

Case Report

Analysis of the circulating myeloid-derived suppressor cells during androgen deprivation therapy for prostate cancer

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Abbreviations & Acronyms

ADT = androgen deprivation therapy
ARs = androgen receptors
CRPC = castration-resistant prostate cancer
DC = dendritic cell
e-MDSC = early-stage myeloid-derived suppressor cells
GnRH = gonadotropin-releasing hormone
MDSCs = myeloid-derived suppressor cells
M-MDSC = monocytic myeloid-derived suppressor cells
MRI = magnetic resonance image
PBMcs = peripheral blood cells
PC = prostate cancer
PMN-MDSC = polymorphonuclear myeloid-derived suppressor cells
PR = partial response
PSA = prostate-specific antigen
STAT = signal transducer and activator of transcription
TUC = transurethral electrocoagulation

Introduction: The present study showed the involvement of immunosuppressive myeloid-derived suppressor cells during the disease progression in a 69-year-old man with a prostate cancer.

Case presentation: The patient with metastatic PC (cT4N1M1ab) was initially treated with primary androgen deprivation therapy for 5 months and then chemotherapy with docetaxel, but he expired at the 8th month. In order to investigate whether myeloid-derived suppressor cells are implicated in the cancer exacerbation during androgen deprivation therapy, we assessed the long-term changes in peripheral blood myeloid-derived suppressor cell fractions by using flow cytometry. While prostate-specific antigen levels decreased after androgen deprivation therapy, the population of each myeloid-derived suppressor cell subsets increased during disease deterioration.

Conclusion: Increase in myeloid-derived suppressor cells populations was correlated with prostate cancer progression.

Key words: androgen antagonists, myeloid-derived suppressor cells, prognosis, prostate-specific antigen, prostatic neoplasms.

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How to cite this article: Kohada, Y., Kaiho Y, Takeda K, *et al.* Analysis of the circulating myeloid-derived suppressor cells during androgen deprivation therapy for prostate cancer. *IJU Case Rep.* 2021; 4: 367–370.

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Received 23 April 2021; accepted 8 July 2021.
Online publication 1 August 2021

Keynote message

We revealed that myeloid-derived suppressor cells more correctly reflected prostate cancer progression than prostate-specific antigen.

Introduction

PC is the most common cancer among men.¹ ADT is the primary treatment of metastatic PC, providing a temporary disease control in the majority of patients. CRPC inevitably develops despite this initial response to ADT.² CRPC still has a poor prognosis although there are some therapeutic options including taxane chemotherapy, androgen receptor axis-targeted therapy, and targeted alpha therapy.

Tumor progression is associated with inflammatory conditions in the context of PC. Previous studies have revealed that inflammation in the tumor microenvironment can induce an immunosuppressive population of immature myeloid cells which are known as MDSCs.³ Furthermore, MDSCs are shown to be associated with disease progression of many tumors and with a poor prognosis.^{4,5} MDSCs are divided into three subsets based on their phenotypic and morphological characteristics.⁶ PMN-MDSC and M-MDSC are phenotypically and morphologically similar to neutrophil and monocyte, respectively. An immature subset of MDSC, which lacks monocytic and granulocytic markers, is called e-MDSC.

MDSCs display phenotypically and morphologically distinct roles, and also have potential predictive and prognostic roles in PC⁷; however, it remains uncertain which MDSC fractions

including e-MDSC relate to the progression of PC. Herein, this study presents the long-term analysis of MDSC subsets in a 69-year-old man with PC during ADT.

Case presentation

Clinical history

A 69-year-old man with an elevated PSA (20.8 ng/mL) presented to the department. The patient was diagnosed with PC (Gleason score, 4 + 4 = 8) by prostatic biopsy. A whole-body magnetic resonance image (MRI) revealed bladder and rectal invasion, and showed para-aortic and pelvic lymph node metastasis in addition to coccyx metastasis (Fig. 1a). Based on these findings, the patient was diagnosed with metastatic PC (cT4N1M1ab) and treated with primary ADT using GnRH antagonist (degarelix). The serum PSA level immediately declined, and whole-body MRI showed the PR at day 125 (Figs 1b and 2). However, the patient developed bladder tamponade bleeding at day 170 of GnRH antagonist treatment, and TUC was performed. The histopathological examination defined poorly differentiated adenocarcinoma, which was negative for PSA, synaptophysin, chromogranin A, and INSM1, but positive for CEA, by

immunohistochemical analysis. To control the bleeding from prostate, radiotherapy for the pelvis was performed from day 173 (2 Gy per day, total of 60 Gy). Whole-body MRI images at day 181 also revealed multiple liver and lung metastases even though PSA levels remained low (Figs 1c, and 2). Therefore, chemotherapy with docetaxel (75 mg/m² on day 1) was immediately administered, but the patient expired at day 246.

