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Research Article

Oct-4 expression maintained stem cell properties in prostate cancer-derived CD133$^+$MDR1$^+$ cells

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Abstract

Purpose: *CD133 (prominin-1), a 5-transmembrane glycoprotein, has recently been considered an important marker that represents the subset population of cancer stem-like cells. The purpose of the present study is to isolate cancerous stem-like cells from normal healthy volunteers and prostate cancer patients (CD133$^+$) which also express MDR1 and to ascertain the influence of Oct-4 on 'stem-ness' and differentiation of these CD133$^+$ cells towards epithelium.*
Methods: *CD133$^+$ cells were isolated using magnetic beads from normal healthy volunteers and prostate cancer patients (NV-CD133$^+$and PC-CD133$^+$). The isolated cells were analyzed using flow cytometry and Western blot technique for CD133, MDR1 and Oct-4. CD133$^+$MDR1$^+$ cells were cultured in presence and absence of antihuman Oct-4 blocking antibody.*
Results: *PC-CD133$^+$ cells displayed higher Oct-4 expression with the ability to self-renew and may represent a reservoir with differentiation potential for generating prostate cancer cells. Furthermore, PC-CD133$^+$ cells highly co-expressed the multiple drug-resistant marker MDR1. The treatment with Oct-4 blocking antibody can specifically block the capability of PC-CD133$^+$ cells to differentiate into prostate epithelial cells bearing CD57.*
Conclusion: *PC-CD133$^+$ cells displayed a higher Oct-4 expression with the ability to self-renew and may represent a reservoir with differentiation potentials for progression of prostate cancer. The MDR1 expression of PC-CD133$^+$ cells in vitro and in vivo is partially due to preferential activation of Oct-4 gene expression.*

Keywords: *Prostate cancer, Cancer stem-like cells, Oct-4, CD133, Multi-drug resistance1 (MDR1)*

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INTRODUCTION

Prostate cancer is one of the leading causes of cancer-related deaths in males. Radiotherapy and chemotherapy play significant and crucial roles in clinical prostate cancer treatment to achieve prolonged patient survival. Autopsy studies show that many older men (and even younger men) who died of other diseases also had prostate cancer that never caused a problem during their lives. These studies showed that seven or eight out of ten men had prostate cancer by the age of 80 years[1]. To improve the patient survival rate, investigation to elucidate the mechanism of tumorigenesis of prostate cancer is needed. Recent data have demonstrated that tumors contain a small subpopulation of cells, i.e., cancer stem-like cells (CSCs) or cancer-initiating cells (CICs), which exhibit a self-renewing capacity and are responsible for tumor maintenance and metastasis. Stem cells have been isolated by their ability to efflux Hoechst 33342 dye[2] and are referred to as the ‘‘side population (SP)’’. Our previous publication[11] showed MDR1 in stem cells, enhanced the migration and homing of cultured bone marrow stem cells.

A transmembrane glycoprotein, CD133 (prominin-1) was first recognized in $CD34^+$ progenitor populations from adult blood, bone marrow, and fetal liver cells. Recently, CD133 has been considered an important marker to represent the subset population of CSCs in leukemia, brain tumors, retinoblastoma, renal tumors, pancreatic tumors, colon carcinoma, prostate carcinoma, and hepatocellular carcinoma. However, the gene regulation mechanisms in maintaining the self-renewal and drug resistant properties in putative cancer stem-like cells of prostate tumors are still unclear.

Oct-4, a member of the family of POU-domain transcription factors, is expressed in pluripotent embryonic stem (ES) and germ cells. Oct-4 activates transcription via octamer motifs, and Oct-4 binding sites have been found in various genes, including *fgf4* (fibroblast growth factor 4) and *pdgfαr* (platelet-derived growth factor α receptor). This suggests that Oct-4 functions as a master switch during differentiation by regulating the pluripotent potentials of the stem cell, and Oct-4[3-5] plays a pivotal role in mammalian development.

In this study, CD133-positive cells ($CD133^+$) were isolated from normal volunteers’ (NV) and prostate cancer patients’ tissue samples (PC) and investigated for their ability to differentiate into prostate cancer epithelial cells bearing CD57.

MATERIALS AND METHODS

All normal and prostate cancer tissues were kindly donated by Drs. Satish Kumar and S. N. Chary (Urologist) from those obtained from volunteers in NRI Hospital, Krishna (Dist), Andhra Pradesh, India. The study was approved by Institutional animal ethics committee (IAEC), India.

