CHAPTER -6

SUMMARY

&

CONCLUSION

CHAPTER- 6

6.1 Summary

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 whereas, PCa is more common in the western world. There is a striking difference in BPH and Prostate cancer risk between different racial and ethnic groups. Disease pathogenesis is still an enigmatic problem in scientific arena and there is no well established biochemical and 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. The cause of BPH and PCa with clear cut discrimination is not still well understood and proper understanding of aetiology and pathogenesis is important for designing diagnostic and therapeutic tools.

Over all maintenance of prostate is dependent on androgens, whose withdrawal through castration demonstrates regression of the prostate gland. 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. Studies suggest a potential role of Cadmium (Cd) in the prostate enlargement due to androgenic and estrogenic mimicking activity. The metal binds with high affinity to the hormone-binding domain of steroid hormone receptors and activates the receptors. Patient data from our lab also has supported the role of cadmium in BPH pathogenesis.

In this context, we hypothesized that whether low dose of Cd exposure induce hyperplasia like condition in rodents. To prove our hypothesis 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*.2002. However, induction of prostate carcinoma by administration of Cd, used higher dose than that used in the present study. 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.

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). ERα (Estrogen Receptor Alpha) antagonist methyl piperidine pyrazole was dissolved in DMSO: 50ug/kg body weight/day, ERβ (Estrogen Receptor β) antagonist 4-hydroxytamoxifen in sesame oil:1mg/kg/day, AR (Androgen Receptor) antagonist nilutamide in DMSO: 10mg/kg/day were administered everyday till 10 days (required time period for BPH development). Animals were sacrificed after 10^{th} day. In antagonist experiment, the results from prostate weight and PAP activity were more significant in the group treated with AR and ERα receptor antagonist than with ERβ receptor antagonist. Providing the fact that Cd would probably mediate its effect by binding to the ERα and AR with more affinity than ERβ receptor. Study suggested Cd induced hyperplasia can be modulated by targeting steroid hormone receptor action, providing, insight for efficient drug design.

In BPH pathogenesis it has been believed that stem cells are playing a crucial role and their reawakening leads to proliferative disorder of prostate. 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. 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, results in alterations of the cell cycle machinery, making the cells susceptible to genomic instability and malignant transformation. There are several non-tumorigenic immortalized human prostate epithelial (HPrE) cell lines established using viral SV- 40Tag or E6/E7 infection including BPH-1, and RWPE-1, 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 showed both basal (p63) as well as secretory epithelial (AR and E-cadherin) cell markers.

Further to understand the role of stem cells in disease progression. Isolated cells were used for stem cell characterization. Interestingly, isolated cells showed presence of embryonic stem cell markers like Oct3/4, Sox-2 and Nanog by mRNA expression, western blotting and flow cytometery. Additionally, 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.

Cadmium is depicted as major culprit for carcinogenesis in many tissues and playing pivotal role in the PCa and making prostatic epithelium suspected as *in vivo* target of cadmium. Population based cohort studies demonstrated increased risk of PCa in BPH patients. Several reports showed *in vitro* transformation of human normal prostate epithelial cells into cancerous prostate cells. However, reports on conversion of BPH condition into PCa are scanty. Our previous lab data showed positive association of Cadmium concentration with increased severity of BPH pathogenesis. Hence, in the present study we tried to understand the link between BPH to PCa conversion using environmental pollutant Cadmium as model in established human BPH epithelial cell line. The cell line has opened new avenues for defining mechanisms in prostate carcinogenesis. In present study we exposed human BPH cells to Cd with different concentrations (i.e 1 nM, 10 nM, 100 nM, 1 µM till eight weeks) for cancer induction. Methyl Nitrosuria (MNU) served as a positive control in our study for PCa induction. Characterization of Cd exposed BPH cells showed subtle morphological changes. The study has been evaluated for conversion of hyperplasia cells into cancer by using flowcytometry, zymography, gene expression and protein profiling.

This is the first report in our knowledge where Cd-induced malignant transformation of human BPH epithelial cells has been demonstrated. The dose dependant effect of the Cd treated BPH cells showed changes in cell cycle, morphology, and gene and protein expression profile towards cancer progression. This study contributed immensely in better understanding of the mechanism involved in Cd induced carcinogenesis which can be used to develop molecular “fingerprint” for identification of Cd-induced prostatic malignancies.

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 report on BPH. Hence, study was further extended 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 200 subjects including control samples were collected for study with proper inclusion and exclusion criteria. In our study we found significant association of the AR and ERβ genes with BPH pathogenesis as compared to control cohort. Additionally, Genotype-phenotype study has provided evidence that gene-environment and gene-gene interactions play an important role in the etiology of BPH. However, a large cohort study is needed from different parts of India to verify the association of snips and the environmental factors that may modify the relationship between genetic polymorphisms and better understanding of disease pathogenesis.

6.2 Conclusion

- One of the major strength of present study is establishment of Cadmium induced rat model for BPH and human BPH cell line. The experimental rodent 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.
- Human BPH cells are behaviorally benign as assessed with histopathological and immunocytochemistry criteria. As such, the cell line represent potentially useful model to investigate mechanisms associated with both benign and malignant prostatic disorders. 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 is likely to be applicable to other epithelial tumurigenesis.

- Polymorphism studies further help in associating genetic basis of disease and can be exploited as potential diagnostic tool. The SNPs under study of the AR and ERβ genes were found significantly associated with the disease. Additionally, Genotype-phenotype study has provided evidences that gene-environment and gene-gene interactions play an important role in the etiology of BPH. The association of cadmium with severity of BPH pathogenesis and transformation into PCa has been also well established. Further this study can be used for future therapies for BPH and to design personalized medicine. The present study warns the mankind to take precautionary measurements against the deleterious effects of environmental pollutants like Cd.

- All the three approaches in the present study provided valuable tool and extended better vision in understanding the BPH pathogenesis and its link with prostate cancer.