Flowcytometric analysis of MDSC subsets

The fractions of MDSCs in the peripheral blood was tested by flow cytometry (Attune NxT Flow Cytometer; Thermo Fisher Scientific, Waltham, MA, USA). After the removal of red blood cells using HetaSep (STEMCELL Technologies, Vancouver, Canada), PBMCs were enriched by density gradient centrifugation (TOMY, Tokyo, Japan). Flow cytometry was conducted using the following antibodies: anti-CD3-FITC (UCHT1; eBioscience, San Diego, CA, USA), anti-CD11b-SuberBright436 (ICRF44, eBioscience), anti-CD14-PE-Cy5 (61D3; eBioscience), anti-CD15-BrilliantViolet711 (W6D3; Biolegend, Sandiego, CA, USA), anti-CD19-FITC (HIB19; Tonbo Biosciences, Sandiego, CA, USA), anti-

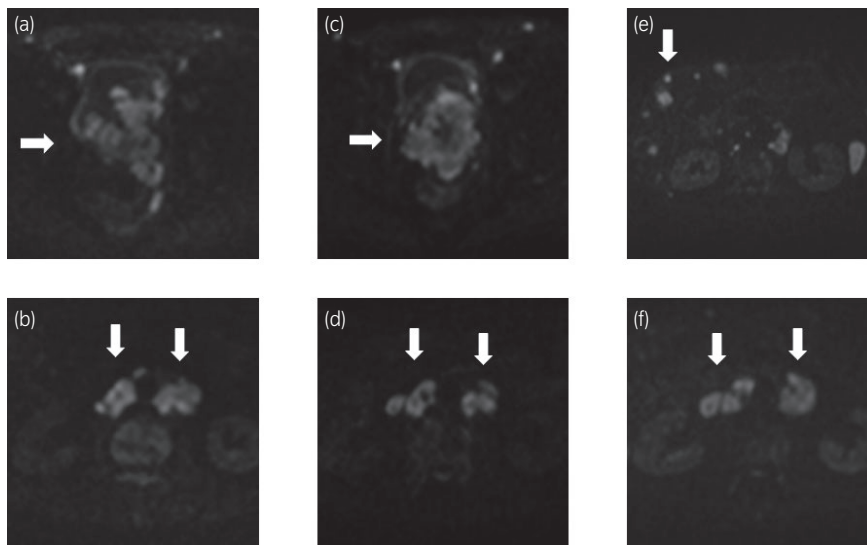


Fig. 1 Axial diffusion-weighted MRI finding. Initial images; (a). Prostate was high signal, enlarged, and had irregular outline; (b). Para-aortic lymph node was enlarged and high signal. (b) Images at 4th month after ADT; (c). Prostate volume was decreased; (d). The volume of high signal para-aortic lymph node was decreased. Images at 5th month after ADT; (e). Multiple liver metastases had expressed; (f). The volume of high signal para-aortic lymph node was increased.

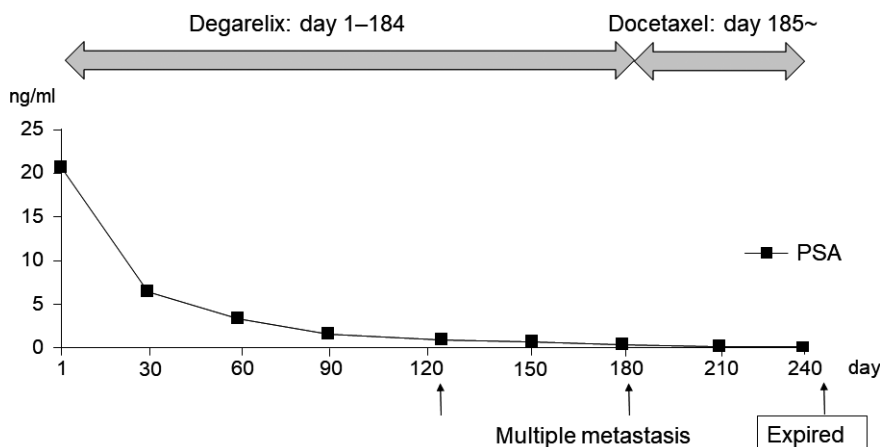


Fig. 2 Time-course changes in therapies and serum PSA levels. Day 1 is the start of treatment with primary ADT of degarelix. Whole-body MRI at day 125 showed the partial response (PR). Whole-body MRI at day 181 showed new multiple liver and lung metastases. The patient expired at day 246.

