MUC1-C integrates type II interferon and chromatin remodeling pathways in immunosuppression of prostate cancer

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ABSTRACT
The oncopgenic MUC1-C protein drives dedifferentiation of castrate resistant prostate cancer (CRPC) cells in association with chromatin remodeling. The present work demonstrates that MUC1-C is necessary for expression of IFNGR1 and activation of the type II interferon-gamma (IFN-γ) pathway. We show that MUC1-C→ARID1A/BAF signaling induces IFNGR1 transcription and that MUC1-C-induced activation of the NuRD complex suppresses FBXW7 in stabilizing the IFNGR1 protein. MUC1-C and NuRD were also necessary for expression of the downstream STAT1 and IRF1 transcription factors. We further demonstrate that MUC1-C and PBRM1/PBAF are necessary for IRF1-induced expression of (i) IDO1, WARS, and PTGES, which metabolically suppress the immune tumor microenvironment (TME), and (ii) the ISG15 and SERPINB9 inhibitors of T cell function. Of translational relevance, we show that MUC1 correlates with expression of IFNGR1, STAT1 and IRF1, as well as the downstream IDO1, WARS, PTGES, ISG15 and SERPINB9 immunosuppressive effectors in CRPC tumors. Analyses of scRNA-seq data further demonstrate that MUC1 correlates with cancer stem cell (CSC) and IFN gene signatures across CRPC cells. Consistent with these results, MUC1 associates with immune cell-depleted “cold” CRPC TMEs. These findings demonstrate that MUC1-C integrates chronic activation of the type II IFN-γ pathway and induction of chromatin remodeling complexes in linking the CSC state with immune evasion.

Introduction
Castration resistant prostate cancer (CRPC) is effectively treated with agents that target the androgen receptor (AR) pathway. However, resistance to this therapy inevitably develops, often from progression to more aggressive disease with neuroendocrine (NE) features. Subsequent treatment options are then limited, emphasizing an unmet need for other therapeutic strategies. Immunotherapy represents an attractive approach for treating advanced PC, although success with immune checkpoint inhibitors (ICIs) has been limited in comparison to other types of cancers. Evaluation of the anti-PI3K inhibitor pembrolizumab in docetaxel-resistant metastatic CRPC (mCRPC) demonstrated modest anti-tumor activity and improvement in overall survival. In a retrospective analysis, pembrolizumab was more effective in the setting of microsatellite instability high (MSI-H) mCRPCs, suggesting that mutational burden may be a contributing factor for response. Other studies have identified the immunosuppressive PC tumor microenvironment (TME) as a critical factor in the lack of response to immunotherapy. Given the complexities of immunosuppressive TMEs, no single mechanism has been attributable to ICI resistance. However, inherently “cold tumors” characterized by depletion or dysfunction of immune effector cells are likely a significant contributing factor.

The MUC1 gene emerged in mammals to provide protection of epithelial niches from loss of homeostasis. MUC1 encodes an N-terminal (MUC1-N) subunit that contributes to a physical mucous barrier and a transmembrane C-terminal (MUC1-C) subunit that is activated by stress. MUC1-C contributes to inflammatory, proliferative and remodeling responses associated with the wound healing response. However, prolonged MUC1-C activation in association with chronic inflammation promotes oncogenesis by driving activation of the epithelial mesenchymal transition (EMT) and epigenetic reprogramming in cancer cells. Upregulation of

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**MUC1** in PC associates with aggressive disease and poor patient outcomes. In PC cells, MUC1-C suppresses the AR axis and activates MYC signaling pathways that drive lineage plasticity in neuroendocrine prostate cancer (NEPC) progression. In this capacity, MUC1-C induces the Yamanaka pluripotency factors that function as pioneer TFs in the reprogramming of embryonic stem cells (ESCs). MUC1-C integrates MYC-induced pluripotency factor expression with activation of E2F1 and induction of the embryonic stem cell esBAF and PBAF chromatin remodeling complexes in driving changes in chromatin accessibility and PC dedifferentiation. Increasing evidence has linked cell fate specification and the cancer stem cell (CSC) state to immune evasion. In this respect, lineage plasticity in cancer represents a major challenge responsible for immune evasion and poor clinical outcomes. These findings have collectively invoked the possibility that MUC1-C-induced lineage plasticity and dedifferentiation in CRPC could contribute to an immunosuppressive TME. In support of this notion, the present work demonstrates that MUC1-C promotes “cold” immune effector cell-depleted CRPC TMEs by chronic activation of tumor intrinsic inflammatory and immunosuppressive pathways.

