PIM Kinases Alter the Prostate Tumor Immune Microenvironment

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Abstract

Prostate cancer is the second leading cause of cancer-related deaths in American men. While prostate cancer patients typically respond to androgen-deprivation therapy and/or taxane chemotherapy, patients inevitably develop resistance, and the disease progresses to an untreatable and lethal form known as castrate resistant prostate cancer (CRPC). Immunotherapy has changed the treatment paradigm for many types of cancer; however, immune checkpoint inhibitors have not shown clinical benefits in CRPC. Tumor associated macrophages (TAMs) secrete factors that promote tumor progression and suppress antitumor immunity, making them a promising therapeutic target. The Proviral Integration site for Moloney murine leukemia virus (PIM) kinases are serine/threonine kinases that are overexpressed in prostate cancer. PIM regulates many signaling pathways that promote cell survival. However, how PIM kinase alters the prostate tumor immune microenvironment and impacts immunotherapy resistance is not well understood.

We analyzed primary prostate and metastatic lymph node samples from treatment-naive metastatic hormone-sensitive prostate cancer patients. Prostate cancer samples with PIM1/PIM2/PIM3-high and -low expression were classified by top and bottom quartile, respectively. PIM high tumors had higher expression of immunostimulatory genes (*IL18*, *TNF*, and *TNFSF13*), increased infiltration of M2 macrophages, B cells, and NK Cells, and a higher T cell inflamed score compared to PIM-low tumors. Due to the changes observed in inflammation, we hypothesized that PIM kinase may regulate macrophage inflammatory signaling. We showed that PIM inhibition suppresses inflammasome signaling and the release of the pro-inflammatory cytokine, IL-1β. Chronic inflammation can lead to the recruitment of TAMs, further promoting resistance to immune checkpoint inhibitors. Utilizing a syngeneic mouse model of prostate cancer, we demonstrated that PIM inhibition in combination with immune checkpoint blockade synergistically decreased tumor growth. Furthermore, immunoprofiling demonstrated that combination treatment enhances T cell activity. Overall, our results suggest that PIM kinase plays an important role in regulating the prostate tumor immune microenvironment and that PIM kinase may be a potential target to enhance the efficacy of immunotherapy for the treatment of prostate cancer.

Introduction

- Hypoxic regions of solid tumors are highly infiltrated by tumor associated macrophages (TAMs)
- TAMs generate an immunosuppressive tumor microenvironment
- TAM infiltration is strongly associated with aggressiveness and poor patient survival in prostate cancer
- PIM kinases are pro-survival kinases that regulate many signaling pathways that promote cell growth, proliferation, and survival
- Recent studies suggest that PIM kinase promotes immune evasion through the regulation of both tumor and immune cells

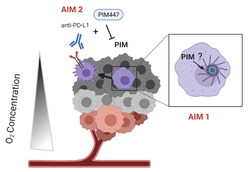

Figure 1. Project Overview. This model demonstrates the two main aims of the project. Aim 1 focuses on examining the effect of PIM kinase on inflammatory signaling in macrophages and the goal of Aim 2 is to test the use of PIM inhibitors in combination with immune checkpoint inhibitors for the treatment of prostate cancer.

PIM Expression Correlates with Macrophage Infiltration in Human Prostatic Adenocarcinoma

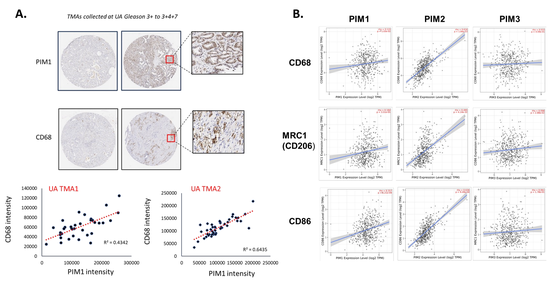

Figure 2. Correlation between PIM and Macrophage Gene Expression. A) Tissue Microarray (TMA) correlating PIM1 gene expression to CD68 (macrophage) expression. B) PIM1, PIM2, and PIM3 gene expression compared to CD68 (pan-macrophage), CD206 (M2 macrophage), and CD86 (M1 Macrophage) expression using The Cancer Genome Atlas (TCGA) prostate adenocarcinoma (PRAD) data (n=498).

