SYNOPSIS

Pre-synopsis abstract

Ph.D. Student: Gaurav Chauhan

Registration: FOS/1921; Date: 13/01/2015

Research Guide: Prof. Sarita Gupta

CVR Hall, Department of Biochemistry

Date: 30/07/2019 Time: 4:15 PM to 5:00 PM

Ph.D. Thesis Title: "**Elucidating the role of Androgen Receptor signaling and stemness in Benign Prostate Hyperplasia**"

Benign Prostate Hyperplasia (BPH) and Prostate Cancer (PCa) are the major prostate related pathologies in men affecting quality of life globally. According to a global data of 2012, rate of incidence is increasing from the beginning of 21st century in majority of the countries. Epidemiological data suggests that PCa is fifth most common cause of mortality and is increasing across in all the countries. Moreover, several indications associate prevalence as well as cellular and molecular signature of BPH to that of PCa. BPH occurrence increases the risk of PCa by 2-3 folds. Both of these diseases are involved hyper activation of Androgen signaling axis in prostate cells and proliferation of stem cells. Hyperactivation of transcriptional activity of AR controls the expression of specific genes pertaining to cell proliferation and differentiation. Additionally, characterization of luminal progenitors exhibited unique expression pattern of stemness markers along with low androgen dependency. Both of these stem cells types undergo proliferative stages during BPH and PCa. The present study demonstrates the role of AR in regulation of stem cells in BPH condition. We have profiled twenty BPH patient derived prostate tissues to develop gene and protein expression signature. Further, to understand the role of AR in development of BPH, the molecular profile of these patients was then correlated with the AR protein expression. The protein levels of stemness markers like LGR4, β-catenin and ΔNP63α were found to be positively correlated with AR protein levels. Also, the patient with higher AR expression had increased transcript and gene levels of these markers.

Bioinformatic analysis of 500 Prostate cancer patients showed similar profile with LGR4 and β-catenin but not with P63. Hence, *in vitro* exploration of AR mediated regulation of these stemness markers was done on BPH epithelial cells. Strategy involved activation (by TP and IGF1) and inhibition of AR in BPH epithelial cells and motor the changes in molecular pattern of these stem cell marker. An indirect activation of AR by growth factors (eg. IGF1) derived from stromal cells can bring phenotypic changes in cells. Since, stromal factors are also under control of AR regulation, we explored the role of stromal AR and its effect on epithelial cells. The study provides compelling evidences on AR mediated regulation of epithelial and stromal cells in BPH pathogenesis.

SPECIFIC OBJECTIVES

Objective-1: Association of Androgen Receptor and stemness in BPH and PCa patients.

Objective-2: To elucidate the role of Androgen Receptor in the maintenance of stemness in benign prostate hyperplasia derived epithelial stem cells.

Objective-3: To explore the role of Androgen Receptor mediated epithelial-stromal crosstalk in the etiopathology of Benign Prostate Hyperplasia.

Objective-1: Association between Androgen Receptor and stemness in BPH and PCa conditions.

A. To correlate AR protein expression with stemness genes and proteins in BPH patients.

As regulation of AR alters upon malignant transformation of normal and benign cells, we expected to have a divergent role of AR in BPH tissues than in TGCA datasets. To achieve this aim, we have assessed twenty BPH patient tissues for the expression of AR and stemness protein levels from total tissue lysates followed by development of correlation between them. We found a positive correlation of AR with LGR4 and β-Catenin protein levels but no correlation with ΔNP63α protein levels. Moreover, androgen independent AR activation have been known to significantly contribute to prostate diseases. A significant positive correlation was observed between AR and IGF1, which is a non-

androgen phosphorylation inducer of AR at Serine-213 residue. AR also showed positive correlation with pARs213 and Ki-67 cell proliferation marker expression in BPH. We have divided patients in two groups based on mean AR protein expression value: patients with high AR (ARhi) expression (n=12) and low AR (ARlo) expression (n=8). To understand transcriptional activity of AR in these two groups, we have assessed two key AR targeted genes; PSA and NKX3.1. Data showed that ARhi patients have decreased transcript levels of *Psa* and increased transcript levels of *Nkx3.1* as compare to ARlo patients. ARhi patients also had increased transcript levels of *cMyc, β-Catenin* and *Lgr4* and decreased transcript levels of *Sox2* as compare to ARlo patients. Moreover, ARhi patients have increased expression of Ki-67 cell proliferation marker which results in early development of BPH in ARhi patients (~62 years) against ARlo patients (~67 years). Hence, increased AR expression in BPH patients is significantly correlated with markers of luminal progenitors.

