Introduction: Prostate cancer is the most frequent form of cancer in males and is the second leading cause of cancer death in men in US alone (1). Over 80% of prostate cancer cases have predilection for bone metastases. Presently there is a great interest in cancer stem cells because stem cell biology and tumorigenesis may be closely linked. The failure to eradicate most cancers may be due to the misidentification of the target cells. We Examined prostate cancer cell lines and tissues and discovered that, cells that express embryonic markers Oct4 and Nanog are present in the cell lines and tissues. These data suggest that stem cell like cells are present in the prostate cancer. In the present study we have employed these markers to identify and to isolate these cells from the prostate cancer cell lines as well as from the prostate tumors.

Methods
Cell Culture and Reagents: The C4-2, LNCaP and PC-3 cell lines were obtained from the American Type Culture Collection and were cultured in a RPMI 1640 supplemented with 10% FBS and antibiotics and maintained in humidified atmosphere of 5% CO2.

Gene expression analysis: Total RNA was prepared using Trizol reagent. The mRNA was reverse transcribed to cDNA using SuperScript™ First-Strand Synthesis System for RT-PCR per the manufacturer’s instructions. Primers specific for Oct4 and Nanog were used to determine the presence of the genes that expresses the ESC cell markers.

Transfection of the cell lines with a plasmid containing the human Oct4 promoter driving GFP (phOct-4-eGFP-Neor): To facilitate in the isolation of the putative prostate cancer stem cells, cell lines were transfected with the plasmid by electroporation. The transfected cells were selected in a medium supplemented with G418 to select for the GFP+ cells.

FACS analysis: Flow cytometry was used to separate the GFP+ cells (Oct4 positive) from the GFP- cells (Oct4 negative) . The recovered GFP+ cells were cultured in RPMI 1640 to determine if the cells undergo differentiation.

Immunocytochemistry: Cells were grown for 2 to 3 days in 8-well chamber slides in RPMI 1640 medium supplemented with 10% FBS. Cells were fixed in freshly prepared cold 2% formaldehyde for 15 min. After blocking for 30 minutes with 25% goat serum in PBS, cells were incubated for 1 hour at room temperature in 25% goat serum in PBS with anti-androgen receptor (AR) TRITC-conjugated antibody. Cells were also treated with PE-conjugated anti cytokeratin 18 followed by FACS analysis.

Prostate cancer tissues: Tissue sections were prepared from the tissue blocks made from the prostate cancer tumors. The sections were treated with specific antibodies to Oct4 and Nanog. Visualization was revealed by secondary antibodies conjugated to Cy3.

Results
mRNA Analysis: The initial screening of cells demonstrated that embryonic stem cell markers (Oct-4 and Nanog) are expressed by some cells in the prostate cancer cell lines (Fig.1A). These data suggested that, cells with embryonic like characteristics are present in the prostate cancer cell lines.

Immunocyto- and immuno-histochemistry: Analysis of prostate cancer cell line and cancer tissues by immunocyto- and immunohisto-chemistry showed immunofluorescence for the Oct-4 and Nanog in the cell lines (Fig.1B) and tissues (Fig.1C). The data indicate that the prostate tumors also contain cells that express the embryonic stem cell markers.

Discussion
Oct-4 and Nanog are two important transcription factors that control the pluripotency and self-renewal of ESC (2, 3). The present study has demonstrated that these factors are expressed by some cells in the prostate cancer cell lines as well as in tumors. The approach used here to isolate the putative prostate cancer stem cells is novel and is specific for the cells that express the ESC markers. Further studies are underway to characterize the nature of these cells in terms of tumor formation. These studies will lead to the development of better treatments for the prostate cancer.

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References:

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