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

Permalink
https://escholarship.org/uc/item/9tp6c26p

Journal
Cell Reports, 17(10)

Authors
Liu, X
Grogan, TR
Hieronymus, H
et al.

Publication Date
2016-12-06

DOI
10.1016/j.celrep.2016.11.010

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

Graphical Abstract

Highlights

- Low expression of CD38 enriches for luminal progenitor cells in human prostate
- CD38lo luminal cells are localized in proximity to prostatic inflammation
- CD38lo luminal cells are target cells for aggressive human prostate cancer
- Low CD38 in prostate cancer is prognostic for biochemical recurrence and metastasis

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.

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,1 Takao Hashimoto,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,5,3,17 Charles L. Sawyers,4 Jack Mottahedeh,1 David Elashoff,3,11 Steve Horvath,12,13 Jiaoti Huang,5,7,11,16 Owen N. Witte,2,6,8,14 and Andrew S. Goldstein1,5,10,11,17,*

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. CD38lo luminal cells are enriched in glands adjacent to inflammatory cells and exhibit epithelial nuclear factor κB (NF-κB) signaling. In response to oncogenic transformation, CD38lo luminal cells can initiate human prostate cancer in an in vivo tissue-regeneration assay. Finally, the CD38lo 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 (Elkahwaji 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., 2014), 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).

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 CD49Fhi 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 CD49flo CD38hi and CD49fhi CD38lo 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 CD38lo compared to CD38hi luminal cells (Figure S1B). Following cell isolation, cell diameters were measured to examine differences in size among the three epithelial populations. CD38lo luminal cells demonstrated an average diameter intermediate between basal and CD38hi luminal cells (Figure 1F). These findings indicate that CD38 stratifies luminal cells into two distinct subpopulations. Importantly, CD38lo luminal cells do not express CD26 by flow cytometry (Figure S1C). These data indicate that CD38lo luminal cells are distinct from CD26hi luminal cells and represent a population not previously investigated in prior studies (Karthauser et al., 2014; Park et al., 2016; Stoyanova et al., 2013).

CD38lo Luminal Cells Are Enriched for Progenitor Activity Compared to CD38hi 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; Karthauser et al., 2014; Park et al., 2016). We used a double-sorting approach to isolate highly purified preparations of CD38lo and CD38hi 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), CD38lo luminal cells exhibited progenitor activity intermediate between the highly clonogenic basal cells and the CD38hi luminal cells (Figures 2B and 2C). In a culture system adapted for the growth of human prostate-like tissue in vitro (Karthauser et al., 2014), CD38lo luminal cells were highly (4- to 5-fold) enriched for organoid formation compared to CD38hi luminal cells.
CD38lo Luminal Cells Contain Both Basal and Luminal Markers

Figure 2D. CD38lo 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 CD38lo luminal cells were capable of generating organoids (Figure 2F), which is considerably greater than the rate of CD38hi luminal cells (1/142) and the 1% rate reported for CD26+ luminal cells (Karthaus et al., 2014; Park et al., 2016).

CD38lo 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 a RNA-seq signature comprised of 449 genes greater than 2-fold enriched in CD38lo luminal cells compared to CD38hi luminal cells and greater than 1.5-fold enriched compared to basal cells (Table S1). We found that CD38lo 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 Ilt8) and chemokines (Cxcl1, Cxcl2, Cxcl3, Cxcl6, Ccl2, and Ccl20) are enriched in CD38lo luminal cells (Figure 3A). We performed pathway analysis using DAVID Bioinformatics (Huang da 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 CD38lo luminal cells by microarray analysis. We confirmed activation of the nuclear factor κB (NF-κB) 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 CD38lo 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 CD38lo luminal cells. We confirmed that TNF is expressed in CD38lo cells but absent from CD38hi luminal cells by immunohistochemistry (Figure S3A), suggesting that TNF may activate NFkB signaling in CD38lo cells. We observed a diminution of p65 phosphorylation and reduced expression of BCL2 in...
CD38lo 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 CD38lo luminal cells to a greater extent than other epithelial subsets (Figure S3C).

**CD38lo 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 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 CD38lo 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 CD38lo surface phenotype (Figures 3G and S3F).

