of pluripotency marker Oct 3/4 in prostate cancer and benign prostate hyperplasia. Prostate 2009;69(9):909-916.
3. Signoretti S, Waltregny D, Dilks J, Isaac B, Lin D, Garraway L, Yang A, Montironi R, McKeon F, Loda M. p63 is a prostate basal cell marker and is required for prostate development. Am J Pathol 2000;157(6):1769-1775.

ACKNOWLEDGEMENT

Author would like to thank Dr. Frenny Sheth (FRIGE House) for technical assistance in Karyotyping.

This work was funded by grant from DBT, India under DBT-MSUB-ILSPARE

SYNOPSIS

Synopsis of the thesis on

To Understand The Etiopathogenesis of Benign Prostate Hyperplasia at Biochemical, Cellular and Molecular level.

To be submitted to

The Maharaja Sayajirao University of Baroda

For the Degree of

Doctor of Philosophy in Biochemistry

By

Akhilesh Kumar

Under Supervision of

Prof. Sarita Gupta

Molecular Endocrinology and Stem cell Research lab

Department of Biochemistry
Faculty of Science
The M.S. University of Baroda
Vadodara-390005

From:
Akhilesh kumar
Dept. of Biochemistry,
Faculty of science,
The M.S. University of Baroda,
Vadodara-390005

16th August, 2013

To
The Registrar (Academic section),
The M.S. University of Baroda,
Vadodara-390002

Subject: Submission of synopsis of the Ph.D work entitled: "To Understand The Etiopathogenesis of Benign Prostate Hyperplasia at Biochemical, Cellular and Molecular Level"

Dear Sir,

Kindly accept the synopsis of my Ph.d work entitled: **"To Understand The Etiopathogenesis of Benign Prostate Hyperplasia at Biochemical, Cellular and Molecular Level"**. My date of registration was 27/08/2009 and registration no. is 468.

Thanking you.

Sincerely yours,

(Akhilesh Kumar)

(Prof. Sarita Gupta)
Guide

Head, Dept. of Biochemistry

Dean, Faculty of Science

Introduction:

The prostate is a fibro-muscular exocrine gland. It is a male accessory reproductive gland which expels a complex proteolytic solution into the urethra during ejaculation. In old age this gland becomes enlarged termed as Benign Prostate Hyperplasia (BPH) (Non-malignant state) or prostatic cancer (PCa) (malignant state). BPH and PCa are a multi-factorial disease associated with hereditary, environmental factors and interplay of androgen and estrogen. Epidemiologically, BPH is more prevalent in Asian population (Denis et al. 1999; Gaynor 2003) whereas, PCa is more common in the western world (Jemal et al. 2009; Shen and Abate-Shen 2010). There is a striking difference in Prostate cancer risk between different racial and ethnic groups, African American men reported incidence rates 40 to 60 fold higher than those reported for Asian men. Disease pathogenesis is still an enigmatic problem in scientific arena and there are no well established biochemical or genetic markers for diagnosis for BPH and PCa. However, there are several reports with the limited understanding of disease pathogenesis with association of heritability, higher risk with race and ethnicity, as well as family history of BPH (Sanda et al. 1994; Platz et al. 2000; Negri et al. 2005). The cause of BPH and Pca with clear cut discrimination is not well understood and proper understanding of aetiology and pathogenesis are still undercover. The development of BPH in men is usually attributed to testicular hormones, aging and stem cells. The principal androgen responsible for prostate development is dihydrotestosterone (DHT) a derivative of testosterone. Testosterone converted by the intervention of a prostate specific enzyme called 5α-reductase. DHT has a very high affinity for binding to androgen receptors. Studies showed that hyperplastic tissues usually have higher concentrations of androgen receptors as compared to normal tissue(Barrack et al. 1983). Testosterone to DHT ratio is supposed to be the major factor for causing BPH (Carson and Rittmaster 2003). Occupational and environmental studies suggest a potential role of cadmium (Cd) in the prostate enlargement (van der Gulden et al. 1992; Vinceti et al. 2007). Cd seems to be implicated in the increased incidence of prostate and other cancers in men exposed to high levels of this metal or its compounds (Anetor et al. 2008). Cd has potent androgenic and estrogenic mimicking activity (Johnson et al. 2003). The metal binds with high affinity to the hormone-binding domain of both Androgen and Estrogen receptors and activates the receptors (Stoica et al. 2000). Earlier reports from our lab demonstrated possible association of Cd content, smoking, Qmax and prostatic acid phosphatase (PAP) level in BPH patients(Pandya et al. 2013a). So far, variety

