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

Akhilesh Prajapati1, Sharad Gupta2, Ramesh Bhonde3 and Sarita Gupta4*

1Department of Biochemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat 390002, India
2Gupta Pathological laboratory, Vadodara, Gujarat, India
3Manipal 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), Bcl-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: sglmescri@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

Copyright: © 2014 Prajapati A, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
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 flow cytometry. 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 cell lineages. 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 consent and ethical approval. Benign prostatic hyperplasia (BPH) patients (underwent TURP) that expresses pluripotency markers.

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) were extra pure and of cell culture and molecular biology respectively. RT-PCR reagents from fermentas, Germany. All the purchased from Sigma-Aldrich, USA and BD Biosciences, USA.

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].

**Table 1: Primers Sequence and annealing temperature.**

| Gene   | Primer sequence                              | Annealing Temp. | Product size |
|--------|----------------------------------------------|-----------------|--------------|
| Oct 3/4| Forward-5AGCTGGAGAAGAGGAGACTGG-3'            | 65.5°C          | 458 bp       |
| Sox-2  | Forward-5GCTACTGAGCATCTTTCTTGAG-3'           | 62°C            | 384 bp       |
| Nanog  | Forward-5GACAAGAGCACTCTTCTGG-3'              | 55.5°C          | 287 bp       |

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 10^5 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 (2) , 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 (passage 7 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 Transcription 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 I (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 blots

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...
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). Primary antibodies were added to blots and incubated overnight at 4°C. Anti-rabbit and Anti-mouse IgG conjugated with HRP were used to detect the blots using Ultra-sensitive enhanced chemiluminescence 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 hr. Cells were then 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 immunofluorescence 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 plate as described above in the presence of neuro-basal medium (Invitrogen) with N2 supplements and 2 mM glutamate for 10 days. Parallel control cells cultured without neuro-basal medium were assessed by immunofluorescence method. Images from Carl Zeiss LSM-710 confocal microscope (Carl Zeiss, Germany) were recorded.

**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 μg/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 IBMx (10 μg/ml),10 mM dexamethasone and 10 mg/l insulin for 8 days. The culture medium was replaced every 3rd day. Parallel control cells were cultured without adipocyte differentiation medium.

Chondrocyte differentiation: Isolated prostate 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 plate 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 epitheloid 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).
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 pluripotential characteristics, cells were further analyzed for the ONS markers by flow cytometry using BD FACS antibodies. Flowcytometry results showed that 11.1% 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. Further 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, 96.3% p63, 89.4%AR and 16.5% CD34 positive cells in isolated prostate cells.
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) Alizarin 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.

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 α2β1high 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 prostate population which showed self-renewal and full differentiation potential characteristics of stem cells. The CD117(+) with CD44(+) phenotype generated functional prostate after transplantation in vivo. Moreover, CD117(+) PCs 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 an 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 tumorigensis.
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 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 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. Bonhoff 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 66: 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. Signorelli S, Wallregn D, Dikos J, Isaac B, Lin D, et al. (2000) p63 is a prostatic 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, Kokawa 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, Daihya 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 cytogentic laboratory manual. (3rd edn), 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, Liddy 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 NH, Rejo 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
38. Isca: 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.

Submit your next manuscript and get advantages of OMICS Group submissions

Unique features:
- User friendly/feasible website-translation of your paper to 50 world’s leading languages
- Audio Version of published paper
- Digital articles to share and explore

Special features:
- 300 Open Access Journals
- 25,000 editorial team
- 21 days rapid review process
- Quality and quick editorial, review and publication processing
- Indexing at PubMed (partial), Scopus, EBSCO, Index Copernicus and Google Scholar etc
- Sharing Option: Social Networking Enabled
- Authors, Reviewers and Editors rewarded with online Scientific Credits
- Better discount for your subsequent articles

Submit your manuscript at: http://www.omicsonline.org/submission