ABSTRACT

Benign Prostate Hyperplasia (BPH) and Prostate cancer (PCa) share some traits like androgen-dependent growth, disease symptoms, and response to anti-androgen therapy. The hyper-proliferative activity of prostate stem cells and Androgen Receptor (AR) signaling pathways have found to be responsible for the pathogenesis of BPH and PCa. But, the precise mechanism and function of AR in stem/progenitor cells are still ambiguous during BPH pathogenesis. In this context, we aimed to understand the role of AR in the regulation of prostate stem/ progenitor cells of BPH and PCa conditions. The assessment of the protein profile of BPH patient tissues (N=20) showed a significant positive correlation between AR and LGR4, β-Catenin, and Ki-67 expressions. Further, patients with AR^{hi} phenotype had increased LGR4, ΔNp63α, β-catenin, Oct3/4 protein levels as compare to AR^{lo}. Moreover, AR^{hi} patients have high transcript levels of *cMYC*, *NKX3.1*, β-*CATENIN*, and *LGR4* against AR^{lo} patients. AR^{hi} patients also had early BPH development (~62 years) as compared to ARlo (~67 Years). A similar profile was also observed in TGCA Prostate Adenocarcinoma patients (N= 498) showing positive correlation of AR with LGR4, β-Catenin, and Ki-67, but not with TP63 gene expression. Further *in vitro* investigations on BPH epithelial stem/progenitor cells suggested that treatment with direct agonist (Testosterone Propionate-TP), indirect agonist (IGF-1), and direct antagonist (Nilutamide-Nil) of AR affects percent population of LGR4 and CD133, but not CD49f and CD117. Activation of AR-enhanced its nuclear localization and euchromatin accessibility in benign stem/progenitor cells. Further, tumorsphere formation ability of the cells was elevated with TP and IGF-1 treatments in BPH stem/progenitor cells with elevated AR and LGR4 expression in the spheres. Additionally, gene and protein expression profile suggested that alteration in AR activity also changes the expression of LGR4 and ΔNP63α in BPH stem/progenitor cells along with altered nuclear localization of ΔNP63α and γ. Additionally, androgen stimulation enhanced the interaction between intracellular AR/β-CATENIN and β-CATENIN/ΔNP63α proteins. We have further discovered AR and β-CATENIN mediated regulation of LGR4 and ΔNP63α promoters upon TP stimulation in BPH stem/progenitor cells. In addition to epithelial stem/progenitor cells, stromal cells derived factors also regulate AR-mediated activities in epithelial stem/progenitor cells. To understand the role of stromal-AR on epithelial stem/progenitor cells, smooth muscle cells (SMCs) from BPH patients were isolated and

exposed to AR agonist and antagonist treatments to prepare conditioned medium. Exposure of conditioned medium suggested that TP induced secretome enhances stemness for a brief time duration, but prolonged incubation caused cell death in epithelial cells. On the contrary, Nil induced secretome causes aggressive growth and survival of these cells controlled by cMYC and AKT activation. The stromal AR activation can increase stemness in these cells but can also antagonize cell growth by inducing cell death. Taken together, these data indicated that AR explicitly regulates the tumorigenicity of BPH stem/progenitor cells through regulation of LGR4/β-CATENIN and ΔNP63α expression, which is under the influence of direct action of epithelial-AR or indirect action of stromal-AR mediated factors.