PIM Kinase Expression Correlates with AR, PSA, and Hypoxia-Gene Expression

A.

	PIM-high	*PIM*-low	p-value
Counts	28	16	
Mean age (SD)	66.15 (10.40)	69.19 (11.14)	0.37
Median age (IQR)	65 [58-74]	69.5 [62.5-76.5]	0.48
Ethnicity Hispanic	10 (37.0%)	9 (56.2%)	0.22
Gleason Grade Group (4-5)	17 (77%)	10 (71%)	0.69
Karnofsky ≥ 90	17 (63%)	5 (31%)	0.04
Tumor Stage			0.36
1	0 (0%)	2 (14 %)	
2	3 (18%)	4 (29 %)	
3	8 (47%)	3 (21%)	
4	6 (35%)	5 (36%)	
Node Positive	16 (76%)	11 (69%)	0.72
Metastasis			0.44
M0	2 (8%)	3 (19%)	
M1a/M1b	14 (54%)	9 (56%)	
M1c	10 (38%)	4 (25%)	
Median PSA [Range]	66.0 [14.0-450.7]	24.0 [6.6-95.0]	0.14

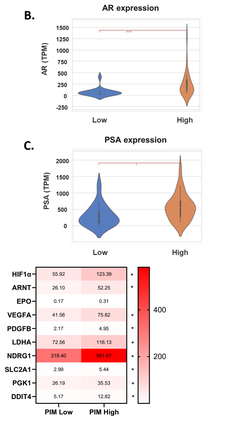

Figure 3. PIM Kinase Expression Correlates with AR, PSA, and Hypoxia-Gene Expression. A) 44 patients of treatment-naive metastatic hormone-sensitive PC pts were analyzed by DNA 592-gene, NextSeq; WES & WTS; NovaSeq (Caris Life Sciences, Phoenix, AZ). PC with PIM1/2/3-high (N=28) and -low (N=16) expressions were classified by top and bottom quartile, respectively. B) Androgen receptor (AR) (118.63 vs 50.89, p=4e-5) and C) Prostate Specific Antigen (PSA) (548.24 vs 213.20, p=0.02) transcripts per million (TPM) in *PIM*-high and –low tumors. D) MAPK pathway activity score (MPAS) (0.85 vs -1.38, p=2e-6) and E) Hypoxia-related gene expression (FC: 1.3-2.4, p<0.05) compared to PIM-low.

PIM Kinases Increase Inflammation and Immune Cell Infiltration in the Prostate Tumor Immune Microenvironment

