CHAPTER-7

7. SUMMARY & CONCLUSIONS

7.1 Summary

The prostate is a fibro-muscular exocrine gland of the reproductive system that nourishes the sperms with a complex proteolytic secretion. The growth of the prostate depends on androgens from fetal to late age and spans through life. Despite the decreasing androgens with age, the growth of the prostate depends on the intracrine stimulus within the prostate gland. Thus, with aging, the gland enlarges with a non-malignant hyperproliferation of the cells obstructing the urethra termed as Benign Prostate Hyperplasia (BPH). During the hyperproliferative stage, due to several genetic, environmental, and/or lifestyle associated factors, some cells acquire the malignant transformation and develop prostate cancer (PCa). Epidemiologically, the prevalence of BPH and PCa is associated with the race of the individual, like BPH is prevalent in the Asian population whereas, PCa is highly prevalent in African and African-American populations. The evidences on the association of BPH with PCa and their coexistence has also been established. The progression of both, BPH and PCa depends on the expression and activation of AR. Yet, due to the vast numbers of interacting partners and abundant gene regulatory efficiency, understanding the enigmatic network of AR has become challenging to the researchers.

The AR expression patterns reveal its presence in both stromal and epithelial cell compartments of the prostate. It has been discovered that the stromal-AR expression is dominated during the gestation period to regulate the development of the prostate via controlling the growth/cytokine factor secretion, followed by the domination of epithelial-AR post-birth which persist throughout life. Regardless of lower AR activation in the cells of the stromal compartment, it regulates a vast network of growth factors and cytokine secretion that exert a paracrine effect on the prostate epithelial cells to stimulate AR and other cell signaling proteins especially during BPH and PCa pathogenesis. In the epithelial compartment, AR is absent or insignificantly expressed in BSCs, and its increased expression in BSCs leads to its differentiation to a secretory luminal epithelial cell. Several investigations have demonstrated AR-mediated positive and negative regulation of BSC-associated proteins, like CD133, CD49b/f, SOX2, NANOG, etc. Further, an intermediate progenitor population exhibiting the characteristics of basal (P63, CD133, PROM2, etc)

and luminal (AR, CK8, NKX3.1, etc) cells, known as luminal progenitors (LPs), are bipotent that can differentiate and dedifferentiate into luminal or basal cell respectively. Although AR expression has been also found in LPs, its functional role in LPs is not well understood. Our previous lab investigations have found the coexistence of stem/progenitor (CD133, CD49f, CD117, OCT4, NANOG) and luminal (AR, NKX3.1) markers in the epithelial cells isolated from BPH patient tissue. In this context, we aimed to investigate the transcriptional role of AR in BPH stem/progenitor cells.

In the present study, we demonstrated the regulatory role of AR over LGR4 and P63 proteins which are vital stem/progenitor markers of the epithelial cells in BPH condition. BPH patient study showed a prominent AR expression in epithelial cells of the BPH tissue. The correlation of AR protein with LGR4/β-CATENIN and OCT4 protein levels showed a positive correlation in the BPH patient tissues. Further, the expression of LGR4/β-CATENIN, OCT4, and ΔNP63α was also found at higher levels in the patient tissues with higher expression of AR with increased *NKX3.1* and decreased *PSA* transcript levels which suggest the presence of increased luminal progenitors in the BPH tissue. Further, articulation of LGR4 and OCT4 markers were also correlated with the AR-V7 and pARs213 levels suggesting its potential regulating role in BPH condition. To correlate BPH and PCa the RNA-Seq contour of the TGCA-PCa dataset has been analyzed which suggested a negative association between stem/progenitor expression and *AR* transcripts. Yet, AR protein levels were positively correlated with LGR4/β-CATENIN expression as similar to BPH patients. Further, negative/ no association was found between AR and *OCT4* and *TP63* transcripts in the PCa patients. The miR expression profile showed decreased levels of miR-27a in the BPH patients with high AR protein levels, whereas the expression of miR-27a also decreased in the TCGA PCa dataset. On the contrary, miR-21 depicted a decreasing trend in BPH tissues, despite its direct regulation by AR but its levels were elevated in the PCa tissue. Interestingly, we have also discovered the increased expression of cell-proliferation marker Ki-67 with a substantially early predisposition of BPH in patients with high AR expression.