**Materials and methods**

**Cell culture**

Human DU-145 cells (ATCC) were cultured in RPMI1640 medium (Thermo Fisher Scientific, Waltham, MA, USA) containing 10% fetal bovine serum (FBS; GEMINI Bio-Products, West Sacramento, CA, USA), 100 μg/ml streptomycin and 100 U/ml penicillin. LNCaP-AI cells were grown in phenol red-free RPMI1640 medium (Thermo Fisher Scientific, Waltham, MA, USA) containing 10% charcoal-stripped FBS (Millipore Sigma, Burlington, MA, USA). Human NCI-H660 NEPC cells (ATCC) were cultured in RPMI1640 medium with 5% FBS, 10 nM β-estradiol (Millipore Sigma), 10 nM hydrocortisone, 1% insulin-transferin-selenium (Thermo Fisher Scientific) and 2 mM L-glutamine (Thermo Fisher Scientific). Cells were treated with 10 ng/ml human recombinant IFN-γ (Stemcell Technologies, Vancouver, BC, Canada). Authentication of the cells was performed by short tandem repeat (STR) analysis every 4 months. Cells were monitored for mycoplasma contamination using the MycoAlert Mycoplasma Detection Kit (Lonza, Rockland, ME, USA) every 3 months.

**Tetracycline-inducible gene silencing**

MUC1shRNA (MISSION shRNA TRCN0000122938; Sigma), MTA1shRNA (MISSION shRNA TRCN0000230496), MBD3shRNA (MISSION shRNA TRCN0000274441), PBRM1shRNA (MISSION shRNA TRCN0000235890), JUNshRNA (sc-29223-SH, Santa Cruz Biotechnologies), ARID1AshRNA (MISSION shRNA TRCN0000590992) was inserted into the pLKO-puro vector. Guide RNA (1#: GATCGTCAAGTTATATCGAG; 2#: TGAACTGTGTTCCACAGTCG) targeting MUC1 exon 4 were inserted into the lentriCRISPR v2 (Plasmid 52961; Addgene). The viral vectors were produced in 293 T cells. Cells transduced with the vectors were selected for growth in 2 μg/ml puromycin. For tet-inducible vectors, cells were treated with 0.1% DMSO as the vehicle control or 500 ng/ml doxycycline (DOX; Millipore Sigma).

**Quantitative reverse-transcription PCR (qRT-PCR)**

Total cellular RNA was isolated using Trizol reagent (Thermo Fisher Scientific). cDNAs were synthesized and amplified as described. Primers used for qRT-PCR are listed in Supplementary Table S1.

**Immunoblot analysis**

Whole cell lysates were prepared in RIPA buffer containing protease inhibitor cocktail (Thermo Fisher Scientific, Waltham, MA, USA). Immunoblotting was performed with anti-MUC1-C (16564, 1:1000 dilution; Cell Signaling Technology (CST), Danvers, MA, USA), anti-STAT1 (9172S, 1:1000 dilution; CST), anti-IRF1 (#8478S, 1:1000 dilution; CST), anti-IFNGR1 (34808, 1:1000; CST), anti-GAPDH (#5174S, 1:1000 dilution; CST) and anti-β-actin (A5441; 1:10000 dilution; Sigma, St. Louis, MO, USA), anti-FBXW7 (ab109617, 1:1000; abcam, Cambridge, MA, USA), anti-MTA1 (5647, 1:1000; CST), anti-MBD3 (14540, 1:1000; CST), anti-IDO1 (86630, 1:1000; CST), anti-WARS (GTX110223, 40037, 1:1000; Gene Tex), anti-PTGES (ab180589, 1:abcam), anti-PBRM1(A301-591A, 1:10000; Bethyl Laboratories, Montgomery, TX, USA), anti-ARID1A (12354, 1:500; CST), anti-IG515 (sc166755, 1:200; Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-SERPINE9 (PA5-51038, 1:2000; Invitrogen, Waltham, MA, USA).

**Coimmunoprecipitation studies**

Nuclear lysates from DU-145 cells were precipitated with a control IgG or anti-MUC1-C. Input and precipitated proteins were immunoblotted with anti-JUN (9165, 1:1000; CST).

**RNA-seq analysis**

Total RNA from cells cultured in triplicates was isolated using Trizol reagent (Invitrogen). TruSeq Stranded mRNA (Illumina, San Diego, CA, USA) was used for library preparation. Raw sequencing reads were aligned to the human genome (GRCh38.74) using STAR. Raw feature counts were normalized and differential expression analysis using DESeq2. Differential expression rank order was utilized for subsequent Gene Set Enrichment Analysis (GSEA), performed using the fgsea (v1.8.0) package in R. Gene sets queried included those from the Hallmark Gene Sets available through the Molecular Signatures Database (MSigDB).