**Reduced Androgen Signaling in CD38lo Luminal Cells**

CD38lo 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 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 CD38lo 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 CD38lo surface phenotype (Figures 3G and S3F).
immunohistochemistry, we confirmed diminished expression of androgen targets PSA, FKBP5, and MSMB in CD38lo luminal cells at the protein level (Figure 4B). Androgen receptor (AR) target NKX3-1 was also reduced in CD38lo luminal cells (Figure S4). We hypothesized that low androgen signaling in CD38lo luminal cells may be due to low levels of AR. While AR is expressed in CD38lo cells, protein levels are reduced compared to CD38hi luminal cells (Figures 1E and 4C).

**CD38lo 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 significantly associated with time to biochemical recurrence (Figures 5A and 5B; Table S3). The lowest expression of CD38 was found in the most advanced tumors based on Gleason grade (Figures 5A and 5B). Immunohistochemical staining was verified using two different anti-CD38 antibodies (Figure S5). While the average CD38 expression level was statistically significantly associated with biochemical recurrence, CD38 expression in cancer specimens was not statistically significantly associated with biochemical recurrence,

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).
To determine whether the CD38lo 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 CD38lo 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 (CD38lo luminal-like) versus all others (Figure S6B; Table S6C). The statistical significance of the CD38lo signature within this restricted group reached p = 0.0007 after adjusting for Gleason sum, age, and cancer percentage (Table S6A). The CD38lo 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 CD38lo signature was independent of the effect 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).

**CD38lo Luminal Cells Express the Therapeutic Target PSCA**

While CD38 expression is low in the most aggressive tumors, we reasoned that genes in the CD38lo 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). Pscsa is enriched in CD38lo 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 (CD38hi PSCA-low) from cancer (CD38lo PSCA-hi) (Figure 6B). We hypothesized that CD38lo PSCA-hi cancers may arise from the transformation of CD38lo PSCA-hi luminal cells.

**CD38lo Luminal Cells Can Regenerate Glands and Initiate Human Prostate Cancer**

To determine the functional capacity of CD38lo 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 CD38lo 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 CD38lo luminal cells were combined with UGSM and implanted into NOD-SCID-IL2Rγnull 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; Stayanova et al., 2013) (Myc, myristoylated AKT, and AR) were introduced into double-sorted CD38lo 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 CD38lo luminal cells (Figure 7C). Regenerated tumors exhibited expression of oncogenes (Figure S7B). Importantly, tumors exhibited the PSCA-hi CD38lo 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...
that CD38\textsuperscript{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, 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, 2010; Garcia et al., 2014).

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\textsuperscript{hi} or CD38\textsuperscript{hi} luminal cell population exhibit organoid-forming activity, \textsim99\% of cells in this subset do not exhibit progenitor features (Figures 2B–2D; Karthaus et al., 2014; Park et al., 2016). In contrast, CD38\textsuperscript{lo} luminal cells are enriched for progenitor activity in 2D and 3D progenitor assays, as 5\% of CD38\textsuperscript{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\textsuperscript{lo} luminal cells may yield mechanisms regulating luminal progenitor activity.

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

CD38\textsuperscript{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\textsuperscript{lo} luminal cells may enhance their resistance to apoptosis (Raffo et al., 1995), suggesting that CD38\textsuperscript{lo} luminal cells may have a survival advantage under low-androgen conditions. Consistent with mouse models demonstrating that prostatic inflammation or epithelial NF-\textkappaB activation is associated with reduced androgen signaling and increased epithelial proliferation (Birbach et al., 2011; Khalili et al., 2010), CD38\textsuperscript{lo} luminal cells express low levels of PSA and other androgen targets. These data suggest that CD38\textsuperscript{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\textsuperscript{lo} luminal cells that retain low expression of CD38.
Alternatively, the activation of a CD38lo 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 CD38lo 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 (CD38lo luminal) is predisposed to initiate aggressive cancer, perhaps due to elevated NF-κB signaling.

CD38lo 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 CD38lo luminal cells into a progenitor-enriched CD38lo luminal-like cell capable of initiating aggressive prostate cancer. Interestingly, CD38lo 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 CD38lo 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 CD38lo 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 to Gapdh, Keratin 8, and Kit3 (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.ingenity.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.
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 antibody, 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-μm 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-κB, ACHP (4547, Tocris) was added to organoid media at a final concentration of 5 μM every 3 days.