Since the positive association of AR was discovered with LGR4/β-CATENIN and ΔNP63α, we aimed to investigate the transcriptional influence of AR on LGR4/β-CATENIN and P63 using BPH epithelial stem/progenitor cells. Both, androgen dependant and independent activation of AR with TP and IGF1 increased the nuclear localization and

euchromatin accessibility of AR in BPH epithelial cells. Further, both of these treatments increased the tumor formation ability of the BPH stem/progenitor cells, with increased AR and LGR4 expression. Further, AR inhibitor and knockdown studies demonstrated that AR expression impacts the expression of LGR4/β-CATENIN and ΔNP63α. LGR4 and ΔNP63α are the key proteins expressed by stem/progenitor cells and are linked to the β-CATENIN actions. LGR4 potentiates the activation of β-CATENIN that localizes in the nucleus and regulates the expression of genes including ΔNP63 TFs. AR-mediated stimulation of LGR4/β-CATENIN and ΔNP63α led us to investigate the linked pathway via three AR regulatory mechanisms; 1) direct interaction with signaling proteins, 2) regulation of gene promoters or enhanced through full/half ARE sites, and 3) regulation of target-specific miRNAs. Assessment of direct interactions of AR upon androgen stimulation showed enhanced interaction with β-CATENIN. Strikingly, androgen stimulation to AR did not directly append to the ΔNP63α, instead, it triggered the interaction of β-CATENIN/ΔNP63α proteins. Thus, the results depicted that the AR activation causes increased LGR4 expression leading to higher levels of active β-CATENIN and its direct interaction with AR, which regulates the promoters of LGR4 and ΔNP63.

miRNA expression profile exhibited substantial evidence of disease-specific miR regulatory role of AR in stem/progenitor cells during BPH and PCa conditions. On stimulation with androgen, the expression of miR-27a and miR-21 in BPH stem/progenitor cells which could be the reason for the high RNA to protein turnover rate, thus enhancing LGR4/β-CATENIN activation. Since the androgen-mediated interaction of AR and β-CATENIN was discovered in BPH epithelial cells, we aimed to understand the gene regulatory role of both the proteins with ChIP. As expected, androgen stimulation amplified the binding of AR and β-CATENIN on the LGR4 promoter. Strikingly, the stimulation showed disengagement of AR from ΔNP63 promoter and binding of only β-CATENIN. The results suggested the direct regulation of LGR4 by AR and β-CATENIN, but the gene regulation of ΔNP63 was achieved by β-CATENIN alone.

Identification of direct AR-mediated regulation as well as the influence of IGF1 on LGR4 and ΔNP63α expression in BPH cells led us to investigate the influence of stromal cells on the expression of epithelial cells. To elucidate the influence of stromal-AR on epithelial cells, androgen and anti-androgen treated stromal cells secretome has been exposed to

epithelial cells. We have demonstrated that secretome derived from stromal-AR activation enhanced stemness with the elevation of LGR4 and ΔNP63α in BPH epithelial cells for a brief time duration. However, chronic exposure of the secretome derived from stromal-AR activation caused cell death in hyperproliferative epithelial stem cells, thereby prevents disease progression. On the contrary, AR inhibited secretome potentiated survival and proliferation of epithelial cells, promoting disease progression with increased cMYC expression. In nutshell present study emphasizes on diverse regulatory effect of AR in the epithelial and stromal-AR on stem/progenitor cells of the prostate gland, which evidently play a role in disease progression and suggesting cautious use of AR inhibitors for BPH and PCa treatment.

