

mRNA EXPRESSION OF PD-1, PD-L1, AND IMMUNOTHERAPY IN BLADDER CANCER

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ABSTRACT

Objective: This study aims to investigate mRNA expression of PD-1 and PD-L1 in patients with bladder cancer. **Material & Methods:** In this study, we examined 30 samples from paraffin embedded tissue blocks, samples were divided into two groups, 15 were NMIBC, and 15 were MIBC according to their histopathological result. mRNA expression of PD-1 and PD-L1 were conducted using Real Time-Polymerase Chain Reaction (qRT-PCR) and statistical significance was set at a p -value < 0.05 . **Results:** Statistical analysis using the Mann-Whitney test found a significant difference in mRNA expression of PD-1 and PD-L1 in NMIBC compared to MIBC groups. **Conclusion:** mRNA expression of PD-1 and PD-L1 were higher in MIBC compared to NMIBC. PD-1 and PD-L1 as immune checkpoints are potential immunotherapy for patients with advance stage bladder cancer. Immunotherapy could be a substitute or combined with other treatments such as chemotherapy or radiotherapy.

Keywords: Bladder cancer, immunotherapy, PD-1, PD-L1.

ABSTRAK

Tujuan: Studi ini bertujuan untuk melakukan investigasi ekspresi mRNA PD-1 dan PD-L1 pada pasien kanker kandung kemih. **Bahan & Cara:** Studi ini menganalisa sampel kanker kandung kemih dari 30 pasien yang didapatkan menggunakan sediaan paraffin blok. Penelitian ini terdiri dari dua kelompok berdasarkan hasil patologi anatomi, yaitu 15 pasien dengan muscle invasive bladder cancer (MIBC) dan 15 pasien non-muscle invasive bladder cancer (NMIBC). Ekspresi mRNA dari PD-1 dan PD-L1 didapatkan menggunakan Real Time-Polymerase Chain Reaction (qRT-PCR) dengan hasil diasumsikan signifikan secara statistik bila $p < 0.05$. **Hasil:** Terdapat peningkatan mRNA PD-1 dan PD-L1 pada pasien MIBC bila dibandingkan dengan pasien NMIBC. **Simpulan:** Terdapat potensi penggunaan PD-1 dan PD-L1 sebagai agen imunoterapi pada pasien dengan stadium lanjut kanker kandung kemih. Imunoterapi dapat menjadi alternatif atau terapi kombinasi dengan kemoterapi atau radioterapi pada tatalaksana kanker kandung kemih.

Kata Kunci: Kanker kandung kemih, imunoterapi, PD-1, PD-L1.

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INTRODUCTION

Bladder cancer is the 10th most commonly occurring cancer worldwide, with an estimated number of about 549.000 new cases and 200.000 deaths according to GLOBOCAN 2018.¹ Bladder cancer is more common in men than in women. Based on invasion to detrusor muscle of the bladder, bladder cancer divided into Non-Muscle Invasive Bladder Cancer (NMIBC) and Muscle Invasive Bladder Cancer (MIBC). After endoscopic resection, most patients with NMIBC have cancer recurrence, 16% to 25% with high-grade cancer.²

Cancer immunotherapeutic therapy or tumor immunotherapy is an anticancer therapy that triggers the immune system.³ In recent years, knowledge of tumor immunology has led to the development of a new class of drugs called "immune checkpoint inhibitors" - some of which have shown impressive antitumor responses to several malignancies, including melanoma, non-small cell lung cancer (NSCLC), and renal cell carcinoma.⁴ Immune Checkpoint inhibitor is immunotherapy which inhibits the antitumor activity mediated by T cells.⁵ Malignant cells can escape from detection through cell surface molecules that interact with

receptors on T cells, this mimic signals issued by healthy cells. The result is an immune system that remains dormant against malignant cells, thus allowing irregular growth and proliferation.⁵ Two target protein checkpoints that have become the main focus of investigation for bladder cancer immunotherapy are programmed cell death protein-1 (PD-1), programmed death-ligand 1 (PD-L1).⁵