**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**

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

**ACCESSION NUMBERS**

**OPEN ACCESS**

Cell Reports 17, 2596–2606, December 6, 2016

2604
REFERENCES


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 initia-
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-medi-
ated Stat3 activation in the malignant transformation of foregut basal progen-
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.
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 IIIA study of AGS-PSCA for castration-resistant prostate can-
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
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., Vencio, R.Z., Page, L.S., Liebeskind, E.S., Shadle, C.P., Troisch,
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,
Raffo, A.J., Periman, H., Chen, M.W., Day, M.L., Streitman, J.S., and Buttyan,
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,
Integrative clinical genomics of advanced prostate cancer. Cell 161, 1215–
1228.
Sato, T., Vries, R.G., Snipper, H.J., van de Wetering, M., Barker, N., Stange,
Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal
Sboner, A., Demichelis, F., Caiza, S., Pawitan, Y., Settur, S.R., Hoshida, Y.,
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 Morri-
son, 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.,
nomogram predicting the 10-year probability of prostate cancer recurrence
tate cancer originating in basal cells progresses to adenocarcinoma propaga-
Taylor, B.S., Schultz, N., Herynynus, H., Gopalan, A., Yao, Y., Carver, B.S.,
Taylor, R.A., Toivanen, R., Frydenberg, M., Pedersen, J., Harewood, L., Collins,
A.T., Maitland, N.J., and Risbridger, G.P.; Australian Prostate Cancer Bio-
source (2012). Human epithelial basal cells are cells of origin of prostate
Cancer Cell 18, 11–22.
van Leenders, G.J., Gage, W.R., Hicks, J.L., van Balken, B., Aalders, T.W.,
prostate epithelium are enriched in proliferative inflammatory atrophy. Am. J.
Pathol. 162, 1529–1537.
Wang, X., Kruithof-de Julio, M., Economides, K.D., Walker, D., Yu, H., Halli,
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.,
reveals their unexpected plasticity and supports a cell-of-origin model for prostate
Luminal cells are favored as the cell of origin for prostate cancer. Cell Rep. 8,
1339–1346.
promotes colorectal cancer stem cell expansion and metastasis in mice.
Gastroenterology 149, 1884–1895.
Supplemental Information

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

Xian Liu, Tristan R. Grogan, Haley Hieronymus, Takao Hashimoto, Jack Mottahedeh, Donghui Cheng, Lijun Zhang, Kevin Huang, Tanya Stoyanova, Jung Wook Park, Ruzanna O. Shkhyan, Behdokht Nowroozizadeh, Matthew B. Rettig, Charles L. Sawyers, David Elashoff, Steve Horvath, Jiaoti Huang, Owen N. Witte, and Andrew S. Goldstein
Low CD38 identifies progenitor-like inflammation-associated luminal cells that can initiate human prostate cancer and predict poor outcome

Xian Liu, Tristan R. Grogan, Haley Hieronymus, Takao Hashimoto, Jack Mottahedeh, Donghui Cheng, Lijun Zhang, Kevin Huang, Tanya Stoyanova, Jung Wook Park, Ruzanna O. Shkhyan, Behdokht Nowroozizadeh, Matthew B. Rettig, Charles L. Sawyers, David Elashoff, Steve Horvath, Jiaoti Huang, Owen N. Witte, Andrew S. Goldstein
Experimental Procedures

Comparison of CD38 expression between histological grades
To compare the CD38 expression levels between the four different prostate regions (Normal, PIN, Gleason < 7, Gleason 7-10), a generalized estimating equation (GEE) model was run with the normal/identity link function. We performed a log+1 transformation prior to analysis due to the skewed distribution of the score. The exchangeable correlation structure was used in the model to account for repeated measures (i.e. multiple cores per person). After the overall effect of different levels of CD38 among the prostate regions was confirmed (p<0.001), follow-up pairwise comparisons were run to determine which regions were statistically significantly different from one another. To control the overall familywise error rate at $\alpha=0.05$ we used Tukey’s adjusted p-values for multiple comparisons.

Summary measure for CD38
Before constructing a prognostic model with CD38 as a predictor, the correlation between the scores across the different histological grades was assessed. The correlation between the average CD38 expression between normal, PIN, and cancer cores was computed using Spearman’s rank correlation coefficient. The correlations between the 3 regions were fairly low (between 0.20-0.30) indicating some biological variation across the different core sample types within an individual. In general the cancer cores expressed lower CD38 levels compared to the PIN and normal regions (Figure 5C). Cox proportional-hazards models were used to determine the prognostic ability of CD38 as a predictor of biochemical recurrence. A statistically significant association between overall mean (low) expression of CD38 and time to biochemical recurrence was observed (p=0.025). All analyses were performed in SAS V9.4 (SAS Institute, Cary, NC) and SPSS V22 (IBM Corp., Armonk, NY). P-values <0.05 were considered statistically significant.