**Chromatin immunoprecipitation (ChIP)**

ChIP was performed on cells crosslinked with 1% formaldehyde for 5 min at 37°C, quenched with 2 M glycine and washed with PBS, and then sonicated in a Covaris E220 sonicator to
generate 300–600 bp DNA fragments. Immunoprecipitation was performed using a control IgG (Santa-Cruz Biotechnology) and antibodies against MUC1-C (D5K91; CST), JUN (32137; CST), ARID1A (12354; CST), EP300 (D2X6N; CST), H3K27ac (ab4729; Abcam), H3K4me1 (ab8895; Abcam) and H3K4me3 (ab8580; Abcam). Precipitated DNAs were detected by PCR using primers listed in Supplemental Table 1. Quantitation was performed on immunoprecipitated DNA using SYBR-green and the CFX384 real-time PCR machine (Bio-Rad, USA). Data are reported as fold enrichment relative to IgG.

**ATAC-seq**

ATAC-seq libraries were generated from three biologically independent replicates per condition. Library preparation and quality control were performed as described. Peak calling for all libraries was performed using MACS2.

**Statistical analysis**

Each experiment was performed at least three times. Data are expressed as the mean ± SD. The unpaired Mann-Whitney U test was used to determine differences between means of groups. A p-value of <0.05 denoted by an asterisk (*) was considered statistically significant.

**Analysis of publicly available cohort data**

TCGA-PRAD, SU2C-CRPC and Beltran cohort expression and clinical annotations were obtained from the Genomic Data Commons (GDC) data portal and processed via TCGAbiolinks package in R using TCGAWorkflow guided practices. Normalized expression and clinical annotations were obtained directly from cBioPortal. Differential expression associated with MUC1 expression (MUC1-high = top quartile; MUC1-low = bottom quartile) within each respective cohort was determined by TCGAbiolinks/edgeR or limma. Gene set enrichment analysis of differential expression was assessed using the clusterProfiler package. Query gene sets derived from the Hallmark, Canonical pathways, and GO Biological Processes Ontology collections were retrieved from the MSigDB. Cell type enrichment within each sample was estimated from bulk expression via xCell and TIP analyses.

**Analysis of CRPC scRNA-seq dataset**

Processed scRNA-seq data comprising cells captured from 14 metastatic CRPC samples were obtained directly from the Broad Institute Single Cell Portal (https://singlecell.broadinstitute.org/single_cell). Normalized expression (transcripts per kilobase million (TPM)) and previously determined cell annotation were utilized. Tumor cells (n = 836) were isolated from prior annotations, and tumor cell gene expression was imputed using MAGIC, implemented via the Rmagic package in R. CRPC cell imputed expression was re-analyzed via Seurat for variable feature selection, dimensionality reduction (PCA), and uniform manifold approximation and projection (UMAP) low-dimensional representation. Single-cell pathway enrichment was performed by AUCell, using select HALLMARK or curated pathways representing AR signaling (AR, FKBP5, TMPRSS2, KLK3, NKX3-1, STEAP4, PMEPA1, PLP1, PART1, ALDH1A3, DPP4) or prostate CSC (KLF4, NANOG, POU5F1, MYC, SOX2, CD44, PSCA, PROM1, EZH2, ABCG2, ALDH1A1, TGM2, KIT, ARID1A) signatures. Associations between MUC1 expression and select genes or signatures within CRPC cells were examined by Pearson correlation analysis.

**Results**

**MUC1 associates with chronic activation of the IFN-γ inflammatory response pathway in prostate carcinomas**

MUC1-C is aberrantly expressed in CRPC and has been linked to CRPC progression. Analysis of the TCGA-PRAD dataset derived from 333 primary PCs demonstrated that MUC1-high PCs significantly associate with activation of the HALLMARK INTERFERON GAMMA RESPONSE gene signature (Figure 1a). Similar results were obtained from analysis of 266 metastatic CRPCs in the SU2C-CRPC dataset (Figure 1b), suggesting that MUC1 may functionally contribute to activation of the type II IFN pathway. Further analysis of the TCGA-PRAD dataset showed that MUC1-high PCs have significantly increased levels of IFNGR1 and IFNGR2 expression as compared to that in MUC1-low tumors (Figure 1c). Stimulation of the IFNGR1 receptor complex by IFN-γ activates the downstream STAT1 and IRF1 transcription factors (TFs) that drive IFN response genes (ISGs) and chronic inflammatory responses in cancer cells. Notably, MUC1-high PCs were significantly associated with upregulation of STAT1 and IRF1 expression (Figure 1d). Moreover, we found that MUC1 significantly associates with the HALLMARK INFLAMMATORY RESPONSE gene signature in the TCGA-PRAD (Figure 1e) and SU2C-CRPC (Figure 1f) datasets, in support of activating the IFN-γ pathway and promoting chronic inflammation in PC cells.