Isolation of $CD133^+$ population from prostate cancer biopsies

The study was approved by the Institutional Ethics Committee/Institutional Review Board of NRI hospital. Biopsy samples from both normal (n=6) and prostate cancer patients (n=10) were digested with Trypsin-collagenase mixture (Sigma) to dissociate cells from tissues. From this fraction, cells were selected for CD133 expression (Miltenyi Biotech) using magnetic beads as described by the manufacturer and were analyzed for the presence of CD133 (AC133, Miltenyi Biotech) and MDR1 (Sigma-Aldrich). The purity of $CD133^+$ cells in the sorted samples was routinely greater than 92% and nearly all these cells also expressed MDR1.

Western blot analysis for Oct-4, CD133 and MDR1

The isolated cells from normal and prostate cancer patients’ biopsies were lysed by adding SDS sample buffer containing 1 mM orthovanadate, and the samples were processed for Western Blotting. The expression patterns of Oct-4, MDR1 (both from Sigma-Aldrich) and CD133 (Miltenyi Biotech) were detected in normal and prostate cancer patients' biopsies.

Isolation of $CD133^+$ population from peripheral blood samples

Mononuclear cells were obtained from normal and prostate cancer patients' buffy coats, on a Ficoll gradient (Biocoll 1077, Sigma). From this fraction, cells were selected for CD133 expression (Miltenyi Biotech) using magnetic beads as described by the manufacturer and were analyzed for the presence of CD133 (AC133, Miltenyi Biotech) and MDR1. The purity of $CD133^+$ cells in the sorted samples was routinely greater than 94% and nearly all these cells also expressed MDR1. The above method is also used for isolation of $CD133^+$ cells from prostate tissues.

Culturing of $CD133^+$ cells

The above isolated cells from peripheral blood as well as from prostate tissues, were cultured on plates coated with fibronectin (50 μg/ml) in Iscove's modified Dulbecco medium (IMDM), BSA (2 mg/ml), bovine transferrin (200 μg/ml), human insulin (10 μg/ml), hydrocortisone (2 μM), hepatocyte growth factor (HGF) (20 ng/ml) and GM-CSF (20 ng/ml). Cells were cultured in serum-free basal medium (Himedia) for 8-10 days after which half of the medium was replaced with fresh medium on alternate days. The cultured cells were trypsinzed and analyzed for various markers like CD133 and MDR1. In some experiments we used blocking rabbit anti-human Oct-4 antibody to block Oct-4 antigen in cell culture. The medium containing the antibody and growth factors was also replaced with fresh medium on alternate days.

Flow cytometry

About 5×10^5 freshly isolated cells from patient biopsies and peripheral blood samples were stained by incubating with 50 μl of diluted primary antibodies at 4^oC for 45 min. The cells were washed and stained with secondary antibody and then again washed and fixed with 0.2% paraformaldehyde. The antibodies used in this study were biotinylated anti-CD133 (Miltenyi Biotech, Labmate (Asia) Pvt., Ltd, India), mouse anti-human MDR1 (Clone F4, Sigma Aldrich), rabbit anti-human Oct-4 (Sigma Aldrich), anti-CD57 (BD Biosciences – Pharmingen. Clone NK-1, labeled with FITC), anti-biotin-PE (Miltenyi Biotech), streptavidin-FITC (Pharmingen), and isotype control antbodies were also procured from Pharmingen. The cells were analyzed on a customized BD FACS Caliber (BD Biosciences, San Jose, CA) using filters of 530/28 BP for FITC and 575/26 BP for PE with a 488-nm argon laser to excite both PE and FITC.

RESULTS

Isolation and characterization of prostate cancer-derived CD133-positive cells

Using the magnetic bead method, we isolated $CD133^+$ cells from tissue samples of six normal healthy volunteers and ten prostate cancer patients. To characterize our isolated NV-$CD133^+$ and PC-$CD133^+$ cells, FACS was used for the detection of the expression profile of cell surface markers. The high percentage (97%) of $CD133^+$ (PC-$CD133^+$) subset was isolated from the PC tissues (Fig 1). Peripheral blood derived $CD133^+$ cells were isolated from normal healthy volunteers and prostate cancer patients. This isolation also gave us 97% pure population of $CD133^+$ cells (Fig 2). Surprisingly prostate cancer patients' tissues and peripheral blood samples derived $CD133^+$ cells showed 80-90% also positive for MDR1 (Figs 1 & 2). We also found that in $CD133^+$ cell fraction, $MDR1^+$ cells were significantly less in tissues of normal non-diseased volunteers than prostate cancer patients (Figs 1 & 2).