Antibodies used

Immunohistochemistry: CD38 (LS-B8131, Lifespan Biosciences; sc-374650, Santa Cruz), CD45 (M0701, Dako), CD4 (MS-1528, Thermo Scientific), CD8 (M7103, Dako), CD11c (ab52632, Abcam), CD68 (M0876, Dako), CD20 (M0755, Dako), CD26 (LS-A9024, Lifespan Biosciences), Keratin 5 (905501, Biolegend), Keratin 8 (904801, BioLegend), p63 (sc-8431, Santa Cruz), AR (M3562, Dako), PSA (A0562, Dako), FKBP5 (ab126715, Abcam), MSMB (ab133296, Abcam), PSCA (LS-B3332, Lifespan Biosciences), NF-kB p65 phosphorylated S536 (ab86299, Abcam), TNF alpha (60291-1-Ig, Proteintech), Myc (ab32072, Abcam), phospho-AKT (3787, Cell Signaling), Ki67 (M7240, Dako), Nkx3-1 (0314, Athena Environmental Sciences).

Immunoblot: p63 (sc-8431, Santa Cruz), CD38 (sc-374650, Santa Cruz), K14 (905301, BioLegend), Histone H3 (9715, Cell Signaling), AR (sc-816, Santa Cruz), NF-kB p65 (ab31481, Abcam), NF-kB p65 phosphorylated S536 (ab86299, Abcam), BCL2 (M0887, Dako).
Fig. S1. Expression of keratins and CD26 in CD38-hi and CD38-lo luminal cells, related to Figure 1. (A-B) Intracellular flow cytometry on benign human prostate glands, gated on CD45- EpCAM+ CD49f-lo luminal cells, further stratified into CD38-hi and CD38-lo subsets and stained with antibodies against Keratin-18 (A) or Keratin-5 (B). (C) Flow cytometry on benign human prostate glands stained with antibodies against CD45, EpCAM, CD49f, CD38 and CD26. CD45- EpCAM+ CD49f-low CD38-low luminal cells do not express CD26.
Fig. S2. p63 expression is absent from sorted CD38-lo luminal cells but evident in organoids derived from CD38-lo luminal cells, related to Figure 2. (A) Single-sorted basal cells and double-sorted CD38-lo luminal cells were plated onto glass slides and cells were stained for the basal antigen p63 (green) and DAPI nuclear counterstain (blue). The percentage of p63+ cells per field is quantified. Statistics represent two-tailed paired t-test, **** represents p < 0.0001. (B) Representative images of p63+ basal cells (left) and p63-CD38-lo luminal cells (right) are shown. Scale bars, 50 microns. (C) Representative staining of CD38-lo luminal cell-derived organoids stained for p63 indicates both p63+ and p63- cells. Scale bars, 200 microns.
Fig. S3. Inflammatory signaling and inflammation-adjacent location of CD38-lo luminal cells, related to Figure 3. (A) Immunohistochemistry on serial sections of benign human prostate stained for CD38 and TNF alpha indicating mutually exclusive staining patterns. Scale bars, 100 microns. (B) Western blot of CD38-lo luminal cells treated for 4 hours with 5 uM NFkB inhibitor ACHP compared to control. (C) 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. (D) Immunohistochemistry on serial slides of benign human prostate stained for CD38 and inflammatory cell markers CD4, CD8, CD11c, CD68 and CD20. All scale bars, 100 microns. (E) Immunohistochemical staining for CD38 in normal and atrophic glands with measurements for distances extending from the basement membrane to the lumen shown. (F) Pearson correlation of % CD45+ immune cells and % of luminal cells expressing CD38-lo phenotype in 29 patient samples. r = 0.4492, ** p = 0.0145.
Fig. S4. CD38 and Nkx3-1 expression are correlated in human prostate, related to Figure 4. Serial sections of benign human prostate showing immunohistochemical staining for CD38 and Nkx3-1. Boxed regions are magnified below to demonstrate stronger staining for Nkx3-1 in CD38-hi compared to CD38-lo luminal cells. Scale bars, 50 microns.
**Fig. S5. Two CD38 antibodies give similar results, related to Figure 5.**
Immunohistochemical staining for CD38 with LifeSpan Biosciences antibody LS-B8131 and Santa Cruz antibody sc-374650 on representative human prostate cancer tissues with higher-power images below. Scale bars, 200 microns.
Fig. S6. Correlation of CD38 expression and CD38-lo luminal signature with common alterations, AR signature score and outcome, related to Figure 5. (A) Using cBioportal, primary tumors with mRNA expression in the MSKCC cohort were stratified based on ERG fusion status (left), ERG mRNA outlier status (middle) or SPINK1 outlier status (right). Statistics represent unpaired t test. (B) 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. (C) Pearson correlation of CD38-lo luminal signature score with AR signature score in MSKCC and TCGA cohorts.
Fig. S7. Fluorescence identifies human epithelial cells in regenerated tissues, related to Figure 7. (A) Schematics of lentiviruses used for in vivo regeneration and transformation related to Figure 3. From top to bottom: FUCRW (RFP), FUCGW (GFP), FU-AR-CGW (AR), FU-AKT-CRW (AKT), FU-Myc-CRW (Myc). (B) CD38-lo luminal cell initiated tumors driven by Myc, AKT and AR stained for oncogenes Myc and AKT. Scale bars, 25 microns. (C) Serial slides stained for H&E or viewed under the fluorescence microscope. In the absence of CD38-lo luminal cells, no epithelial structures are present corresponding to an absence of fluorescence signal. Normal glands in the RFP/GFP group and malignant glands in the Myc/AKT/AR group express varying red fluorescence confirming human epithelial origin. Scale bars, 100 microns.
Table S1. CD38-lo luminal gene signature and RPKM values, related to Figure 3. Table S1A indicates genes listed by official gene symbol or ID and the average fold change for CD38-lo luminal cells compared to CD38+ luminal (middle) or basal cells (right) from three distinct individuals. The signature is restricted to genes with expression in CD38-lo luminal cells that is 2-fold greater than CD38+ luminal and 1.5-fold greater than basal cells. Table S1B (second tab) includes all RPKM values from three patients and fold changes within each patient. See excel file.
Table S2. Patient characteristics of UCLA Tissue Microarray, related to Figure 5. Table indicates the breakdown of patients based on age, serum levels of prostate-specific antigen (PSA), Gleason score, extracapsular extension on pathological analysis, positive surgical margin, seminal vesicle invasion, Pathologic stage at diagnosis and recurrence status.