of growth factors and hormonal factors, including androgens and estrogens, have been described in the hyperplastic development of the prostate gland (McConnell 1991; Marcelli and Cunningham 1999; Mimeault and Batra 2006) .

Prostate gland consist two types of cellular compartments namely stromal and epithelial cells. Both human and animal studies have shown that stromal cells are essential for functional and morphological differentiation of prostatic epithelium. It has been hypothesized that the basal layer is the proliferative compartment of the prostate, containing a stem cell population, which can differentiate into secretory epithelium and transit amplifying (TA) cells. Prostatic stem cells are present within the epithelium and are capable of regenerating the adult organ(Litvinov et al. 2006; Prajapati et al. 2013). Several investigations based on stem cell models have elegantly defined role of stem cells in cellular turnover and morphology in normal human prostate(Isaacs 2008). To further support the role of basal stem cells in prostate development, an experiment on p63 null mice was performed and the resultant progeny of these animals were born devoid of prostate gland.(Mills et al. 1999; Signoretti et al. 2000; Mills et al. 2002). As the stem cells are key target for mutagenic changes and tumurogenesis in human prostate, an urge arises to understand more about their status in normal and disease prostate tissue and the cellular and molecular mechanism of BPH pathogenesis.

To date several functional and non- functional genetic polymorphisms have been reported to have positive association with prostatic growth, but genetic polymorphisms associated with BPH are yet to be investigated.

In light of this, the present study was proposed to understand the etiopathogenesis of BPH in Indian population with respect to altered genetic and cellular functions. This study was designed into two different aspects, wherein one focused on establishment of BPH animal model and other by studying the cellular status and the associated polymorphisms of prostatic tissue obtained from BPH patients underwent TURP (Trans Urethral Resection of Prostate) surgery. Characterization at cellular and molecular level of isolated cellular compartments performed from animal and human diseased Prostatic tissue shed a light, on disease progression and pathogenesis of BPH. Association of novel SNP in Indian population would be used as marker in early diagnosis of BPH and to differentiate from PCa and will help in formulating new therapy.

Specific objectives: Major Objectives of the present study are.

Objective-1: Establishment of BPH rat model & validation of progression of diseased condition in *in-vivo* model.

Objective-2: Isolation of epithelial & stromal cells from Benign Prostate Hyperplasia patient's.

a. Analysis of cellular and molecular status of both cell types and to understand the role of stem cell in benign prostate hyperplasia tissue.
b. To study the link between BPH and PCa by using Human BPH epithelial cell-line using Cadmium as model.

Objective-3: To understand the genetic association of single nucleotide polymorphism in human prostate genes from benign prostate hyperplasia patients.

Objective-1: Establishment of BPH rat model & validation of progression of diseased condition in *in-vivo* model.

Over all maintenance of prostate is dependent on androgens, whose withdrawal through castration demonstrates regression of the prostate gland (Coffey and Isaacs 1981). Rat and mice prostates have been documented to respond to hormone and chemical carcinogen treatment. However, only the dorso-lateral lobe of the rodent prostate is ontogenetically comparable to the human prostate. There are several factors responsible for disease pathogenesis including environmental pollutant and endocrine disruptor cadmium. Studies suggest a potential role of cadmium (Cd) in the prostate enlargement due to androgenic and estrogenic mimicking activity (Vinceti et al. 2007) (Johnson et al. 2003). The metal binds with high affinity to the hormone-binding domain of steroid hormone receptors and activates the receptors (Stoica et al. 2000).