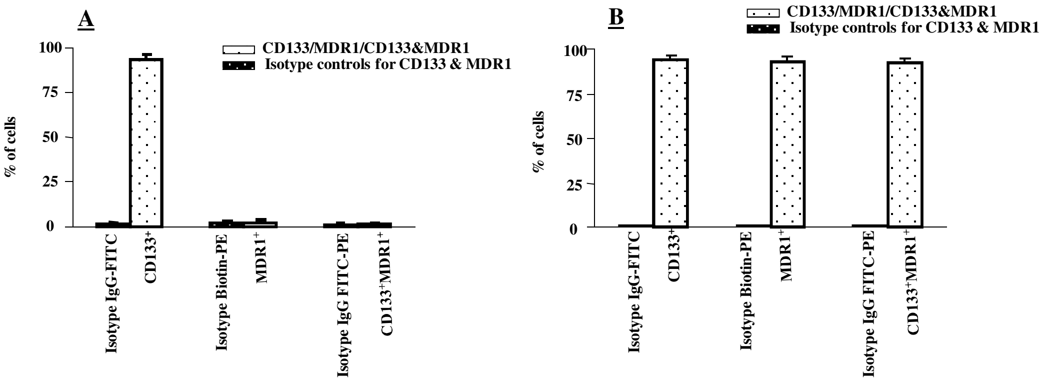

Figure 1: Cellular characterization of CD133/ MDR1/ CD133 & MDR1 in prostate tissues of normal and cancer patients

Note: *Fig 1A represents isotype controls for CD133 and MDR1 in normal healthy volunteers' prostate tissue samples (n=6) and the number of CD133*$^{+}$*, MDR1*$^{+}$ *and CD133*$^{+}$*MDR1*$^{+}$ *cells in normal healthy volunteers' prostate tissue samples (n=6). Results are expressed in mean ± SEM. Fig 1B represents isotype controls for CD133 and MDR1 in prostate cancer patients' tissue samples (n=10) and the number of CD133*$^{+}$*, MDR1*$^{+}$ *and CD133*$^{+}$*MDR1*$^{+}$ *cells in prostate cancer patients' tissue samples(n=10). Results are expressed in mean ± SEM.*

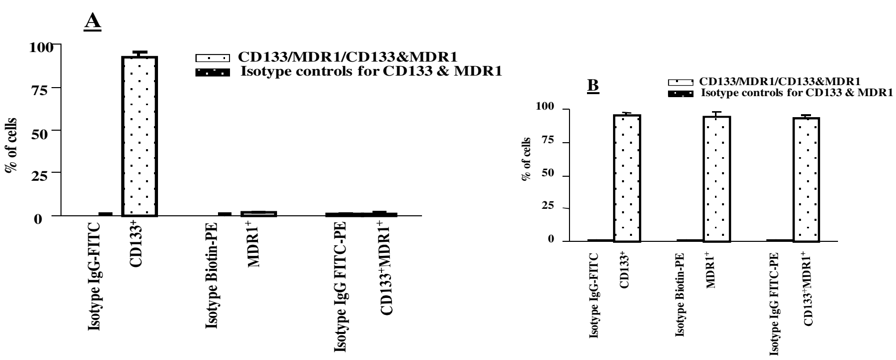

Figure 2: Cellular characterization of CD133/ MDR1/ CD133 & MDR1 in peripheral blood samples of normal and cancer patients

Note: *Fig 2A represents isotype controls for CD133 and MDR1 in normal healthy volunteers' peripheral blood samples (n=6) and the number of CD133*$^{+}$*, MDR1*$^{+}$ *and CD133*$^{+}$*MDR1*$^{+}$ *cells in normal healthy volunteers' peripheral blood samples (n=6). Results are expressed in mean ± SEM. Fig 2B represents isotype controls for CD133 and MDR1 in prostate cancer patients' peripheral blood samples (n=10) and the number of CD133*$^{+}$*, MDR1*$^{+}$ *and CD133*$^{+}$*MDR1*$^{+}$ *cells in prostate cancer patients' peripheral blood samples (n=10). Results are expressed in mean ± SEM.*

Blotting analysis for Oct-4, MDR1 and CD133

Western blot results suggested that the expression level of Oct-4, self-renewal and stem-ness protein in PC-CD133^{+} cells was significantly up-regulated than that in normal healthy volunteers (Fig 3A). To validate this finding, we examined the expression of Oct-4 in peripheral blood also. The amounts of Oct-4 protein in isolated PC-CD133^{+} cells were significantly more than in normal healthy volunteers (Fig 3B).