<table>
<thead>
<tr>
<th>Age_GT65</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid &lt;65</td>
<td>150</td>
<td>58.4</td>
<td>58.4</td>
<td>58.4</td>
</tr>
<tr>
<td>&gt;=65</td>
<td>111</td>
<td>41.6</td>
<td>41.6</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>267</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PSAcat</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid &lt;5</td>
<td>56</td>
<td>21.0</td>
<td>21.0</td>
<td>21.0</td>
</tr>
<tr>
<td>5-10</td>
<td>110</td>
<td>41.2</td>
<td>41.2</td>
<td>62.2</td>
</tr>
<tr>
<td>&gt;10</td>
<td>101</td>
<td>37.8</td>
<td>37.8</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>267</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GleasonScorecat</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid &lt;=6</td>
<td>140</td>
<td>52.4</td>
<td>52.4</td>
<td>52.4</td>
</tr>
<tr>
<td>7</td>
<td>113</td>
<td>42.3</td>
<td>42.3</td>
<td>94.8</td>
</tr>
<tr>
<td>&gt;=8</td>
<td>14</td>
<td>5.2</td>
<td>5.2</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>267</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ECE on path analysis</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid No</td>
<td>236</td>
<td>88.4</td>
<td>88.7</td>
<td>88.7</td>
</tr>
<tr>
<td>Yes</td>
<td>30</td>
<td>11.2</td>
<td>11.3</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>266</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>Missing System</td>
<td>1</td>
<td>.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>267</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Positive surgical margin</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid No</td>
<td>159</td>
<td>59.8</td>
<td>60.2</td>
<td>60.2</td>
</tr>
<tr>
<td>Yes</td>
<td>105</td>
<td>39.3</td>
<td>39.8</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>264</td>
<td>99.6</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>Missing System</td>
<td>3</td>
<td>.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>267</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SV invasion</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid No</td>
<td>236</td>
<td>88.4</td>
<td>88.7</td>
<td>88.7</td>
</tr>
<tr>
<td>Yes</td>
<td>30</td>
<td>11.2</td>
<td>11.3</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>266</td>
<td>99.6</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>Missing System</td>
<td>1</td>
<td>.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>267</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CsigCat</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid T1</td>
<td>112</td>
<td>41.9</td>
<td>41.9</td>
<td>44.9</td>
</tr>
<tr>
<td>T2</td>
<td>145</td>
<td>54.7</td>
<td>54.7</td>
<td>99.6</td>
</tr>
<tr>
<td>T3</td>
<td>1</td>
<td>.4</td>
<td>.4</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>267</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Recurrence</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid No</td>
<td>159</td>
<td>59.8</td>
<td>60.2</td>
<td>60.2</td>
</tr>
<tr>
<td>Yes</td>
<td>105</td>
<td>39.3</td>
<td>39.8</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>264</td>
<td>99.9</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>Missing System</td>
<td>3</td>
<td>1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>267</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>
Table S3. Statistical comparison of CD38 expression between histological grades, related to Figure 5. Statistical analysis comparing the CD38 expression levels between the four different prostate regions (NL, PIN, Gleason < 7, Gleason 7-10) indicates a statistically significant difference between CD38 levels in normal tissue and diseased states.