**MUC1-C activates the IFNGR1 distal enhancer-like sequence**

In extending these observations linking MUC1-C to regulation of the IFN-γ pathway, we silenced MUC1-C in DU-145 CRPC cells with a tet-inducible MUC1shRNA and found that MUC1-C is necessary for expression of IFNGR1 transcripts (Figure 2a) and protein (Figure 2b). Stable MUC1-C-silencing with single guide RNAs (sgRNAs) confirmed that MUC1-C is necessary for IFNGR1 expression (Figure 2c). In addition, similar results were obtained in (i) LNCAP cells that were derived from androgen-dependent LNCaP cells selected for growth under androgen-independent (AI) conditions and overexpress MUC1-C (Supplemental Figs. S1a and S1b), (ii) PC3 CRPC cells (Supplemental Fig. S1c) and (iii) NCI-H660 NEPC cells (Supplemental Fig. S1d). In investigating the mechanism responsible for MUC1-C-induced IFNGR1 expression, we assessed the effects of silencing MUC1-C on the IFNGR1 gene
at a distal enhancer-like signature (dELS) that includes a putative AP-1 binding motif (Figure 2d). Along these lines, nuclear MUC1-C forms complexes with JUN/AP-1 (Supplemental Fig. S1e) and we detected occupancy of MUC1-C and JUN on the IFNGR1 dELS region (Figure 2d). Consistent with JUN-mediated recruitment of the BAF chromatin remodeling complex, we also detected occupancy of ARID1A (Figure 2d). Of significance for MUC1-C-mediated IFNGR1 activation, silencing MUC1-C decreased JUN and ARID1A occupancy (Figure 2e). Moreover, silencing JUN and ARID1A suppressed IFNGR1 expression (Supplemental Figs. S1f-S1i).

Silencing MUC1-C also decreased H3K4me3 levels (Figure 2f) and chromatin accessibility of the dELS (Figure 2g), indicating that MUC1-C activates IFNGR1 by a mechanism involving JUN, ARID1A and remodeling of chromatin.

**MUC1-C suppresses FBXW7 to promote IFNGR1 expression**

The FBXW7 ubiquitin E3 ligase induces proteasomal IFNGR1 degradation and thereby downregulation of the IFN-γ signaling pathway. In determining whether MUC1-C also contributes to regulation of IFNGR1

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**Figure 1.** Expression of MUC1 in PC tumors associates with chronic activation of the type II IFNG pathway. (a and b). Enrichment plots for the HALLMARK INTERFERON GAMMA RESPONSE pathway, comparing MUC1-high to MUC1-low PC tumors in the TCGA-PRAD (a) and SU2C-CRPC (b) cohorts. (c and d). Normalized expression data for the TCGA-PRAD cohort were downloaded from cBioPortal, and median expression used to group samples into MUC1-high and MUC1-low groups. Expression of IFNGR1 and IFNGR2 (c), and downstream STAT1 and IRF1 (d). genes was assessed in MUC1-high and MUC1-low groups using a Wilcoxon rank-sum test. Boxplots represent the 1st quartile, median and 3rd quartile of each distribution. Whiskers extend to the maximum and minimum values up to 1.5*interquartile range (IQR). (e and f). Enrichment plots for the HALLMARK INFLAMMATORY RESPONSE pathway, comparing MUC1-high to MUC1-low PC tumors in the TCGA-PRAD (e) and SU2C-CRPC (f) cohorts.
through this pathway, we found that silencing MUC1-C results in the induction of FBXW7 mRNA and protein (Figure 3a–b; Supplemental Fig. S2a-S2c). In a gain-of-function study, we also found that overexpression of MUC1-C suppresses FBXW7 and upregulates IFNGR1 expression (Supplemental Fig. S2d). MUC1-C represses gene expression by activation of the nucleosome remodeling and deacetylation (NuRD) complex that consists in part of MTA1 and MBD3.66 Silencing MUC1-C resulted in suppression of MTA1 and MBD3 expression in DU-145 and LNCaP-AI cells (Figure 3c; Supplemental Fig. S2e). Moreover, silencing MTA1 or MBD3 was associated with induction of FBXW7 and downregulation of IFNGR1 levels (Figure 3d–e; Supplemental Fig. S2f). In
extending these results, suppression of IFNGR1 levels by silencing MUC1-C (Figure 3f), MTA1 (Figure 3g) or MBD3 (Figure 3h) was abrogated by treatment with the proteasome inhibitor MG-132, confirming the involvement of MUC1-C and the NuRD complex in regulating IFNGR1 stability. Taken together, these results indicate that MUC1-C drives IFNGR1 expression by transcriptional and posttranscriptional mechanisms.