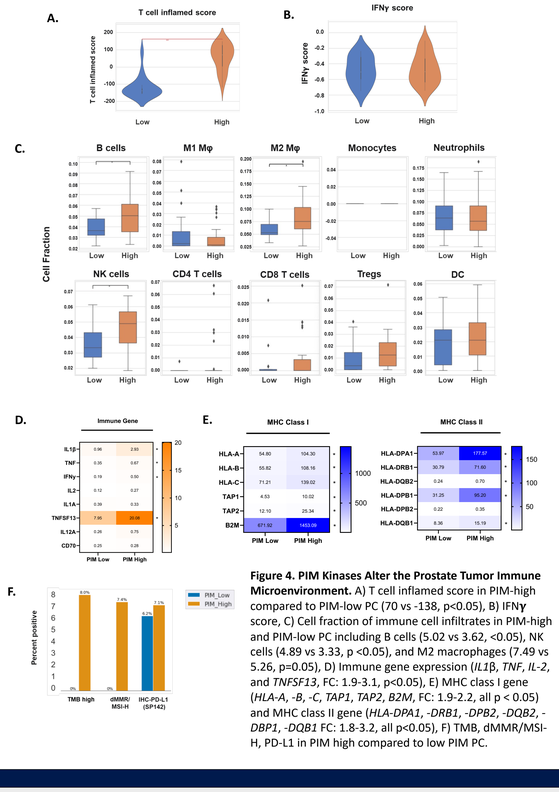

Figure 4. PIM Kinases Alter the Prostate Tumor Immune Microenvironment. A) T cell inflamed score in PIM-high compared to PIM-low PC (70 vs -138, p<0.05), B) IFNγ score, C) Cell fraction of immune cell infiltrates in PIM-high and PIM-low PC including B cells (5.02 vs 3.62, <0.05), NK cells (4.89 vs 3.33, p <0.05), and M2 macrophages (7.49 vs 5.26, p=0.05), D) Immune gene expression (*IL1β*, *TNF*, *IL-2*, and *TNFSF13*, FC: 1.9-3.1, p<0.05), E) MHC class I gene (*HLA-A*, *-B*, *-C*, *TAP1*, *TAP2*, *B2M*, FC: 1.9-2.2, all p < 0.05) and MHC class II gene (*HLA-DPA1*, *-DRB1*, *-DPB2*, *-DQB2*, *-DBP1*, *-DQB1* FC: 1.8-3.2, all p<0.05), F) TMB, dMMR/MSI-H, PD-L1 in PIM high compared to low PIM PC.

PIM Expression in Tumor Associated Macrophages Promotes MyC-Cap Prostate Tumor Growth

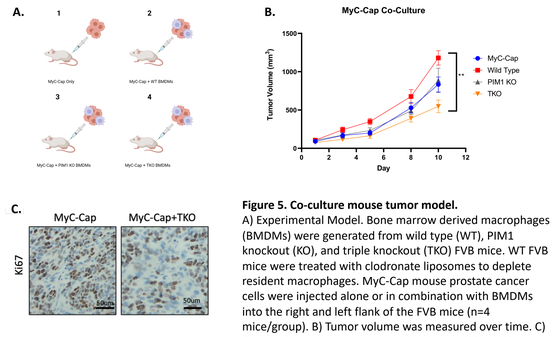

Figure 5. Co-culture mouse tumor model.
A) Experimental Model. Bone marrow derived macrophages (BMDMs) were generated from wild type (WT), PIM1 knockout (KO), and triple knockout (TKO) FVB mice. WT FVB mice were treated with clodronate liposomes to deplete resident macrophages. MyC-Cap mouse prostate cancer cells were injected alone or in combination with BMDMs into the right and left flank of the FVB mice (n=4 mice/group). B) Tumor volume was measured over time. C) Proliferation was assessed via Ki67 staining **p<.001

PIM Inhibition Suppresses Inflammasome Activation in Bone Marrow Derived Macrophages

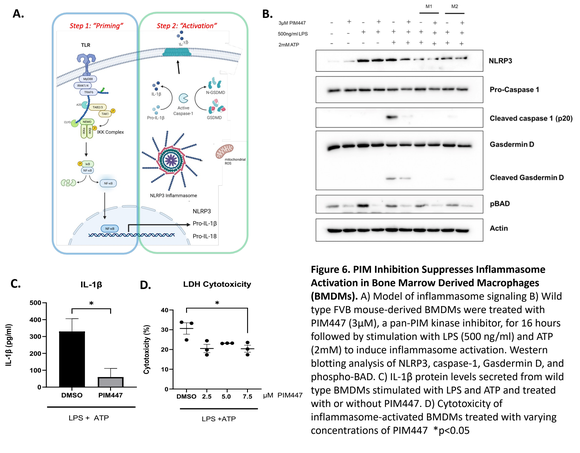

Figure 6. PIM Inhibition Suppresses Inflammasome Activation in Bone Marrow Derived Macrophages (BMDMs). A) Model of inflammasome signaling B) Wild type FVB mouse-derived BMDMs were treated with PIM447 (3μM), a pan-PIM kinase inhibitor, for 16 hours followed by stimulation with LPS (500 ng/ml) and ATP (2mM) to induce inflammasome activation. Western blotting analysis of NLRP3, caspase-1, Gasdermin D, and phospho-BAD. C) IL-1β protein levels secreted from wild type BMDMs stimulated with LPS and ATP and treated with or without PIM447. D) Cytotoxicity of inflammasome-activated BMDMs treated with varying concentrations of PIM447 *p<0.05