B. To evaluate AR expression with stemness in PCa datasets and compare with BPH.

Cancer genomics data analysis was performed on Prostate Adenocarcinoma patients using an open-access cancer database on cBioPortal (http://www.cbioportal.org/).(20, 21) The dataset analysis is comprised of about 21 genes AR and its co-activators (NCOA1, NCOA2, FOXA1) BSC Markers (CD44, CD49f(ITGA6), CD133(PROM1), CD44, CK5, CK14, LGR4, TP63, OCT4, SOX2, NANOG, cMYC, KLF4), key regulatory signaling network genes (AKT1, SMAD2/3/4, β-CATENIN) and cell proliferation marker (MKI67) in The Cancer Genome Atlas (TCGA) PRAD study consisting of 500 patients. Analysis of gene expression datasets showed two distinct groups, one with very low or no AR and another with moderate to high AR expression. Group of Patients' with low or no AR expression seemed to have higher transcripts of BSC positive markers and pluripotency markers. Patients with higher AR transcripts had increased transcripts of AR coregulators with amplified transcripts of LGR4, β-CATENIN and CD49f stem cell markers. The evidence from dataset suggest that AR expression is more abundant in luminal cancer cells than in basal stem cells.

Objective 2: To elucidate the role of Androgen Receptor in the maintenance of stemness in Benign Prostate Hyperplasia derived epithelial stem cells.

As observed in previous objective, we aimed to explore the regulatory effect of AR over stemness of luminal progenitors. In this objective, the BPH patient derived epithelial stem cells were exposed to the treatment of testosterone (TP) (AR agonist) and Nilutamide (Nil) (AR Antagonist) and cellular localization of AR was monitored. Further, gene and protein expressions were assessed of stemness markers on BPH patient derived epithelial stem cells with 24 hours of treatments of TP and Nil. Flowcytometric analysis showed increased the percent cell population of CD133 and LGR4 with TP Treatment, whereas decreased with Nil treatment in these cells. Gene expression study depicted increased transcripts of cMyc, Lgr4, and ΔNP63α upon TP treatment. Similar results were found with protein expression study representing increased protein expression of LGR4 and ΔNP63α markers with TP treatment.

Further, exploration in Wnt, Akt and Smad2/3 signaling with TP treatment suggested, 1) the down regulation of Smad2/3, ultimately suppressing TGF-β mediated epithelial-mesenchymal transition, 2) decreased Akt levels, suggesting a direct inhibition of PI3K by AR and 3) increased expression of β-catenin in these stem cells. Hence, AR potentiated Wnt/β-catenin, with increased the expression of LGR4. As the expression of both of these TFs were found to be increased by AR which in turn increased nuclear localization of ΔNP63α in BPH epithelial stem cells. Co-immunoprecipitation showed that β-catenin interacts with both AR and ΔNP63α, and the interaction is enhanced with TP treatment. Further, we will be assessing AR dependent gene regulation of these stemness genes. Thus, our results confirm that luminal progenitor markers (LGR4, β-CATENIN, ΔNP63α) are under positive regulation of AR in BPH condition.

