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Growth Kinetics of CD133-Positive Prostate Cancer Cells

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Abstract

BACKGROUND—In the adult human prostate CD133 expression is thought to mark rare prostate epithelial stem cells and malignant tumor stem/initiating cells. Such putative stem cell populations are thought to proliferate slowly, but possess unlimited proliferative potential. Based on this, we hypothesized that CD133^{pos} prostate cancer cells proliferate slower than CD133^{neg} cells.

METHODS—Human prostate cancer cell lines were analyzed for CD133 expression and DNA content using flow cytometry. Rates of cell division and DNA synthesis were determined using CFSE cell tracing and BrdU uptake, respectively. Changes in cell cycle distribution and the percentage of CD133^{pos} cells were assayed under conditions of different cell density and AR-pathway modulation. Lastly, we over-expressed lentiviral CD133 to measure whether CD133 regulates the cell cycle.

RESULTS—The cell cycle distribution differs between CD133^{pos} and CD133^{neg} cells in all three human prostate cancer cell lines studied. CD133^{pos} cells have a greater proportion of cells in G₂ and proliferate faster than CD133^{neg} cells. High cell density increases the percentage of CD133^{pos} cells without changing CD133^{pos} cell cycle progression. Treatment with the AR agonist R1881, or the anti-androgen MDV3100, significantly changed the percentage and proliferation of CD133^{pos} cells. Finally, ectopic over-expression of CD133 had no effect on cell cycle progression.

CONCLUSIONS—Contrary to our hypothesis, we demonstrate that CD133^{pos} cells proliferate faster than CD133^{neg} cells. This association of CD133 expression with increased cell proliferation is not directly mediated by CD133, suggesting that surface CD133 is a downstream target gene of an undefined pathway controlling cell proliferation.

Keywords

CD133; androgen receptor; prostate cancer; cancer stem cell; castration-resistance; cell cycle; G₂-phase

INTRODUCTION

Prostate cancer is the most common non-cutaneous malignancy in men, and even with increased early detection and treatment stemming from aggressive screening programs, prostate cancer remains the second leading cause of cancer mortality in men [1]. Thus, new concepts are needed to understand the mechanisms leading to tumor progression and relapse of malignant prostate cancer after hormone therapy. As such, the paradigms of malignant tumor stem/initiating cells present novel avenues to understand prostate cancer progression and castration-resistant tumor growth. There is a growing literature supporting that cancer lethality is the result of the hierarchical expansion of tumor stem/initiating cells which function as stem-like cells to maintain malignant growth [2]. Defining characteristics of such cells are that they proliferate infrequently yet possess an unlimited proliferative capacity, they are present at low frequency, undergo self-renewal, and produce phenotypically heterogeneous progeny with only a limited proliferative potential. With regard to prostate cancer, recent experimental studies suggest that the hierarchical expansion of tumor stem/initiating cells drives both the lethality and heterogeneity of prostate cancer [3–9]. The concept of tumor stem/initiating cells offers an intriguing explanation for the development of castration-resistant disease, whereby tumor stem/initiating cells possess the ability to resist and survive during androgen deprivation therapy and eventually generate a new class of castration-resistant malignant cancer cells.

The normal stem cell marker CD133 is a marker for prostate cancer stem cells whose function is very poorly understood [10]. CD133 (a.k.a. Prominin-1 or AC133) is a membrane glycoprotein with an N-terminal extracellular domain, five transmembrane loops with two large extracellular loops containing eight putative N-linked glycosylation sites, and a cytoplasmic tail [10]. Very little is known, however, about the biological function of CD133 except that it is localized to membrane protrusions where it interacts with membrane cholesterol and marks cholesterol-based lipid microdomains [11]. Adult stem cells often express CD133 as a surface marker [10,12,13], and it is thought that CD133-marked cholesterol microdomains function to maintain stem cell properties by suppressing differentiation within the stem cell niche [14]. In the adult human prostate, CD133 expression has been documented to mark normal prostate epithelial stem cells [6,15–18] and is thought to mark rare malignant tumor stem/initiating cells [3,6]. Functional studies have demonstrated that human prostate cancer cell lines do contain a rare population (~5.0%) of CD133^{pos} cells which have the defining characteristics of tumor stem/initiating cells, since they are present at low frequency, self-renew, exhibit unlimited proliferative capacity, and give rise to phenotypically different progeny [6]. Moreover, such rare CD133^{pos} populations are positive for AR [6]. However, the growth kinetics of CD133^{pos} populations are generally unknown, as well as our understanding of what factors impact the prevalence of CD133^{pos} populations [10]. In this study, we sought to understand how various conditions, including

cell density and AR-pathway modulation, impact the cell cycle distribution and percentage of CD133^{pos} populations within prostate cancer cell lines. Additionally, we aimed to delineate whether CD133 expression functions to regulate cell cycle progression. Our data documents that CD133^{pos} cells progress through the cell cycle at a more rapid rate compared to CD133^{neg} cells; this rate of proliferation was not dependent upon cell density and AR signaling pathway activation. Furthermore, over-expression of CD133 using a lentiviral construct did not impact CD133 cycle progression, suggesting that CD133 did not play an active role in cell proliferation.

MATERIALS AND METHODS

Cell Lines and Materials

R1881 was purchased from Sigma (St. Louis, MO), MDV3100 was purchased from Selleck Chemicals (Houston, TX); all drugs were stored at -20°C in ethanol. The LNCaP, LAPC4, and CWR-R1 cell lines were generously provided by Dr. John Isaacs at The Johns Hopkins University; these lines were cultured as previously described [6,19]. All cultures were routinely screened for the absence of mycoplasma contamination using the ATCC Universal Mycoplasma Detection Kit (Manassas, VA). To modulate cellular AR signaling, cells were cultured in charcoal-stripped FCS (Atlanta Biologicals, Lawrenceville, GA) for 24 hr and then treated with either 1nM R1881, 10 μM MDV3100, or both for 5 days.

Flow Cytometry

All antibody incubations, washes, and flow cytometric analyses were performed using ice cold $1\times$ PBS in minimal light using previously reported protocols [6]. One million cells were stained with Live/ Dead Fixable Green Dead Cell Stain Kit (Invitrogen, Grand Island, NY) per the manufacturer's instructions. After washing, cells were incubated with FcR Blocking Reagent (Miltenyi Biotec, Cologne, Germany, 1:20 dilution for 10 min), and then labeled with CD133/2 (293C3)-APC-conjugated human monoclonal antibody (Miltenyi Biotec) or isotype control mouse IgG2b-APC (Miltenyi Biotec) at a 1:10 dilution for 30 min. The cells were then washed and fixed for 15 min with 3.2% Ultra Pure EM Grade Formaldehyde (Polysciences, Inc., Warrington, PA). After fixation, the cells were washed and stained with FxCycle Violet (Invitrogen, 1:1,000 dilution). Analysis was conducted on a Becton Dickinson LSR II, and a minimum of 175,000 counts was acquired for each experimental condition. Alternatively, cells were analyzed on an ImageStream X (Amnis, Seattle, WA). Staining and analyses were conducted in at least three biological replicates, and FACSDiVa Software was used for data acquisition while FlowJo software was used for analysis. ImageStream X analyses was conducted using IDEAS software (Amnis). The Watson-Pragmatic model on FlowJo flow cytometry software was used to determine the cell cycle distribution of CD133^{pos} and CD133^{neg} cells in all cell lines.

CFSE Cell Tracing and BrdU Incorporation

To track the rate of cell proliferation, cells were washed twice with PBS and labeled with 2.5 μM of CFSE from the Cell Trace Proliferation Kit (Invitrogen) at room temperature for 5 min. The cells were then quenched and washed twice with complete media. After 3 days of incubation, flow cytometry was performed according to the aforementioned flow cytometry

protocol. To measure BrdU incorporation, $5E^5$ cells were plated per well of a 6-well plate, and cells were incubated with 10 μ M BrdU (BD Biosciences, San Jose, CA) for 2, 12, and 24 hr. Cells were then trypsinized and stained for CD133 cell surface marker as previously described, followed by BrdU intra-cellular staining according to the manufacturer's instructions (anti-BrdU-FITC flow kit; BD Biosciences). Staining and analyses were conducted in at least three biological replicates, and FACSDiVa Software was used for data acquisition while FlowJo software was used for analysis.

Quantitative Real-Time PCR (Q-RT-PCR) Analyses

RNA was purified using the Qiagen RNeasy Mini Kit with the optional DNase digestion kit (Qiagen, Valencia, CA) and quality tested using an Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA). For standard Q-RT-PCR, extracted RNA was converted to cDNA by reverse transcription using SuperScript[®] III Reverse Transcriptase (Invitrogen). Levels of PSA and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcript were quantified using *Power SYBR[®] Green Master Mix* (Invitrogen) using custom primers for PSA [5'-TCATCCTGTCTCGGA-TTGTG-3'(forward), 5'-ATATCGTAGAGCGGGTG-TGG-3'(reverse)] and GAPDH [5'-GAGTCAACGG-ATTTGGTCGT-3'(forward), 5'-TTGATTTTGGAGG-GATCTCG-3'(reverse)]. Standard curves were used to assess primer efficiency and average change in threshold cycle (C_T) values was determined for each of the samples relative to endogenous GAPDH levels and compared to vehicle control (C_T). Experiments were performed in triplicate to determine mean standard error, and Student's *t*-tests performed with normalization to control to obtain *P* values.

Lentiviral Infection

Lentiviral CD133 (Accession # BC012089.1) and Control vectors (pReceiver-Lv105) were purchased from GeneCopoeia (Rockville, MD). High-titer lentivirus was made by co-transfecting HEK293T cells with ViraPower Lentiviral packaging mix (Invitrogen) and Lenti-X Concentrator (Clontech, Mountain View, CA) according to manufacturer's instructions. Target prostate cancer cells were infected with virus and selected in 1.0 ng/ml puromycin.

Statistical Analysis

Data was analyzed using Sigma Plot 11 software using a one way ANOVA with Pairwise Multiple Comparison Procedures (Holm-Sidak method).

RESULTS

Cell Cycle Distribution Differs Between CD133^{pos} and CD133^{neg} Prostate Cancer Cells

Because CD133^{pos} cells are associated with a tumor stem/initiating cell phenotype [20], we hypothesized that CD133^{pos} cells would proliferate more slowly than CD133^{neg} cells. This is based upon the notion that rare tumor stem/initiating cells are proliferatively quiescent, giving rise to rapidly proliferating transit-amplifying populations. These transit-amplifying populations have a limited proliferative capacity, but compose the bulk of the tumor. To test our hypothesis, we utilized three AR-positive human prostate cancer cell lines (LAPC-4, CWR-R1, and LNCaP). These cell lines were chosen as they are representative of the

majority of lethal metastatic prostate cancers since they express and depend upon AR signaling for their continued growth and survival [19,21–26]. As expected, we observe the presence of rare but viable CD133^{pos} cells in all three cultures (Fig. 1A and B) [6]. When comparing CD133^{pos} versus CD133^{neg} populations for DNA content, we observe that CD133^{pos} cells show a statistically significant increase in G₂-phase compared to CD133^{neg} controls in all three cell lines (Fig. 1A–C). In LAPC-4 cells, there is also a significant difference in G₁-phase distribution; CWR-R1 cells demonstrate a significant difference in the quantity of cells in S-phase (Fig. 1C). Thus, CD133^{pos} cells have a different cell cycle distribution relative to CD133^{neg} cells in human prostate cancer cell lines, with an increased G₂ fraction within the CD133^{pos} populations in all three lines.

CD133^{pos} Cells Proliferate Faster Than CD133^{neg} Cells

The increased proportion of CD133^{pos} cells in G₂ could be due to either an increased rate of proliferation or a G₂-specific cell cycle arrest. To test whether CD133^{pos} cells were proliferating at a different rate than CD133^{neg} cells, we used a cell tracing assay in which cells were stained with the cell tracer CFSE and allowed to divide in culture for 3 days. At the initial time of labeling, all cells were uniformly high for CFSE.

After 3 days, however, rapidly dividing CD133^{pos} cells retain significantly less CFSE than slower dividing CD133^{neg} populations. These studies document that 77.9% of CD133^{pos} LAPC-4 cells had divided while only 13.1% of CD133^{neg} cells had divided (Fig. 2A and B). Similarly, 59.4% of CD133^{pos} CWR-R1 cells had divided while only 10.7% of CD133^{neg} cells had divided (Fig. 2A and B). To further validate this surprising finding, we measured BrdU incorporation of CD133^{pos} versus CD133^{neg} LAPC-4 and CWR-R1 cells over a period of 2, 12, or 24 hr. These data document that CD133^{pos} cells incorporate BrdU into their genomic DNA at a faster rate than CD133^{neg} cells, further demonstrating that such CD133^{pos} cells progress through the cell cycle more rapidly than CD133^{neg} cells (Fig. 3). These combined data demonstrate that CD133^{pos} prostate cancer cells proliferate more rapidly than CD133^{neg} cells, implying that they may not be a quiescent cell population that gives rise to rapidly proliferating transit-amplifying CD133^{neg} cells.

Cell Density Alters CD133 Populations and Cell Cycle Distribution

During tumor dissemination and metastasis, the prevalence of tumor stem/initiating cells and their transit-amplifying progeny is likely to be dependent upon tumor cell density. This is based upon the idea that tumor stem/initiating cells comprise the bulk of early metastatic lesions (i.e., micro-metastasis), and their transit-amplifying progeny proliferate as secondary tumors grow into macroscopic metastases. To determine how cell density affects the cell cycle distribution in CD133^{pos} cells, we plated LAPC-4 cells at low (4,000 cells/cm²), medium (30,000 cells/cm²), and high (160,000 cells/cm²) density, and analyzed CD133 populations after 3 days in culture. Our data documents that, at low and medium densities, CD133^{pos} cells have a G₂-distribution of 20.3% and 21.6%, respectively. At high density, however, CD133^{pos} cells have a significantly smaller G₂-distribution of 13.6% (Fig. 4A and B), but CD133^{pos} cells maintain a significantly increased fraction in G₂ compared to CD133^{neg} cells (Fig. 4A and B). Next, we wanted to determine how the percentage of CD133^{pos} cells was impacted by cell density. Interestingly, high cell density significantly

increases the percentage of CD133^{pos} LAPC-4 cells (Fig. 4C). This increase in overall CD133^{pos} cells also occurs in LNCaP cells grown under similar conditions ($P = 0.031$, data not shown). Thus, our data demonstrates that CD133 expression is influenced by cell density, whereby at high cell density the proliferation of CD133^{pos} cells decreases but the overall percentage of CD133^{pos} cells increases.

The Effect of AR Modulation on CD133^{pos} Cells

Androgens, mainly testosterone, are important for proper function and development of the prostate [27], and for the maintenance and progression of prostate cancer [28]. Advanced prostate cancers inevitably progresses to a castration-resistant state, whereby cells utilize numerous mechanisms to maintain oncogenic AR signaling even in the near absence of ligand [29,30]. Moreover, these mechanisms include overcoming direct inhibition of the AR using anti-androgens [29]. Because CD133^{pos} prostate cancer cells have been shown to express AR [31], we sought to elucidate the effects of various levels of AR stimulation upon CD133 expression and the cell cycle distribution of CD133^{pos} cells. To address this, we treated cells with the AR agonist R1881 (1 nM) or the AR antagonist MDV3100 (10 μ M) for 5 days in the presence of charcoal-stripped FCS. As expected, treatment of AR-dependent LAPC-4 and CWR-R1 cell lines results in an increased production of PSA mRNA in the presence of R1881; this increase in PSA mRNA is blocked by the addition of MDV3100 with R1881 (Fig. 5A). In the presence of the anti-androgen MDV3100 alone, PSA mRNA levels decrease in LAPC-4 cells but did not within CWR-R1 cells; this lack of PSA response in CWR-R1 cells is likely attributed to the presence of a form of AR lacking the ligand-binding domain (Fig. 5A) [23]. Treatment of LAPC-4 cells with either R1881 or MDV3100 significantly increases the percentage of CD133^{pos} cells (Fig. 5B). In CWR-R1 cells, treatment with R1881 alone or in combination with MDV3100 results in a marked decrease in CD133^{pos} cells (Fig. 5B). In both cell lines, treatment with R1881 significantly increases the G₂ distribution of CD133^{pos} cells compared to untreated controls, indicating that R1881 preferentially increases the proliferation of CD133^{pos} cells (Fig. 5C). In LAPC-4 cells, CD133^{pos} cells treated with MDV3100 or a combination of R1881/MDV3100 maintains an increased G₂ fraction compared to treated CD133^{neg} cells (Fig. 5C). Castration-resistant CWR-R1 cells grown only in charcoal-stripped serum, however, show a significantly reduced G₂ distribution of CD133^{pos} cells, which is reversed in the presence of R1881 or combined R1881/MDV3100 (Fig. 5C). Thus, CD133^{pos} cells respond differently to AR pathway modulation than their CD133^{neg} counterparts, and in castration-sensitive LAPC-4 cells, both pro and antiAR stimuli lead to an increase in CD133^{pos} cells without decreases in G₂ distribution. These data indicate that the proliferation of CD133^{pos} cells is impacted differently than CD133^{neg} cells in the presence of AR pathway modulation.

Ectopic CD133 Over-Expression Does Not Alter Cell Cycle Distribution

Since CD133^{pos} proliferate faster than CD133^{neg} cells, we asked whether CD133 plays an active or passive role in the cell cycle progression and proliferation of CD133^{pos} cells. To answer this, we lentivirally over-expressed CD133 (LV-CD133) or a control vector (LV-Control) into LAPC-4 and CWR-R1 cells. After successfully infecting cells with LV-CD133 and documenting cell surface expression of CD133 (Fig. 6A), we analyzed the cell cycle distribution of CD133-positive LAPC-4 and CWR-R1 cells. We hypothesized that if CD133

was playing an active role in cell cycle progression, cells infected with LV- CD133 would display a phenotype similar to that of normal, non-infected LAPC-4 and CWR-R1 cells, with a higher G₂-phase distribution relative to CD133^{neg} controls (Fig. 1A and B). However, the cell cycle analysis of LAPC-4 and CWR-R1 cells over-expressing CD133 reveals no significant differences in G₁-, S-, and G₂-phases relative to lentiviral controls (Fig. 6B). Thus, our data indicates that CD133 plays a passive role in cell cycle progression and proliferation, and may be a downstream target of pathways that regulate the cell cycle.

DISCUSSION

The objective of this study was to characterize the cell cycle dynamics of CD133^{pos} cells and to determine how their environment influenced the quantity and cell growth kinetics of CD133^{pos} cells. Here we show that, contrary to our hypothesis, putative CD133^{pos} tumor stem/initiating cells proliferate faster than CD133^{neg} cells. These results indicate that either: (i) CD133^{pos} cells are not cancer stem/initiating cells; (ii) that they do not behave like cancer stem/initiating cells under the in vitro culture conditions examined; or (iii) that CD133^{pos} populations may contain a smaller sub-population of cells that behave like bona fide cancer stem/initiating cells. Our results are in line with a previous study by Shepherd et al. [32] who compared the gene expression profile of CD133^{pos} versus CD133^{neg} epithelial cells from freshly isolated benign human prostate tissue and CRPC. Their study identified various genes that positively correlate with cell proliferation and were up-regulated in CD133^{pos} cells from benign human prostate tissue samples compared to CD133^{neg} controls, including PBK, CDC25A, CDKAL1, and MCM5 [32]. Moreover, their study also showed that in CRPC samples, CD133^{pos} cells had increased expression of CDK7 and CDC2, both of which are known to play a positive role in cell cycle progression [33]. Another study by Rowehl et al. [34] utilized genome-wide microarray analysis to study the genes in CD133^{pos} and CD133^{neg} cells in PC3MM2 human prostate cancer cells. Their results showed that many genes were up-regulated in CD133^{pos} cells relative to CD133^{neg} cells, including cell cycle regulation and proliferation genes Cyclin B, CCNB1, CDC2, CDC6, and CDCA2, 3, 5, and 8 [34]. Thus, our findings that CD133^{pos} cancer cells proliferate faster than CD133^{neg} cells reflects the expression array data of other groups which suggested that CD133^{pos} cells have an increased expression of genes involved in cell cycle progression and proliferation. However, future experiments are needed to determine which gene or set of genes are specifically contributing to the increased proliferation of CD133^{pos} cells and what role, if any, CD133 expression has in this process.

Previous work has documented that CD133 expression also marks a multipotent stem cell population in normal prostate epithelial cell cultures [6]. The expression of CD133 on normal prostate stem cells is likely associated with a molecular profile and growth phenotype that is distinct from malignant CD133^{pos} prostate cancer cells. Thus, these data highlight the experimental consideration that a single marker, such as CD133, is not sufficient to define a stem cell population spanning normal and malignant stem cell populations, and other markers are required to more accurately define and characterize putative prostate cancer stem cells.

The increased rate of CD133^{pos} cell proliferation compared to CD133^{neg} cells also lends support to previous observations in prostate and other tumor models that CD133^{pos} cells are able to initiate tumor xenografts more efficiently than their CD133^{neg} counterparts [35–37]. Our data suggests, however, that this phenomenon of enhanced tumor initiation may simply be due to an association between CD133 expression and rapid cell proliferation. Thus, these data support a notion that within prostate tumors CD133^{pos} cells may be more susceptible to cytotoxic drugs targeting dividing cells.

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Abbreviations

ADT	androgen deprivation therapy
AR	androgen receptor
CRPC	castration-resistant prostate cancer
CSCs	cancer stem cells

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Fig. 1.

Cell cycle distribution differs between CD133^{pos} and CD133^{neg} cells. **A:** ImageStream X capturing images of individual CD133^{pos} or CD133^{neg} LAPC-4 cells in G₁-, S-, or G₂-phase. Dead cells stain positive using the live/dead cell stain. **B:** CD133^{pos} cells contain a greater proportion of cells in G₂. Dot plot showing the percentage of CD133 expressing cells in three human prostate cancer cell lines (LAPC-4, LNCaP, and CWR-R1) relative to isotype control (IgG-APC). The histogram shows the cell cycle distribution (G₁, S, and G₂) of CD133^{pos} versus CD133^{neg} cells. **C:** Bar graph showing the cell cycle distribution comparing CD133^{pos} versus CD133^{neg} cells. Stars represent statistical significance (**P* < 0.05; ***P* < 0.02; ****P* < 0.005).

^a The percentage of cells from the original sample which divided at least once.

Fig. 2.

CD133^{pos} cells proliferate faster than CD133^{neg} cells. **A:** Histogram of LAPC-4 and CWR-R1 cells showing unstained, CD133^{pos} and CD133^{neg} cells 3 days after CFSE labeling. CD133^{pos} cells have less CFSE than CD133^{neg} cells, documenting that they underwent more cell divisions over a 3-day period. **B:** Table showing the number of cells from the original LAPC-4 or CWR-R1 cell culture that divided over 3 days (percent divided). *P* values represent a paired two-tailed *t*-test.

Fig. 3.

CD133^{pos} cells incorporate BrdU at a faster rate than CD133^{neg} cells. Genomic BrdU incorporation into CD133^{pos} versus CD133^{neg} LAPC-4 (**left panel**) or CWR-R1 (**right panel**) prostate cancer cells was measured by culturing cells for 2, 12, or 24 hr in the presence of BrdU and measuring incorporation using an anti-BrdU-FITC antibody. Statistical comparisons document that CD133^{pos} cells incorporate more BrdU over 2, 12, or 24 hr than CD133^{neg} cells.

Fig. 4.

Cell density increases the percentage of CD133^{pos} cells. **A:** Cell cycle distribution of CD133^{pos} and CD133^{neg} LAPC-4 cells at low (~4,000 cells/cm²), medium (~30,000 cells/cm²), and high (~160,000 cells/cm²) density. **Upper panel:** Images of cells at low, medium, and high density. **Lower panels:** cell cycle distribution of CD133^{pos} and CD133^{neg} cells. **B:** Graphical representation comparing cell cycle distribution between CD133^{pos} and CD133^{neg} cells grown at low, medium, and high density (**P* < 0.05; ***P* < 0.02; ****P* < 0.005). **C:** Overall percentage of CD133^{pos} cells when cultured at different cell densities (***P* < 0.02).

Fig. 5.

AR pathway modulation of CD133^{pos} cells. **A:** PSA mRNA expression resulting from AR stimulation or inhibition. LAPC-4 and CWR-R1 cells were grown in media containing charcoal-stripped serum and treated with the synthetic androgen R1881 or the anti-androgen MDV3100. After 5 days, cells were harvested for mRNA, CD133, and cell cycle analyses. As expected, PSA mRNA levels were indicative of AR pathway activity in LAPC-4 cells (wild-type AR) and MDV3100 did not inhibit PSA expression in CWR-R1 cells (wild-type and mutant AR; $*P < 0.05$). **B:** Modulation of the AR signaling pathway increases the percentage of CD133^{pos} cells in LAPC-4 cells and decreases the percentage of CD133^{pos} cells in CWR-R1 cells ($*P < 0.05$). **C:** CD133^{pos} cells continue to have a greater G₂ fraction even in the presence of AR pathway modulation ($*P < 0.05$).

Fig. 6. CD133 over-expression does not alter cell cycle distribution. **A:** Histogram showing detectable cell-surface CD133 expression in the vast majority lentiviral infected CD133 (LV-CD133) cells. Unstained and vector alone (LV-Control) cells were used as negative controls. **B:** Cell cycle distribution of CWR-R1 and LAPC-4 cell over-expressing CD133. Ectopic expression of CD133 does not increase the fraction of cells in G₂ as observed within endogenous rare CD133pos populations.