To prove our hypothesis that low dose of Cd exposure induce hyperplasia like condition in rodents, we performed an experiment with different age group of Charles foster rat. A significant increase in prostate weight with characteristic histological features in five month old animals treated with single dose of 20 µg Cd /kg body weight developed BPH like

condition within ten days. Cd exposure induces cell proliferation, depicted by increased prostate weight. The current findings suggest that, single dose of Cd causes 1.62 fold increase in the prostate weight compared to control, which are in concordance to earlier reports by Martin *et. al* (Martin et al. 2002). However, induction of prostate carcinoma by administration of Cd, used were much higher dose than that used in the present study (Benbrahim-Tallaa et al. 2007). Moreover, histological studies suggest the present condition is BPH, since the ductal morphology is maintained unlike in prostate cancer where unorganized growth in the cells is observed. Also presence of basal cells, a characteristic of BPH further strengthens the cadmium induced BPH rat model. .we are the first to report Cd induced, cost effective and less time consuming animal model for BPH study.

Cadmium is well known endocrine disruptor. In our above study we used Cd to establish BPH like condition in rodent but the molecular mechanism behind BPH induction was not known. In this context, we further aimed to find out whether cadmium binds to steroid hormone receptors and modulate the downstream signals which eventually lead to cell proliferation and BPH like condition in rat model. To reveal the precise role of Cd an experiment was performed with 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 (20µg/kg body weight). ER-α (Estrogen Receptor Alpha) antagonist methyl piperidine pyrazole in DMSO: 50ug/kg body weight/day (Davis et al. 2008), ER-β (Estrogen Receptor Beta)antagonist 4-hydroxytamoxifen in sesame oil :1mg/kg/day (Reed et al. 2005), AR (Androgen Receptor) antagonist nilutamide in DMSO: 10mg/kg/day (Horsmans et al. 1991; Huang et al. 2008) were administered everyday till 10 days (required time period for BPH development). Animals were sacrificed after 10th day. In antagonist experiment, the results from prostate weight and PAP activity were more significant in the group treated with AR and ER-α receptor antagonist along with Cd. While the group treated with ER-β receptor antagonist along with Cd, did not show significant results, providing the fact that Cadmium would probably mediate its effect by binding to the ER-α and AR with more affinity than ER-β receptor. Previous studies suggest that cadmium binds to hormone- binding domain of ER-α and AR with high affinity and activate receptors (Stoica et al. 2000; Martin et al. 2002) . The steroid hormone receptor antagonist study suggested Cd induced hyperplasia like condition by modulating steroid hormone receptor action in rats.

Objective-2: Isolation of epithelial & stromal cells from Benign Prostate Hyperplasia patient's.

a. Analysis of cellular and molecular status of both cell types and to understand the role of stem cell in benign prostate hyperplasia tissue.

Molecular mechanism and pathways involved in hyperplastic prostate differentiation, especially stem cell differentiation, are poorly understood due to the lack of availability of *in vitro* and *in vivo* models. In this context, we have standardized isolation protocol for both human and rat prostate cells as a model system using modified method of Chapronie R. et al 1986 with DMEM-F12 medium and collagenase type I enzyme digestion(Chaproniere and McKeehan 1986). Later we were successful to culture human prostate epithelial cells from BPH patients underwent TURP and use them for further investigations towards disease pathogenesis. Characterization of isolated cells were positive for mesenchymal markers vimentin, nestin, CD117,CD34 and epithelial markers like p63, E-cadhrin, Ki-67, CK19 and AR by immunocytochemistry, flowcytometry, western blotting and mRNA expression profile. We were also successful to isolate stromal cells. Immunocytochemistry staining of stromal cells showed positive for mesenchymal markers vimentin, nestin and AR.

To pursue studies relevant to normal human prostate biology with associated disorders, there is an urgent need for human prostate cell lines that show phenotypes similar to human tissue samples. To best of knowledge human BPH cell line has been established for the first time in the present study. Many efficient methods have been used to establish cell lines using viral oncogenes, overexpression of human TERT or knockdown of specific proteins, but results alterations to the cell cycle machinery, making the cells susceptible to genomic instability and malignant transformation (Gudjonsson et al. 2004; Bhatia et al. 2008; Wieser et al. 2008). There are several non-tumorigenic immortalized human prostate epithelial (HPrE) cell lines established using viral SV- 40Tag or E6/E7 infection including BPH-1(Hayward et al. 1995), and RWPE-1(Bello et al. 1997), none of these accurately recapitulate normal human prostatic growth and function. In this study, we present new BPH cell line, with a self-renewing stem/ progenitor population on the basis of expression of stem and basal cell markers in vitro. Interestingly, the cell line showing both basal as well as secretory epithelial cellular markers expression which was previously characterized including p63, AR, and E-cadherin (Uzgare et al. 2004; Tokar et al. 2005; Prajapati et al. 2013)