**MUC1-C induces STAT1 and IRF1 expression**

Stimulation of IFNGR1 by IFN-γ activates the STAT1→IRF1 pathway. Consistent with MUC1-C-mediated induction of IFNGR1, we found that silencing MUC1-C decreases STAT1 and IRF1 transcripts (Figure 4a; Supplemental Fig. S3a) and protein (Figure 4b–c; Supplemental Figs. S3b and S3c). In addition, MUC1-C was necessary for induction of STAT1 and
IRF1 expression in the response to IFN-γ stimulation (Figure 4d). As found for IFNGR1, silencing MTA1 or MBD3 suppressed STAT1 and IRF1 expression (Figure 4e). In addition, silencing MUC1-C decreased chromatin accessibility of the STAT1 (Figure 4f) and IRF1 (Figure 4g) genes, in support of a MUC1-C-driven pathway that drives IFNGR1, STAT1 and IRF1.

**MUC1-C and PBRM1 are necessary for induction of the metabolic IDO1, WARS and PTGES immunosuppressive factors**

The finding that MUC1-C induces IRF1 invoked the possibility that MUC1-C is necessary for activation of downstream effectors in the type II IFN pathway. To identify type II IFN genes...
that are regulated by MUC1-C, RNA-seq was performed on DU-145/tet-MUC1shRNA cells treated with vehicle or DOX and then stimulated with IFN-γ. Analysis of the datasets demonstrated that MUC1-C drives IFN-γ-induced activation and repression of the HALLMARK INTERFERON GAMMA RESPONSE gene signature (Figure 5a; Supplemental Fig. S4a) and other enriched pathways that include the HALLMARK INTERFERON ALPHA RESPONSE (Supplemental Figs. S4b and S4c). Specifically, we found that MUC1-C is necessary for IFN-γ-induced STAT1 and IRF1 expression (Figure 5b). As determined by Epigenetic Landscape in Silico deletion Analysis (LISA), MUC1-C induced genes were enriched for STAT1 and IRF1 regulation (Supplemental Fig. S4d), indicating that MUC1-C drives the IFN-γ response by activating STAT1→IRF1 signaling. Consistent with this notion, we found that MUC1-C is necessary for expression of immunosuppressive indoleamine-2,3-dioxygenase-1 (IDO1), tryptophanyl-tRNA synthetase (WARS), and PTGES effectors (Figure 5b). IRF1 induces (i) IDO1, which reduces tryptophan (Trp) levels in the TME that are necessary for T cell proliferation and function; (ii) WARS that is associated with IDO1 expression and protects cancer cells from Trp depletion; and (iii) PTGES, which is required for the synthesis of PGE2, an inhibitor of T cell function. Analysis of the TCGA-PRAD and SU2C-CRPC datasets further demonstrated that MUC1-high PCs significantly associate with upregulation of IDO1 (Figure 5c; Supplemental Fig. S5a), as well as IDO2 and tryptophan-2,3-dioxygenase (TD2), which also degrades Trp (Supplemental Figs. S5a and S5b). In addition, MUC1 was significantly associated with WARS and PTGES expression.
(Figure 5c; Supplemental Fig. S5c). In support of these observations, we confirmed that MUC1-C is necessary for constitutive (Figure 5d–e; Supplemental Figs. S6a–S6d) and IFN-γ-induced (Figure 5f; Supplemental Figs. S6e and S6f) expression of IDO1, WARS and PTGES transcripts and protein. The polybromo-associated BAF (PBAF) chromatin remodeling complex, which includes BRG1, PBRM1/BAF180, ARID2/BAF200 and BRD7, is activated by the MUC1-C→E2F1 signaling pathway in CRPC cells.55 PBRM1 has been associated with conferring resistance to T cell-mediated killing of tumor cells.53–55 Of interest in that regard, we found that PBRM1 is dispensable for constitutive STAT1 and IRF1 expression (Figure 5g). However, and as found for MUC1-C, PBRM1 was necessary for expression of IDO1, WARS and PTGES (Figure 5g). PBRM1 was also necessary for IFN-γ-stimulated upregulation of IDO1, WARS and PTGES (Figure 5h), indicating that the MUC1-C→PBRM1 pathway functions in promoting induction of these IRF1 target genes.

