Original Study

Immune Characterization of the Programmed Death Receptor Pathway in High Risk Prostate Cancer

Wesley Baas,[1] Svetlana Gershburg,[2] Danuta Dynda,[1] Kristin Delfino,[2] Kathy Robinson,[3] Daotai Nie,[4] Jennifer Holmes Yearley,[5] Shaheen Alanee[6]

Abstract

The objective of this study was to determine the expression of programmed cell death-1 (PD-1) and programmed cell death ligand-L1 (PD-L1) in high-grade prostate cancer tissues, and correlate the expression with disease and patient characteristics. Of the 25 samples, 2 (8%) scored high for PD-1 expression, 2 (8%) scored high for PD-L1 expression, and 18 (72%) scored high for CD3 expression.

Background: Programmed cell death-1 (PD-1), a T-cell inhibitory receptor, and its ligand, PD-L1, have been reported to be expressed in many tumor types, and this expression has led to the development of many drugs targeting the PD-1 pathway. The objective of this study was to determine the expression of PD-1 and PD-L1 in high-grade prostate cancer tissues, and correlate the expression with disease and patient characteristics. **Materials and Methods:** Immunohistochemistry for PD-1 (CD279), PD-L1 (B7-H1), and CD3 was performed and scored from 0 to 5 on prostatectomy/biopsy tissue samples taken from 25 men with high-grade prostate cancer. Charts were then retrospectively reviewed for numerous patient and disease characteristics. Statistical analyses were done to investigate the association of these patient and disease characteristics with PD-1, PD-L1, and CD3 expression. **Results:** A score of 3 to 5 on the semiquantitative 0 to 5 score was deemed "high" expression whereas a score of 0 to 2 was deemed "low" expression. Of the 25 samples, 2 (8%) scored high for PD-1 expression, 2 (8%) scored high for PD-L1 expression, and 18 (72%) scored high for CD3 expression. There was no statistically significant difference between high and low expression groups of PD-1, PD-L1, or CD3 for any of the variables we collected. **Conclusion:** An overall low expression of PD-1 and PD-L1, and a concurrent high expression of CD3+ T cells was found in high-risk prostate cancer tissue. No significant association was found between expression of PD-1, PD-L1, or CD3, and patient or disease characteristics. Because of this, one might be able to question the role of PD-L1 in local immune suppression in prostate cancer.

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Introduction

Prostate cancer (PCa) is the most common nondermatologic cancer in US men with more than 2.6 million men being diagnosed in the past 20 years. Whereas most men with PCa do not die from their disease, PCa still ranks as the second leading cause of cancer death in US men.[1] PCa is broken down into 4 risk levels: very low, low, intermediate, and high. High-risk PCa is defined as having prostate-specific antigen (PSA) $>$ 20 ng/mL at diagnosis, Gleason score $\geq$ 8, or clinical stage T2c disease. Patients with high-risk disease are of particular interest because they have been historically difficult to treat and suffer poor prognosis with approximately 28% of such patients presenting with bony metastasis.[2]

There has been a recent resurgence in immunotherapy as a way of combatting cancer. Of particular interest is the usage of immunomodulation via numerous immune response pathways. Many novel strategies for immunotherapy are potentially changing the landscape of treatment of genitourinary malignancies, including autologous cell therapy, and checkpoint inhibitors such as anticytotoxic

[1]Division of Urology
[2]Center for Clinical Research
[3]Cancer Institute
[4]Department of Med Micro, Immunology, and Cell Biology, Southern Illinois University School of Medicine, Springfield, IL
[5]Merck Research Laboratories, Palo Alto, CA
[6]Vattikuti Urology Institute, Henry Ford Health System, Detroit, MI

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Address for correspondence: Shaheen Alanee, MD, MPH, MBA, Vattikuti Urology Institute, Henry Ford Health System, 2799 W Grand Blvd, Detroit, MI 48202
E-mail contact: salanee1@hfhs.org

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T–lymphocyte-associated protein 4 (anti-CTLA-4), anti-programmed cell death 1 (anti-PD1), and anti-programmed cell death ligand 1 (anti-PDL1). Agents active in these pathways are producing impressive results in clinical trials, but their efficacy has not been consistent. The programmed cell death-1 (PD-1) receptor–ligand interaction is a major pathway that has been implicated in tumor immune evasion.[3] PD-1 protein is a T-cell coinhibitory receptor expressed by activated T cells and serves as a modulator to prevent excessive immune response under normal conditions.[4-6] PD-1 has 2 known ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC), which are expressed by tumor cells, stromal cells, or both.[4,7] There is little expression of PD-L1 in healthy tissues, but interestingly, numerous cancers have been reported to have high levels of this T-cell inhibitory ligand.[8-11] Because PD-L1 is a PD-1 ligand upregulated in solid tumors, and the blockage of the PD-1 and PD-L1 interaction has been shown to potentiate immune responses, it has sparked interest as a potential target in cancer immunotherapy.[3,12]

What determines a solid tumor response to an anti-PD1 agent is still to be elucidated. Recent reports on drugs acting on the PD-1 pathway indicated a possible association between differences in PD-1 and PD-L1 expression in tumor tissue and response to treatment. Therefore, the goal of this study was to understand the expression of PD-1, PD-L1, and CD3 in high-grade PCa tissue, and correlate the expression with aggressive pathologic characteristics and treatment outcomes in these patients. The hypothesis of this study was that there might be increased expression of PD-1 and PD-L1 in high-risk PCa, because of its presence in other cancers, and that high expression might correlate with some patient characteristics or treatment outcomes.