PIM Kinases Regulate NfκB Signaling

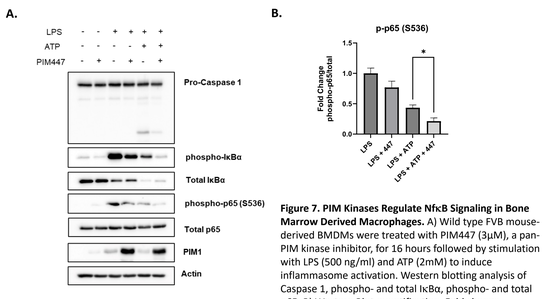

Figure 7. PIM Kinases Regulate NfκB Signaling in Bone Marrow Derived Macrophages. A) Wild type FVB mouse-derived BMDMs were treated with PIM447 (3μM), a pan-PIM kinase inhibitor, for 16 hours followed by stimulation with LPS (500 ng/ml) and ATP (2mM) to induce inflammasome activation. Western blotting analysis of Caspase 1, phospho- and total IκBα, phospho- and total p65. B) Western Blot quantification. Fold change phospho-p65/total. *<0.05

PIM Inhibition Enhances PD-L1 Blockade

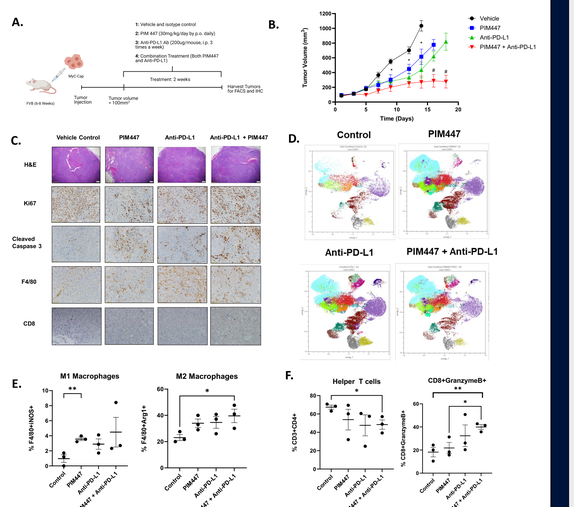

Figure 8. PIM Inhibition Enhances PD-L1 blockade. A) Experimental Design**.** MyC-Cap mouse prostate cells were injected into the right and left flank of FVB mice. Mice were treated with vehicle/isotype control, PIM447, Anti-PD-L1, or combination for two weeks. B) Tumor volume (n=10/group) was measured over time (* significant vs control, # significant vs ctrl, PD-L1 or PIM447 alone, p<.05). C) IHC of Ki67 (proliferation), cleaved caspase 3 (cell death), F4/80 (macrophage) and CD8 (Cytotoxic T cell). D) UMAP and flow Analysis (n=3/group) of E) M1 and M2 macrophages, F) Helper T cells and granzyme B+ CD8+ T cells.

Conclusions

- PIM1/2/3 upregulation correlates with increased inflammation and immunosuppressive immune cell infiltration
- PIM kinase expression in TAMs promotes tumor progression
- PIM inhibition suppresses inflammasome activation
- PIM inhibition decreases tumor growth and decreases macrophages in syngeneic mouse prostate tumors
- PIM inhibition enhances PD-L1 blockade
- PIM inhibition alters the prostate tumor immune microenvironment

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