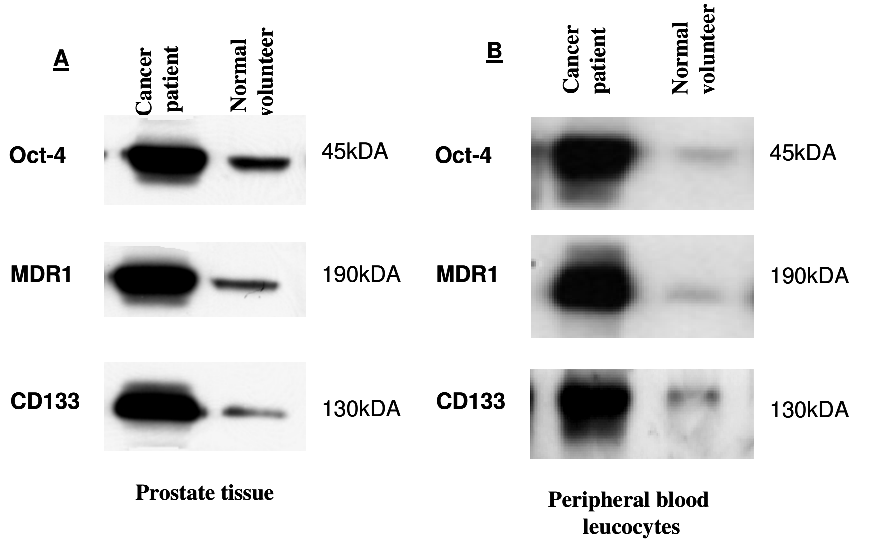

Figure 3: Western blot analysis of Oct-4, MDR1 and CD133 of prostate tissues in both normal healthy volunteer and prostate cancer patients.

Note: *Fig 3A represents the samples are from prostate tissues of prostate cancer patients and normal healthy volunteers. Fig 3B represents the samples from peripheral blood leucocytes of prostate cancer patients and normal healthy volunteers.*

Role of Oct-4 Expression in CD133$^+$ differentiation towards prostate epithelium

To investigate whether Oct-4 expression plays a role in maintaining self-renewal or cancer stem-like properties in PC-CD133$^+$cells, we used the rabbit anti-human Oct-4 blocking antibody (3µg/ml), from day 1 to 8-10 days of culture of tissue and peripheral blood derived CD133$^+$ cells of both normal healthy volunteers and prostate cancer patients. We found that treatment of blocking antihuman Oct-4 antibody in CD133$^+$ cell differentiation could significantly interfere with the capabilities of differentiation towards prostate epithelium bearing CD57. However, this was not the case with NV-CD133+ differentiation (Figs 4A and 4B).

Statistical analysis

Data were analyzed by one–way ANOVA followed by Dunnett's t-test using computerized Graph Pad Instat® version 3.05 (Graph Pad software, U.S.A.).

DISCUSSION

Oct-4 has been suggested as one of four major factors that render the reprogramming capability[3-5] of adult cells into germline-competent-induced pluripotent stem cells. Recently, Oct-4 transcript can be consistently detected in human embryonal carcinomas, testicular germ cell tumors[6-9], seminomas, and bladder carcinomas. The expression of Oct-4 has further been shown in human breast cancer stem-like cells[10], suggesting that its expression may be implicated in self-renewal and tumorigenesis via activating its downstream target genes. Here we report the isolation of CD133-positive cells (PC-CD133$^+$) from clinical tissue samples and peripheral blood samples. PC-CD133$^+$ cells showed strong stem-ness and differentiation in vitro and in vivo (Figs 1, 2, and 4). PC-CD133$^+$ cells also displayed significant expression profiles of Oct-4 and MDR1 (Fig 3). We also demonstrated that Oct-4 regulated differentiation into prostate epithelium expressing CD57 in PC-CD133$^+$ (Fig 4).