Objective:3 To explore the role of Androgen Receptor mediated epithelial-stromal crosstalk in the etiopathology of Benign Prostate Hyperplasia

As the role of stromal AR is largely unknown in BPH condition, we aimed to control AR actions in stromal cells and observe its effect on epithelial cells. To achieve this work, we

isolated and characterised stromal cells by demonstrating the presence of Vimentin, α-Smooth Muscle Actin (α-SMA) and CD90. The isolated primary stromal cells were treated with TP and Nil to achieve AR activation and inhibition. Further, to investigate the role of stromal AR for altering stromal secretome, TP and Nil treated stromal cells secretome was collected. The collected secretome then treated to epithelial stem cells. Treatment with TP treated stromal secretome (TP-t-SS) showed proliferation and increased LGR4 and ΔNP63α stemness marker expression at 48 in BPH epithelial stem cells. However, at 48 hours, Nil treated stromal secretome (Nil-t-SS) demonstrated slow cell proliferation at 48 hours but gained proliferative potential at 72 hours. Nil-t-SS also depicted increased cMyc transcripts and pAkt protein levels, which can lead them to survival and proliferation. Then, we evaluated the clonogenicity of epithelial cells with TP-t-SS and Nil-t-SS treatments, which showed rapid cell proliferation upto 6 days with the treatment with Nil-t-SS. On the contrary, TP-t-SS treated epithelial cells did not survive post-3 days of its treatment. Hence, its is clear that secretome derived from stromal cells with active stromal AR actually have protective effect by inhibiting rapid expansion of BPH epithelial stem cells.

CONCLUSION

AR expression in luminal progenitors correlated with the expression of LGR4, ΔNP63α and β-Catenin in BPH patient tissues. Also, *in vitro* study depicted positive regulation of LGR4, β-Catenin and ΔNP63α by AR in BPH epithelial cells. Secretome derived from AR activated stromal cells enhanced stemness of BPH epithelial cells for a brief period of time. But later, AR activated stromal cell secretome causes cell death in hyperproliferative epithelial cells, hence prevents disease progression. On the contrary, AR inhibited secretome potentiates survival and proliferation of epithelial cells, promoting disease progression. In nutshell present study emphasizes on diverse regulatory effect of AR in epithelial and stromal cell of prostate gland, playing evident role in disease progression and suggesting cautious use of AR inhibitors for BPH treatment.

PUBLICATIONS

1. **Gaurav Chauhan**, Abhishek Pethe, Steffi Verghese, Akhilesh Prajapati, Sarita Gupta; "AR targets LGR4 and NP63 through β-Catenin for stem cell regulation in benign prostate tumors" **-Under Preparation**
2. **Gaurav Chauhan**, Ragitha Chruvattil, Akhilesh Prajapati, Abhishek Pethe, Sarita Gupta: "Intricacies of Androgen Receptor in dynamics of prostate cancer stem cells" **- Communicated**
3. Akhilesh Prajapati, **Gaurav Chauhan**, Sharad Gupta, Parth Pandya, Sukhbir Kaur and Sarita Gupta; "Analysis of AR, PSA (KLK) and ER-β Genetic Variants and Benign Prostate Hyperplasia (BPH) Pathogenesis in Indian Population." Biomedical Research Journal; 2016; 3(1):88–103
4. Manit Gandhi, Priyanka Bhatt, **Gaurav Chauhan**, Sarita Gupta, Ambikanandan Misra, Rajashree Mashru; "IGF-II conjugated nano carrier for brain targeted delivery of p11 gene for depression" AAPS PharmSciTech, 2019
5. Prachi D. Karia, Laxmi A. Patil, Mitul S. Vakani, **Gaurav Chauhan**, Sarita S. Gupta, S. P. Rathod, Kirti V. Patel; "Chemoprevention of breast cancer by Psidium guajava Linn." Asian Journal of Pharmacy and Pharmacology, 2018

ABSTRACTS PUBLISHED THROUGH POSTER PRESENTATIONS

1. **Poster Presentation:** Akhilesh Prajapati, **Gaurav Chauhan**, Sharad Gupta, Sarita Gupta; "Association of Single Nucleotide polymorphic candidate genes with Benign Prostate Hyperplasia in Western Part of Indian Population: A genomic approach"; Presented at International symposium on Recent Trends in Cancer Research: From OM to OMICS at Gujarat Cancer Research Institute, Ahmedabad, Gujarat held on 24th November, 2014
2. **Poster Presentation: Gaurav Chauhan**, Dievya Gohil, Akhilesh Prajapati, Sarita Gupta; "Unraveling the potentials of novel therapy for Benign Prostate Hyperplasia and Prostate Cancer: An in vitro study". Presented at 6th International Translational