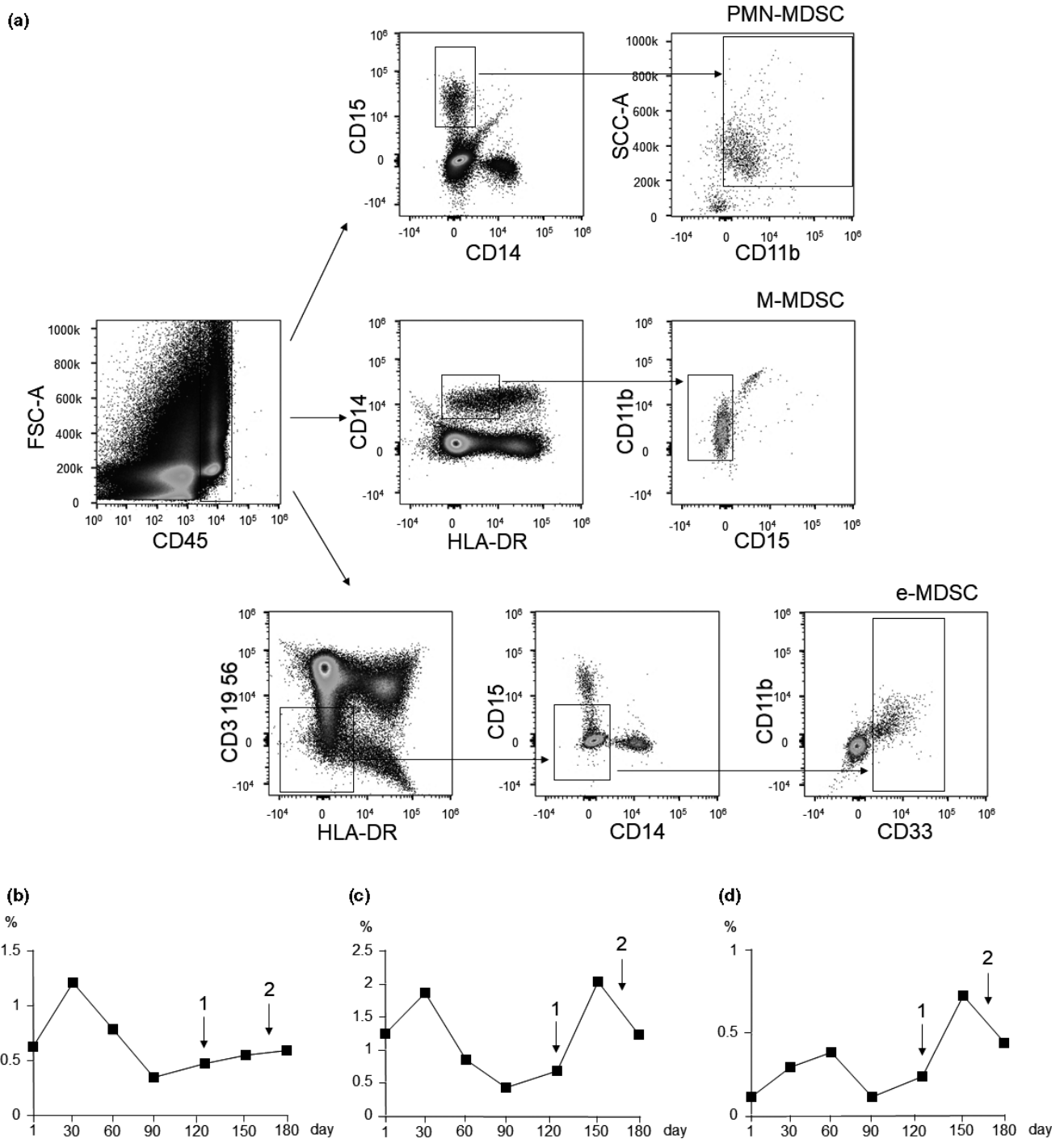


Fig. 3 Changes in MDSC fractions during GnRH antagonist therapy. (a). Gating strategy for the identification of peripheral MDSC subsets by flow cytometry. PMN-MDSC; CD11b⁺CD14⁻CD15⁺, M-MDSC; CD11b⁺CD14⁺CD15⁻HLA-DR⁻, e-MDSC; Lin(CD3/14/15/19/56)⁻ CD33⁺HLA-DR⁻ B–C: Changes in MDSC fractions. (b) PMN-MDSC, C. M-MDSC, and D. e-MDSC. Down arrows indicate as follows: 1. Confirmation date of partial response by whole-body MRI. 2. Onset of bladder tamponade.