The concept of stem/progenitor cells with the capacity for self-renewal and multi-lineage differentiation have been important to understand the molecular mechanisms of normal development and functional homeostasis(Wang et al. 2001; Li et al. 2009). This is also very important to understand how tissues are remodelled during inflammatory repair, or in carcinogenesis resulting from genomic insult, oxidative stress, inflammation or metabolic insult(Coffey and Isaacs 1981; Tsujimura et al. 2002). In BPH pathogenesis it has been believed that stem cells are playing a crucial role and their reawakening leads to proliferative disorder of prostate. In light of this we isolated and established a candidate population of prostatic stem/progenitor cells from BPH patients underwent TURP. Characterization of isolated cells showed presence of embryonic stem cell markers like Oct 3/4, Sox-2 and Nanog by mRNA expression, western blotting and flow cytometery. Further these cells were also found positive for mesenchymal and other stem cell markers such as CD49b, CD44, CD117, CD34, p63 and prostatic tissue specific marker like Androgen Receptor. In-vitro differentiation of the cells demonstrated osteocyte, adipocyte, chondrocyte and neural cell lineage differentiation, and In-vivo teratoma formation in balb/c mouse with presence of tri-germinal layer representative in excised teratoma. Our results clearly throw a light that BPH is stem cell associated disorder.

b. To study the link between BPH and PCa by using Human BPH epithelial cell-line using Cadmium as model.

Cd is depicted as major factor for carcinogenesis in many tissues and playing pivotal role in the prostate cancer as well (Waalkes 2003). Population based cohort studies demonstrated increased risk of PCa in BPH patients (Orsted et al. 2011) Several reports also showed *in vitro* transformation of human normal prostate epithelial cells into cancerous prostate cells (Achanzar et al. 2001; Nakamura et al. 2002). However, the underlying mechanisms involved in Cd carcinogenesis remain unclear. Our previous lab data showed positive association of Cd concentration with increased severity of BPH pathogenesis(Pandya et al. 2013b). In the present study we successfully demonstrated malignant transformation of human BPH epithelial cell line by chronic exposure to Cd to understand the link between BPH to PCa.

Objective-3: To understand the genetic association of single nucleotide polymorphism in human prostate genes from benign prostate hyperplasia patients.

Steroid hormones are involved in normal prostate growth and carcinogenesis. They maintain the homeostasis of cell survival & cell death in the prostate gland. Various factors are attributed to the pathogenesis of BPH. But till now there is no early diagnostic genetic marker for the pathogenesis of BPH. Single nucleotide polymorphisms (SNPs) are considered very promising genetic markers for a better understanding of the genetic basis for various complex diseases like Breast cancer, Lung Cancer etc. Remarkably, several independent studies from India, and other populations have reported, a significant association of CAG repeats with prostate cancer but no reports on BPH (Giovannucci et al. 1999; Mishra et al. 2005; Krishnaswamy et al. 2006; Alptekin et al. 2012).

The purpose of our study was to investigate the susceptibility of polymorphic candidate (Androgen receptor, Prostate Specific Antigen & Estrogen Receptor-β) genes with BPH risk of Indian population in Western part. Patients' detailed demographic and anthropometric data were collected using a structured questionnaire. Patients were asked several questions about their other disorders, dietary habits, addictive habits, and environmental pollutant exposure status at their place of residence and work and whether they had any genetic lineage of BPH from their family background. A total of 133 subjects including control samples were collected for study with proper inclusion and exclusion criteria. In our study, we found that there was a significant increase in A/G genotype of Prostate Specific Antigen(ORs=2.7, 95% CI 0.5-6.2), A/G genotype of Androgen receptor(ORs=2, 95% CI 0.5-6.2) and A/G genotype of Estrogen Receptor-β (ORs=3.7, 95% CI 1.1-15.4).

In conclusion, the association of BPH pathogenesis can be due to the alteration in multiple candidate prostate genes. Our results need to be confirmed with larger number of cohorts from different part of India.

Conclusion:

One of the major strength of present study is establishment of Cadmium induced rat model for BPH and human BPH cell line, which are potentially valuable tools for investigating the genesis of BPH and will expand our vision to understand disease pathogenesis. Human BPH cells are behaviourally benign as assessed with histopathological and immunocytochemistry criteria. As such, this cell line represent potentially useful model to investigate mechanisms associated with both benign and malignant prostatic disorders. Polymorphism studies further help in associating genetic basis of disease and can be exploited as potential diagnostic tool.

References:

Achanzar WE, Diwan BA, Liu J, Quader ST, Webber MM, Waalkes MP. 2001. Cadmium-induced malignant transformation of human prostate epithelial cells. *Cancer Res* **61**: 455-458.

Alptekin D, Izmirli M, Bayazit Y, Luleyap HU, Yilmaz MB, Soyupak B, Erkoc MA, Tansug Z. 2012. Evaluation of the effects of androgen receptor gene trinucleotide repeats and prostate-specific antigen gene polymorphisms on prostate cancer. *Genet Mol Res* **11**: 1424-1432.

Anetor JI, Ajose F, Anetor GO, Iyanda AA, Babalola OO, Adeniyi FA. 2008. High cadmium / zinc ratio in cigarette smokers: potential implications as a biomarker of risk of prostate cancer. *Niger J Physiol Sci* **23**: 41-49.

Barrack ER, Bujnovszky P, Walsh PC. 1983. Subcellular distribution of androgen receptors in human normal, benign hyperplastic, and malignant prostatic tissues: characterization of nuclear salt-resistant receptors. *Cancer Res* **43**: 1107-1116.

Bello D, Webber MM, Kleinman HK, Wartinger DD, Rhim JS. 1997. Androgen responsive adult human prostatic epithelial cell lines immortalized by human papillomavirus 18. *Carcinogenesis* **18**: 1215-1223.

Benbrahim-Tallaa L, Liu J, Webber MM, Waalkes MP. 2007. Estrogen signaling and disruption of androgen metabolism in acquired androgen-independence during cadmium carcinogenesis in human prostate epithelial cells. *Prostate* **67**: 135-145.

Bhatia B, Jiang M, Suraneni M, Patrawala L, Badeaux M, Schneider-Broussard R, Multani AS, Jeter CR, Calhoun-Davis T, Hu L et al. 2008. Critical and distinct roles of p16 and telomerase in regulating the proliferative life span of normal human prostate epithelial progenitor cells. *J Biol Chem* **283**: 27957-27972.

Carson C, 3rd, Rittmaster R. 2003. The role of dihydrotestosterone in benign prostatic hyperplasia. *Urology* **61**: 2-7.

Chaproniere DM, McKeehan WL. 1986. Serial culture of single adult human prostatic epithelial cells in serum-free medium containing low calcium and a new growth factor from bovine brain. *Cancer Res* **46**: 819-824.

Coffey DS, Isaacs JT. 1981. Control of prostate growth. *Urology* **17**: 17-24.

Davis AM, Mao J, Naz B, Kohl JA, Rosenfeld CS. 2008. Comparative effects of estradiol, methyl-piperidino-pyrazole, raloxifene, and ICI 182 780 on gene expression in the murine uterus. *J Mol Endocrinol* **41**: 205-217.

Denis L, Morton MS, Griffiths K. 1999. Diet and its preventive role in prostatic disease. *Eur Urol* **35**: 377-387.

Gaynor ML. 2003. Isoflavones and the prevention and treatment of prostate disease: is there a role? *Cleve Clin J Med* **70**: 203-204, 206, 208-209 passim.

Giovannucci E, Stampfer MJ, Chan A, Krithivas K, Gann PH, Hennekens CH, Kantoff PW. 1999. CAG repeat within the androgen receptor gene and incidence of surgery for benign prostatic hyperplasia in U.S. physicians. *Prostate* **39**: 130-134.

Gudjonsson T, Villadsen R, Ronnov-Jessen L, Petersen OW. 2004. Immortalization protocols used in cell culture models of human breast morphogenesis. *Cell Mol Life Sci* **61**: 2523-2534.

Hayward SW, Dahiya R, Cunha GR, Bartek J, Deshpande N, Narayan P. 1995. Establishment and characterization of an immortalized but non-transformed human prostate epithelial cell line: BPH-1. *In Vitro Cell Dev Biol Anim* **31**: 14-24.

Horsmans Y, Lannes D, Larrey D, Tinel M, Letteron P, Loeper J, Pessayre D. 1991. Nilutamide inhibits mephenytoin 4-hydroxylation in untreated male rats and in human liver microsomes. *Xenobiotica* **21**: 1559-1570.

Huang D, Zhang Y, Qi Y, Chen C, Ji W. 2008. Global DNA hypomethylation, rather than reactive oxygen species (ROS), a potential facilitator of cadmium-stimulated K562 cell proliferation. *Toxicol Lett* **179**: 43-47.

Isaacs JT. 2008. Prostate stem cells and benign prostatic hyperplasia. *Prostate* **68**: 1025-1034.

Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. 2009. Cancer statistics, 2009. *CA Cancer J Clin* **59**: 225-249.

Johnson MD, Kenney N, Stoica A, Hilakivi-Clarke L, Singh B, Chepko G, Clarke R, Sholler PF, Lirio AA, Foss C et al. 2003. Cadmium mimics the in vivo effects of estrogen in the uterus and mammary gland. *Nat Med* **9**: 1081-1084.

Krishnaswamy V, Kumarasamy T, Venkatesan V, Shroff S, Jayanth VR, Paul SF. 2006. South Indian men with reduced CAG repeat length in the androgen receptor gene have an increased risk of prostate cancer. *J Hum Genet* **51**: 254-257.

Li H, Jiang M, Honorio S, Patrawala L, Jeter CR, Calhoun-Davis T, Hayward SW, Tang DG. 2009. Methodologies in assaying prostate cancer stem cells. *Methods Mol Biol* **568**: 85-138.

Litvinov IV, Vander Griend DJ, Xu Y, Antony L, Dalrymple SL, Isaacs JT. 2006. Low-calcium serum-free defined medium selects for growth of normal prostatic epithelial stem cells. *Cancer Res* **66**: 8598-8607.

Marcelli M, Cunningham GR. 1999. Hormonal signaling in prostatic hyperplasia and neoplasia. *J Clin Endocrinol Metab* **84**: 3463-3468.

Martin MB, Voeller HJ, Gelmann EP, Lu J, Stoica EG, Hebert EJ, Reiter R, Singh B, Danielsen M, Pentecost E et al. 2002. Role of cadmium in the regulation of AR gene expression and activity. *Endocrinology* **143**: 263-275.

McConnell JD. 1991. The pathophysiology of benign prostatic hyperplasia. *J Androl* **12**: 356-363.

Mills AA, Qi Y, Bradley A. 2002. Conditional inactivation of p63 by Cre-mediated excision. *Genesis* **32**: 138-141.

Mills AA, Zheng B, Wang XJ, Vogel H, Roop DR, Bradley A. 1999. p63 is a p53 homologue required for limb and epidermal morphogenesis. *Nature* **398**: 708-713.

Mimeault M, Batra SK. 2006. Recent advances on multiple tumorigenic cascades involved in prostatic cancer progression and targeting therapies. *Carcinogenesis* **27**: 1-22.

Mishra D, Thangaraj K, Mandhani A, Kumar A, Mittal R. 2005. Is reduced CAG repeat length in androgen receptor gene associated with risk of prostate cancer in Indian population? *Clin Genet* **68**: 55-60.

Nakamura K, Yasunaga Y, Ko D, Xu LL, Moul JW, Peehl DM, Srivastava S, Rhim JS. 2002. Cadmium-induced neoplastic transformation of human prostate epithelial cells. *Int J Oncol* **20**: 543-547.