Cancer Research Conference: "Prevention and Treatment of Cancer" Ahmedabad, India on 5th February, 2016

3. **Poster Presentation: Gaurav Chauhan**, Abhishek Pethe, Sharad Gupta, Sarita Gupta; "Androgen Receptor intervenes the expression of LGR4 through miR-27a in benign prostate tumors"; Presented at International conference on "Proteins, Exosomes and miRNA" from 11-13th December, 2018 hosted by Department of Biochemistry, Faculty of Science, The M. S. University of Baroda, Vadodara – 390002
4. **Poster Presentation: Gaurav Chauhan**, Steffi Verghese, Abhishek Pethe, Sharad Gupta, Sarita Gupta "Androgen Receptor mediated regulation of ΔNP63α stem cells in Benign Prostate tumours". Presented at "The Tumour Cell: Plasticity, Progression and Therapy" conference organized by Memorial Sloan Kettering Cancer Center, Nature, Nature Cancer and Nature Reviews Cancer at Zuckerman Research Center at Memorial Sloan Kettering Cancer Center, New York, NY, USA from March 3-6, 2019

AWARDS

1. **University Grants Commission -National eligibility Test** (UGC-NET-JRF/LS -2012) in Forensic Science.
2. **Best poster Presented at 6th International Translational Cancer Research Conference: "Prevention and Treatment of Cancer"** held on 5th February 2016 organized by GCRI, Ahmedabad, Gujarat, India.
3. **Received Ph.D. Travel Grant from UGC** for attending "Bioinformatics Workshop on RNASeq Data Analysis (10th to 13th January 2018)" at Guwahati, Assam, India
4. **Received Registration waiver from Thermo-Fisher Scientific** for attending "Bioinformatics Workshop on RNASeq Data Analysis (10th to 13th January 2018)" at Guwahati, Assam, India
5. Received **Department of Biotechnology (DBT)-CTEP, Govt. of India, Travel Grant** for attending & presenting poster in international conference "The Tumour Cell: Plasticity, Progression and Therapy" organised by Zuckerman Research Center and Nature Publishing Group at Memorial Sloan Kettering Cancer Center, New York, NY, USA to be held on March 3-6, 2019.

WORKSHOP

1. Actively Participated in Organizing the Four Day Workshop on "Advanced Techniques in Stem cell Research" Jointly Organized by DBT-MSUB-ILSPARE and Department of Biochemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara held on 31st December 2014 to 3rd January 2015.

2. Attended "Bioinformatics Workshop on "RNASeq Data Analysis" at Gauhati University, Guwahati, Assam, India from 10th to 13th January 2018

DEPARTMENT OF BIOCHEMISTRY, FACULTY OF SCIENCE,

THE M. S. UNIVERSITY OF BARODA, VADODARA

DOCTORAL COMMITTEE REPORT OF Ph. D. STUDENTS

Assessment for Pre-synopsis of **Mr. Gaurav Chauhan** for the year **2017-2018** Date, time and venue of assessment/interview: **30th July, 2019 at 04:15 PM to 5:00 PM, CVR Seminar Hall.**

Doctoral Committee

1. External member : Dr. Sharad Gupta
Designation : MD Pathology
Department : Gupta Pathology Lab, Dandiya Bazar, Vadodara

2. Internal member (01) : Prof. Rajesh Singh
Designation : Professor
Department : Department of Biochemistry
University : The M.S University of Baroda

3. Internal member (02) : Dr. Ravi Vijayvargia
Designation : Assistant Professor
Department : Department of Biochemistry
University : The M.S University of Baroda

4. Guide/supervisor **:** Prof. Sarita Gupta (HEAD)

Committee Report

Remarks / Suggestions

Signature:

Name: