

AWARD NUMBER: W81XWH-13-1-0470

TITLE: Identification and Targeting of Candidate Preexisting Lurker Cells That Give Rise to Castration-Resistant Prostate Cancer

PRINCIPAL INVESTIGATOR: Andrew Goldstein

CONTRACTING ORGANIZATION:
University of California, Los Angeles
Los Angeles, CA 90095-1406

REPORT DATE: December 2017

TYPE OF REPORT: Final

PREPARED FOR:
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:
Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

*Form Approved
OMB No. 0704-0188*

The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing the burden, to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.

PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

1. REPORT DATE (<i>DD-MM-YYYY</i>)	2. REPORT TYPE	3. DATES COVERED (<i>From - To</i>)
---	-----------------------	--

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S) Email: AGoldstein@mednet.ucla.edu	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)	8. PERFORMING ORGANIZATION REPORT NUMBER
---	---

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER (<i>Include area code</i>)

TABLE OF CONTENTS

	<u>PAGE</u>
1. INTRODUCTION	2
2. KEYWORDS	2
3. ACCOMPLISHMENTS	2
4. IMPACT	13
5. CHANGES/PROBLEMS	14
6. PRODUCTS	14
7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS	15
8. SPECIAL REPORTING REQUIREMENTS	16
9. APPENDICES	16

1. INTRODUCTION

Castration-resistant prostate cancer (CRPC) is a lethal disease. We lack standard biomarkers to predict hormonal sensitivity of tumors, and we need new targets for therapy to prevent or treat CRPC. The adaptation theory predicts that pre-existing androgen-independent prostate cancer cells (termed lurker cells) contribute to CRPC, as they are not targeted by androgen-deprivation therapy and may be capable of regenerating the tumor. Therefore, targeting these predicted lurker cells in combination with androgen-deprivation therapy would likely prevent or delay the onset of CRPC. The goals of this proposal are to provide functional evidence for intermediate luminal progenitor cells as the pre-existing “lurker” cells in primary prostate tumors, to evaluate potential therapeutic targets in intermediate luminal progenitor cells, and to define candidate biomarkers in intermediate luminal progenitor cells that can predict prognosis and response to hormonal therapy.

2. KEYWORDS

Prostate, epithelial, Androgen-deprivation therapy, Castration-resistant prostate cancer, luminal progenitor, intermediate cell, lurker cell, CD38, inflammation

3. ACCOMPLISHMENTS

What were the major goals of the project?

Specific Aim 1. Interrogation of human I/LP cells as candidate lurker cells. Time frame: months 1- 24. This aim is complete.

Task 1. Test the in vivo effects of castration on human luminal cancer subsets isolated from primary regenerated prostate tumors.

Task 2. Test the in vivo growth capacity of human luminal cancer subsets isolated from primary clinical prostate tumors.

Task 3. Test the in vivo effects of castration on human luminal cancer subsets isolated from primary clinical prostate tumors.

Specific Aim 2. Proof of principle therapeutic targeting of I/LP cells to prevent CRPC. Time frame: months 1-36. This aim is complete.

Task 4. Determine if I/LP cancer cells from primary clinical human prostate tumors express genes associated with CRPC.

Task 5. Determine regulators of I/LP clonogenic activity in vitro.

Task 6. Target I/LP cells in vivo.

Specific Aim 3. Diagnostic potential of I/LP cells as lurker cells to predict CRPC development. Time frame: months 1-36. This aim is complete.

Task 7. Determine whether I/LP cell transcriptional signature can predict patient prognosis.

Task 8. Testing of candidate I/LP biomarkers on prostate cancer tissue.

Task 9. Determine if I/LP biomarkers can predict hormone sensitivity.

What was accomplished under these goals?

Specific Aim 1. Interrogation of human I/LP cells as candidate lurker cells.

- 1) major activities: Fluorescence Activated Cell Sorting (FACS) was used to isolate distinct cellular subsets, followed by functional assays to characterize progenitor activity. Immunohistochemistry was performed on clinical tissues with distinct androgen states (intact/castrated). Organoid-forming progenitor assays were performed in the presence or absence of DHT. (1.1) We determined that differential expression of CD38 stratifies luminal cells into two subsets with differential progenitor capacity. CD38-lo luminal cells are enriched for 2D colony-forming activity as well as 3D sphere-forming and organoid-forming activity. (1.2) We evaluated clinical tissues from patients undergoing hormonal therapy compared to control patients with no prior therapy and analyzed the frequency of cells with a mature luminal or intermediate/progenitor phenotype, allowing us to measure the castration-resistance of each subset in humans. (1.3) We measured castration-resistance of isolated subsets in vitro in the presence or absence of DHT in an organoid-forming assay. (1.4) We determined that CD38-lo progenitor cells could initiate aggressive, highly-proliferative tumors upon transplantation into immune-deficient mice.
- 2) specific objectives: (1) Test the in vivo effects of castration on human luminal cancer subsets isolated from primary regenerated prostate tumors, (2) Test the in vivo growth capacity of human luminal cancer subsets isolated from primary clinical prostate tumors, (3) Test the in vivo effects of castration on human luminal cancer subsets isolated from primary clinical prostate tumors
- 3) significant results or key outcomes:

(1.1) We demonstrated that CD38-lo luminal cells are enriched for progenitor activity using 2D and 3D clonogenic assays. Figure 1 shows post-sort purity for CD38-lo and CD38-hi luminal subsets (1A-B), results of organoid formation (C) and representative organoids formed from single CD38-lo progenitor cells (D).

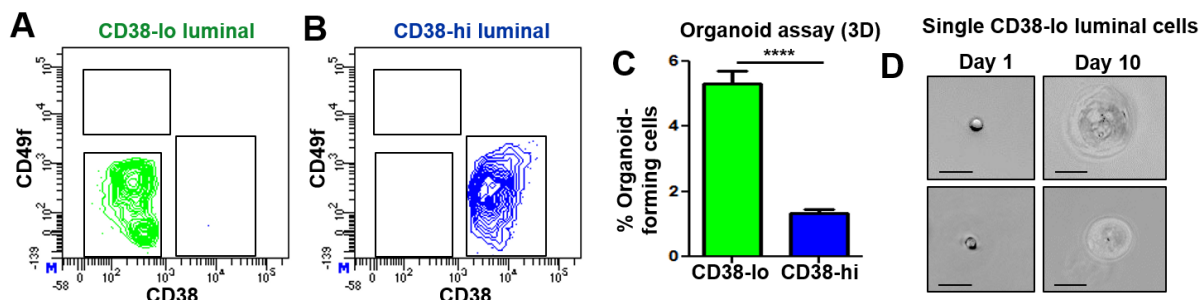


Fig. 1. Low expression of CD38 identifies luminal progenitor cells from human prostate. (A-B) Fluorescence Activated Cell Sorting (FACS) analysis of isolated CD38-lo (A) and CD38-hi (B) luminal cells. (C) Results of organoid-forming assay, representative of five independent patient samples. Statistics represent Two-tailed unpaired t test with **** $p < 0.0001$. (D) Representative images of organoids forming from single CD38-lo luminal cells taken at day 1 and day 10. Scale bars, 100 microns.

(1.2) We evaluated expression of CD38 in human prostate tissues representing intact or castrate-resistant states. In benign human prostate tissues from 6 patients undergoing radical prostatectomy, an average of 38% of luminal cells were CD38-low. In contrast, an average of 89% of luminal cells were CD38-low in benign glands adjacent to cancer from 7 patients treated with anti-androgen therapy (including Bicalutamide and Lupron) for a range of 5 months up to 20 years prior to biopsy or radical prostatectomy (Figure 2A-B). These results indicate that CD38-lo cells are preferentially castration-resistant.

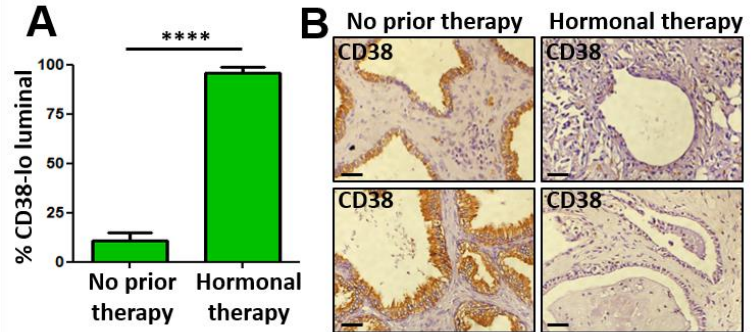


Fig. 2. Evidence for in vivo castration-resistance of CD38-lo luminal progenitor cells. (A) Percentage of luminal cells with CD38-lo phenotype in benign glands from 7 patients undergoing hormonal therapy compared to 7 patients without prior therapy. Two-tailed unpaired T-test with **** $p = 0.0002$. (B) Immunohistochemical staining for CD38 in representative glands from patient specimens corresponding to plot in (A). Scale bars, 50 microns.

(1.3) We plated CD38-lo and CD38-hi luminal subsets into organoid-forming conditions in the presence or absence of exogenous DHT and determined the sensitivity or resistance to in vitro castration. While CD38-hi cells formed few organoids in the absence of DHT, CD38-lo progenitor cells generated the same number of organoids regardless of DHT suggestive of in vitro castration-resistance (Figure 2C).

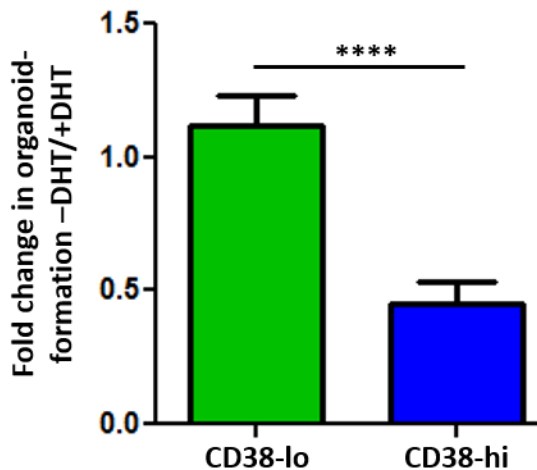


Fig. 3. Evidence for in vitro castration-resistance of CD38-lo luminal progenitor cells. Fold change in organoid formation for CD38-lo and CD38-hi luminal subsets without DHT compared to DHT-containing media, demonstrating castration-resistance of CD38-lo cells.

(1.4) Using the in vivo regeneration and transformation assay that we have previously developed to regenerate glands and convert human prostate epithelium to malignancy (Figure 4A), we found that CD38-lo progenitor cells could generate basal and luminal cells in vivo (Figure 4B), and could respond to oncogene expression and initiate aggressive human prostate tumors (Figure 4C).

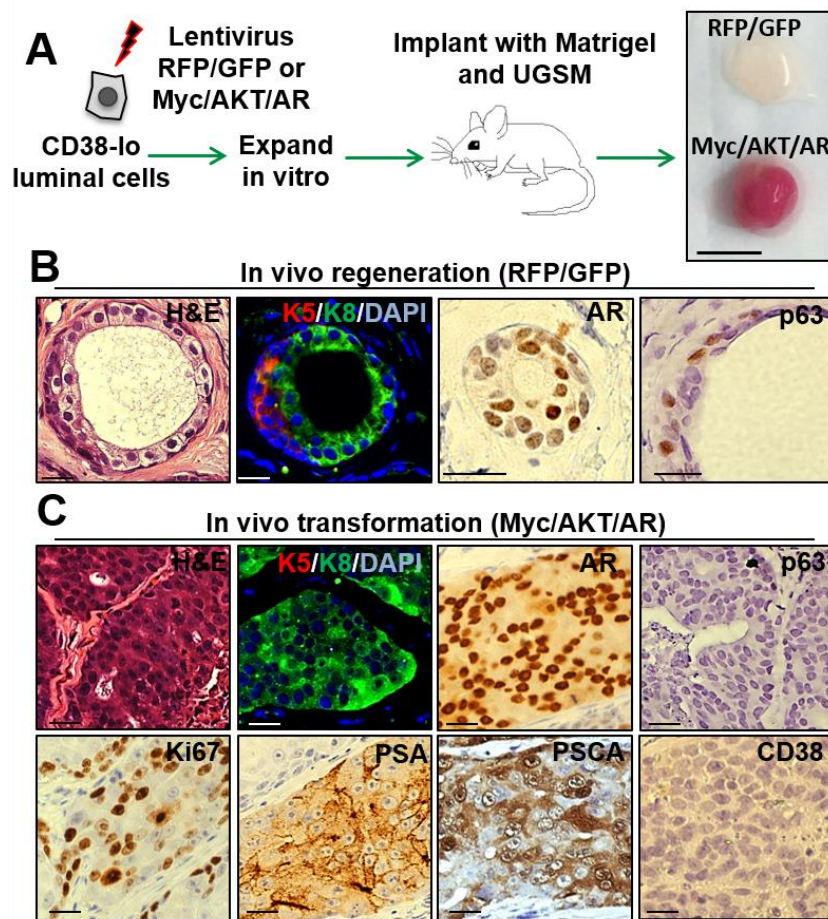


Fig. 4. Tumor initiation from CD38-lo progenitor cells. (A) Schematic: CD38-lo luminal cells are transduced with lentivirus, expanded in vitro 7–10 days, combined with UGSM and Matrigel and implanted in vivo. Regenerated tissues from representative experiments are shown on the right. Scale bars, 5 microns. (B) Representative in vivo regeneration of CD38-lo luminal cell-derived organoids transduced with control fluorescent GFP and red fluorescent protein (RFP) stained for H&E, K5, K8, DAPI nuclear counterstain, AR, and p63. Scale bars, 50 microns. (C) Representative adenocarcinoma in regenerated tumor tissues from CD38-lo luminal cells transduced with Myc, AKT, and AR stained for H&E, K5, K8, DAPI nuclear counterstain, AR, p63, Ki67, PSA, PSCA, and CD38. Scale bars, 25 microns.

4) other achievements: Nothing to report

Specific Aim 2. Proof of principle therapeutic targeting of I/LP cells to prevent CRPC.

- 1) major activities: Transcriptional profiling was performed in distinct subsets to identify differentially expressed genes. Western blotting on sorted cells and immunohistochemistry on fixed tissue were used to determine expression of genes associated with CRPC in CD38-lo progenitor cells. Small molecule inhibitors were used in organoid culture to block signaling pathways elevated in progenitor cells. (2.1) We determined that CD38-lo progenitor cells exhibit reduced androgen signaling. (2.2) We determined that CD38-lo progenitor cells express the anti-apoptotic factor BCL2 which has previously been implicated in castration-resistance. (2.3) We determine that CD38-lo progenitor cells express PSCA, previously implicated in castration-resistance. (2.4) We found that CD38-lo progenitor cells exhibit NFkB signaling. (2.5) We found that CD38-lo progenitor cells are sensitive to NFkB inhibition in an organoid-forming assay.
- 2) specific objectives: (1) Determine if I/LP cancer cells from primary clinical human prostate tumors express genes associated with CRPC. (2) Determine regulators of I/LP clonogenic activity in vitro. (3) Target I/LP cells in vivo.

3) significant results or key outcomes:

(2.1) We determined that CD38-lo progenitor cells exhibit reduced androgen signaling. Transcriptional profiling between CD38-lo and CD38-hi luminal cells from three distinct patient samples (Figure 5A) revealed differences in androgen receptor (AR) target genes including *Klk3* which encodes Prostate-Specific Antigen (PSA). Quantitative real-time PCR validated differential expression of *Klk3* (PSA) whereas no significant difference was found in expression levels of luminal gene Keratin 8 (K8) (Figure 5B). Immunohistochemistry on serial sections demonstrates low expression of AR targets PSA, FKBP5 and MSMB in CD38-lo luminal cells (Figure 5C). AR expression is low in CD38-lo progenitor cells (Figure 5D)

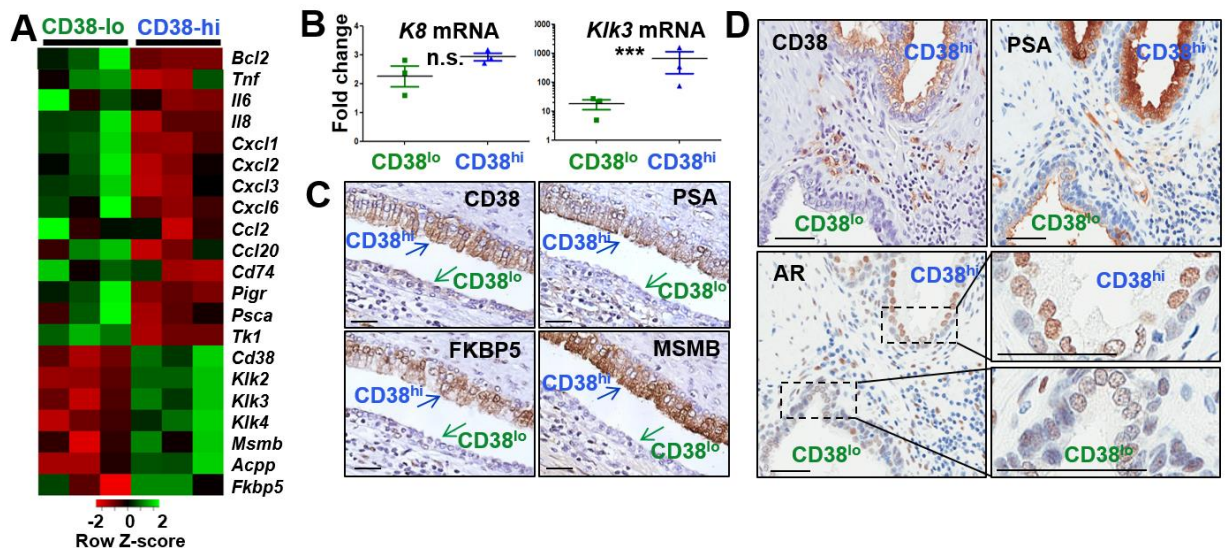


Fig. 5. Reduced AR and AR targets in CD38-lo progenitor cells. (A) Heatmap of RNA-sequencing results of CD38^{lo} luminal cells and CD38^{hi} luminal cells from three independent patients. (B) Relative expression of *Klk3* or *K8* compared to *Gapdh* in basal cells as measured by qPCR in CD38^{lo} and CD38^{hi} luminal cells isolated from three independent patient samples. Data are presented as mean \pm SEM with replicate points shown. *Klk3* is shown on a log scale. Two-tailed unpaired t test between CD38^{lo} and CD38^{hi} luminal subsets; ***p = 0.0004. (C) Immunohistochemical analysis of benign human prostate stained for CD38 and AR targets PSA, FKBP5, and MSMB. Scale bars, 100 microns. (D) Immunohistochemistry for CD38, PSA, and AR distinguishes CD38^{hi} and CD38^{lo} regions. AR expression is magnified to demonstrate reduced expression in CD38^{lo} cells. Scale bars, 50 microns

(2.2) We determined that CD38-lo progenitor cells express the anti-apoptotic factor BCL2 which has previously been implicated in castration-resistance. RNA sequencing identified elevated BCL2 expression in CD38-lo progenitors (Figure 5A). Expression was validated by western blot (Figure 6A) and immunohistochemistry (Figure 6B).

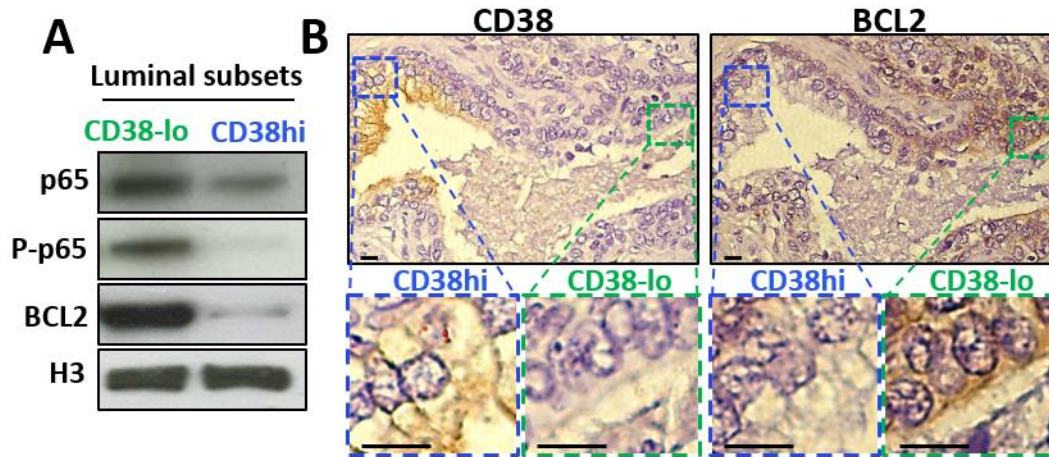


Fig. 6. Anti-apoptotic factor associated with castration-resistance (BCL2) is expressed in CD38-lo progenitor cells. (A) Western blot analysis of CD38lo and CD38hi luminal cells from a representative human prostate sample stained for total p65, phosphorylated p65 (P-p65 S536), BCL2, and histone H3 as a loading control. (B) Immunohistochemical analysis of human prostate stained for CD38 and BCL2. Insets identify regions of high CD38/low BCL2 (left) and low CD38/high BCL2 (right). Scale bars, 25 microns.

(2.3) RNA sequencing identified elevated PSCA expression (associated with castration-resistance) in CD38-lo progenitors (Figure 5A) and expression was validated by immunohistochemistry (Figure 7) in benign and cancer tissues.

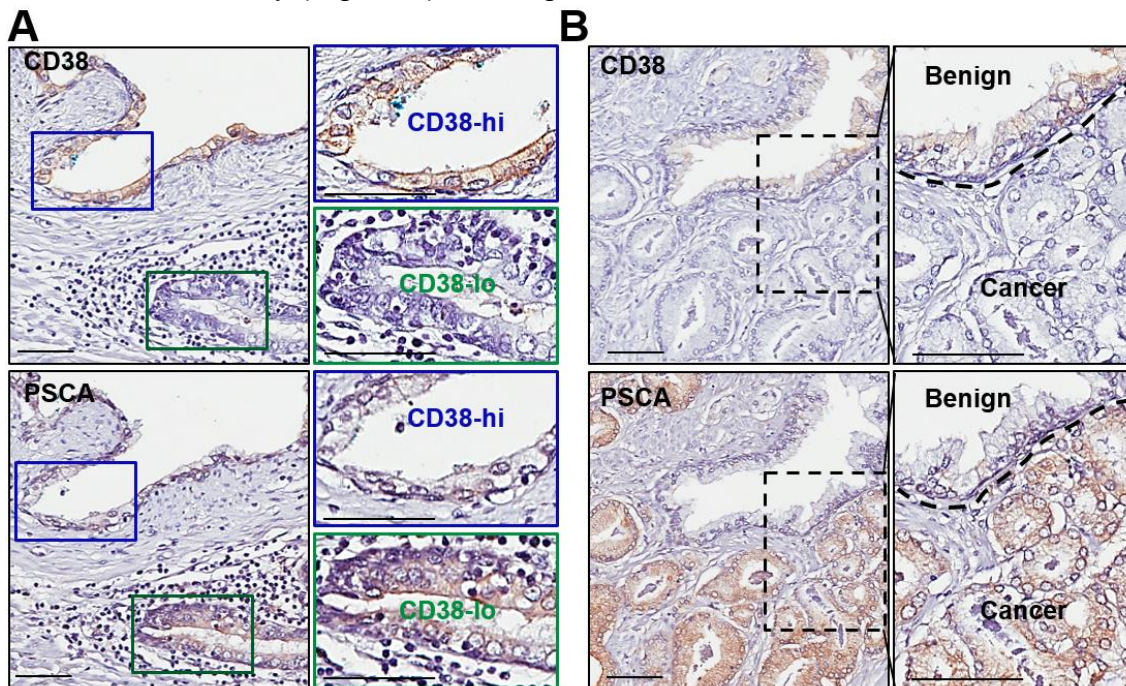


Fig. 7. Prostate stem cell antigen (PSCA) is expressed in CD38-lo progenitor cells. (A) Serial sections of benign human prostate stained for CD38 and PSCA with boxed regions to magnify distinct CD38hi PSCA-lo glands and inflammation-associated CD38lo PSCA-hi cells. (B) Serial sections of human prostate tissue stained for CD38 and PSCA with boxed region magnifying CD38hi PSCA-lo benign tissue separated by a dotted line from CD38lo PSCA-hi cancer. Scale bars, 50 microns.

(2.4) RNA sequencing identified inflammatory genes elevated in CD38-lo progenitor cells including TNF, IL6, IL8 and various chemokines (Figure 5A). Western blotting of CD38-lo and CD38-hi luminal cells identified increased phosphorylated p65 in CD38-lo progenitor cells, indicative of active NFkB signaling (Figure 6A). Immunohistochemistry confirmed NFkB activation, which is associated with castration-resistance, in CD38-lo progenitor cells (Figure 8).

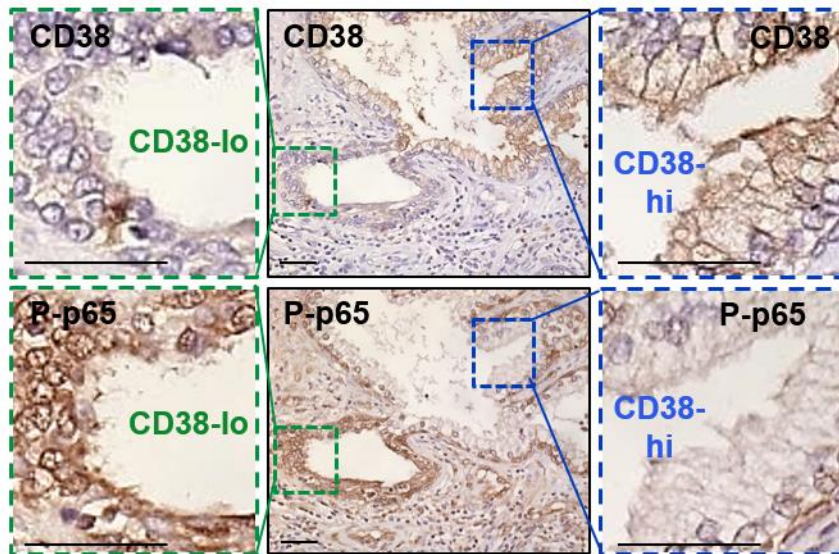


Fig. 8. NFkB activation in CD38-lo progenitor cells. Immunohistochemical analysis of benign human prostate stained for CD38 and phosphorylated p65 (S536) on serial sections indicating an inverse relationship between expression of CD38 and P-p65. Scale bars, 50 microns.

(2.5) We found that CD38-lo progenitor cells are sensitive to NFkB inhibition in an organoid-forming assay. The small molecule ACHP inhibits NFkB activation. Western blotting for phosphorylated p65 verified the activity of ACHP (Figure 9A). Organoid-forming assays revealed that CD38-lo progenitor cells are more sensitive to ACHP treatment than CD38-hi luminal cells (Figure 9B).

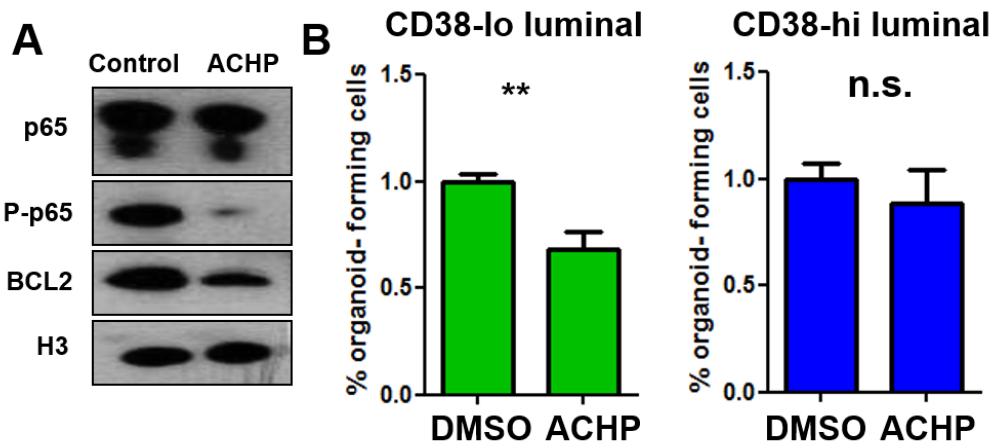


Fig. 9. NFkB inhibition depletes CD38-lo progenitor activity. (A) Western blot of CD38-lo luminal cells treated for 4 hours with 5 uM NFkB inhibitor ACHP compared to control. (B) NFkB inhibition reduces organoid-formation of epithelial subsets cells in response to 5 uM ACHP compared to control. Data represent two-tailed unpaired t-test. * $p = 0.0441$, ** $p = 0.0015$, n.s. not significant.

4) other achievements: Nothing to report

Specific Aim 3. Diagnostic potential of I/LP cells as lurker cells to predict CRPC development.

1) major activities: Transcriptional signatures of progenitor cells were evaluated with respect to patient outcome. Immunohistochemical staining for progenitor-related markers were characterized in tissue microarrays linked to patient outcome. (3.1) We found that the CD38-lo progenitor signature was associated with poor outcome in prostate cancer using the Swedish Watchful Waiting Cohort. (3.2) We found that low CD38 mRNA in prostatectomy tissues was prognostic for biochemical recurrence and metastasis. (3.3) We determined that CD38 expression is low in castration-resistant prostate cancer. (3.4) We determined that CD38 protein expression in prostate cancer is associated with recurrence.

2) specific objectives: (1) Determine whether I/LP cell transcriptional signature can predict patient prognosis. (2) Testing of candidate I/LP biomarkers on prostate cancer tissue. (3) Determine if I/LP biomarkers can predict hormone sensitivity.

3) significant results or key outcomes:

(3.1) CD38-lo luminal signature was used to evaluate associations with outcome in the Swedish Watchful Waiting Cohort. We performed a multivariate cox model adjusting for Gleason sum, age, and percentage of cancer. When including all patients in the analysis, we found the mean of the scaled expression values of the CD38-lo luminal genes is significantly ($p=0.037$) associated with all-cause mortality. When we restricted analysis to patients with Gleason scores greater than or equal to 7 and a follow-up within 5 years, the mean of the scaled expression values of the CD38-lo luminal genes is significantly ($p=0.0007$) associated with all-cause mortality. Kaplan-Meier plots are shown (Figure 10).

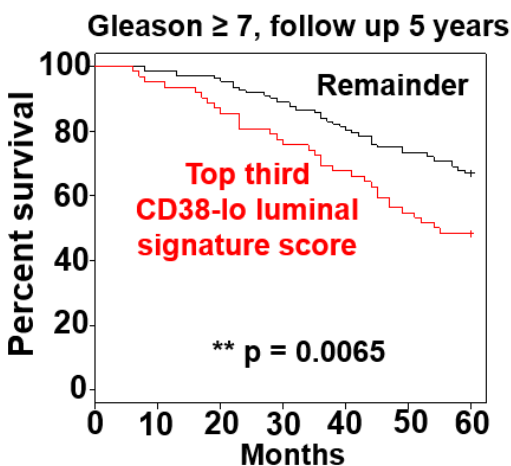


Fig. 10. CD38-lo signature is associated with outcome in prostate cancer. Survival analysis measuring overall survival of the top third tumors with CD38-lo luminal gene signature (red) compared to the remainder (black) from the Swedish Watchful Waiting cohort, restricted to patients with Gleason greater than or equal to 7 and follow up within 5 years. Log rank $p=0.0065$.

(3.2) Using the Memorial Sloan Kettering Cancer Center dataset, we found that low CD38 mRNA in prostatectomy-derived prostate cancer samples is prognostic for biochemical recurrence (Figure 11A) and metastasis (Figure 11B). Low CD38 mRNA is also prognostic for recurrence in TCGA datasets (Figure 11C).

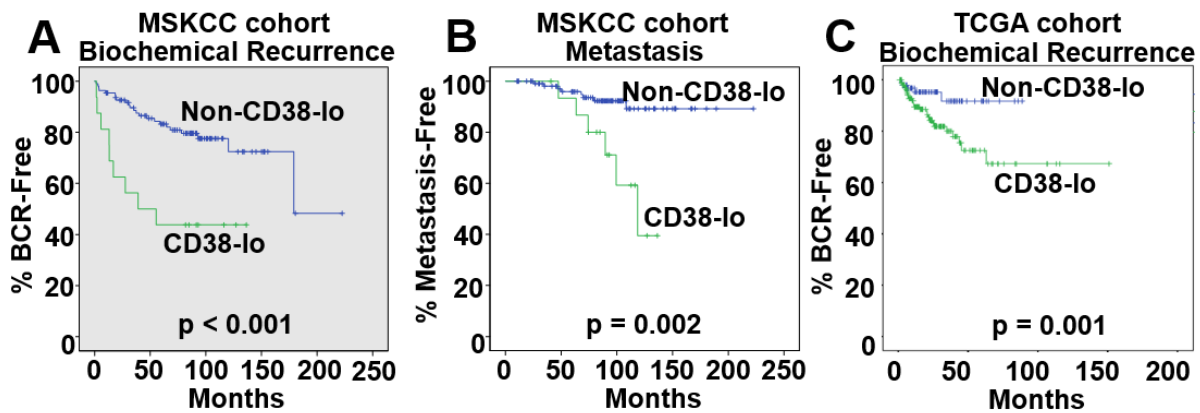


Fig. 11. CD38 mRNA is associated with outcome in prostate cancer. Survival analysis from MSKCC cohort measuring biochemical recurrence (A) and metastasis (B) for tumors containing CD38 mRNA 1 SD below the mean compared to the remainder (non-CD38lo). Log rank (Mantel Cox) $p < 0.001$ (recurrence in A) and $p = 0.002$ (metastasis in B). (C) Survival analysis from the TCGA cohort measuring biochemical recurrence comparing CD38 expression less than (CD38lo) or greater than (non-CD38lo) the mean Z score. Log rank (Mantel Cox) $p = 0.001$.

(3.3) In collaboration with Beltran and Sboner, we evaluated CD38 mRNA in tissues representing benign prostate, localized cancer, or castration-resistant prostate cancer separated into histological/molecular phenotypes of adenocarcinoma (Adeno) and small cell neuroendocrine prostate cancer (NEPC). CD38 expression is lower in CRPC than localized cancer with the lowest mean expression in NEPC (Figure 12).

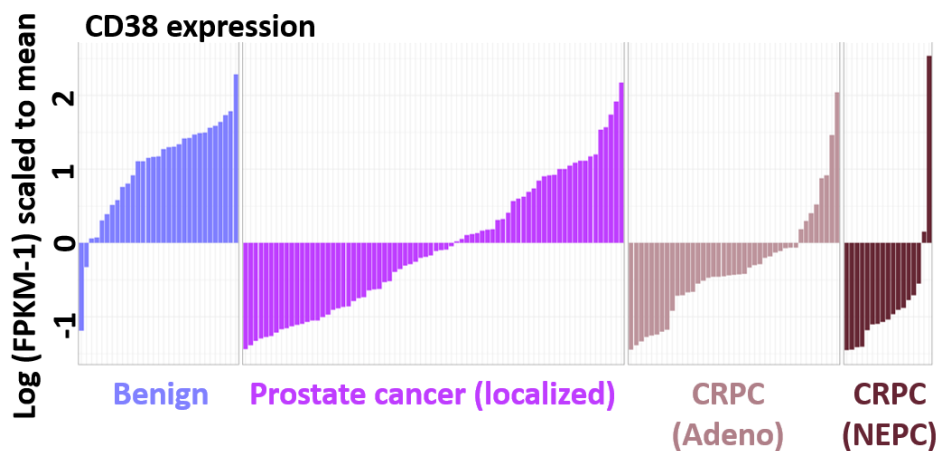
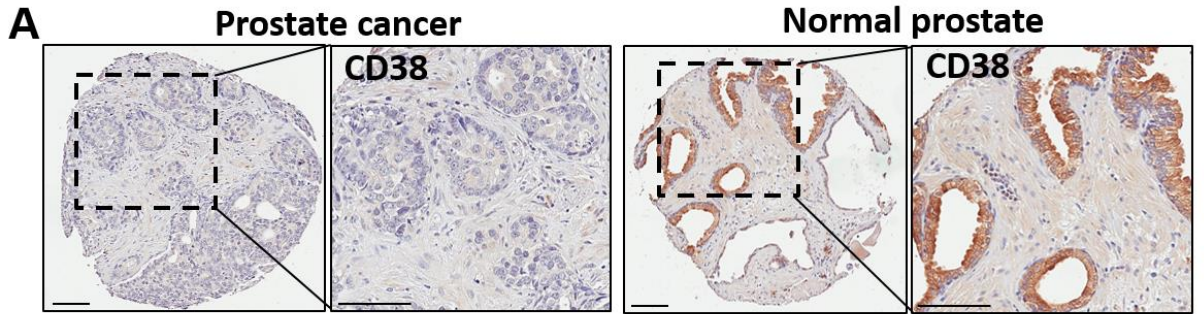


Fig. 12. Low CD38 mRNA in castration-resistant prostate cancer. Waterfall plot indicating relative CD38 mRNA expression from RNA sequencing of benign, localized prostate cancer, metastatic castration-resistant prostate cancer (CRPC) with an adenocarcinoma or neuroendocrine prostate cancer phenotype. Expression levels are presented as Log of FPKM-1 (Fragments Per Kilobase Million) values and scaled to the mean of all values shown.

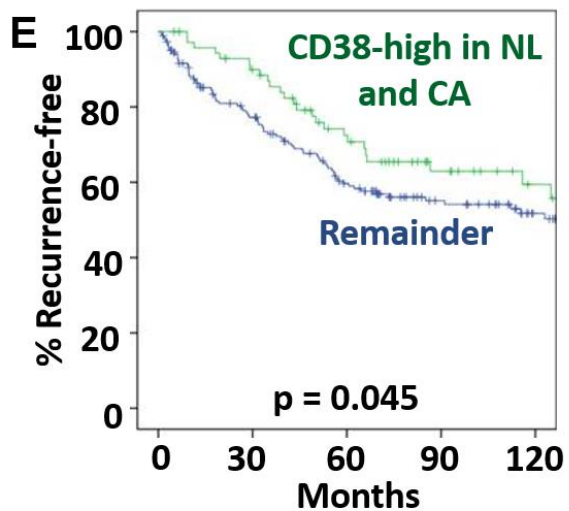
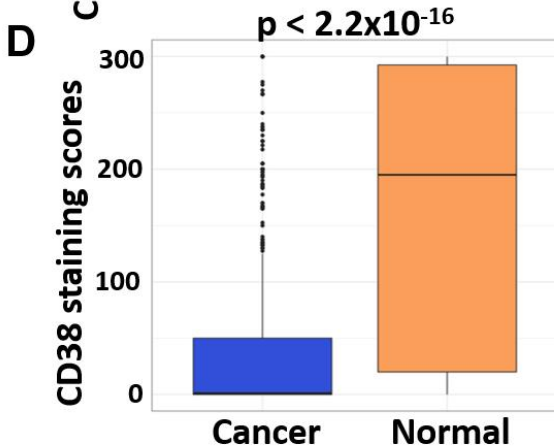
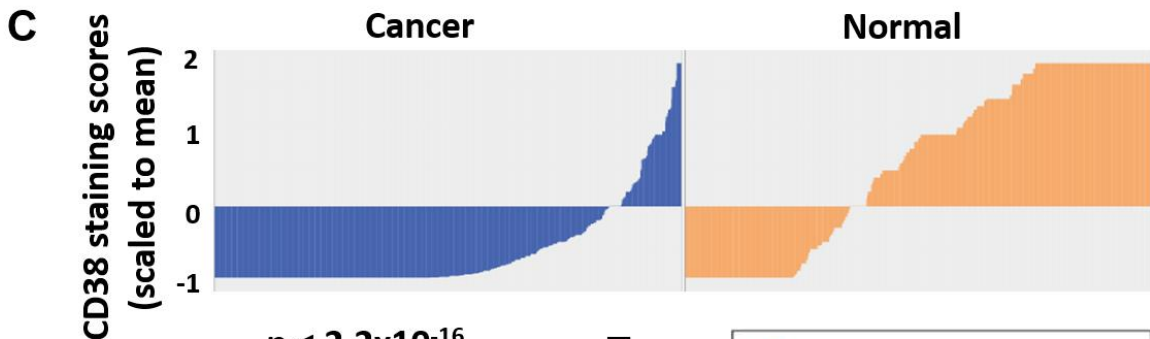
(3.4) Using immunohistochemistry on tissue microarrays with normal and cancer cores linked to patient outcome (Figure 13A), we evaluated the expression of CD38 and association with recurrence. We evaluated CD38 expression in the PSA progression tissue microarray (TMA) which contains prostatectomy tissues from greater than 500 patients who developed biochemical recurrence as well as more than 500 control patients who did not develop recurrence, matched based on age, race, pathological stage (T2, T3a, T3b, etc.) and Gleason sum. When cancer samples were split into two groups defined as above or below the median CD38 staining score, we found that higher CD38 (above the median) in cancer specimens was inversely correlated with risk of biochemical recurrence in a statistically significant manner ($p=0.031$, OR=0.76, 95% CI 0.59-0.98) (Figure 13B). Overall, cancer tissues contained significantly lower CD38 staining scores than normal prostate (Figure 13 C-D). These data are consistent with mRNA expression (Figure 11), indicating that low CD38 is associated with risk of recurrence. When combining CD38 scores in normal and cancer, we found that high expression of CD38 (above the median) in both normal and cancer tissues is significantly associated with a reduced risk of recurrence ($p=0.0176$) when compared to all other combinations (above median cancer and below median normal, below median cancer and above median normal, below median cancer and below median normal). After directly controlling for age, race, Gleason sum and pathological stage, we found that high expression of CD38 in normal and cancer tissues decreases the risk of recurrence by 21% (Odds ratio 0.79, 95% CI 0.63-0.98, $p=0.0369$) compared to patients who do not have high CD38 in both. We analyzed a separate TMA for CD38 expression and observed a similar trend, with high (above the median) expression for CD38 in both benign and cancer tissues associated with reduced risk of recurrence (Figure 13E).

Fig. 13. CD38 protein expression is associated with risk of recurrence in prostate cancer. (A) Representative images of CD38 staining in normal and cancer cores from the PSA progression tissue microarray. Scale bars represent 100 microns. (B) The distribution of the average CD38 scores between cancer/benign biopsy cores were compared using the non-parametric Wilcoxon signed-rank test. The effect of CD38 on our outcome of interest (recurrence) was assessed using conditional logistic regression, taking into account the patching factors (age, race, gleason, s-stage) and adjusting for pre-op PSA and surgical margins. Three models were run, each with the covariates mentioned above and CD38 as: above the median in PCa cores, above the median in Benign cores, and above the median in both. Statistical tests were two-sided and p-values <0.05 were considered statistically significant. (C) Waterfall plot representing composite CD38 staining scores (intensity multiplied by percentage positive cells) in normal and cancer cores scaled to the mean of all values. (D) Box plot of composite CD38 staining scores. Statistics represent Welch Two Sample t-test. (E) Kaplan-Meier plot of biochemical recurrence-free survival for patients with CD38 staining scores greater than the mean in both normal and cancer cores compared to all other patients (remainder). Breslow test statistics are shown, with a hazard ratio of 0.71.



B

Characteristic	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value
Age at surgery	0.81 (0.70 - 0.93)	0.003	0.81 (0.71 - 0.93)	0.003	0.81 (0.71 - 0.93)	0.003
Caucasian	0.09 (0.02 - 0.41)	0.002	0.10 (0.02 - 0.45)	0.003	0.11 (0.02 - 0.49)	0.004
High Gleason Sum (7,8)	1.44 (0.08 - 27.4)	0.819	1.80 (0.10 - 34.1)	0.715	1.41 (0.07 - 26.7)	0.831
S Stage	2.09 (0.26 - 16.59)	0.487	2.06 (0.25 - 17.00)	0.502	2.31 (0.25 - 21.07)	0.457
Pre-op PSA	1.01 (0.99 - 1.03)	0.227	1.01 (0.99 - 1.03)	0.282	1.01 (0.99 - 1.03)	0.274
Surgical margins +	2.33 (1.65 - 3.30)	<0.001	2.25 (1.60 - 3.17)	<0.001	2.37 (1.68 - 3.36)	<0.001
CD38 above med. CA	0.69 (0.52 - 0.91)	0.007	XX	XX	XX	XX
CD38 above med. Be	XX	XX	0.91 (0.69 - 1.20)	0.505	XX	XX
CD38 above for both	XX	XX	XX	XX	0.65 (0.48 - 0.88)	0.006



- 4) other achievements: We identified CD74 as a candidate target expressed in CD38^{lo} cells and evaluated the role of CD38 in cell proliferation. We determined that CD74 expression is inversely correlated with AR activity and PSA expression. CD74 is uniquely expressed in AR-negative, PSA-negative prostate cancer cells, further supporting an association with castration-resistance. We found that CD74 expression can be detected in a subset of tumor tissues prior to castration, suggesting that these tumors may preferentially resist castration. The role of CD38 was interrogated using inducible over-expression in benign and malignant human prostate cell lines. Over-expression of CD38 did not alter cell proliferation or intracellular NAD⁺ levels. In contrast, CD38 expression regulated extracellular NAD⁺ levels, both in cell lines and in mouse tissues (manuscript to be submitted)

What opportunities for training and professional development has the project provided? The Principal Investigator was invited to participate in the 2016 DOD Impact Meeting in Maryland and the SBUR Spring meeting at the AUA meeting in Boston during May, 2017. Opportunities for collaborations with numerous groups came out of these meetings, including co-authorship in the manuscript to be submitted soon with groups from Weill-Cornell (Beltran, Sboner) and Johns Hopkins (De Marzo, Platz, Isaacs, Yegansubramanian)

How were the results disseminated to communities of interest?

Major findings were reported in a publication in Cell Reports in addition to press releases by UCLA's Jonsson Comprehensive Cancer Center and a video on youtube: https://www.youtube.com/watch?v=2zcei5_af5M. More recent results on CD38 are written in a manuscript that will soon be submitted.

What do you plan to do during the next reporting period to accomplish the goals?
N/A (Final report)

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?

Our Cell Reports publication highlights a role for inflammation in the expansion of progenitor cells that are precursors for prostate cancer. Our more recent manuscript (which will soon be submitted) defines the expression of CD38, regulation of CD38 expression by methylation, and role of CD38 in metabolism and NAD⁺ levels.

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS

Changes in approach and reasons for change

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to Report

Changes that had a significant impact on expenditures

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS

Publications, conference papers, and presentations

Work describing the role of luminal progenitor-like cells in prostate cancer and the CD38 biomarker were reported in a 2016 Cell Reports paper. Data was presented at the Society for Basic Urological Research 2017 Spring Meeting in Boston. Recent work has been written up as a manuscript to be submitted in April 2018.

Journal publications

Liu X, Grogan T, Hieronymus H, Hashimoto T, Mottahedeh J, Cheng D, Zhang L, Huang K, Stoyanova T, Park JW, Shkhyan RO, Nowroozizadeh B, Rettig MB, Sawyers CL, Elashoff D, **Horvath S**, Huang J, Witte ON, **Goldstein AS**. Low CD38 identifies progenitor-like inflammation-associated luminal cells that can initiate human prostate cancer and predict poor outcome. Cell Reports (2016) 17:2596-2606.

Crowell PD, **Goldstein AS**. Functional evidence that progenitor cells near sites of inflammation are precursors for aggressive prostate cancer. *Mol Cell Oncol* (2017), 4:e1279723.

Mottahedeh J, Haffner MC, Grogan TR, Hashimoto T, Crowell PD, Beltran H, Sboner A, Bareja R, Esopi D, Isaacs WB, Yegansubramanian S, Rettig MB, Elashoff D, Platz EA, De Marzo AM, Teitell MA, **Goldstein AS**. CD38 is methylated in prostate cancer and regulates extracellular NAD⁺. *In Preparation*.

Books or other non-periodical, one-time publications

Nothing to Report

Other publications, conference papers, and presentations

The Principal Investigator Andrew Goldstein presented his work “Precursors for human prostate cancer” in the breakout session: Cell of Origin in Urology, Controversies and Convergences at the Society for Basic Urological Research 2017 Spring Meeting, Boston, MA. The PI also presented the work “Investigating cancer susceptibility in the prostate” at the UCLA Molecular Biology Institute Faculty Seminar Series, Los Angeles, CA in 2017. Dr. Goldstein presented his work “Regulation of prostate epithelial cells” at the 2017 UCLA Molecular, Cell & Developmental Biology Department Retreat in Lake Arrowhead, CA. Dr. Goldstein presented “Prostate epithelial progenitor cells and their metabolic regulation” in 2018 in the UCLA Jonsson Comprehensive Cancer Center Research Seminar. Finally, Dr. Goldstein was the UCLA Spotlighted Researcher and presented “Role of CD38 in prostate tumorigenesis” at the 2018 American Cancer Society Los Angeles Area Research Reception.

Website(s) or other Internet site(s)

Major findings were described in a video generated by the UCLA Jonsson Comprehensive Cancer Center: https://www.youtube.com/watch?v=2zcei5_af5M

Technologies or techniques

Nothing to Report

Inventions, patent applications, and/or licenses

Nothing to Report

Other Products

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Andrew Goldstein

Project Role: PI

Nearest Person Month Worked: 9

Contribution to Project: Supervision of the project, immunohistochemical analysis.

Name: Takao Hashimoto

Project Role: Technician/Staff Research Associate

Nearest Person Month Worked: 2

Contribution to Project: Performed immunohistochemical staining

Name: Xian Liu

Project Role: Technician/Staff Research Associate

Nearest Person Month Worked: 14

Contribution to Project: Organoid-forming assays, cell sorting, small molecule inhibition studies

Name: Steve Horvath

Project Role: Co-investigator

Nearest Person Month Worked: 1.2

Contribution to Project: Statistics and bioinformatics

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Yes, the PI has acquired new funding from the American Cancer Society to further study the role of CD38 in prostate cancer.

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

N/A

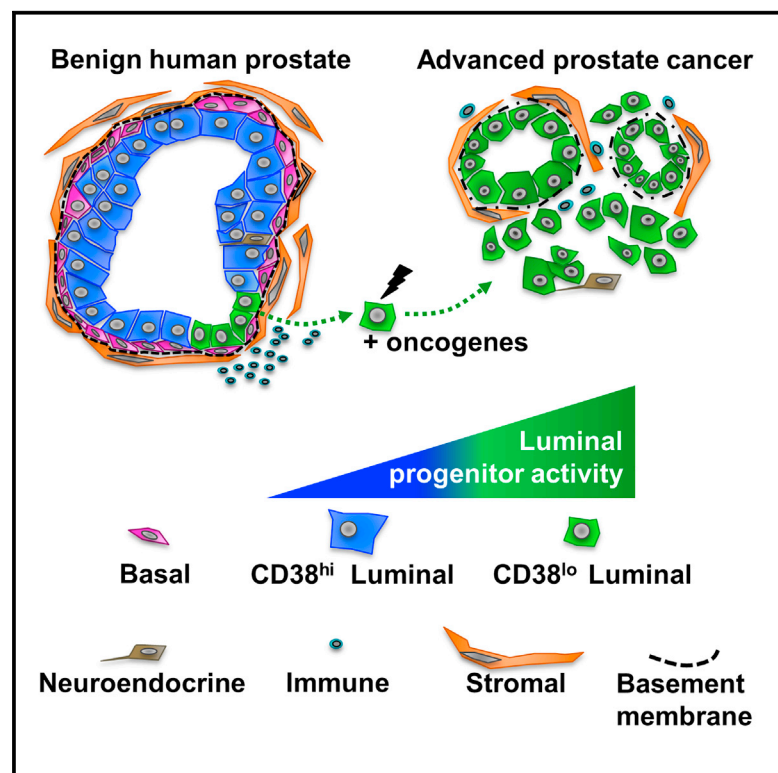
9. APPENDICES

Cell Reports Article (2016)

Molecular Cellular Oncology "Author's view" (2017)

Low CD38 Identifies Progenitor-like Inflammation-Associated Luminal Cells that Can Initiate Human Prostate Cancer and Predict Poor Outcome

Graphical Abstract



Authors

Xian Liu, Tristan R. Grogan, Haley Hieronymus, ..., Jiaoti Huang, Owen N. Witte, Andrew S. Goldstein

Correspondence

agoldstein@mednet.ucla.edu

In Brief

Chronic inflammation of the prostate is a risk factor for cancer, but how inflammation increases disease risk remains poorly defined. Liu et al. show that luminal progenitor cells expressing low levels of CD38 are expanded around inflammation, and these progenitors are target cells that can initiate human prostate cancer.

Highlights

- Low expression of CD38 enriches for luminal progenitor cells in human prostate
- CD38^{lo} luminal cells are localized in proximity to prostatic inflammation
- CD38^{lo} luminal cells are target cells for aggressive human prostate cancer
- Low CD38 in prostate cancer is prognostic for biochemical recurrence and metastasis

Accession Numbers

GSE89050



Low CD38 Identifies Progenitor-like Inflammation-Associated Luminal Cells that Can Initiate Human Prostate Cancer and Predict Poor Outcome

Xian Liu,¹ Tristan R. Grogan,³ Haley Hieronymus,⁴ Takao Hashimoto,¹ Jack Mottahedeh,¹ Donghui Cheng,⁵ Lijun Zhang,² Kevin Huang,² Tanya Stoyanova,^{6,15} Jung Wook Park,⁶ Ruzanna O. Shkhyan,¹ Behdokht Nowroozizadeh,⁷ Matthew B. Rettig,^{8,9,10,11} Charles L. Sawyers,⁴ David Elashoff,^{3,11} Steve Horvath,^{12,13} Jiaoti Huang,^{5,7,11,16} Owen N. Witte,^{2,5,6,11,14} and Andrew S. Goldstein^{1,5,10,11,17,*}

¹Molecular, Cell and Developmental Biology, University of California, Los Angeles, Los Angeles, CA 90095, USA

²Molecular and Medical Pharmacology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA 90095, USA

³Department of Medicine Statistics Core, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA 90095, USA

⁴Programs in Human Oncology and Pathogenesis, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA

⁵Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, University of California, Los Angeles, Los Angeles, CA 90095, USA

⁶Microbiology, Immunology and Molecular Genetics, University of California, Los Angeles, Los Angeles, CA 90095, USA

⁷Pathology and Laboratory Medicine, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA 90095, USA

⁸Division of Hematology-Oncology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA 90095, USA

⁹VA Greater Los Angeles Healthcare System, Los Angeles, CA 90024, USA

¹⁰Urology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA 90095, USA

¹¹Jonsson Comprehensive Cancer Center, University of California, Los Angeles, Los Angeles, CA 90095, USA

¹²Human Genetics, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA 90095, USA

¹³Biostatistics, UCLA Fielding School of Public Health, University of California, Los Angeles, Los Angeles, CA 90095, USA

¹⁴Howard Hughes Medical Institute, University of California, Los Angeles, Los Angeles, CA 90095, USA

¹⁵Present address: Department of Radiology, Canary Center at Stanford for Cancer Early Detection, Stanford University School of Medicine, Palo Alto, CA 94304, USA

¹⁶Present address: Department of Pathology, Duke University School of Medicine, Durham, NC 27710, USA

¹⁷Lead Contact

*Correspondence: agoldstein@mednet.ucla.edu

<http://dx.doi.org/10.1016/j.celrep.2016.11.010>

SUMMARY

Inflammation is a risk factor for prostate cancer, but the mechanisms by which inflammation increases that risk are poorly understood. Here, we demonstrate that low expression of CD38 identifies a progenitor-like subset of luminal cells in the human prostate. CD38^{lo} luminal cells are enriched in glands adjacent to inflammatory cells and exhibit epithelial nuclear factor κ B (NF- κ B) signaling. In response to oncogenic transformation, CD38^{lo} luminal cells can initiate human prostate cancer in an *in vivo* tissue-regeneration assay. Finally, the CD38^{lo} luminal phenotype and gene signature are associated with disease progression and poor outcome in prostate cancer. Our results suggest that prostate inflammation expands the pool of progenitor-like target cells susceptible to tumorigenesis.

INTRODUCTION

An inflammatory microenvironment is a critical component driving tumorigenesis, from cancer initiation to metastasis to

end-stage treatment-resistant lethal disease (Das Roy et al., 2009; Gurel et al., 2014; Liu et al., 2013; Wang et al., 2015). In many adult tissues, cancers originate in sites of chronic inflammation (Coussens and Werb, 2002). It is hypothesized that sustained proliferative signals from inflammatory cells can cooperate with oncogenic events to promote tumorigenesis (De Marzo et al., 2007). Mouse models have been developed that recapitulate features of inflammation in the tumor microenvironment and demonstrate a role for defined immune cell types and inflammatory cytokines in cancer initiation and progression (Ammirante et al., 2010; Garcia et al., 2014). Few studies have investigated the functional consequences of inflammation in human epithelial tissues.

Chronic inflammation of the prostate is a risk factor for aggressive prostate cancer (Gurel et al., 2014; Sfanos and De Marzo, 2012; Shafique et al., 2012), as men with chronic inflammation in benign tissues have greater than double the risk for developing high-grade disease compared to men with no inflammation in their benign biopsy cores (Gurel et al., 2014). Groundbreaking work from De Marzo and colleagues has defined a series of histological changes associated with chronic inflammation in the human prostate known as proliferative inflammatory atrophy (PIA) as a likely precursor for prostate cancer (De Marzo et al., 1999, 2007). In PIA, the luminal epithelial layer in close proximity to infiltrating immune cells is

described as having an atrophic appearance with an increased proliferative index, suggesting a regenerative response (De Marzo et al., 2003). Luminal cells associated with PIA exhibit reduced androgen signaling and increased expression of the anti-apoptotic factor BCL2 (De Marzo et al., 1999, 2003). PIA cells are thought to exhibit an intermediate state of differentiation between basal and luminal cells and are predicted to serve as target cells in prostate tumorigenesis (van Leenders et al., 2003). Several groups have modeled inflammation in the mouse prostate using a variety of approaches, including bacterial infection (Elkhwaji et al., 2009; Khalili et al., 2010; Kwon et al., 2014), high-fat diet (Kwon et al., 2016), and adoptive transfer of prostate-targeting T cells (Haverkamp et al., 2011), demonstrating that prostatic inflammation is associated with increased epithelial proliferation. However, mouse models may not recapitulate the complex environment of aged human prostate tissues exposed to chronic inflammation for years prior to the development of prostate cancer (Gurel et al., 2014).

Lineage tracing in the mouse has demonstrated that both basal and luminal cells are sufficient to initiate prostate cancer following *Pten* deletion, with differences in tumor outcome depending on the genetic background and status of *Nkx3-1* (Choi et al., 2012; Lu et al., 2013; Wang et al., 2013). Luminal cells can initiate murine prostate cancer in diverse genetically engineered mouse models (Wang et al., 2014), while purified basal cells can respond to a range of oncogene combinations to generate murine tumors using a tissue-regeneration approach (Lawson et al., 2010). In the human prostate, we and others have demonstrated that basal cells isolated from benign human prostate can give rise to tumors following oncogenic transformation (Goldstein et al., 2010; Stoyanova et al., 2013; Taylor et al., 2012). To date, luminal cells in the human prostate have only been shown to initiate indolent-like tumors with limited proliferation (Park et al., 2016), perhaps due to the low rate of proliferation among luminal cells (De Marzo et al., 1999). In contrast, luminal cell proliferation is increased in regions associated with inflammation in human prostate (De Marzo et al., 1999), suggesting that luminal cells isolated from regions with inflammation may exhibit progenitor cell features and give rise to aggressive prostate cancer.

Here, we report that expression of the luminal cell marker CD38 is heterogeneous in the human prostate. We used low expression of CD38 to isolate a subset of p63-negative, keratin 14 (K14)-negative, keratin 18 (K18)-positive luminal cells from primary benign human prostate with unique functional and molecular properties compared to CD38^{hi} luminal cells. Human prostate CD38^{lo} CD38^{lo} luminal cells are expanded in regions with inflammation, express markers of PIA, and are enriched for progenitor activity. In human tissues, CD38 is inversely correlated with prostate cancer progression and low CD38 mRNA in tumors is prognostic for biochemical recurrence and metastasis. Finally, CD38^{lo} luminal cells can initiate prostate cancer in response to oncogenic transformation using a human tissue regeneration model in immune-deficient mice. These collective results support inflammation-associated luminal cells as a target cell for aggressive prostate cancer.

RESULTS

Differential Expression of CD38 Enables Isolation of Two Distinct Luminal Subsets

CD38 is reported as a marker of luminal cells in the human prostate (Kramer et al., 1995), and gene expression studies demonstrate that CD38 mRNA can distinguish basal from luminal cells (Smith et al., 2015). We measured cell-surface expression of CD38 by flow cytometry and confirmed that within the EpCAM⁺ CD45⁻ epithelial fraction, CD38 is expressed by CD26⁺ luminal cells, but not by CD49f^{hi} basal cells (Figure 1A). Upon performing immunohistochemical staining for CD38 in human prostate tissue, we noted heterogeneous staining within the luminal layer, including areas where CD38 expression was reduced within individual glands (Figure 1B).

We set out to viably isolate luminal cells based on variations in surface expression of CD38 using fluorescence-activated cell sorting (FACS). After removing CD45⁺ immune cells and gating on EpCAM⁺ epithelial cells, we observed three cell populations based on expression of CD49f and CD38 (Figure 1C) in preparations of dissociated cells isolated from benign human prostate tissue. Intracellular flow cytometry revealed that both CD49f^{lo} CD38^{hi} and CD49f^{lo} CD38^{lo} subsets expressed high levels of the luminal marker K18 (Figures 1D and S1A). Western blot analysis demonstrated that basal markers p63 and K14 are not expressed in either luminal subset (Figure 1E). We noted elevated expression of keratin 5 (K5), a marker of a proposed intermediate-type luminal cell (van Leenders et al., 2003), in CD38^{lo} compared to CD38^{hi} luminal cells (Figure S1B). Following cell isolation, cell diameters were measured to examine differences in size among the three epithelial populations. CD38^{lo} luminal cells demonstrated an average diameter intermediate between basal and CD38^{hi} luminal cells (Figure 1F). These findings indicate that CD38 stratifies luminal cells into two distinct subpopulations. Importantly, CD38^{lo} luminal cells do not express CD26 by flow cytometry (Figure S1C). These data indicate that CD38^{lo} luminal cells are distinct from CD26⁺ luminal cells and represent a population not previously investigated in prior studies (Karthauss et al., 2014; Park et al., 2016; Stoyanova et al., 2013).

CD38^{lo} Luminal Cells Are Enriched for Progenitor Activity Compared to CD38^{hi} Luminal Cells

In order to test the functional capacity of distinct luminal subsets, it is important to remove any potential contaminating basal cells that are highly clonogenic in progenitor assays (Goldstein et al., 2008; Karthauss et al., 2014; Park et al., 2016). We used a double-sorting approach to isolate highly purified preparations of CD38^{lo} and CD38^{hi} luminal cells from benign human prostate tissue and confirmed an absence of basal cells based on lack of expression of CD49f (Figure 2A) and p63 (Figures S2A and S2B). In a two-dimensional colony-forming assay and a three-dimensional sphere-forming assay (Lawson et al., 2010; Lukacs et al., 2010), CD38^{lo} luminal cells exhibited progenitor activity intermediate between the highly clonogenic basal cells and the CD38^{hi} luminal cells (Figures 2B and 2C). In a culture system adapted for the growth of human prostate-like tissue in vitro (Karthauss et al., 2014), CD38^{lo} luminal cells were highly (4- to 5-fold) enriched for organoid formation compared to CD38^{hi} luminal cells

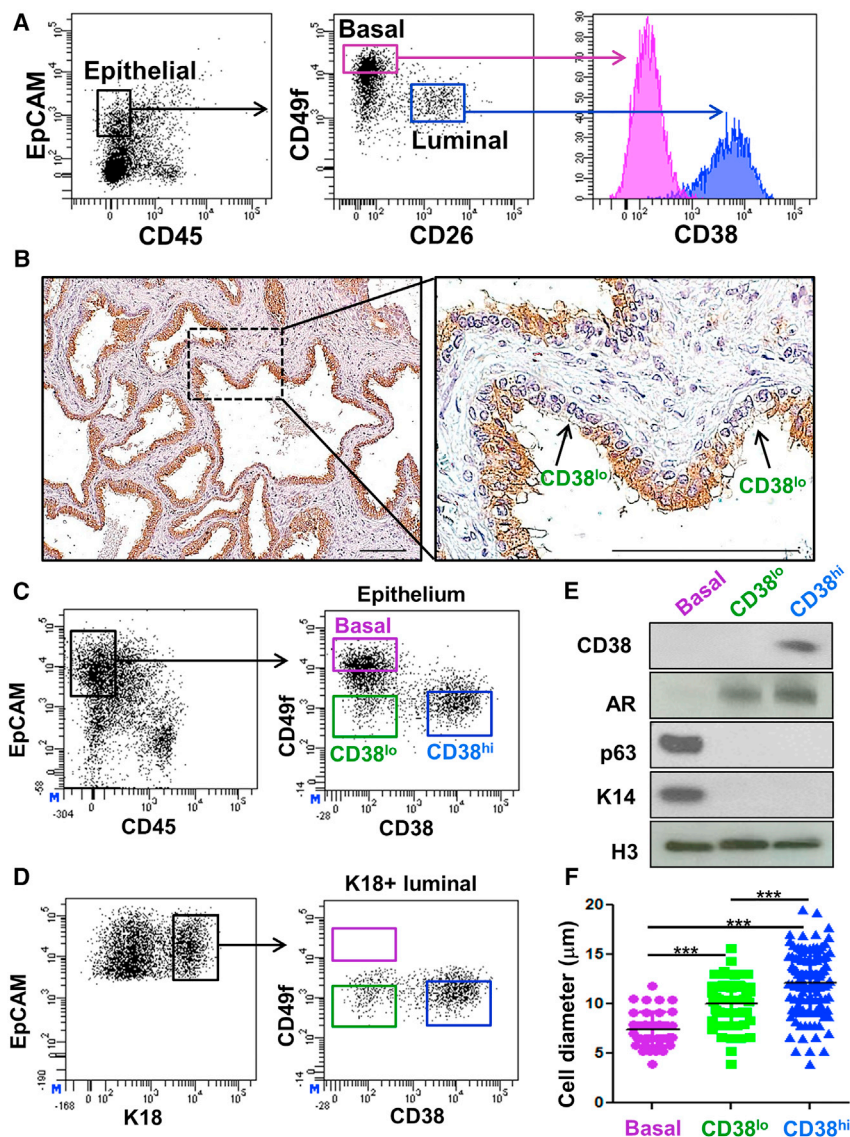


Figure 1. Differential Expression of CD38 Enables Isolation of Discrete Luminal Subsets from Fresh Human Prostate Tissue

(A) Fluorescence-activated cell sorting analysis of dissociated benign human prostate cells demonstrates CD38 expression in EpCAM⁺ CD45⁻ CD26⁺ luminal cells, but not in EpCAM⁺ CD45⁻ CD49f⁻hi basal cells.

(B) Immunohistochemical analysis of benign human prostate glands stained with anti-CD38 antibodies. Boxed region is magnified in the right. Arrows identify rare CD38^{lo} cells lining the gland. Scale bars, 50 μ m.

(C) Fluorescence-activated cell sorting analysis of dissociated benign human prostate cells gated on EpCAM⁺ CD45⁻ cells and further stained with antibodies against CD49f and CD38.

(D) Intracellular flow cytometry for Keratin 18 (K18) and surface staining for CD38 and CD49f. Gating on K18⁺ luminal cells demonstrates two luminal populations in right plot.

(E) Western blot analysis of three epithelial subsets from representative patient sample stained with antibodies against CD38, AR, p63, keratin 14 (K14), and histone H3 as a loading control.

(F) Sorted cells were counted in a hemocytometer, and average cell diameter (in microns) was quantified for each population from multiple patient samples.

Data represent mean \pm SEM with replicate points shown. Newman-Keuls multiple comparison test with *** p < 0.0001.

(Figure 2D). CD38^{lo} luminal cell-derived organoids contained cells expressing both basal (K5 and p63) and luminal (K8) markers (Figures 2E and S2C), similar to what has been reported for CD26⁺ luminal cells (Karthaus et al., 2014). When double-sorted cells were plated at single-cell density and visually confirmed, 5% (9/177) of single CD38^{lo} luminal cells were capable of generating organoids (Figure 2F), which is considerably greater than the rate of CD38^{hi} luminal cells (1/142) and the 1% rate reported for CD26⁺ luminal cells (Karthaus et al., 2014; Park et al., 2016).

CD38^{lo} Luminal Cells Exhibit an Inflammatory Signature

To investigate potential mechanisms regulating differential progenitor activity, we performed RNA sequencing (RNA-seq) and microarray analysis. We generated an RNA-seq signature comprised of 449 genes greater than 2 fold-enriched in CD38^{lo} luminal cells compared to CD38^{hi} luminal cells and greater

than 1.5-fold enriched compared to basal cells (Table S1). We found that CD38^{lo} luminal cells express elevated levels of BCL2, a gene previously associated with PIA (De Marzo et al., 1999) (Figure 3A). A number of inflammatory-related genes including cytokines (*Tnf*, *Il6*, and *Il8*) and chemokines (*Cxcl1*, *Cxcl2*, *Cxcl3*, *Cxcl6*, *Ccl2*, and *Ccl20*) are enriched in CD38^{lo} luminal cells (Figure 3A). We performed pathway analysis using DAVID Bioinformatics (Huang et al., 2009) and found the most significant keywords included “inflammatory signaling” and “immune response” (Figure 3B). The significant pathways and inflammatory-related genes were confirmed to be upregulated in CD38^{lo} luminal cells by microarray analysis. We confirmed activation of the nuclear factor κ B (NF- κ B) pathway at the protein level based on phosphorylated p65 and expression of BCL2 by western blot (Figure 3C). In tissue sections, we found that phosphorylated p65 was highly expressed in CD38^{lo} luminal cells in a pattern inversely correlated with CD38 (Figure 3D). Ingenuity pathway analysis identified tumor necrosis factor alpha (TNF) as a likely upstream regulator of CD38^{lo} luminal cells. We confirmed that TNF is expressed in CD38^{lo} cells but absent from CD38^{hi} luminal cells by immunohistochemistry (Figure S3A), suggesting that TNF may activate NF κ B signaling in CD38^{lo} cells. We observed a diminution of p65 phosphorylation and reduced expression of BCL2 in

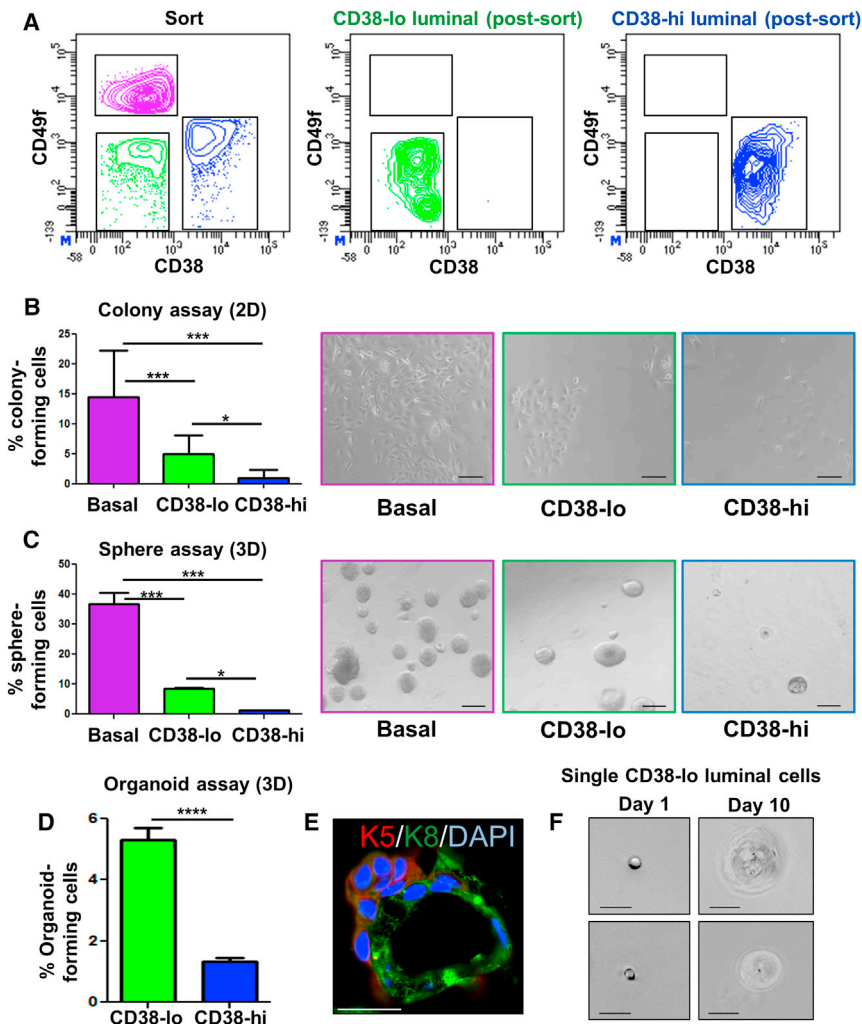


Figure 2. CD38^{lo} Cells Exhibit Greater Progenitor Activity than CD38^{hi} Luminal Cells In Vitro

(A) Flow cytometry analysis for CD49f and CD38 dissociated human prostate epithelium as gated during the sort (left), and post-sort analysis of CD38^{lo} and CD38^{hi} luminal cells prior to in vitro culture.

(B and C) Primary human benign prostate epithelial cells (EpCAM⁺ CD45⁻) were sorted into basal cells, CD38^{lo} and CD38^{hi} luminal subsets and plated for in vitro colony and sphere forming assays. Results from in vitro two-dimensional colony-forming assay (B) and three-dimensional sphere-forming assay (C) measured 10–14 days after plating benign human prostate epithelial subsets. Data represent pooled experiments from three independent patients. Mean ± SEM is shown. Statistics represent Newman-Keuls multiple comparison test with *p < 0.05, ***p < 0.0005. Representative images of outgrowths from each epithelial subset are taken at 10–14 days. Scale bars, 250 μm.

(D) CD38^{lo} luminal cells generate significantly more organoids than CD38^{hi} luminal cells, four replicates per sample, representative of five independent patient samples. Statistics represent Two-tailed unpaired t test with ****p < 0.0001.

(E) Representative staining of CD38^{lo} luminal cell-derived organoids for K5, K8, and DAPI nuclear counterstain. Scale bars, 50 μm.

(F) Representative images of organoids forming from single CD38^{lo} luminal cells taken at day 1 and day 10. Scale bars, 100 μm.

CD38^{lo} cells following treatment with the NF-κB pathway inhibitor ACHP (Figure S3B). In a functional assay, ACHP treatment blocked the organoid-forming progenitor activity of CD38^{lo} luminal cells to a greater extent than other epithelial subsets (Figure S3C).

CD38^{lo} Luminal Cells Are Expanded in Glands Adjacent to Inflammation

Based on their gene expression profiling, including genes associated with PIA and inflammation, we investigated expression of CD38 in regions containing inflammatory infiltration (Figure 3E). Staining for CD38 and CD45 on serial slides indicates that CD38 expression is reduced adjacent to CD45⁺ cells (Figure 3F). We stained for a panel of immune cell subsets and found evidence of CD4⁺ and CD8⁺ T cells, CD11c⁺ and CD68⁺ myeloid cells, and CD20⁺ B cells adjacent to regions of the gland with low expression of CD38 (Figure S3D). In order to better define the relationship between CD38 expression and inflammation, we stratified glands into three categories: normal, inflamed, and atrophic, as classified by an expert pathologist. Inflamed glands were identified by pathologic criteria and confirmed with CD45

staining. Atrophic glands were identified by pathologic criteria and generally measured less than 20 μm from the basement membrane to the lumen in contrast to normal glands (Figure S3E). Loss of CD38 expression was measured in 95% (38/40) of inflamed glands, 88% (35/40) of atrophic glands, and only 10% (4/40) of normal glands from representative patient samples. Similar results were obtained for TNF, with strong expression in inflamed (38/40) and atrophic glands (33/40) with rare expression in normal glands (1/40). We further explored the connection between immune cells and CD38^{lo} luminal cells by flow cytometry in benign human prostate tissues from 29 distinct patient samples. We found a statistically significant association between the percentage of CD45⁺ immune cells and the proportion of luminal cells exhibiting a CD38^{lo} surface phenotype (Figures 3G and S3F).

Reduced Androgen Signaling in CD38^{lo} Luminal Cells

CD38^{lo} luminal cells express lower levels of *Klk3*, the gene for prostate-specific antigen (PSA) and other androgen-target genes (*Klk2*, *Klk4*, *Msmb*, *Acpp*, and *Fkbp5*) (Figure 3A), consistent with reduced androgen signaling observed in PIA lesions (De Marzo et al., 1999). Similar results were observed by microarray analysis. qPCR confirmed differences in *Klk3* mRNA between the luminal subsets (Figure 4A). In contrast, levels of the luminal gene *K8* were not significantly different (Figure 4A). Using

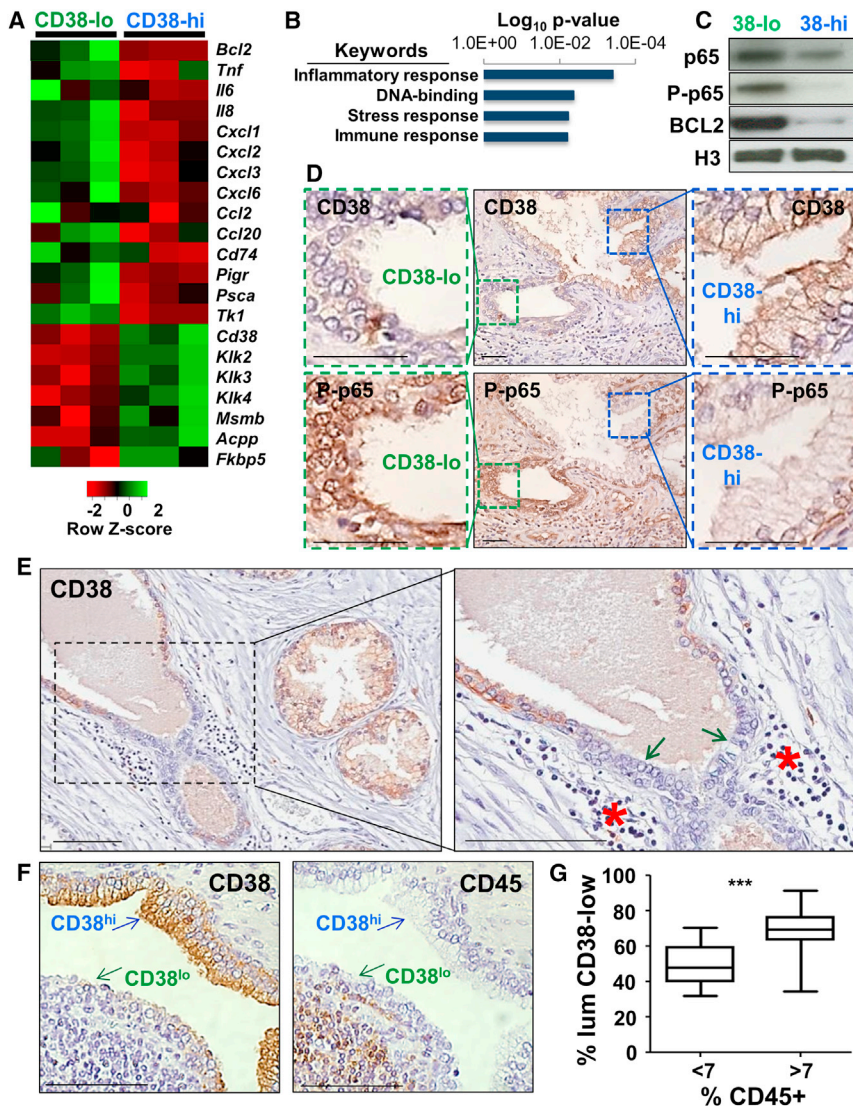


Figure 3. CD38^{lo} Luminal Cells Are Associated with Inflammation

(A) Heatmap of RNA-sequencing results of CD38^{lo} luminal cells and CD38^{hi} luminal cells from three independent patients.

(B) DAVID bioinformatics analysis (SP_PIR_KEYWORDS) of the CD38^{lo} luminal gene signature with log(10) p values plotted.

(C) Western blot analysis of CD38^{lo} and CD38^{hi} luminal cells from a representative benign human prostate sample stained for total p65, phosphorylated p65 (P-p65 S536), BCL2, and histone H3 as a loading control.

(D) Immunohistochemical analysis of benign human prostate stained for CD38 and phosphorylated p65 (S536) on serial sections indicating an inverse relationship between expression of CD38 and P-p65. Scale bars, 50 μ m.

(E) Immunohistochemical analysis of benign human prostate glands stained with anti-CD38 antibodies. Boxed regions are magnified in the bottom row. Arrows denote regions of epithelium with low expression of CD38 in close proximity to red asterisk identifying areas with immune cell infiltration. Scale bars, 100 μ m.

(F) Immunohistochemistry on serial slides stained for CD38 and CD45. Scale bars, 100 μ m.

(G) Increased proportion of luminal cells with CD38^{lo} phenotype from 29 samples plotted as CD45⁺ cells less than or greater than 7% of total prostate. Statistics represent two-tailed t test; ***p = 0.0003.

immunohistochemistry, we confirmed diminished expression of androgen targets PSA, FKBP5, and MSMB in CD38^{lo} luminal cells at the protein level (Figure 4B). Androgen receptor (AR) target NKX3-1 was also reduced in CD38^{lo} luminal cells (Figure S4). We hypothesized that low androgen signaling in CD38^{lo} luminal cells may be due to low levels of AR. While AR is expressed in CD38^{lo} cells, protein levels are reduced compared to CD38^{hi} luminal cells (Figures 1E and 4C).

CD38^{lo} Luminal Phenotype Is Associated with Disease Progression and Poor Outcome

CD38 expression was previously reported to be reduced in a small cohort of prostate cancer specimens compared to benign prostate tissues (Kramer et al., 1995). We examined expression of CD38 in a tissue microarray containing tissue cores from 267 patients with prostate cancer (Gollapudi et al., 2013) (Table S2) and found the highest protein expression of CD38 in benign glands, with reduced expression in PIN and prostate cancer

CD38 expression in cancer specimens was not statistically significant (Table S4).

Using the Memorial Sloan Kettering Cancer Center (MSKCC) dataset (Hieronymus et al., 2014; Taylor et al., 2010), we found that low CD38 mRNA in prostatectomy-derived prostate cancer samples is prognostic for biochemical recurrence and metastasis (Figures 5C and 5D) in a statistically significant manner (Table S5). Importantly, low CD38 mRNA status is statistically significantly associated with both biochemical recurrence and metastasis after correction for Stephenson nomogram score (Stephenson et al., 2005), which includes both clinical and pathological variables (Table S5). Low CD38 mRNA is also prognostic for biochemical recurrence in the Cancer Genome Atlas (TCGA) dataset (Network, 2015) (Figure 5E). Upon stratifying tumors based on genotype, we found that low CD38 mRNA expression was associated with SPINK1 outlier status, but not ETS-related gene (ERG) rearrangements, in the MSKCC cohort (Figure S6A).

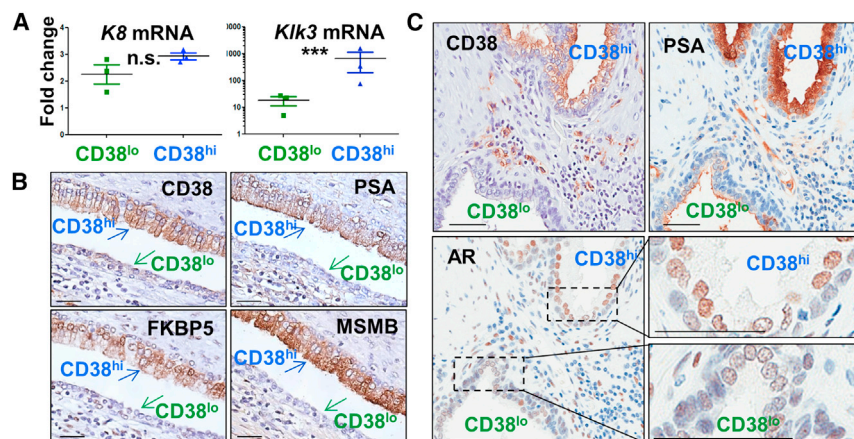


Figure 4. CD38^{lo} Luminal Cells Exhibit Reduced Androgen Signaling

(A) Relative expression of *Klk3* or *K8* compared to *Gapdh* in basal cells as measured by qPCR of basal, CD38^{lo}, and CD38^{hi} luminal cells isolated from three independent patient samples. Data are presented as mean ± SEM with replicate points shown. *Klk3* is shown on a log scale. Two-tailed unpaired t test between CD38^{lo} and CD38^{hi} luminal subsets; ***p = 0.0004.

(B) Immunohistochemical analysis of benign human prostate stained for CD38 and AR targets PSA, FKBP5, and MSMB. Scale bars, 100 μm.

(C) Immunohistochemistry for CD38, PSA, and AR distinguishes CD38^{hi} and CD38^{lo} regions. AR expression is magnified to demonstrate reduced expression in CD38^{lo} cells. Scale bars, 50 μm.

To determine whether the CD38^{lo} luminal gene signature could provide value in predicting patient survival, we turned to a Swedish watchful waiting cohort containing outcome data for 281 men (Sboner et al., 2010). The mean of the scaled expression values of the CD38^{lo} luminal genes was significantly (p = 0.037) associated with all-cause mortality after adjusting for Gleason sum, age, and cancer percentage (Table S6A). When we analyzed the patients most at risk for prostate cancer-related mortality, with a Gleason score greater than or equal to 7 and follow-up within 5 years, we found a statistically significant difference (p = 0.007) between patients whose expression values lie in the top third (CD38^{lo} luminal-like) versus all others (Figure S6B; Table S6C). The statistical significance of the CD38^{lo} signature within this restricted group reached p = 0.0007 after adjusting for Gleason sum, age, and cancer percentage (Table S6B). The CD38^{lo} luminal gene signature is inversely correlated with the AR signature score (Hieronymus et al., 2006) in prostate tumor specimens in a statistically significant manner in multiple cohorts (Figure S6C). We confirmed that the effect of the CD38^{lo} signature was independent of the AR signature (Hieronymus et al., 2006; Nelson et al., 2002) and a previously reported immune signature (Jin et al., 2014) (Tables S6D and S6E).

CD38^{lo} Luminal Cells Express the Therapeutic Target PSCA

While CD38 expression is low in the most aggressive tumors, we reasoned that genes in the CD38^{lo} luminal cell signature may be expressed in advanced disease and might serve as positive markers of this cell population. We used immunohistochemistry to investigate expression of prostate stem cell antigen (PSCA), a therapeutic target expressed in aggressive, metastatic, and castration-resistant prostate cancer (Gu et al., 2000). *PscA* is enriched in CD38^{lo} cells (Figure 3A) but has not been previously associated with PIA. In tissue sections, PSCA expression was observed more commonly in inflamed (28/40) glands than atrophic (15/40) or benign (12/40) glands, in an expression pattern inversely correlated with CD38 (Figure 6A). In some regions, these two markers can distinguish benign glands (CD38^{hi} PSCA-low) from cancer (CD38^{lo} PSCA-hi) (Figure 6B). We hy-

pothesized that CD38^{lo} PSCA-hi cancers may arise from the transformation of CD38^{lo} PSCA-hi luminal cells.

CD38^{lo} Luminal Cells Can Regenerate Glands and Initiate Human Prostate Cancer

To determine the functional capacity of CD38^{lo} luminal cells in tissue-regeneration and disease-initiation, we turned to an in vivo regeneration assay where human prostate epithelial cells are combined with urogenital sinus mesenchyme cells (UGSM) in Matrigel and transplanted subcutaneously into immune-deficient mice (Goldstein et al., 2010, 2011). To track human epithelial cells, we introduced genes for red and green fluorescent proteins into CD38^{lo} luminal cells prior to in vivo transplantation (Figures 7A and S7A). Due to low cell numbers insufficient for direct in vivo implantation, we utilized a recently published approach to expand isolated cells in organoid culture for 1–2 weeks prior to transplantation (Park et al., 2016). An average of 200 lentiviral-transduced organoids derived from CD38^{lo} luminal cells were combined with UGSM and implanted into NOD-SCID-IL2Rγ^{null} mice. 8 weeks post-transplantation, we identified prostatic glands containing both luminal cells expressing K8 and AR and basal cells expressing K5 and p63 (Figure 7B). Lentiviral transduced human epithelial structures in regenerated tissues could be identified based on fluorescent signal, whereas control grafts without human cells lacked any epithelial structures or fluorescence (Figure S7C).

Oncogenes previously shown to transform primary human prostate (Goldstein et al., 2010; Stoyanova et al., 2013) (Myc, myristoylated AKT, and AR) were introduced into double-sorted CD38^{lo} luminal cells via lentiviral delivery prior to in vitro expansion and in vivo transplantation. We identified features of human prostate adenocarcinoma, including expression of the luminal markers K8, PSA, and AR and an absence of the basal markers K5 and p63 in regenerated tumors derived from oncogene-expressing CD38^{lo} luminal cells (Figure 7C). Regenerated tumors exhibited expression of oncogenes (Figure S7B). Importantly, tumors exhibited the PSCA-hi CD38^{lo} luminal phenotype and a high frequency of cells expressed the proliferative marker Ki67 (>40% Ki67⁺) (Figure 7C). Results were confirmed using tissue from four independent patient samples. These findings indicate

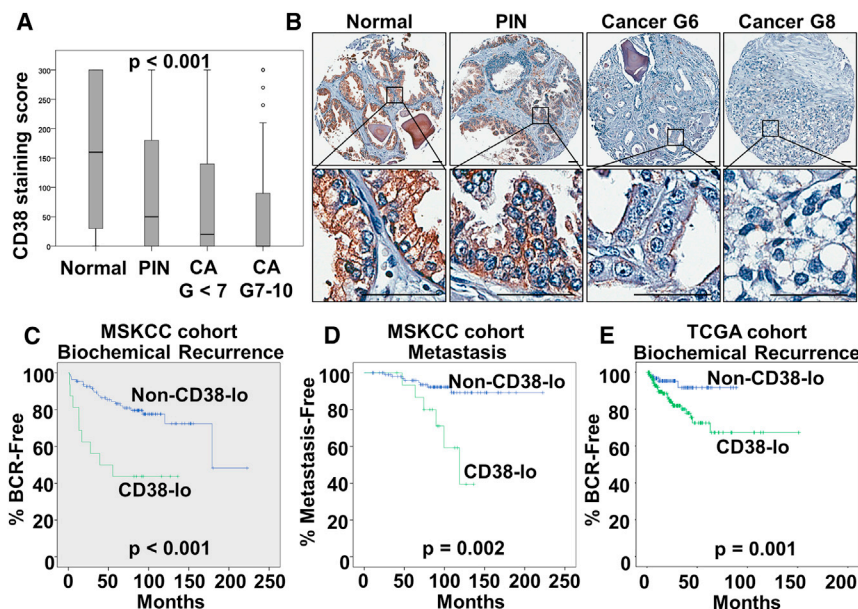


Figure 5. Association of CD38^{lo} Phenotype with Prostate Cancer Progression

(A) Plot of composite CD38 staining score in tissues histologically classified as normal, prostate intra-epithelial neoplasia (PIN), or prostate cancer (CA) with Gleason scores less than 7 or 7–10. Middle bar represents mean value. The overall effect of different levels of CD38 among the prostate regions was confirmed ($p < 0.001$).

(B) Representative immunohistochemical images of normal, PIN, Gleason 6, and Gleason 8 cancer stained for CD38, with high-power images below. Scale bars, 50 μm .

(C and D) Survival analysis from MSKCC cohort measuring biochemical recurrence (C) and metastasis (D) for tumors containing CD38 mRNA 1 SD below the mean compared to the remainder (non-CD38^{lo}). Log rank (Mantel Cox) $p < 0.001$ (recurrence in C) and $p = 0.002$ (metastasis in D).

(E) Survival analysis from the TCGA cohort measuring biochemical recurrence comparing CD38 expression less than (CD38^{lo}) or greater than (non-CD38^{lo}) the mean Z score. Log rank (Mantel Cox) $p = 0.001$.

that CD38^{lo} luminal cells can initiate human prostate cancer in vivo.

DISCUSSION

Chronic inflammation is associated with cancer initiation, progression, metastasis, and treatment resistance in a range of tumor types including prostate cancer (Das Roy et al., 2009; Gurel et al., 2014; Liu et al., 2013; Wang et al., 2015). In mouse models, several groups have established a tumor-promoting functional contribution of distinct immune cell types to prostate cancer, including B cells and myeloid-derived suppressor cells (Ammirante et al., 2010; Garcia et al., 2014). However, little is known about the influence of inflammation on the function of human prostate epithelium prior to tumor formation. De Marzo and colleagues have proposed that inflammation may be an inciting event in prostate transformation and that PIA cells may serve as target cells for tumor initiation (De Marzo et al., 1999, 2003, 2007). Our results demonstrate that PIA-like CD38^{lo} luminal cells can initiate human prostate cancer.

Several groups have isolated human prostate luminal cells based on low expression of CD49f or high expression of CD26 for functional studies (Goldstein et al., 2010; Karthaus et al., 2014; Park et al., 2016; Stoyanova et al., 2013). We found that CD26 and CD38 identify an analogous population of prostate luminal cells (Figure S1C). While rare cells within the CD26⁺ or CD38^{hi} luminal cell population exhibit organoid-forming activity, ~99% of cells in this subset do not exhibit progenitor features (Figures 2B–2D; Karthaus et al., 2014; Park et al., 2016). In contrast, CD38^{lo} luminal cells are enriched for progenitor activity in 2D and 3D progenitor assays, as 5% of CD38^{lo} luminal cells are capable of generating organoids (Figure 2D), similar to the rate of *Lgr5*-GFP-hi intestinal stem cells (Sato et al., 2009). Therefore, identifying the signaling pathways active in CD38^{lo} luminal cells may yield mechanisms regulating luminal progenitor activity.

We found that CD38^{lo} and CD38^{hi} luminal cells have distinct transcriptional signatures. While androgen signaling is active in CD38^{hi} luminal cells (Figure 4), CD38^{lo} luminal cells exhibit elevated NF- κ B signaling (Figure 3). Previous studies demonstrate that NF- κ B activation can synergize with MYC overexpression or *Pten* heterozygosity to accelerate murine prostate cancer initiation and the NF- κ B activation signature in human tumors can predict poor outcome (Birbach et al., 2011; Jin et al., 2014). These data suggest that NF- κ B activity may predispose CD38^{lo} luminal cells to tumor initiation. More work is necessary to determine whether strategies to reduce inflammation or small molecules that block epithelial NF- κ B signaling can prevent prostate cancer initiation by targeting CD38^{lo} luminal cell survival and progenitor activity.

CD38^{lo} luminal cells express PSCA, which is being evaluated as a therapeutic target in clinical trials using monoclonal antibodies (Morris et al., 2012). BCL2 expression in CD38^{lo} luminal cells may enhance their resistance to apoptosis (Raffo et al., 1995). Both BCL2 and PSCA are associated with castration resistance (Gu et al., 2000; Raffo et al., 1995), suggesting that CD38^{lo} luminal cells may have a survival advantage under low-androgen conditions. Consistent with mouse models demonstrating that prostatic inflammation or epithelial NF- κ B activation is associated with reduced androgen signaling and increased epithelial proliferation (Birbach et al., 2011; Khalili et al., 2010), CD38^{lo} luminal cells express low levels of PSA and other androgen targets. These data suggest that CD38^{lo} luminal-like cancer cells may contribute to castration-resistant prostate cancer.

Distinguishing indolent from aggressive prostate cancer remains a critical goal of the field. We found that low expression of CD38 mRNA in prostatectomy specimens is prognostic for biochemical recurrence in two independent cohorts (Figures 5C–5E). These data suggest that aggressive tumors may arise in CD38^{lo} luminal cells that retain low expression of CD38.

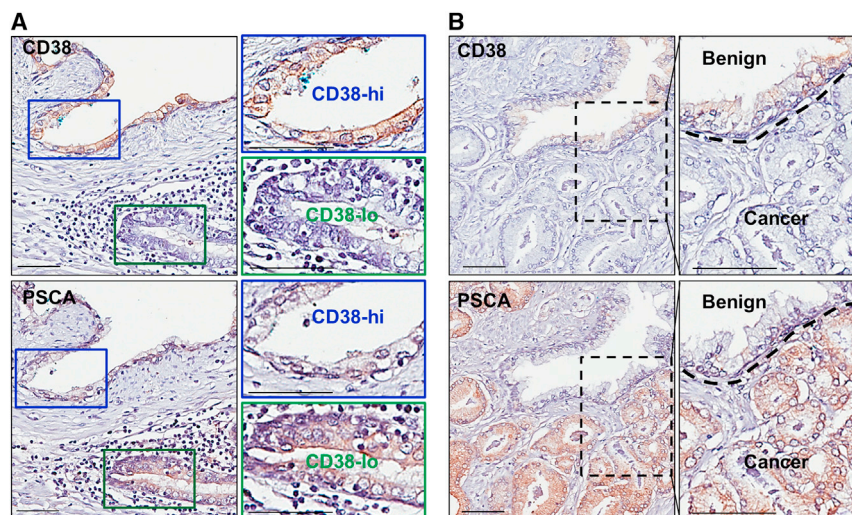


Figure 6. PSCA Is Expressed in CD38^{lo} Luminal Cells

(A) Serial sections of benign human prostate stained for CD38 and PSCA with boxed regions to magnify distinct CD38^{hi} PSCA^{lo} glands and inflammation-associated CD38^{lo} PSCA^{hi} cells. (B) Serial sections of human prostate tissue stained for CD38 and PSCA with boxed region magnifying CD38^{hi} PSCA^{lo} benign tissue separated by a dotted line from CD38^{lo} PSCA^{hi} cancer. Scale bars, 50 μm.

Alternatively, the activation of a CD38^{lo} luminal-like signature during tumorigenesis may promote an aggressive phenotype, regardless of the cell of origin. Several studies have looked at the cellular origins of cancer to distinguish indolent from aggressive prostate cancer with different results using distinct mouse models (Choi et al., 2012; Lu et al., 2013; Wang et al., 2009, 2013, 2014). In human prostate models, basal cells have been shown to generate aggressive, highly proliferative tumors, while luminal cells can initiate only indolent tumors (Goldstein et al., 2010; Park et al., 2016; Stoyanova et al., 2013). In this study, we demonstrate that CD38^{lo} luminal cells can generate highly proliferative prostate cancer (>40% Ki67+). Taken together, we hypothesize that one target cell (basal) is predisposed to the initiation of aggressive cancer under normal conditions. In the context of chronic inflammation, a second target cell (CD38^{lo} luminal) is predisposed to initiate aggressive cancer, perhaps due to elevated NF-κB signaling.

CD38^{lo} luminal cells may arise from basal to luminal differentiation, as has been demonstrated using lineage tracing in mouse models of inflammation (Kwon et al., 2014, 2016). Alternatively, inflammation may alter the differentiation of luminal cells by enhancing NF-κB signaling and reducing androgen signaling. Future studies will determine whether exposure to inflammatory cytokines can reprogram CD38^{hi} luminal cells into a progenitor-enriched CD38^{lo} luminal-like cell capable of initiating aggressive prostate cancer. Interestingly, CD38^{lo} luminal cells express cytokines and chemokines known to recruit pro-inflammatory cells and promote tumorigenesis in other tissues like *Ccl2* (Qian et al., 2011) and *Cxcl1/2* (Jablonska et al., 2014), indicating that CD38^{lo} luminal cells may influence their microenvironment to promote inflammation and maintain proliferative cues.

Our findings are consistent with previous reports of low CD38 expression in prostate cancer (Kramer et al., 1995; Pascal et al., 2009). While rare deletions and mutations of CD38 can be identified in metastatic castration-resistant prostate cancer (Grasso et al., 2012; Robinson et al., 2015), transcriptional repression of CD38 is the likely mechanism causing low expression in the vast majority of prostate tumors. CD38 is an ectoenzyme that

consumes the cofactor nicotinamide adenine dinucleotide (NAD) (Howard et al., 1993), suggesting that loss of CD38 may serve to increase pools of NAD required for cellular metabolism in prostate cancer. Further study of the functional role of CD38 in tumorigenesis and the cellular mechanisms driving CD38^{lo} luminal cells may yield new predictive markers and therapeutic targets for aggressive disease.

EXPERIMENTAL PROCEDURES

qPCR, RNA Sequencing, Bioinformatics, and Microarray

RNA was extracted from cell pellets using the RNeasy mini Kit (QIAGEN/SA Biosciences). qPCR was carried out as previously described (Goldstein et al., 2010) using commercial primers for *Gapdh*, *Keratin 8*, and *Klk3* (QIAGEN/SA Biosciences). RNA-seq libraries were prepared with the Ovation RNA-Seq System V2 (Nugen). Double-stranded cDNA was generated with a mixture of random and poly(T) priming, followed by fragmentation, generation of blunt ends by end repair, A-tailing, ligation of adaptors (different adaptors for multiplexing samples), PCR amplification, and sequencing (pair read 100 run) on Illumina HiSeq 2500. Illumina SAV was used for data quality check, and Illumina CASAVA 1.8.2 was used for de-multiplexing. Reads were mapped to the most recent UCSC transcript set with Bowtie2 version 2.1.0. RSEM v1.2.15 was used to estimate level of gene expression. Gene expression was normalized by TPM (transcript per million) or RPKM (reads per kilobase per million mapped reads). DeSeq was used to identify differentially expressed genes. FASTQ files were not available at the time of publication. For that reason, the full RPKM values are provided in Table S1. Upstream regulator analysis was performed using QIAGEN's Ingenuity Pathway Analysis (<http://www.ingenuity.com>). For microarray analysis, total RNA was isolated from sorted cells with RNeasy Micro Kit and amplified with NuGen Pico kit according to standard manufacturer protocols. Biotinylated cDNA were prepared from total RNA using the standard Affymetrix protocol and hybridized to the Affymetrix Human Genome U133 Plus 2.0 Chip. Chips were scanned using an Affymetrix GeneChip Scanner 7G and data were analyzed with Microarray Suite version 5.0 (MAS 5.0) using Affymetrix default analysis settings and global scaling as normalization method. Data are available at the GEO repository under accession number GEO: GSE89050.

Primary Cell Preparation and Cell Separation

Human prostate tissue samples were provided in a de-identified manner deemed by the institutional review board to not qualify as human subjects. Tissue was provided by the UCLA Translational Pathology Core Laboratory, and benign specimens were processed as described in detail previously (Goldstein et al., 2011). For antibody staining of primary benign dissociated cells, see Supplemental Information. Intracellular flow cytometry has been described previously (Goldstein et al., 2010). Antibodies are listed in Supplemental Information.

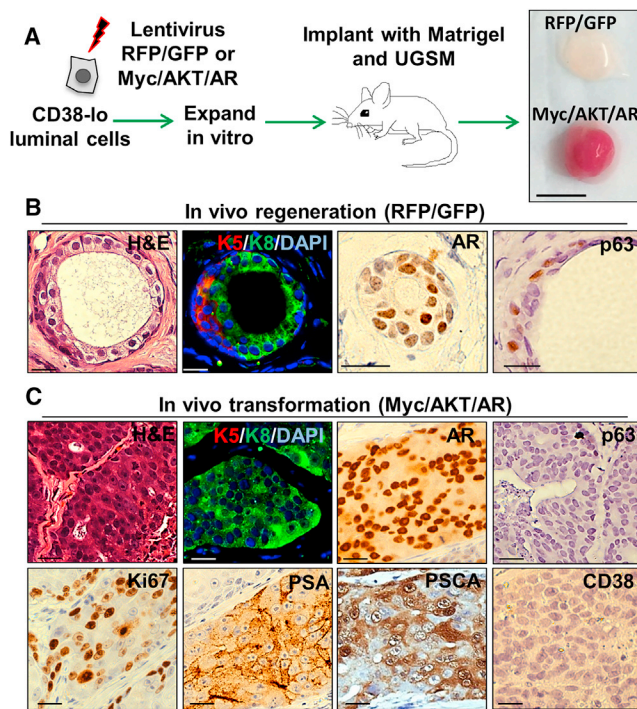


Figure 7. CD38^{lo} Luminal Cells Can Generate Glands and Initiate Human Prostate Cancer In Vivo

(A) Schematic representing experimental flow where CD38^{lo} luminal cells are transduced with lentivirus, expanded in vitro 7–10 days, combined with UGSM and Matrigel and implanted in vivo. Regenerated tissues from representative experiments are shown on the right. Scale bars, 5 mm.

(B) Representative in vivo regeneration of CD38^{lo} luminal cell-derived organoids transduced with control fluorescent GFP and red fluorescent protein (RFP) stained for H&E, K5, K8, DAPI nuclear counterstain, AR, and p63. Scale bars, 50 μ m.