Programmed cell death protein-1 (PD-1) is one of the most studied checkpoint targets for immunotherapy treatment of bladder cancer besides programmed death-ligand 1 (PD-L1) and cytotoxic T-lymphocyte associated protein 4 (CTLA-4).⁵ PD-1 is a type I transmembrane receptor of the immunoglobulin subfamily, PD-1 pathway consists of the receptor PD-1 and its 2 ligands, PD-L1, and PD-L2. Activation of the PD-1 pathway results in a cascade of events that reduce immune activity, cytokine secretion, T-cell activation, and target cell lysis which ultimately leading to decreased autoimmunity and prevents collateral tissue damage.⁶ PD-1 and PD-L1 have been altered in a significant proportion of urothelial cancers and, its modulation could be a desirable target for bladder cancer treatment.⁷ Anti-PD-1 are humanized monoclonal antibodies that bind the PD-1 receptor. They prevent the engagement of PD-1 to its ligand on the tumor cells (PD-L1 and PD-L2) and provide the anti-tumor effect. Drugs targeting PD-1 are nivolumab, pembrolizumab, and pidilizumab, all of which block the interaction between PDL-1 and PD-1 so that T-cells can continue to be active.⁷⁻⁸

Programmed cell death-ligand 1 (PD-L1) is the 40kDA-transmembrane protein that is expressed on activated T cells, myeloid cells, and B cells. PD-L1 binding to PD-1 leads to inactivation of T-cell by controlling signaling pathways, for example, NF-KB signaling.⁸ PDL-1 is expressed very high by tumor cells, T-lymphocytes, epithelial cells, or fibroblasts. Oversecretion of PD-L1 can

protect tumor cells from CD8 + T-cells mediating tumor cell lysis.⁹ Programmed death-ligand 1 (PD-L1) has become the main focus of investigation for bladder cancer immunotherapy. In addition to bladder cancer cells, PD-L1 is expressed on the cell surface of many types of cancer, including melanoma, kidney cell carcinoma, lung cancer, head and neck cancer, ovarian cancer, and hematological malignancies. Agents which inhibit PD-L1 include durvalumab, atezolizumab, and avelumab.⁵

OBJECTIVE

This study aims to investigate mRNA expression of PD-1 and PD-L1 in patients with bladder cancer.

MATERIAL & METHODS

In this study we examined 30 samples from paraffin embedded tissue blocks, samples were divided into two groups, 15 were MIBC and 15 were NMIBC according to their histopathological result. This study was conducted in the Anatomical Pathology Laboratory of the Faculty of Medicine, Gadjah Mada University, Yogyakarta. This study received ethical approval from Gadjah Mada University Ethical Review Board.

mRNA expression of PD-1 and PDL-1 were conducted using Real Time-Polymerase Chain Reaction (qRT-PCR). RNA purification from bladder cancer tissue was conducted using Ribospin™ II (GeneAll®) kit and NEXpro™ 1-step qRT-PCR 2x Master Mix (SYBR) was used in this study. All of the procedures followed the manufacturer's recommendations and statistical analyses were performed using SPSS Version 23 and GraphPad Prism 7. Statistical significance was set at a p-value < 0.05 in this study.

The median age of the samples was 62 (41 - 96), 62 (41-78) for MIBC and NMIBC, respectively.

Table 1. Demographic characteristics of the selected samples.

	MIBC	NMIBC
Subject	15	15
Age (minimum – maximum, median) [years]	41 – 96.62	41 – 78.62
Gender [%]		
Male	93.3	66.7
Female	6.7	33.3

MIBC: Muscle Invasive Bladder Cancer

NMIBC: Non-Muscle Invasive Bladder Cancer.

The gender distributions in MIBC were 93.3% male and 6.7% female. In NMIBC the gender distributions were 66.7% male and 33.3% female.

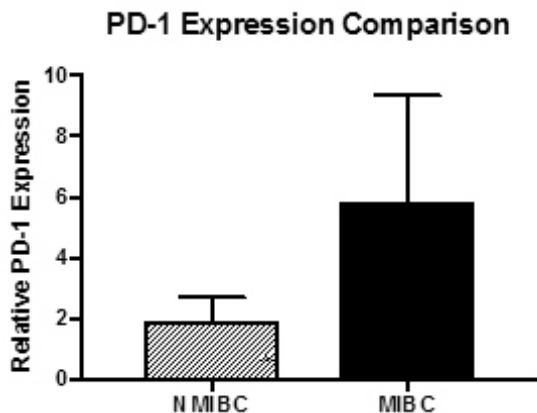


Figure 1. The mean expression comparison of PD-1 between NMIBC and MIBC.