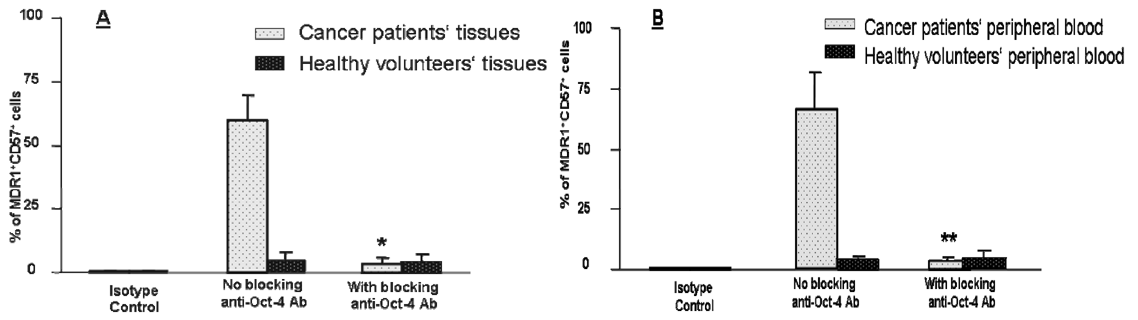

Figure 4: Cellular characterization of cultured CD133$^+$ cells

Note: *Fig 4A represents expression levels of MDR1$^+$CD57$^+$ of normal healthy volunteers' (n=6) and prostate cancer patients' (n=6) tissue derived CD133$^+$MDR1$^+$ cells cultured for 8-10 fays. Results are expressed in mean ± SEM. (n=6) significance levels * P<0.05, as compared with the respective control. Fig 4B represents expression levels of MDR1$^+$CD57$^+$ of normal healthy volunteers'(n=6) and prostate cancer patients' (n=6) peripheral blood derived CD133$^+$MDR1$^+$ cells cultured for 8-10 fays. Results are expressed in mean ± SEM. (n=6) significance levels ** P<0.01, as compared with the respective control. In all the experiments (3µg/ml) of anti-human Oct-4 blocking antibody was used through out culturing for 8 to 10 days.*

Indeed, Oct-4 functions as a master switch during differentiation[31–33] by regulating the pluripotent potential in stem cells.

Using the blocking antihuman Oct-4 blocking antibody in PC-CD133$^+$ cells in culture, our data showed that the treatment of Oct-4 blocking antibody can block the differentiation of PC-CD133$^+$ cells towards epithelial cells bearing CD57 (Fig 4). To our knowledge, this is the first study to show that Oct-4 expression plays a crucial role in maintaining self-renewal and cancer stem-like properties in PC-CD133$^+$ cells. The property of resistance to chemotherapy (MDR1) and irradiation treatment is the major clinical criterion to characterize "cancer stem-like cells (CSCs)".

The existence of cancer stem-like cells may explain why conventional anti-cancer therapies[1,12,13] are able only to suppress or shrink a tumor but often cannot completely eradicate it, resulting in eventual recurrence. These results indicate that the up-regulated expression of Oct-4 in PC-CD133$^+$ cells may contribute to the development of chemo-radio resistance in patients with prostate cancer. Recent studies have revealed that the human ABCG2 transporter is a P-glycoprotein that causes multidrug resistance (MDR1)[13,14] including mitoxantrone, doxorubicin, and topoisomerase I inhibitors of irinotecan, topotecan, and 7-ethyl-10-hydroxycamp-tothecin (topoisomerase inhibitor) and gefitinib (an inhibitor of EGF receptor) in patients with lung cancer. Monzani and colleagues[15] further showed that cancer stem-like cells derived from the melanoma cell line highly coexpressed CD133 and MDR1 markers with enhanced tumorigenic potential. In this study, we found that PC-CD133$^+$ cells highly co-expressed with MDR1 showing resistance to conventional treatment methods compared with NV-CD133$^+$ (Figs 1 and 2). Interestingly, a significant down-regulating of MDR1 expression and differentiation marker CD57 expression of PC-CD133$^+$ were observed when the antihuman Oct-4 blocking antibody treatment was given (Fig 4). Thus, more studies are needed to investigate whether over-expression of Oct-4, CD133, and/or MDR1 play a role in progression of prostate cancer.

CONCLUSION

We demonstrated that PC-CD133$^+$ cells display a higher Oct-4 expression with the ability to self-renew and may represent a reservoir with differentiation potentials for progression of prostate cancer. The MDR1 expression of PC-CD133$^+$ cells *in vitro* and *in* *vivo* is partially due to preferential activation of Oct-4 gene expression. In addition, these data support that the up-regulated expression of Oct-4 play an important role in the tumorigenesis of patients with prostate cancer.

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