**MUC1-C→PBRM1 signaling induces the ISG15 and SERPINB9 inhibitors of CTL function**

Analysis of the IFN-γ-stimulated DU-145 cell dataset further demonstrated that MUC1-C is necessary for induction of (i) ISG15, which encodes a ubiquitin-like protein involved in innate immunity that suppresses CTL function in the TME,56–58 and (ii) SERPINB9, a member of the serpin family encoding a granzyme B inhibitor that confers resistance to CTL killing.59,60 Analysis of the TCGA-PRAD dataset demonstrated that MUC1 is associated with ISG15 and SERPINB9 expression (Figure 6a). Studies in DU-145 and LNCaP-AI cells further showed that silencing MUC1-C downregulates ISG15 and SERPINB9 mRNA (Supplemental Fig. S7a) and protein (Figure 6b–c) levels. We also found that PBRM1 is necessary for ISG15 and SERPINB9 expression (Figure 6d; Supplemental Fig. S7b), supporting involvement of the MUC1-C→PBRM1 pathway. Consistent with these results, MUC1-C (Figure 6e–f; Supplemental Fig. S7c) and PBRM1 (Figure 6f; Supplemental Fig. S7d) were necessary for IFN-γ-induced expression of these suppressive effectors of CTL function.

**MUC1 associates with suppression of the CRPC tumor immune microenvironment**

In support of MUC1-C involvement in promoting suppression of the PC TME, analysis of the TCGA-PRAD dataset demonstrated that MUC1 associates with enrichment of REACTOME INTERLEUKIN 10 SIGNALING and GO RESPONSE TO TRANSFORMING GROWTH FACTOR BETα pathways (Figure 7a). GSEA confirmed that MUC1-high tumors significantly associate with activation of the IL-10 (Supplemental Fig. S8a) and TGFβ1 (Supplemental Fig. S8b) gene signatures, which function as negative regulators of the immune TME.61 We also found that MUC1-high tumors associate with expression of CCL5 (Supplemental Fig. S8c), an inflammatory chemokine that recruits TAMs, MDSCs and T-reggs into the TME and inhibits CTL activity.62 Consistent with these findings, MUC1 was significantly associated with negative regulation of (i) T cell and NK cell mediated immunity, (ii) T cell proliferation, and (iii) B cell activation (Figure 7b). In extending these analyses to the Beltran cohort (67 CRPC/NEPC samples),53 tumors were stratified by MUC1-high and MUC1-low expression. Hierarchical clustering based on cell type estimation (xCell)34 demonstrated that MUC1-high clusters associate with decreased estimates of immune cell infiltration (Figure 7c). Further analysis by immune cell type demonstrated that MUC1-high tumors significantly associate with decreases in CD4+ memory T cells, Th2 cells, B cells and M2 macrophages, among others, as well as the ImmuneScore (Figure 7d; Supplemental Fig. S8d).

![Figure 6](https://example.com/figure6.png)

*Figure 6.* MUC1-C and PBRM1/PBAF drive ISG15 and SERPINB9 expression. (a) Expression of ISG15 and SERPINB9 in the TCGA-PRAD cohort was assessed in MUC1-high and MUC1-low groups using a Wilcoxon rank-sum test. (b) Lysates from DU-145/tet-MUC1shRNA treated with vehicle or DOX for 7 days were immunoblotted with antibodies against the indicated proteins. (c) Lysates from DU-145/ChshRNA or DU-145/MUC1shRNA1 were immunoblotted with antibodies against the indicated proteins. (d) Lysates from DU-145/CshRNA and DU-145/PBRM1shRNA cells were immunoblotted with antibodies against the indicated proteins. (e and f) DU-145/tet-MUC1shRNA cells treated with vehicle or DOX for 7 days were stimulated with 10 ng/ml IFN-γ for 24 hours. Analysis of the indicated genes in vehicle- and DOX-treated cells showing significant differences in mRNA levels (e). Lysates were immunoblotted with antibodies against the indicated proteins (f). (g) Lysates from DU-145/CshRNA and DU-145/PBRM1shRNA cells stimulated with 10 ng/ml IFN-γ for 24 hours were immunoblotted with antibodies against the indicated proteins.
MUC1 associates with intratumoral CSC and IFN signatures in CRPCs