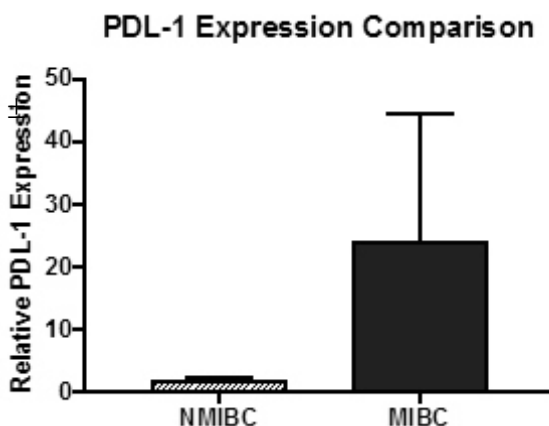


Figure 2. The mean expression comparison of PD-L1 between NMIBC and MIBC.

In this study, the mean mRNA expression of PD-1 in MIBC and NMIBC were 5.79 3.55 and 1.87 0.83, respectively. The mean mRNA expression of PD-L1 in MIBC and NMIBC were 23.79 20.72 and 1.66 0.72, respectively.

Table 2. P-value of Mann-Whitney Test.

Statistical test	P-value
PD-1	< 0.0001
PD-L1	< 0.0001

PD-1 : Programmed cell death protein-1
 PD-L1 : Programmed death ligand-1

In table 2, statistical analysis using the Mann-Whitney test found a significant difference, mRNA expression of PD-1 and PDL-1 were higher in MIBC compared to NMIBC.

DISCUSSION

The potential of immunotherapy provides new ideas about research from this field, with a variety of therapeutic modalities being investigated. One of the most promising is the use of monoclonal antibodies to block negative co-signaling molecules that prevent effective immune responses.⁵

Mean mRNA expression of PD-1 in the MIBC group was higher compared to the NMIBC group, 5.79 ± 3.55 compared to 1.87 ± 0.83 , respectively. These studies indicated that PD-1 plays a major role in bladder cancer progression and could be a desirable target for bladder cancer treatment. Previous studies showed that PD-1 expression in bladder cancer was significantly associated with higher stage disease.¹⁰ Study from Nakanishi et al. demonstrated that the expression of PD-L1 was substantial in all tested urothelial cancer and its interaction with PD-1 resulting in tumor progression.¹¹ These studies indicated that PD-1 plays a major role in bladder cancer progression and could be a desirable target for muscle-invasive bladder cancer.

Mean mRNA expression of PD-L1 in the MIBC group was higher compared to the NMIBC group, 23.79 ± 20.72 compared to 1.66 ± 0.72 , respectively. These studies indicate that high expression of PD-L1 is an important prognostic marker and a therapeutic target for bladder cancer. Several previous studies showed that there was a significant difference in mRNA PD- L1 gene expression in NMIBC relative to MIBC. Specimens of muscle-invasive bladder cancer expressed considerably more of PD-L1. The PD-L1 expression levels were significantly higher with a higher T-stage and also substantially higher with a higher tumor N-stage.¹² PD-L1 was more highly expressed in high-grade bladder cancer than in low-grade bladder cancer.¹³ Other studies showed that high PD-L1 expression in bladder cancer.

The expression of PD-L1 was well linked with pathological grade, clinical level, recurrence, and postoperative bladder cancer prognosis.¹⁴ PD-L1, a negative coregulatory molecule that prevents antigen-specific T-cell function, serves as a predictive marker for progression in the local tumor

stage, independent of tumor type in bladder cancer.¹⁵ The aberrant expression of PD-L1 in urothelial cancer of the bladder is associated with aggressive tumors. Hence, modulation of tumor-associated PD-L1 may become a beneficial target for immunotherapy.¹¹

CONCLUSION

mRNA expression of PD-1 and PDL-1 were higher in MIBC compared to NMIBC. PD-1 and PDL-1 as immune checkpoints are potential immunotherapy for patients with advance stage bladder cancer. Immunotherapy could be a substitute or combined with other treatments such as chemotherapy or radiotherapy. Therefore, it could be an appropriate alternative for the management of bladder cancer.

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