Materials and Methods

Study Design

In this study, we obtained prostatectomy or biopsy tissue samples (either a biopsy or prostatectomy specimen was used for each patient, not both) from 25 men with high-grade Gleason 8-10 PCa for analysis using PD-1, PD-L1, and CD3 immunohistochemistry (IHC). Patients were required to be male and older than the age of 18 years with documented Gleason 8-10 PCa. Patients were excluded if medical charts contained incomplete data, or if the patients were found to be prisoners at the time of initial tissue collection.

The following information was gathered from charts: age at diagnosis, race, Gleason score, PSA level at diagnosis, number of positive cores at biopsy, volume of tissue on biopsy and/or prostatectomy involved by cancer, clinical Tumor, Node, Metastases (TNM) stage, pathologic TNM stage, treatment and dates, any biochemical recurrence, and metastases. Tissue samples and clinical data were obtained according to an institutional review board-approved study protocol.

Immunohistochemistry

Programmed cell death-1, PD-L1, and CD3 IHC was performed at Merck Research Laboratories (Palo Alto, CA) on formalin-fixed paraffin-embedded sections of prostatectomy/biopsy tissue from all subjects. Sections were baked for 45 minutes at 60°C, then deparaffinized and rehydrated with serial passage through changes of xylene and graded ethanols. All slides were then subjected to heat-induced epitope retrieval in either Envision FLEX Target Retrieval Solution, High pH (cat K8012, Dako, Carpinteria, CA) or Target Retrieval Solution, pH 6.1 (cat S1699, Dako). Endogenous peroxidase in tissues was blocked by incubation of slides in 3% hydrogen peroxide solution before incubation with primary antibody (anti-PD-L1 clone 22C3; Merck Research Laboratories; anti-PD-1 clone NAT105, Cell Marque, Rocklin, CA; or anti-CD3 clone F7.2.38, Dako) for 60 minutes. Antigen–antibody binding was detected using polymer-based methods and visualized by application of 3,3′ diaminobenzidine chromogen (K4368; Dako). Stained slides were counterstained with hematoxylin and coverslipped for review and scoring by a pathologist on a semiquantitative 0 to 5 scale. This scale was on the basis of subjective score placed by a single pathologist (J.H.Y.) who analyzed all samples. These stained slides contained tumor cells as well as infiltrating immune cells, which were both stained and used as part of the analyses. Internal positive and negative controls were used by the pathologist.

Table 1 Patient Demographic Characteristics

Characteristic	n	Mean ± SD (Range)
Age at Diagnosis	25	64 ± 7.2 (50-79)
PSA at Diagnosis	23	13.9 ± 14.3 (2.4-68.9)
Percentage of Cores (+)	21	42.81 ± 24.95 (11-90)

Abbreviation: PSA = prostate-specific antigen.

Statistical Analysis

Descriptive statistics were computed for all study variables. Continuous variables are described with measures of central tendency (mean) and dispersion (range, SD). Categorical variables are summarized as frequencies and percentages. χ^2 Tests of independence or Fisher exact tests were used to compare categorical variables. Independent *t* tests were used to compare continuous patients and tumor variables. Linear regression was used to estimate the effect of various predictors on the outcome IHC score for each of the 3 (PD-1, PD-L1, and CD3) tumor markers. Pearson correlation coefficient was used to estimate the correlations between variables. Differences were considered statistically significant when the *P* value (2-sided) was $< .05$.

Results

Twenty-five men met inclusion criteria and had samples analyzed for PD-1, PD-L1, and CD3 expression. The average patient age was 64 ± 7.23 years, with a PSA of 13.9 ng/mL (range, 2.4-68.9), a Gleason score was 8 in 17 patients, 9 in 7 patients, and 10 in 1

Table 2 Immunohistochemical Expression in Samples

Expression	PD-L1 (%)	PD-1 (%)	CD3 (%)
Low	23 (92)	23 (92)	7 (28)
High	2 (8)	2 (8)	18 (72)

Samples were examined by a pathologist (J.H.Y.) and scored semiquantitatively on a 0 to 5 scale. A score of 0 to 2 was deemed "low" expression, whereas a score of 3 to 5 was deemed "high."
Abbreviations: PD-1 = programmed death 1; PD-L1 = programmed death ligand 1.