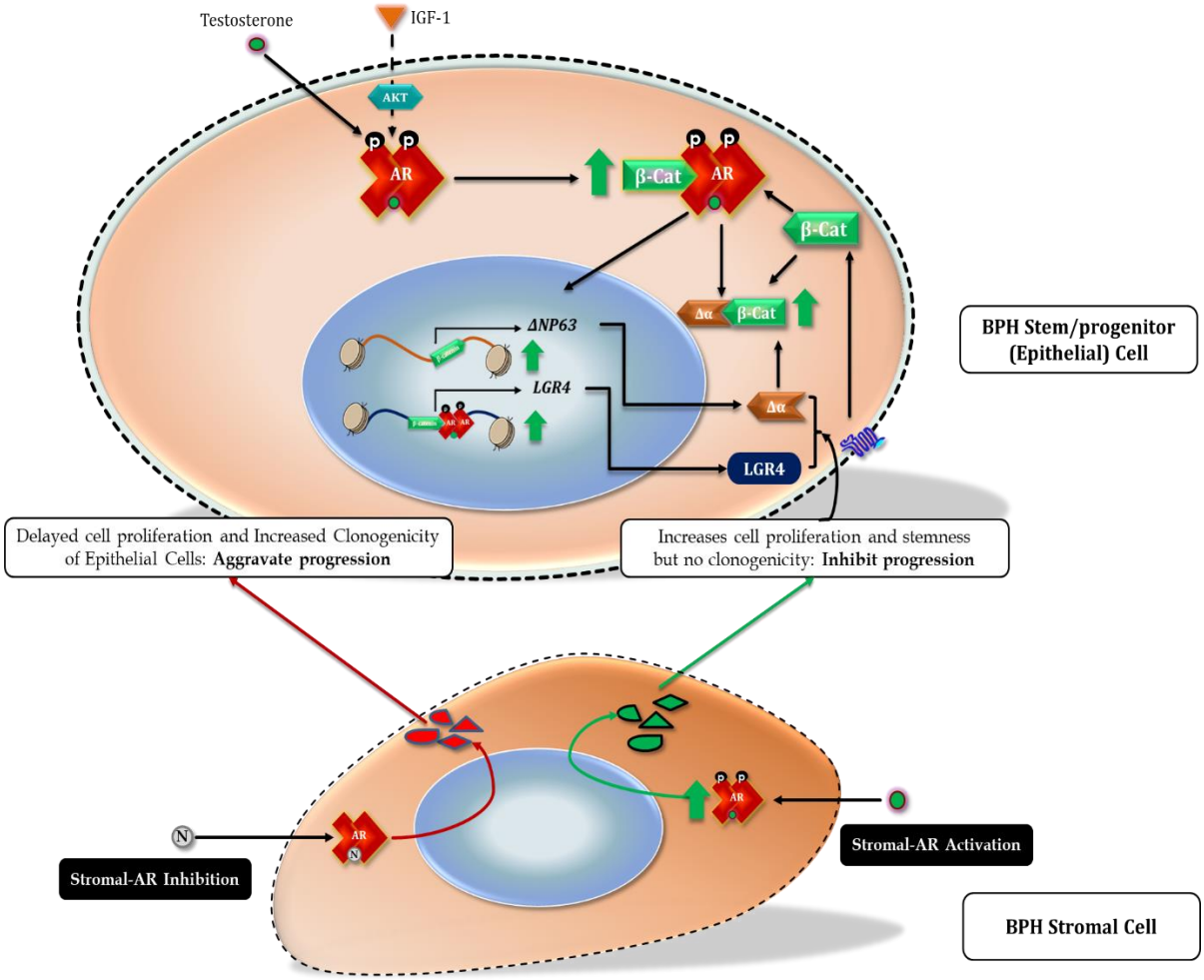

Figure 7. 1: Overall summary of the present work.

7.2 Conclusions

- Elevated levels of AR in BPH patients vitally affect the aggressiveness of the BPH condition via enhanced cell proliferation of LPs and causing early BPH development.
- The LGR4/β-CATENIN expression is substantially correlated with AR expression in BPH and PCa patients, which could be a coercing element for the malignant divergence of the BPH condition.
- Correlation of OCT4 and ΔNP63α stem/progenitor markers were associated with AR in BPH tissue but not in PCa tissue, suggesting intrigue molecular actions of AR depending on BPH and PCa condition.
- Disease-specific expression signature of AR regulated miR-27a and miR-21 exhibited differential expression of miR-21 in BPH and PCa tissues.
- Ligand-induced AR activation and its implication in the regulation sphere formation with elevated LGR4 levels was unrevealed in the BPH stem/progenitor cells.
- Testosterone induced novel protein-protein interaction between AR-β-CATENIN and β-CATENIN-ΔNP63α was discovered in the BPH stem/progenitor cells.
- AR and β-CATENIN mediated regulation of LGR4 and ΔNP63α promoters have been revealed for the first time in BPH stem/progenitor cells.
- Testosterone induction to stromal-AR alters the secretory profile of the BPH patient-derived stromal cells, that strongly influence the expression of stem/progenitor associated LGR4 and ΔNP63α proteins in BPH epithelial cells.
- Stomal factors are substantially involved in the BPH development, where activation of stromal-AR exhibit protective effect via inducing cells death in proliferative stem/progenitor epithelial cells, whereas its inhibition causes aggressive disease progression.

7.3 Future directions

- Role of AR-V7 expression in stem/progenitor cells of the prostate and its role in the development of BPH and PCa.
- Identification of the sub-sets of the various populations in the epithelial compartment of the human prostate and degree of AR and AR-V7 expression for better therapeutic interventions.

- The functional consequence of androgen stimulated β-CATENIN/ΔNP63α interaction in stem/progenitor cells of the prostate.
- Identification of AR controlled secretory factors from stromal cells that potentially control the stem/progenitor population of the prostate gland.