(C) Representative adenocarcinoma in regenerated tumor tissues from CD38^{lo} luminal cells transduced with Myc, AKT, and AR stained for H&E, K5, K8, DAPI nuclear counterstain, AR, p63, Ki67, PSA, PSCA, and CD38. Scale bars, 25 μ m.

Immunohistochemical Analysis and Immunofluorescence

Paraffin-embedded tissue sections were incubated in a 60°C oven for 1 hr and de-paraffinized in three changes of xylene, followed by 100% alcohol twice for 3 min each. Then the slides were transferred once in 95% and 70% alcohol, each for 3 min. A heat antigen retrieval step was used to unmask the antigenic epitopes. The remaining procedure was performed according to the manufacturer's protocol (R&D Cell and Tissue Staining Kit HRP-DAB system, R&D Systems). Primary antibodies are listed in [Supplemental Information](#). Biotinylated anti-mouse and anti-rabbit secondary antibodies are supplied by HRP-DAB system for Mouse (CTS002, R&D Systems) and Rabbit Kit (CTS005, R&D Systems). Double-sorted cells were plated onto a glass slide coated with poly-L-lysine (Sigma), and cells were allowed to attach overnight. The following day, cells were fixed in 4% paraformaldehyde, washed three times in 1 \times PBS and stained with primary antibody. Alexa-488-conjugated goat anti-mouse secondary antibody (A-11001, Invitrogen) was used followed by DAPI nuclear counterstain (H-1200, Vector Laboratories).

Immunoblot Analysis

Isolated cell populations were lysed in RIPA buffer (150mM NaCl, 1% NP-40, 50 mM Tris-HCl [pH 8.0], 0.5% sodium deoxycholate, and 0.1% SDS) with protease inhibitor cocktail tablet (Complete, Roche) on ice 15 min followed by spin at maximum speed for 15 min. Supernatants were boiled in loading buffer and

membranes probed with antibodies (see [Supplemental Information](#)). Primary rabbit, mouse, or goat antibodies were detected with horseradish peroxidase (HRP)-conjugated secondary antibodies (Pierce).

Tissue Microarray and Scoring

The tissue microarray was constructed from 332 men who underwent radical prostatectomy surgery at the West Los Angeles Veteran's Administration Hospital from 1991 to 2003 and has been described previously ([Gollapudi et al., 2013](#)). At least three cores of each histological type are included per patient. Follow-up patient information for up to 120 months was used for analysis. Biochemical recurrence refers to PSA values greater than 0.2, two readings at 0.2, or additional treatment for high post-operative PSA. 4- μ m sections were stained for CD38 and scanned using the AperioSlide scanner, and staining was evaluated in a blinded fashion by the pathologists (B.N. and J.H.). Scoring was assessed based on staining intensity from 0 (no staining) to 3 (strong) and percentage of tumor cell expression (1%–100%), creating a composite score from 0 to 300. For statistical methods, see [Supplemental Information](#).

Cell Culture

For the in vitro organoid-forming assay, double-sorted FACS-isolated cell populations were plated in a 96-well plate (Corning Incorporated) at a cell density of one cell per well, and those wells with a single cell were visually confirmed and marked for continued analysis. Prostate organoid growth conditions were based on established protocols ([Karthaus et al., 2014](#)). Primary single-cell organoids were dissociated in 1 mg/mL Dispase (Gibco) at 37°C for 1 hr and then treated with 0.05% Trypsin-EDTA (Life Technologies) in order to passage. For the colony assay, primary cells were plated on Matrigel-coated six-well dishes as described previously ([Lukacs et al., 2010](#)) and grown in PrEGM media. Colonies containing more than two cells were quantified after 10–14 days. For the sphere assay, cells were resuspended in a PrEGM/Matrigel mixture and plated around the edges of the well in a 12-well dish as described previously ([Lukacs et al., 2010](#)). Spheres were quantified after 10–14 days. For inhibition of NF- κ B, ACHP (4547, Tocris) was added to organoid media at a final concentration of 5 μ M every 3 days.

In Vivo Regeneration and Transformation and Animal Care

In vivo tissue-regeneration and transformation methods have been described in detail ([Goldstein et al., 2011](#)). Transduced cells were expanded in organoid culture ([Karthaus et al., 2014](#)) prior to transplantation as described previously ([Park et al., 2016](#)). All human tissues were transplanted subcutaneously into NOD-SCID-IL2R γ ^{null} mice. Animals have been purchased from the Jackson Laboratory and are now housed and bred under the care of the Division of Laboratory Animal Medicine at the University of California, Los Angeles, using protocols approved by the Animal Research Committee. Lentiviral vectors were previously described ([Goldstein et al., 2010](#); [Stoyanova et al., 2013](#)).

ACCESSION NUMBERS

The accession number for the data reported in this paper is GEO: GSE89050.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, seven figures, and six tables and can be found with this article online at <http://dx.doi.org/10.1016/j.celrep.2016.11.010>.

AUTHOR CONTRIBUTIONS

X.L. and A.S.G. performed the majority of experiments and wrote and edited the manuscript. T.H., J.M., D.C., L.Z., K.H., T.S., and R.O.S. performed experiments. T.R.G., D.E., S.H., H.H., and C.L.S. performed statistical analysis. J.W.P. developed organoid transformation methods. B.N. and J.H. provided pathology expertise. M.B.R. provided tissue microarrays. J.H. and O.N.W. edited and contributed to writing of the manuscript.

ACKNOWLEDGMENTS

This work was supported by Department of Defense Idea Development Award W81XWH-13-1-0470 (A.S.G. and S.H.), a Prostate Cancer Foundation Young Investigator Award (A.S.G. and T.S.), the Broad Stem Cell Research Center (A.S.G. and J.H.), an AACR/PCF/Stand Up 2 Cancer West Coast Dream Team Award (O.N.W., J.H., M.R., and A.S.G.), a National Institute of Health/National Cancer Institute grant (P50CA092131/UCLA SPORE in Prostate Cancer; D.E., S.H., O.N.W., J.H., M.R., and A.S.G.), a NIH/National Center for Advancing Translational Science (NCATS) UCLA CTSI grant (UL1TR000124; T.R.G. and D.E.), a National Institute of Health/National Cancer Institute K99 Pathway to Independence Award (4R00CA184397; T.S.), and the Howard Hughes Medical Institute (O.N.W.). M.R. and funding for TMA creation were supported by the Department of Defense (PC030686).

Received: June 17, 2016

Revised: September 27, 2016

Accepted: October 28, 2016

Published: December 6, 2016

REFERENCES

- Ammirante, M., Luo, J.L., Grivennikov, S., Nedospasov, S., and Karin, M. (2010). B-cell-derived lymphotoxin promotes castration-resistant prostate cancer. *Nature* *464*, 302–305.
- Birbach, A., Eisenbarth, D., Kozakowski, N., Ladenhauf, E., Schmidt-Suppran, M., and Schmid, J.A. (2011). Persistent inflammation leads to proliferative neoplasia and loss of smooth muscle cells in a prostate tumor model. *Neoplasia* *13*, 692–703.
- Choi, N., Zhang, B., Zhang, L., Ittmann, M., and Xin, L. (2012). Adult murine prostate basal and luminal cells are self-sustained lineages that can both serve as targets for prostate cancer initiation. *Cancer Cell* *21*, 253–265.
- Coussens, L.M., and Werb, Z. (2002). Inflammation and cancer. *Nature* *420*, 860–867.
- Das Roy, L., Pathangey, L.B., Tinder, T.L., Schettini, J.L., Gruber, H.E., and Mukherjee, P. (2009). Breast-cancer-associated metastasis is significantly increased in a model of autoimmune arthritis. *Breast Cancer Res.* *11*, R56.
- De Marzo, A.M., Marchi, V.L., Epstein, J.I., and Nelson, W.G. (1999). Proliferative inflammatory atrophy of the prostate: implications for prostatic carcinogenesis. *Am. J. Pathol.* *155*, 1985–1992.
- De Marzo, A.M., Meeker, A.K., Zha, S., Luo, J., Nakayama, M., Platz, E.A., Isaacs, W.B., and Nelson, W.G. (2003). Human prostate cancer precursors and pathobiology. *Urology* *62* (5, Suppl 1), 55–62.
- De Marzo, A.M., Platz, E.A., Sutcliffe, S., Xu, J., Grönberg, H., Drake, C.G., Nakai, Y., Isaacs, W.B., and Nelson, W.G. (2007). Inflammation in prostate carcinogenesis. *Nat. Rev. Cancer* *7*, 256–269.
- Elkhwaji, J.E., Hauke, R.J., and Brawner, C.M. (2009). Chronic bacterial inflammation induces prostatic intraepithelial neoplasia in mouse prostate. *Br. J. Cancer* *101*, 1740–1748.
- Garcia, A.J., Ruscetti, M., Arenzana, T.L., Tran, L.M., Bianci-Frias, D., Sybert, E., Priceman, S.J., Wu, L., Nelson, P.S., Smale, S.T., and Wu, H. (2014). Pten null prostate epithelium promotes localized myeloid-derived suppressor cell expansion and immune suppression during tumor initiation and progression. *Mol. Cell. Biol.* *34*, 2017–2028.
- Goldstein, A.S., Lawson, D.A., Cheng, D., Sun, W., Garraway, I.P., and Witte, O.N. (2008). Trop2 identifies a subpopulation of murine and human prostate basal cells with stem cell characteristics. *Proc. Natl. Acad. Sci. USA* *105*, 20882–20887.
- Goldstein, A.S., Huang, J., Guo, C., Garraway, I.P., and Witte, O.N. (2010). Identification of a cell of origin for human prostate cancer. *Science* *329*, 568–571.
- Goldstein, A.S., Drake, J.M., Burnes, D.L., Finley, D.S., Zhang, H., Reiter, R.E., Huang, J., and Witte, O.N. (2011). Purification and direct transformation of epithelial progenitor cells from primary human prostate. *Nat. Protoc.* *6*, 656–667.
- Gollapudi, K., Galet, C., Grogan, T., Zhang, H., Said, J.W., Huang, J., Elashoff, D., Freedland, S.J., Rettig, M., and Aronson, W.J. (2013). Association between tumor-associated macrophage infiltration, high grade prostate cancer, and biochemical recurrence after radical prostatectomy. *Am. J. Cancer Res.* *3*, 523–529.
- Grasso, C.S., Wu, Y.M., Robinson, D.R., Cao, X., Dhanasekaran, S.M., Khan, A.P., Quist, M.J., Jing, X., Lonigro, R.J., Brenner, J.C., et al. (2012). The mutational landscape of lethal castration-resistant prostate cancer. *Nature* *487*, 239–243.
- Gu, Z., Thomas, G., Yamashiro, J., Shintaku, I.P., Dorey, F., Raitano, A., Witte, O.N., Said, J.W., Loda, M., and Reiter, R.E. (2000). Prostate stem cell antigen (PSCA) expression increases with high gleason score, advanced stage and bone metastasis in prostate cancer. *Oncogene* *19*, 1288–1296.
- Gurel, B., Lucia, M.S., Thompson, I.M., Jr., Goodman, P.J., Tangen, C.M., Kristal, A.R., Parnes, H.L., Hoque, A., Lippman, S.M., Sutcliffe, S., et al. (2014). Chronic inflammation in benign prostate tissue is associated with high-grade prostate cancer in the placebo arm of the prostate cancer prevention trial. *Cancer Epidemiol. Biomarkers Prev.* *23*, 847–856.
- Haverkamp, J.M., Charbonneau, B., Crist, S.A., Meyerholz, D.K., Cohen, M.B., Snyder, P.W., Svensson, R.U., Henry, M.D., Wang, H.H., and Ratliff, T.L. (2011). An inducible model of abacterial prostatitis induces antigen specific inflammatory and proliferative changes in the murine prostate. *Prostate* *71*, 1139–1150.
- Hieronymus, H., Lamb, J., Ross, K.N., Peng, X.P., Clement, C., Rodina, A., Nieto, M., Du, J., Stegmaier, K., Raj, S.M., et al. (2006). Gene expression signature-based chemical genomic prediction identifies a novel class of HSP90 pathway modulators. *Cancer Cell* *10*, 321–330.
- Hieronymus, H., Schultz, N., Gopalan, A., Carver, B.S., Chang, M.T., Xiao, Y., Heguy, A., Huberman, K., Bernstein, M., Assel, M., et al. (2014). Copy number alteration burden predicts prostate cancer relapse. *Proc. Natl. Acad. Sci. USA* *111*, 11139–11144.
- Howard, M., Grimaldi, J.C., Bazan, J.F., Lund, F.E., Santos-Argumedo, L., Parkhouse, R.M., Walseth, T.F., and Lee, H.C. (1993). Formation and hydrolysis of cyclic ADP-ribose catalyzed by lymphocyte antigen CD38. *Science* *262*, 1056–1059.
- Huang da, W., Sherman, B.T., and Lempicki, R.A. (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat. Protoc.* *4*, 44–57.
- Jablonska, J., Wu, C.F., Andzinski, L., Leschner, S., and Weiss, S. (2014). CXCR2-mediated tumor-associated neutrophil recruitment is regulated by IFN- β . *Int. J. Cancer* *134*, 1346–1358.
- Jin, R., Yi, Y., Yull, F.E., Blackwell, T.S., Clark, P.E., Koyama, T., Smith, J.A., Jr., and Matusik, R.J. (2014). NF- κ B gene signature predicts prostate cancer progression. *Cancer Res.* *74*, 2763–2772.
- Karthauss, W.R., Iaquinta, P.J., Drost, J., Gracanin, A., van Boxtel, R., Wongvipat, J., Dowling, C.M., Gao, D., Begthel, H., Sachs, N., et al. (2014). Identification of multipotent luminal progenitor cells in human prostate organoid cultures. *Cell* *159*, 163–175.
- Khalili, M., Mutton, L.N., Gurel, B., Hicks, J.L., De Marzo, A.M., and Bieberich, C.J. (2010). Loss of Nkx3.1 expression in bacterial prostatitis: a potential link between inflammation and neoplasia. *Am. J. Pathol.* *176*, 2259–2268.
- Kramer, G., Steiner, G., Födinger, D., Fiebiger, E., Rappersberger, C., Binder, S., Hofbauer, J., and Marberger, M. (1995). High expression of a CD38-like molecule in normal prostatic epithelium and its differential loss in benign and malignant disease. *J. Urol.* *154*, 1636–1641.
- Kwon, O.J., Zhang, L., Ittmann, M.M., and Xin, L. (2014). Prostatic inflammation enhances basal-to-luminal differentiation and accelerates initiation of prostate cancer with a basal cell origin. *Proc. Natl. Acad. Sci. USA* *111*, E592–E600.
- Kwon, O.J., Zhang, B., Zhang, L., and Xin, L. (2016). High fat diet promotes prostatic basal-to-luminal differentiation and accelerates initiation of prostate