Negri E, Pelucchi C, Talamini R, Montella M, Gallus S, Bosetti C, Franceschi S, La Vecchia C. 2005. Family history of cancer and the risk of prostate cancer and benign prostatic hyperplasia. *Int J Cancer* **114**: 648-652.

Orsted DD, Bojesen SE, Nielsen SF, Nordestgaard BG. 2011. Association of clinical benign prostate hyperplasia with prostate cancer incidence and mortality revisited: a nationwide cohort study of 3,009,258 men. *Eur Urol* **60**: 691-698.

Pandya C, Gupta S, Pillai P, Bhandarkar A, Khan A, Bhan A, Prajapati A, Gupta S. 2013a. Association of Cadmium and Lead with Antioxidant Status and Incidence of Benign Prostatic Hyperplasia in Patients of Western India. *Biol Trace Elem Res*.

-. 2013b. Association of cadmium and lead with antioxidant status and incidence of benign prostatic hyperplasia in patients of Western India. *Biol Trace Elem Res* **152**: 316-326.

Platz EA, Kawachi I, Rimm EB, Willett WC, Giovannucci E. 2000. Race, ethnicity and benign prostatic hyperplasia in the health professionals follow-up study. *J Urol* **163**: 490-495.

Prajapati A, Gupta S, Mistry B, Gupta S. 2013. Prostate Stem Cells in the Development of Benign Prostate Hyperplasia and Prostate Cancer: Emerging Role and Concepts. *BioMed Research International* **2013**: 10.

Reed CA, Berndtson AK, Nephew KP. 2005. Dose-dependent effects of 4-hydroxytamoxifen, the active metabolite of tamoxifen, on estrogen receptor-alpha expression in the rat uterus. *Anticancer Drugs* **16**: 559-567.

Sanda MG, Beaty TH, Stutzman RE, Childs B, Walsh PC. 1994. Genetic susceptibility of benign prostatic hyperplasia. *J Urol* **152**: 115-119.

Shen MM, Abate-Shen C. 2010. Molecular genetics of prostate cancer: new prospects for old challenges. *Genes Dev* **24**: 1967-2000.

Signoretti S, Waltregny D, Dilks J, Isaac B, Lin D, Garraway L, Yang A, Montironi R, McKeon F, Loda M. 2000. p63 is a prostate basal cell marker and is required for prostate development. *Am J Pathol* **157**: 1769-1775.

Stoica A, Katzenellenbogen BS, Martin MB. 2000. Activation of estrogen receptor-alpha by the heavy metal cadmium. *Mol Endocrinol* **14**: 545-553.

Tokar EJ, Ancrile BB, Cunha GR, Webber MM. 2005. Stem/progenitor and intermediate cell types and the origin of human prostate cancer. *Differentiation* **73**: 463-473.

Tsujimura A, Koikawa Y, Salm S, Takao T, Coetzee S, Moscatelli D, Shapiro E, Lepor H, Sun TT, Wilson EL. 2002. Proximal location of mouse prostate epithelial stem cells: a model of prostatic homeostasis. *J Cell Biol* **157**: 1257-1265.

Uzgare AR, Xu Y, Isaacs JT. 2004. In vitro culturing and characteristics of transit amplifying epithelial cells from human prostate tissue. *J Cell Biochem* **91**: 196-205.

van der Gulden JW, Kolk JJ, Verbeek AL. 1992. Prostate cancer and work environment. *J Occup Med* **34**: 402-409.

Vinceti M, Venturelli M, Sighinolfi C, Trerotoli P, Bonvicini F, Ferrari A, Bianchi G, Serio G, Bergomi M, Vivoli G. 2007. Case-control study of toenail cadmium and prostate cancer risk in Italy. *Sci Total Environ* **373**: 77-81.

Waalkes MP. 2003. Cadmium carcinogenesis. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* **533**: 107-120.

Wang Y, Hayward S, Cao M, Thayer K, Cunha G. 2001. Cell differentiation lineage in the prostate. *Differentiation* **68**: 270-279.