CD33-PE (WM53; eBioscience), anti-CD45-PerCP/Cy5.5 (HI30, Biolegend), anti-CD56-FITC (TULY56; Thermo, Tokyo, Japan), and anti-HLA-DR-APC-eFlyor780 (LN3; eBioscience). The data of 10 000–100 000 events were analyzed using the FlowJo software programs (BD bioscience, San Jose, CA, USA). MDSC subsets were defined as following surface markers⁶: PMN-MDSCs; CD11b⁺, CD14⁻,

CD15⁺, M-MDSC; CD11b⁺, CD14⁺, CD15⁻, HLA-DR⁻, and e-MDSC; and Lineage (CD3/14/15/19/56)⁻, CD33⁺, and HLA-DR⁻. As shown in Fig. 3, the populations of each MDSC subsets decreased after the start of ADT; however, MDSC subsets increased after 4th month of ADT. In particular, the fraction of M-MDSC and e-MDSC was rapidly elevated before the treatment or even more.

Discussion

This study reported for the first time the long-term alteration in MDSC subsets in the case of PC progression after first ADT.

MDSCs are myeloid-derived heterogeneous cells consisting of immature macrophages, DCs, and granulocytes. Numerous papers have been demonstrated that MDSC subsets expand in the peripheral blood and intra-tumor in patients with malignant disorders.⁸ Previous studies have also shown that the significant increases in PMN-MDSC and M-MDSC subsets were found in the peripheral blood of CRPCs^{9–11}; however, analysis of the e-MDSC fraction has not yet been reported. In addition, the kinetics of the MDSCs during the treatment, including ADT, is still uncertain.

Our study demonstrated the temporal analysis of three MDSC subsets during PC progression. The population of all MDSC subsets transiently declined upon the start of treatment, whereas the fractions of M-MDSC and e-MDSC expanded after 4 months of treatment with disease exacerbation (Fig. 3c,d), suggesting the correlation between MDSC populations and PC progression, albeit it is necessary to accumulate number of cases. On the other hand, the temporary increase in MDSCs was observed at day 30 and/or 60 days after ADT. Although it remains uncertain whether ADT directly involved in the differentiation of MDSCs, the effect of MDSC growth inhibition may have been delayed compared to the decrease in PSA. Furthermore, the decreases in M- and e-MDSC fractions were observed at 6th month, which presumed to be due to the radiotherapy and deterioration of performance status (Eastern Cooperative Oncology Group performance status 2). Regarding the mechanism of PC progression, previous study has shown that interleukin-6 secreted from PC activates STAT3 and ARs, which leads to the transformation of PC from hormone sensitive to hormone resistant.¹² In addition, a recent murine study provided the interesting result showing that an AR antagonist promotes MDSC-mediated immune suppression via its enhancement of glycolysis, suggesting that GnRH antagonist could vary the characteristics of human MDSCs.¹³ Although it remains unknown which MDSC subsets are affected by STAT3 and/or AR-mediated signaling pathways, future studies are also needed to determine how ADT contributes to the gene alternation involved in metabolism as well as cytokines in MDSCs from patients with PC.

Many established immunotherapies, such as immune checkpoints and immune modulators, for other malignant diseases have been tried for PC patients¹⁴; however, there are still no approved immunotherapies for PC, except for an autologous DC vaccine called sipuleucel-T.¹⁵ Our present case study suggested that the expansion of MDSC fractions was implicated in the progression of PC during ADT. The inhibition of membrane proteins and/or signaling molecules that are specifically expressed in tumor-associated MDSC subsets could be a novel treatment for PC.

Acknowledgments

The authors thank Y. Atobe and I. Nakashima for technical assistance with the experiments of this study.

Conflict of interest

The authors declare no conflict of interest.

Approval of the research protocol by an Institutional Reviewer Board

Not applicable.

Informed consent

Not applicable.

Registry and the registration no. of the study/trial

Not applicable.

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