<table>
<thead>
<tr>
<th>Difference</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NL Pin</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NL &lt; 7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NL 7-10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PIN &lt; 7</td>
<td>0.037</td>
</tr>
<tr>
<td>PIN 7-10</td>
<td>0.001</td>
</tr>
<tr>
<td>7-10 &lt; 7</td>
<td>0.070</td>
</tr>
</tbody>
</table>

Table S4. Association between low protein expression of CD38 and biochemical recurrence, related to Figure 5. Cox proportional-hazards model was used to calculate the association between low expression of CD38 and time to biochemical recurrence. P-values <0.05 were considered statistically significant.

**Univariate model (biochemical recurrence)**

<table>
<thead>
<tr>
<th>CD38 Aggregation</th>
<th>HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean CA</td>
<td>0.9994 (0.9971, 1.002)</td>
<td>0.595</td>
</tr>
<tr>
<td>Overall mean</td>
<td>0.9967 (0.9939, 0.9996)</td>
<td>0.025</td>
</tr>
</tbody>
</table>
Table S5. Statistics evaluating CD38 mRNA with patient outcome, related to Figure 5. Table indicates Cox regression analysis for low CD38 (z-score less than 1) or CD38 expression as a continuous variable in the Memorial Sloan-Kettering dataset (Taylor et al, Cancer Cell, 2010; Hieronymus et al, PNAS 2014) and TCGA dataset (TCGA, Cell, 2015). Sig: statistical significance. HR: hazard ratio. Lower/Upper represents 95% confidence interval. Nomogram refers to Stephenson et al prediction for 10-year probability of disease recurrence after prostatectomy including both clinical and pathological variables (Stephenson et al. J Clin Oncol 2005).