Wieser M, Stadler G, Jennings P, Streubel B, Pfaller W, Ambros P, Riedl C, Katinger H, Grillari J, Grillari-Voglauer R. 2008. hTERT alone immortalizes epithelial cells of renal proximal tubules without changing their functional characteristics. *Am J Physiol Renal Physiol* **295**: F1365-1375.

Publications:

1. Chirayu Pandya, Sharad Gupta, Prakash Pillai, Ajay Bhandarkar, Arif Khan, Arunodya Bhan, **Akhilesh Prajapati**, Sarita Gupta. Association of Cadmium and Lead with Antioxidant Status and Incidence of Benign Prostatic Hyperplasia in Patients of Western India. *Biol Trace Elem Res* 2013. 152:316–326.

2. **Akhilesh Prajapati,** Sharad Gupta, Bhavesh Mistry, Sarita Gupta: Prostate Stem Cells in the Development of Benign Prostate Hyperplasia and Prostate Cancer: Emerging Role and Concepts. *BioMed Research International;* 2013:10.

Manuscript under communication:

1. **Akhilesh Prajapati,** Akshay Rao, Jhanvi Patel, Sharad Gupta, Sarita Gupta: A single low dose of cadmium exposure induce Benign Prostate Hyperplasia like condition in rat: a novel BPH rodent model. 2013

Manuscript under preparation:

1. **Akhilesh Prajapati**, Nidheesh Dadheech, Sharad Gupta, R.R. Bhonde, Sarita Gupta Human Prostate Cell Population Derived From Benign Hyperplasia Specimen Demonstrate Pluripotent Stem Cells Properties. 2013.

Abstract Published and Poster Presented:

1. **Akhilesh Kumar Prajapati**, Nidheesh Dadheech, Sharad Gupta and Sarita Gupta. **Human Prostate Cell Population Derived From Benign Hyperplasia Specimen Demonstrate Pluripotent Stem Cells Properties.** Abstract book, 11th annual meeting, ISSCR, Boston, MA, USA. 12-15th June, 2013.

2. **Akhilesh Kumar Prajapati**, Jhanvi Patel, Sharad Gupta and Sarita Gupta. "**To Assess The Possible Role of Cadmium in BPH Pathogenesis via Steroid Hormone Receptor Blocker**", in International conference on **Reproductive Health with emphasis on strategies for family planning & 22nd annual meeting of the Indian society for the study of reproduction and fertility(ISSRF) " ICMR Centenary celebration 1911-2011,** organized by AIIMS, New Delhi. 19 – 21st Feb, 2012.

3. Nidheesh Dadheech, **Akhilesh Kumar Prajapati**, Sanket Soni and Sarita Gupta. **Identification of novel Population of Nestin positive cells from rat Prostate for insulin Producing cell differentiation**. Abstract book, 9th annual meeting, ISSCR, Toranto, Canada 2011.

4. **Akhilesh Kumar Prajapati**, Akshay Rao, Sharad Gupta and Sarita Gupta. "**Cadmium: a Potent Benign Prostate hyperplasia inducer in rat** ",in International conference on **MOLECULAR MEDICINE,** organized by Charotar University of Science and Technology, change, Gujarat. Jan-2011

5. **Akhilesh Prajapati**, Chirayu Pandya, Sharad Gupta, Prakash Pillai, Ajay Bhandarkar, Arif Khan, Arunodya Bhan, Sarita Gupta "**Association of Benign Prostate Hyperplasia with Respect to environmental Pollutant Cadmium**", in the SRBCE Sponsored **'international symposium on endocrinology and Reproduction: molecular Mechanisms to Molecular Medicine'** Organized by Jawahar Lal Nehru University, New Delhi. Feb-2010.

Achievements:

1. DBT-CTEP International Travel Grant to attend International Society for Stem Cell Research (ISSCR) 11th annual meeting, Boston, MA, USA from 12-15 June, 2013.
2. Awarded Department of Biotechnology, Govt. of India-Junior Research Fellowship under DBT-MSUB-ILSPARE Project 2011.
3. Qualify PET exam conducted by MS University of Baroda, 2010**.**

Date: 16th August, 2013

(Akhilesh Kumar) (Prof. Sarita Gupta)
Guide

Head, Dept. of Biochemistry Dean, Faculty of Science