To examine MUC1 as it relates to tumor cell heterogeneity and the CSC state, we analyzed a scRNA-seq dataset containing 836 CRPC cells obtained from 14 patients with metastatic disease to assess the distribution of MUC1 expression (Figure 8a–b; Supplemental Fig. S9a). This dataset comprises CRPC cells of both adenocarcinoma and small cell morphology, thus spanning an array of CRPC transcriptional phenotypes. Single-cell pathway enrichment was performed to examine the cellular distributions of AR, CSC and IFN signaling pathways (Figure 8c, Fig. S9b–c). The CSC signature was strongly enriched in CRPC cells of small cell phenotype, as well as subsets of adenocarcinoma cells, which was inversely correlated with AR signaling (Figure 8c). Notably, IFN and inflammatory signatures were significantly and positively associated with CSC enrichment. Similarly, MUC1 expression significantly associated with CSC and IFN signatures across CRPC cells, typified by significant associations with CSC-related (SOX2, NANOG, KIT, EZH2, KL4, ARID1A) and to a lesser extent IFN-related (IRF1, STAT1, IFNGR1, IFNGR2) genes (Figure 8d). MUC1 was inversely correlated with AR and AR target genes (KLK3, TMPRSS2, NKX3-1, DPP4) (Figure 8d). These results suggest that MUC1, as well as IFN, signatures, reside in CRPC cells with CSC characteristics rather than being homogeneously distributed across all CRPC cells.

Discussion

The type II IFN-γ pathway plays opposing roles in tumor immune surveillance and evasion that are dependent on context of both the tumor cell and TME. Intrinsic activation of the IFN-γ pathway in cancer cells contributes to chronic inflammation, immune evasion and progression. Despite this importance, little is known about induction of the IFNGRI gene, which is essential for activating this pathway. Analysis of the TCGA-PRAD and SU2C-CRPC RNA-seq datasets revealed that MUC1 associates with IFNGR1, as well as IFNGR2, expression. Moreover, we found that MUC1-high PC
tumors significantly associate with activation of the HALLMARK INTERFERON GAMMA RESPONSE pathway. These findings invoked the possibility that MUC1-C is necessary for induction of the IFNGR1 gene and the IFN-γ pathway (Figure 9). To address this notion, we studied CRPC and NEPC cells and found that MUC1-C is necessary for expression of IFNGR1 transcripts. MUC1-C→E2F1 signaling induces ARID1A and other components of the esBAF chromatin remodeling complex in NEPC progression. ARID1A mutations, which drive cancer development, limit chromatin accessibility and expression of IFN-responsive genes. MUC1-C forms complexes with JUN and ARID1A in promoting chromatin accessibility of NOTCH1 and other stemness-associated genes in CRPC cells. In the present work, we found that MUC1-C, JUN and ARID1A form a complex on the IFNGR1 eLMS region that induces chromatin accessibility, H3K4 trimethylation and IFNGR1 expression. These findings provided direct evidence for a MUC1-C-induced pathway that integrates induction of ARID1A/BAF with intrinsic activation of the CSC state and IFN-γ pathway in CRPC progression (Figure 9).

While performing these experiments, we recognized that MUC1-C is also playing a role in the posttranscriptional regulation of IFNGR1 expression. In this regard, other work had demonstrated that the FBXW7 ubiquitin ligase promotes degradation of the IFNGR1 protein. Those studies showed that the ELF5 TF induced FBXW7 expression and in turn IFNGR1 destabilization. There is no known link between MUC1-C and the regulation of FBXW7. MUC1-C activates the NuRD complex, which includes MTA1 and MBD3, in suppressing gene expression and has been linked to the regulation of FBXW7 transcription. We found that MUC1-C downregulates FBXW7 by a mechanism involving MTA1 and MBD3. MUC1-C, MTA1 and MBD3 were necessary for suppression of FBXW7 and, as a result, stabilization of the IFNGR1 protein (Figure 9). Taken together with the effects of MUC1-C on IFNGR1 transcription, these
findings uncovered another previously unrecognized MUC1-C-driven pathway that increases IFNGR1 expression by a posttranscriptional mechanism. Stimulation of IFNGR1 by IFN-γ activates the downstream STAT1 and IRF1 effectors of the type II IFN pathway. Consistent with MUC1-C-induced upregulation of IFNGR1, we found that MUC1 is associated with STAT1 and IRF1 expression in CRPC/NEPC cells (Figure 9). In addition, silencing MUC1-C, MTA1 and MBD3 in CRPC cells decreased chromatin accessibility and expression of STAT1 and IRF1, indicating that MUC1-C is necessary for activation of the IFNGR1→STAT1/IRF1 pathway (Figure 9).