<table>
<thead>
<tr>
<th>Table</th>
<th>Univariate Cox Regression, Biochemical Recurrence, MSKCC cohort</th>
<th>Multivariate Cox Regression with nomogram, Biochemical Recurrence</th>
<th>Univariate Cox Regression, Metastasis, MSKCC cohort</th>
<th>Multivariate Cox Regression with nomogram, Metastasis</th>
<th>Univariate Cox Regression, Biochemical Recurrence, TCGA cohort</th>
<th>Multivariate Cox Regression with nomogram, Metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>low CD38 (z-score less than 1)</td>
<td></td>
<td>low CD38 (z-score less than 1)</td>
<td>low CD38 (z-score less than 1)</td>
<td>CD38 expression (z-score, continuous)</td>
<td>CD38 expression (z-score, continuous)</td>
</tr>
<tr>
<td>Sig.</td>
<td>0.001</td>
<td></td>
<td>0.006</td>
<td>0.004</td>
<td>0.001</td>
<td>0.006</td>
</tr>
<tr>
<td>HR</td>
<td>3.665</td>
<td></td>
<td>3.023</td>
<td>4.729</td>
<td>0.517</td>
<td>0.412</td>
</tr>
<tr>
<td>Lower</td>
<td>1.685</td>
<td></td>
<td>1.381</td>
<td>1.640</td>
<td>0.352</td>
<td>0.218</td>
</tr>
<tr>
<td>Upper</td>
<td>7.972</td>
<td></td>
<td>6.619</td>
<td>13.635</td>
<td>0.759</td>
<td>0.778</td>
</tr>
<tr>
<td>Nomogram score</td>
<td>0.000</td>
<td></td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>0.955</td>
<td></td>
<td>0.960</td>
<td>0.960</td>
<td>0.959</td>
<td>0.977</td>
</tr>
<tr>
<td></td>
<td>0.942</td>
<td></td>
<td>0.943</td>
<td>0.977</td>
<td>0.942</td>
<td>0.977</td>
</tr>
<tr>
<td></td>
<td>0.968</td>
<td></td>
<td>0.968</td>
<td>0.977</td>
<td>0.977</td>
<td>0.977</td>
</tr>
<tr>
<td></td>
<td>0.000</td>
<td></td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>0.968</td>
<td></td>
<td>0.968</td>
<td>0.977</td>
<td>0.977</td>
<td>0.977</td>
</tr>
</tbody>
</table>
### All patients

<table>
<thead>
<tr>
<th></th>
<th>coef</th>
<th>se (coef)</th>
<th>Z</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top Third CD38-lo luminal-like</td>
<td>0.3125</td>
<td>0.1498</td>
<td>2.086</td>
<td>0.03699</td>
</tr>
<tr>
<td>Gleason Sum</td>
<td>0.5348</td>
<td>0.0693</td>
<td>7.715</td>
<td>1.21E-14</td>
</tr>
<tr>
<td>Age</td>
<td>0.0508</td>
<td>0.0111</td>
<td>4.566</td>
<td>4.97E-06</td>
</tr>
<tr>
<td>Cancer %</td>
<td>0.0095</td>
<td>0.0031</td>
<td>3.061</td>
<td>0.00221</td>
</tr>
</tbody>
</table>

#HR for tumors in the top third: 1.37 (95% CI: 1.019,1.833)

### Gleason >=7, follow up within 5 years

<table>
<thead>
<tr>
<th></th>
<th>coef</th>
<th>se (coef)</th>
<th>Z</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top Third CD38-lo luminal-like</td>
<td>0.8061</td>
<td>0.2387</td>
<td>3.377</td>
<td>0.0007</td>
</tr>
<tr>
<td>Gleason Sum</td>
<td>0.4358</td>
<td>0.1171</td>
<td>3.723</td>
<td>0.0002</td>
</tr>
<tr>
<td>Age</td>
<td>0.0492</td>
<td>0.0175</td>
<td>2.815</td>
<td>0.0049</td>
</tr>
<tr>
<td>Cancer %</td>
<td>0.0088</td>
<td>0.0043</td>
<td>2.057</td>
<td>0.0397</td>
</tr>
</tbody>
</table>

#HR for tumors in the top third: 2.056 (95% CI: 1.322,3.198)

### Gleason >=7, follow up within 5 years (corresponding to Fig S7B)

<table>
<thead>
<tr>
<th></th>
<th>coef</th>
<th>se (coef)</th>
<th>Z</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top Third CD38-lo luminal-like</td>
<td>0.6203</td>
<td>0.2315</td>
<td>2.68</td>
<td>0.0074</td>
</tr>
</tbody>
</table>

#HR for tumors in the top third: 1.86 (95% CI: 1.181, 2.927)

Concordance= 0.574 (se = 0.026 )
Rsquare= 0.034  (max possible= 0.981 )
Likelihood ratio test= 6.84  on 1 df,  p=0.008928
Wald test        = 7.18  on 1 df,  p=0.007371
Score (logrank) test = 7.41  on 1 df,  p=0.006479
Table S6. Statistical analysis of CD38-lo luminal gene signature associated with overall survival in Swedish Watchful Waiting cohort, related to Figure 5. (A-B) We performed a multivariate cox model adjusting for Gleason sum, age, and percentage of cancer. (A) 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. (B) 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. (C) Analysis corresponding to Figure S6B comparing the survival of patients in the top third compared to the remainder. (D-E) Multivariate cox model including AR signature score and NARP21 immune signature score related to A-B.