- epithelial hyperplasia originated from basal cells. *Stem Cell Res. (Amst.)* **16**, 682–691.
- Lawson, D.A., Zong, Y., Memarzadeh, S., Xin, L., Huang, J., and Witte, O.N. (2010). Basal epithelial stem cells are efficient targets for prostate cancer initiation. *Proc. Natl. Acad. Sci. USA* **107**, 2610–2615.
- Liu, K., Jiang, M., Lu, Y., Chen, H., Sun, J., Wu, S., Ku, W.Y., Nakagawa, H., Kita, Y., Natsugoe, S., et al. (2013). Sox2 cooperates with inflammation-mediated Stat3 activation in the malignant transformation of foregut basal progenitor cells. *Cell Stem Cell* **12**, 304–315.
- Lu, T.L., Huang, Y.F., You, L.R., Chao, N.C., Su, F.Y., Chang, J.L., and Chen, C.M. (2013). Conditionally ablated Pten in prostate basal cells promotes basal-to-luminal differentiation and causes invasive prostate cancer in mice. *Am. J. Pathol.* **182**, 975–991.
- Lukacs, R.U., Goldstein, A.S., Lawson, D.A., Cheng, D., and Witte, O.N. (2010). Isolation, cultivation and characterization of adult murine prostate stem cells. *Nat. Protoc.* **5**, 702–713.
- Morris, M.J., Eisenberger, M.A., Pili, R., Denmeade, S.R., Rathkopf, D., Slovin, S.F., Farrelly, J., Chudow, J.J., Vincent, M., Scher, H.I., and Carducci, M.A. (2012). A phase I/IIA study of AGS-PSCA for castration-resistant prostate cancer. *Ann. Oncol.* **23**, 2714–2719.
- Nelson, P.S., Clegg, N., Arnold, H., Ferguson, C., Bonham, M., White, J., Hood, L., and Lin, B. (2002). The program of androgen-responsive genes in neoplastic prostate epithelium. *Proc. Natl. Acad. Sci. USA* **99**, 11890–11895.
- Network, T.R.; Cancer Genome Atlas Research Network (2015). The molecular taxonomy of primary prostate cancer. *Cell* **163**, 1011–1025.
- Park, J.W., Lee, J.K., Phillips, J.W., Huang, P., Cheng, D., Huang, J., and Witte, O.N. (2016). Prostate epithelial cell of origin determines cancer differentiation state in an organoid transformation assay. *Proc. Natl. Acad. Sci. USA* **113**, 4482–4487.
- Pascal, L.E., Vêncio, R.Z., Page, L.S., Liebeskind, E.S., Shadle, C.P., Troisch, P., Marzolf, B., True, L.D., Hood, L.E., and Liu, A.Y. (2009). Gene expression relationship between prostate cancer cells of Gleason 3, 4 and normal epithelial cells as revealed by cell type-specific transcriptomes. *BMC Cancer* **9**, 452.
- Qian, B.Z., Li, J., Zhang, H., Kitamura, T., Zhang, J., Campion, L.R., Kaiser, E.A., Snyder, L.A., and Pollard, J.W. (2011). CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis. *Nature* **475**, 222–225.
- Raffo, A.J., Perlman, H., Chen, M.W., Day, M.L., Streitman, J.S., and Buttyan, R. (1995). Overexpression of bcl-2 protects prostate cancer cells from apoptosis in vitro and confers resistance to androgen depletion in vivo. *Cancer Res.* **55**, 4438–4445.
- Robinson, D., Van Allen, E.M., Wu, Y.M., Schultz, N., Lonigro, R.J., Mosquera, J.M., Montgomery, B., Taplin, M.E., Pritchard, C.C., Attard, G., et al. (2015). Integrative clinical genomics of advanced prostate cancer. *Cell* **161**, 1215–1228.
- Sato, T., Vries, R.G., Snippert, H.J., van de Wetering, M., Barker, N., Stange, D.E., van Es, J.H., Abo, A., Kujala, P., Peters, P.J., and Clevers, H. (2009). Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* **459**, 262–265.
- Sboner, A., Demichelis, F., Calza, S., Pawitan, Y., Setlur, S.R., Hoshida, Y., Perner, S., Adami, H.O., Fall, K., Mucci, L.A., et al. (2010). Molecular sampling of prostate cancer: a dilemma for predicting disease progression. *BMC Med. Genomics* **3**, 8.
- Sfanos, K.S., and De Marzo, A.M. (2012). Prostate cancer and inflammation: the evidence. *Histopathology* **60**, 199–215.
- Shafique, K., Proctor, M.J., McMillan, D.C., Qureshi, K., Leung, H., and Morrison, D.S. (2012). Systemic inflammation and survival of patients with prostate cancer: evidence from the Glasgow Inflammation Outcome Study. *Prostate Cancer Prostatic Dis.* **15**, 195–201.
- Smith, B.A., Sokolov, A., Uzunangelov, V., Baertsch, R., Newton, Y., Graim, K., Mathis, C., Cheng, D., Stuart, J.M., and Witte, O.N. (2015). A basal stem cell signature identifies aggressive prostate cancer phenotypes. *Proc. Natl. Acad. Sci. USA* **112**, E6544–E6552.
- Stephenson, A.J., Scardino, P.T., Eastham, J.A., Bianco, F.J., Jr., Dotan, Z.A., DiBlasio, C.J., Reuther, A., Klein, E.A., and Kattan, M.W. (2005). Postoperative nomogram predicting the 10-year probability of prostate cancer recurrence after radical prostatectomy. *J. Clin. Oncol.* **23**, 7005–7012.
- Stoyanova, T., Cooper, A.R., Drake, J.M., Liu, X., Armstrong, A.J., Pienta, K.J., Zhang, H., Kohn, D.B., Huang, J., Witte, O.N., and Goldstein, A.S. (2013). Prostate cancer originating in basal cells progresses to adenocarcinoma propagated by luminal-like cells. *Proc. Natl. Acad. Sci. USA* **110**, 20111–20116.
- Taylor, B.S., Schultz, N., Hieronymus, H., Gopalan, A., Xiao, Y., Carver, B.S., Arora, V.K., Kaushik, P., Cerami, E., Reva, B., et al. (2010). Integrative genomic profiling of human prostate cancer. *Cancer Cell* **18**, 11–22.
- Taylor, R.A., Toivanen, R., Frydenberg, M., Pedersen, J., Harewood, L., Collins, A.T., Maitland, N.J., and Risbridger, G.P.; Australian Prostate Cancer Bioresource (2012). Human epithelial basal cells are cells of origin of prostate cancer, independent of CD133 status. *Stem Cells* **30**, 1087–1096.
- van Leenders, G.J., Gage, W.R., Hicks, J.L., van Balken, B., Aalders, T.W., Schalken, J.A., and De Marzo, A.M. (2003). Intermediate cells in human prostate epithelium are enriched in proliferative inflammatory atrophy. *Am. J. Pathol.* **162**, 1529–1537.
- Wang, X., Kruihof-de Julio, M., Economides, K.D., Walker, D., Yu, H., Halili, M.V., Hu, Y.P., Price, S.M., Abate-Shen, C., and Shen, M.M. (2009). A luminal epithelial stem cell that is a cell of origin for prostate cancer. *Nature* **461**, 495–500.
- Wang, Z.A., Mitrofanova, A., Bergren, S.K., Abate-Shen, C., Cardiff, R.D., Califano, A., and Shen, M.M. (2013). Lineage analysis of basal epithelial cells reveals their unexpected plasticity and supports a cell-of-origin model for prostate cancer heterogeneity. *Nat. Cell Biol.* **15**, 274–283.
- Wang, Z.A., Toivanen, R., Bergren, S.K., Chambon, P., and Shen, M.M. (2014). Luminal cells are favored as the cell of origin for prostate cancer. *Cell Rep.* **8**, 1339–1346.
- Wang, D., Fu, L., Sun, H., Guo, L., and DuBois, R.N. (2015). Prostaglandin E2 promotes colorectal cancer stem cell expansion and metastasis in mice. *Gastroenterology* **149**, 1884–1895.

Functional evidence that progenitor cells near sites of inflammation are precursors for aggressive prostate cancer

Preston D. Crowell & Andrew S. Goldstein

To cite this article: Preston D. Crowell & Andrew S. Goldstein (2017) Functional evidence that progenitor cells near sites of inflammation are precursors for aggressive prostate cancer, *Molecular & Cellular Oncology*, 4:2, e1279723, DOI: [10.1080/23723556.2017.1279723](https://doi.org/10.1080/23723556.2017.1279723)

To link to this article: <https://doi.org/10.1080/23723556.2017.1279723>



Accepted author version posted online: 13 Jan 2017.
Published online: 26 Feb 2017.



[Submit your article to this journal](#)



Article views: 215



[View related articles](#)



[View Crossmark data](#)



Citing articles: 2 [View citing articles](#)

AUTHOR'S VIEW

Functional evidence that progenitor cells near sites of inflammation are precursors for aggressive prostate cancer

Preston D. Crowell^{a,b} and Andrew S. Goldstein^{a,c,d,e}

^aMolecular, Cell and Developmental Biology, University of California, Los Angeles, Los Angeles, CA, USA; ^bMolecular Biology Institute, University of California, Los Angeles, Los Angeles, CA, USA; ^cEli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, University of California, Los Angeles, Los Angeles, CA, USA; ^dUrology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, USA; ^eJonsson Comprehensive Cancer Center, University of California, Los Angeles, Los Angeles, CA, USA

ABSTRACT

While chronic inflammation has been causally associated with several epithelial malignancies, whether it causally contributes to the development of prostate cancer has remained unclear. We recently reported that progenitor-like inflammation-associated luminal cells marked by low expression of Cluster of Differentiation 38 (CD38) can initiate human prostate cancer and predict poor outcome.

ARTICLE HISTORY

Received 31 December 2016
Revised 31 December 2016
Accepted 2 January 2017

KEYWORDS

CD38; Luminal; Prostate cancer; stem cell

In 2016, an estimated 180,890 new cases of prostate cancer will be diagnosed, and approximately 26,120 men will die of the disease.¹ Despite extensive research efforts, the prevalence of prostate cancer remains a mystery. Chronic inflammation has been causally associated with the development of epithelial malignancies such as stomach, large intestine, liver, and urinary bladder cancers. Recent studies have identified an association between inflammation and the development of prostate cancer.²

De Marzo, Nelson, and colleagues have reported changes in the morphology of epithelial cells associated with chronic inflammation in the human prostate known as proliferative inflammatory atrophy (PIA).³ Luminal cells in PIA have an atrophic appearance, exhibit an imbalance between proliferation and apoptosis, and show signs of oxidative stress. Additionally, these cells show an increase in the anti-apoptotic factor B-cell lymphoma 2 (BCL2) and a decrease in androgen receptor (AR) signaling. PIA is commonly observed in close spatial proximity to premalignant and malignant tissue, suggesting that PIA may represent a precursor to prostate cancer.³ However, the functional role of these cells in prostate cancer had not been investigated due to an inability to isolate PIA-like and non-PIA epithelial cells from viable human prostate tissue.

We identified a unique population of luminal cells marked by low expression of Cluster of Differentiation 38 (CD38lo) that exhibit many hallmarks of cells associated with PIA, including increased BCL2 expression and reduced androgen signaling.⁴ Gene expression analysis of CD38lo cells revealed enrichment of many inflammatory-related genes, and immunohistochemical staining of human prostate tissue confirmed that CD38lo luminal cells are predominantly localized in glands adjacent to inflammation.

Having established that CD38lo luminal cells represent an inflammation-associated cell population with hallmarks of PIA, we sought to assess their proliferative potential in three

progenitor assays (colony-forming, sphere-forming, organoid-forming). CD38lo luminal cells isolated from freshly dissociated primary human prostate tissue exhibited a level of progenitor activity in between the stem-like basal cells and the differentiated CD38hi luminal cells. Importantly, the inflammation-associated CD38lo luminal cells represent an enriched luminal progenitor subset.

In previous studies, we have shown that basal cells from human prostate tissue can generate tumors following oncogenic transformation.⁵ In contrast, *ex vivo*-expanded luminal cells only give rise to indolent-like tumors with limited proliferative potential.⁶ In our recent study, CD38lo luminal progenitor cells were lentivirally transduced with oncogenes (Myc, AKT1, and AR), expanded in organoid culture, and transplanted subcutaneously with Matrigel and urogenital sinus mesenchyme cells into immune-deficient mice. Transplanted oncogene-expressing CD38lo luminal cells developed features of highly proliferative prostate adenocarcinoma, suggesting that they can initiate aggressive human prostate cancer *in vivo*.⁴

Our development of an approach to isolate PIA-like luminal cells based on low expression of CD38 allowed us to functionally test their capacity. Our demonstration that PIA-like luminal cells isolated from human prostate tissue can initiate prostate cancer³ provides functional evidence to support the model that PIA may represent a precursor to prostate cancer (Fig. 1). Furthermore, our ability to isolate PIA-like luminal cells allowed us to profile and characterize them in a deeper way than in previous studies. We can now show that PIA-like cells exhibit elevated nuclear factor kappa B (NF- κ B) signaling,⁴ which has been associated with aggressive prostate cancer.⁷ Moreover, since CD38lo cells display decreased androgen signaling and elevated BCL2 expression, they would be predicted to respond poorly to hormonal therapy.

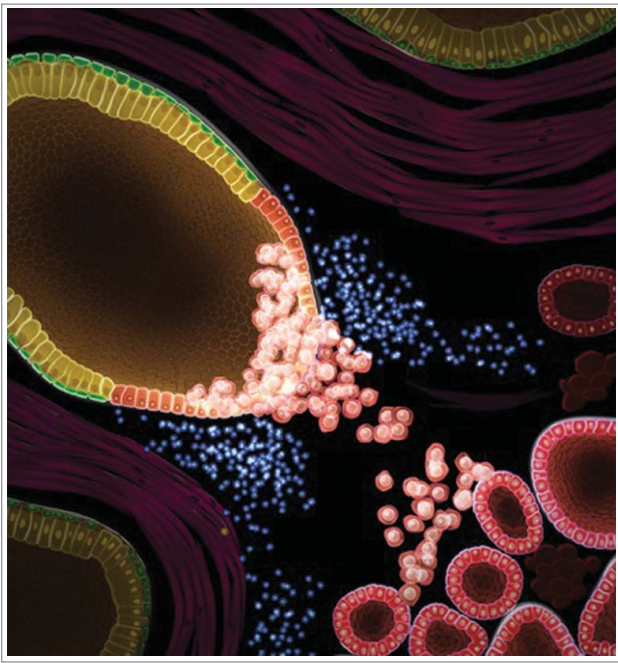


Figure 1. Initiation of aggressive prostate cancer by oncogene-expressing Proliferative Inflammatory Atrophy (PIA)-like luminal cells. PIA-like luminal cells (red) are associated with regions that contain inflammation (blue). When lentivirally transduced with oncogenes (*Myc*, *AKT1*, and androgen receptor), these cells expand and can initiate aggressive prostate cancer (bottom right corner). Visualization by Kandeo Studios.

Previous studies in mouse models have demonstrated that acute inflammation promotes an expansion of progenitor-like cells,⁸ and an increase in basal-to-luminal differentiation.⁹ While we show that PIA-like luminal cells are enriched in regions of inflammation, an inability to study kinetic processes in human tissue prevents us from uncovering the sequence of events that links inflammation and PIA-like luminal progenitor cells. Serial biopsies of benign human prostate tissue containing chronic inflammation may help uncover the precise order of events involved in PIA. Interestingly, our gene expression analysis revealed that PIA-like cells upregulate several pro-inflammatory cytokines and chemokines,⁴ suggesting that PIA-like cells may actively recruit inflammation. The assays used to measure progenitor activity require that epithelial cells be removed from their native environment, dissociated into single cells, and grown in a culture system lacking several of the key cell types found in the prostate microenvironment including inflammatory cells. We hope that future studies will be able to incorporate more of these microenvironmental cell types and may enable us to study the influence of inflammation on human prostate epithelial development and malignant transformation in culture.

A key goal of prostate cancer research is to uncover the biology that drives aggressive prostate cancer to allow us to distinguish indolent from aggressive disease and develop therapeutic targets to treat advanced stage disease. In this study, we illustrate that low *CD38* mRNA levels in prostatectomy specimens from two separate cohorts are prognostic for biochemical recurrence, suggesting that *CD38*lo aggressive cancers may arise from PIA-like *CD38*lo luminal cells. Given that *CD38*lo luminal cells and *CD38*lo tumors exhibit progenitor-like features, we hypothesize that *CD38* loss may be functionally significant. *CD38* consumes cellular nicotinamide adenine

dinucleotide (NAD),¹⁰ suggesting that loss of *CD38* may increase the pool of NAD available for cellular metabolism in *CD38*lo luminal cells and *CD38*lo prostate cancer. Future studies should investigate if an increased pool of NAD provides *CD38*lo luminal cells with a proliferative advantage. Finally, NF- κ B signaling and other signaling pathways found to be important in *CD38*lo luminal cells should be investigated as therapeutic targets to prevent the initiation and progression of *CD38*lo luminal cell-derived aggressive prostate cancer.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors thank Angelo De Marzo for discussions about PIA and the role of inflammation in prostate cancer. Due to space limitations, many important studies and research articles were not able to be referenced.

References

1. Siegel RL, Miller KD and Jemal A. Cancer statistics, 2016. *CA: Cancer J Clin* 2016; 66:7-30; PMID: 26742998; <http://dx.doi.org/10.3322/caac.21332>
2. Gurel B, Lucia MS, Thompson IM Jr., Goodman PJ, Tangen CM, Kristal AR, Parnes HL, Hoque A, Lippman SM, Sutcliffe S, et al. Chronic inflammation in benign prostate tissue is associated with high-grade prostate cancer in the placebo arm of the prostate cancer prevention trial. *Cancer Epidemiol. Biomarkers Prev* 2014; 23:847-56; PMID: 24748218; <http://dx.doi.org/10.1158/1055-9965.EPI-13-1126>
3. De Marzo AM, Marchi VL, Epstein JI, and Nelson WG Proliferative inflammatory atrophy of the prostate: implications for prostatic carcinogenesis. *Am J Pathol* 1999 155:1985-92; PMID: 10595928; [http://dx.doi.org/10.1016/S0002-9440\(10\)65517-4](http://dx.doi.org/10.1016/S0002-9440(10)65517-4)
4. Liu X, Grogan TR, Hieronymus H, Hashimoto T, Mottahedeh J, Cheng D, Zhang L, Huang K, Stoyanova T, Park JW, et al. Low *CD38* identifies progenitor-like inflammation-associated luminal cells that can initiate human prostate cancer and predict poor outcome. *Cell Rep* 2016 17(10):2596-606; PMID: 27926864; <http://dx.doi.org/10.1016/j.celrep.2016.11.010>
5. Goldstein AS, Huang J, Guo C, Garraway IP, Witte ON. Identification of a cell of origin for human prostate cancer. *Science* 2010; 329:568-71; PMID: 20671189; <http://dx.doi.org/10.1126/science.1189992>
6. Park JW, Lee JK, Phillips JW, Huang P, Cheng D, Huang J, and Witte ON. Prostate epithelial cell of origin determines cancer differentiation state in an organoid transformation assay. *Proc Natl Acad Sci USA* 2016; 113:4482-7; PMID:27044116; <http://dx.doi.org/10.1073/pnas.1603645113>
7. Jin R, Yi Y, Yull FE, Blackwell TS, Clark PE, Koyama T, Smith JA Jr, Matusik RJ. NF- κ B gene signature predicts prostate cancer progression. *Cancer Res* 2014; 74:2763-72; PMID: 24686169; <http://dx.doi.org/10.1158/0008-5472.CAN-13-2543>
8. Wang H, Wang L, Jerde TJ, Chan BD, Savran CA, Burcham GN, Crist S, and Radliff TL. Characterization of autoimmune inflammation induced prostate stem cell expansion. *Prostate* 2015; 75(14):1620-31; PMID: 26174474; <http://dx.doi.org/10.1002/pros.23043>
9. Kwon OJ, Zhang L, Ittmann MM, and Xin L. Prostatic inflammation enhances basal-to-luminal differentiation and accelerates initiation of prostate cancer with a basal cell origin. *Proc Natl Acad Sci USA* 2014; 111:E592-600; PMID:27044116; <http://dx.doi.org/10.1073/pnas.1318157111>
10. Howard M, Grimaldi JC, Bazan JF, Lund FE, Santos-Argumedo L, Parkhouse RM, Walseth TF, and Lee HC. Formation and hydrolysis of cyclic ADP-ribose catalyzed by lymphocyte antigen *CD38*. *Science* 1993; 262:1056-9; PMID: 8235624; <http://dx.doi.org/10.1126/science.8235624>