IRF1 is an essential regulator of downstream effectors, such as IDO1,49 WARS50,51 and PTGES,52 that promote immunosuppression of the TME by metabolically inhibiting T cell functions. IRF1 also drives ISG1556–58 and SERPINB959,60 that confer resistance of cancer cells to CTL killing. Consistent with the demonstration that MUC1-C is necessary for induction of IRF1, we found that silencing MUC1-C in CRPC/NEPC cells suppresses expression of these immunosuppressive effectors. In addition, MUC1-high PC tumors significantly associated with upregulation of IDO1, WARS, PTGES, ISG15 and SERPINB9. As reported for ARID1A/BAF,21 MUC1-C→E2F1 signaling activates the PBAF chromatin remodeling complex, integrating the ARID1A/BAF and PBRM1/PBAF pathways in CRPC cells.22 Unexpectedly, we found that PBRM1 is necessary for induction of IDO1, WARS, PTGES, ISG15 and SERPINB9 (Figure 9). PBRM1 has been associated with conferring resistance to T cell-mediated killing of melanoma cells.73 In addition, PBRM1 deficiency has been associated with (i) clinical benefit to ICI treatment,74 and, in contrast, (ii) a less immunogenic TME and ICI resistance.55 Our findings that the MUC1-C→E2F1→PBRM1/PBAF signaling induces effectors that inhibit CTL functions support involvement of this pathway in immune evasion (Figure 9).

MUC1-C drives lineage plasticity and dedifferentiation in the progression of CRPC and NEPC cells.19 MUC1-C-induced activation of the BAF and NuRD complexes has been linked to dedifferentiation and progression of CSCs.21,46 MUC1-C also activates the PBRM1/PBAF complex in regulating redox balance and lineage plasticity in CSCs.22 These and the present findings lend support to a model in which MUC1-C integrates activation of (i) the BAF, NuRD and PBAF complexes, (ii) IFNGR1 and the immunosuppressive IFN-γ pathway, and (iii) dedifferentiation and CSC progression (Figure 9). In this regard, cancer cell stemness is intimately associated with resistance to treatment with immunotherapeutic agents, albeit by mechanisms that remain unclear.24–27,68 Our findings in individual CRPC cells with a small cell phenotype demonstrate that MUC1-C associates with CSC and IFN gene signatures. These results suggest that MUC1 is selectively expressed in CRPC cells with CSC characteristics and chronic inflammatory signaling, linking stemness with immune evasion. In further support of the notion that MUC1-C integrates the CSC state with immune suppression, MUC1-C drives dedifferentiation in triple-negative breast cancer (TNBC) cells23,46 and contributes to suppression of the TNBC immune TME.69

Of potential translational relevance, antibodies and vaccines have been developed against the MUC1-N subunit with a particular emphasis on targeting the VNTR region.10 Clinical trials of these agents have demonstrated the induction of immune responses, but not effective anti-tumor activity.70 CAR T cells directed against the MUC1-N VNTR are now being evaluated in the clinic [Immunity Therapeutics; NCT04025216]. MUC1-N is shed from the cancer cell surface and circulates at increased levels in cancer patients, posing potential obstacles for directing anti-MUC1-N CAR T cells to tumors.12 In contrast, MUC1-C is not shed from the surface of cancer cells and the MUC1-C extracellular domain thus represents another potential target for CAR T cells.12 Nonetheless, treating solid tumors with CAR T cells has had limited success to date71 and, based on the present results and those in TNBCs,69 the effects of MUC1-C on suppression of the immune TME could represent a significant challenge for this field. In this regard, targeting the MUC1-C extracellular domain with antibody-drug conjugates72 and the MUC1-C intracellular domain with the GO-203 inhibitor12 provide alternative approaches for
killing MUC1-C-expressing tumor cells and reversing the associated immunosuppressive TME that could be used in combination with other immunotherapeutics.

**Author contributions**

Conceptualization, MH, AF, MDL, DK; Methodology, MH, AF, NY, YM; Investigation, MH, AF, NY, YM; Bioinformatics Analysis, AF, HGW, QH, TL, MDL, SL; Writing-Original Draft, D.K.; Writing-Review and Editing, MH, MDL, DK; Funding Acquisition, MO, SL, KKW, DK.

**Disclosure statement**

DK has equity interests in Genus Oncology, Reata Pharmaceuticals, and HillstreamBioPharma, and is a paid consultant to Reata and CanBas.

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**Data availability**

The accession numbers for the RNA-seq data are GEO Submission GSE139335 and GSE184896.

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