Case Reports in Oncology

Case Rep Oncol 2022;15:1114–1120
DOI: 10.1159/000528328
Received: August 12, 2022
Accepted: November 11, 2022
Published online: December 27, 2022

Case Report

Serum CCL22 Increased in Advanced Melanoma Patients with Liver Metastases: Report of 5 Cases

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Keywords
Melanoma · Liver metastasis · CCL22 · Prognosis

Abstract
Advanced melanoma patients with liver metastases show a limited response to immunotherapy by the induction of regulatory T cells and depletion of effector cells, which leads to a poor prognosis. Tumor-associated macrophages (TAMs) induce apoptosis of activated antigen-specific CD8+ T cells in melanomas, leading to induction of tolerance to immune checkpoint inhibitors. In addition, TAMs produce various chemokines, and several serum pro-inflammatory chemokines measured at baseline are useful for the prediction of the efficacy of immuno-modulatory drugs. In this study, serum levels of CCL22, CXCL5, and CXCL10 were evaluated by ELISA at baseline in 10 melanoma patients, 5 with liver metastases and 5 with lung metastases, treated with anti-PD1 Abs. Serum levels of CCL22, but not CXCL5 and CXCL10, were increased in patients with liver metastases compared to those with lung metastases or historical controls. The present data suggest that elevated serum CCL22 levels might be a biomarker for liver metastases in melanoma patients.

Introduction

Immune checkpoint inhibitors (ICIs) such as anti-PD1 antibodies (Abs) significantly prolong survival in patients with metastatic melanoma [1], and co-administration with ipilimumab also leads to improved outcomes, but severe immune-related adverse events (irAEs) are a problem in patients with advanced melanoma, especially with combined therapy

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[1–3]. Therefore, biomarkers to predict efficacy and irAEs have recently been widely investigated [4–6]. Recent reports suggest that evaluation of serum chemokine levels that correlate with the recruitment of tumor-infiltrating leukocytes, including regulatory T cells (Tregs), is useful to predict the efficacy and irAEs of anti-PD1 Abs in patients with unresectable advanced melanoma [7–11].

Metastatic cancer in the liver induces immune tolerance by various pathways [1], including induction of Tregs [12–14] and effector T-cell elimination [15]. Recently, Sideras et al. [13] reported that the intra-tumoral CD8+/Foxp3+ ratio is an independent predictor of survival in colorectal cancer metastasis in the liver. Another report also suggested that the reduction of the immune response by liver metastasis was associated with activated Tregs and CD11b+ monocytes in the tumor, which leads to development of tolerance to anti-PD1 Ab [15]. Notably, this tolerance to anti-PD1 Ab could be recovered by the depletion of Tregs by anti-CTLA4 Ab [15]. These reports suggest the importance of Tregs in the induction of tolerance in patients with liver metastases.

Case Presentation

Serum CCL22, CXCL5, and CXCL10 Levels

The baseline serum levels of CCL22, CXCL5, and CXCL10 were evaluated in 10 patients with advanced melanoma, 5 with liver metastases and 5 with lung metastases. The serum levels of each chemokine were determined by an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocol (R&D Systems, Minneapolis, MN, USA).

Results

Demographic Data

Patients' demographic data are shown in Table 1. The patients were 7 men and 2 women, with a mean age of 57.8 years. All patients were administered nivolumab (2 mg/kg/3 weeks or 240 mg/2 weeks) as first-line therapy between September 2014 and December 2018. Of the 5 cases with liver metastases and 5 cases with lung metastases, the subtype of cutaneous melanoma was 5 cases of cumulative sun damage, 2 cases of non-cumulative sun damage, and 3 cases of acral melanoma. Eight patients had melanoma without BRAF mutation, and 2 patients had BRAF-mutated melanoma. All patients were de novo metastatic patients and had not received adjuvant therapy previously. Median drug duration time was 12 weeks in each cohort. In cases 7 and 8, the nivolumab monotherapy was switched to another protocol before progressive disease because of irAEs (Table 2). The CARE checklist has been completed by the authors for this case report, attached as online supplementary material (for all online suppl. material, see www.karger.com/doi/10.1159/000528328).

Serum Levels of CCL22, CXCL5, and CXCL10

Since several serum chemokines at baseline are useful for the prediction of the efficacy of immunomodulatory drugs such as anti-PD1 Abs and bexarotene [7, 8], and since liver metastases, but not lung metastases, abrogate the efficacy of ICIs such as anti-PD1 Abs [15], we hypothesized that such serum chemokine levels might differ between melanoma patients with liver and those with lung metastases. To test this hypothesis, the baseline serum levels of CCL22, CXCL5, and CXCL10 were evaluated in melanoma patients, 5 with liver metastases and 5 with lung metastases, treated with anti-PD1 Abs. The results showed that the serum levels of CCL22 were significantly higher in melanoma patients with liver metastases than in

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Table 1. Patients' characteristics (age, sex, subtype, location, BRAF states)

Case	Age	Sex	Subtype	Location	BRAF mutation
Liver metastasis					
1	60	F	CSD	Lower lip	Wild type
2	68	M	CSD	Ear	Wild type
3	46	M	Acral	Sole	Wild type
4	34	F	CSD	Lower leg	Wild type
5	33	F	CSD	Lower leg	V600E
Lung metastasis					
6	60	M	Non-CSD	Back	Wild type
7	69	M	Non-CSD	Femur	V600E
8	79	M	CSD	Neck	Wild type
9	67	M	Acral	Toe	Wild type
10	62	M	Acral	Toe	Wild type

CSD, cumulative sun damage.

Table 2. Efficacy and drug duration time (DDT) of nivolumab monotherapy

Case	Efficacy	DDT (week)	Next treatment
Liver metastasis			
1	PD	12	Nivolumab plus ipilimumab
2	PD	18	Nivolumab plus ipilimumab
3	PD	10	Nivolumab plus ipilimumab
4	PD	12	BSC
5	SD	12	Dabrafenib + trametinib
Lung metastasis			
6	PD	18	BSC
7	PR	12 (switch)	Dabrafenib + trametinib
8	PR	12 (switch)	Ipilimumab
9	SD	60	BSC
10	PD	12	Ipilimumab

DDT, drug duration time; PD, progressive disease.

patients with lung metastases and in historical controls [7] (Fig. 1a). On the other hand, the baseline serum levels of CXCL5 and CXCL10 at the start of anti-PD1 Abs monotherapy were not significantly different (Fig. 1b, c).

Discussion

Advanced melanoma patients with liver metastases show a limited response to immunotherapy, which leads to a poor prognosis [15]. Indeed, previous reports suggested that liver metastases can induce peripheral tolerance by T-cell apoptosis or by the induction of Tregs [14]. More recently, Yu et al. [15] reported that tumor-associated macrophages (TAMs) induce apoptosis of activated antigen-specific CD8+ T cells in B16F10 melanomas, leading to

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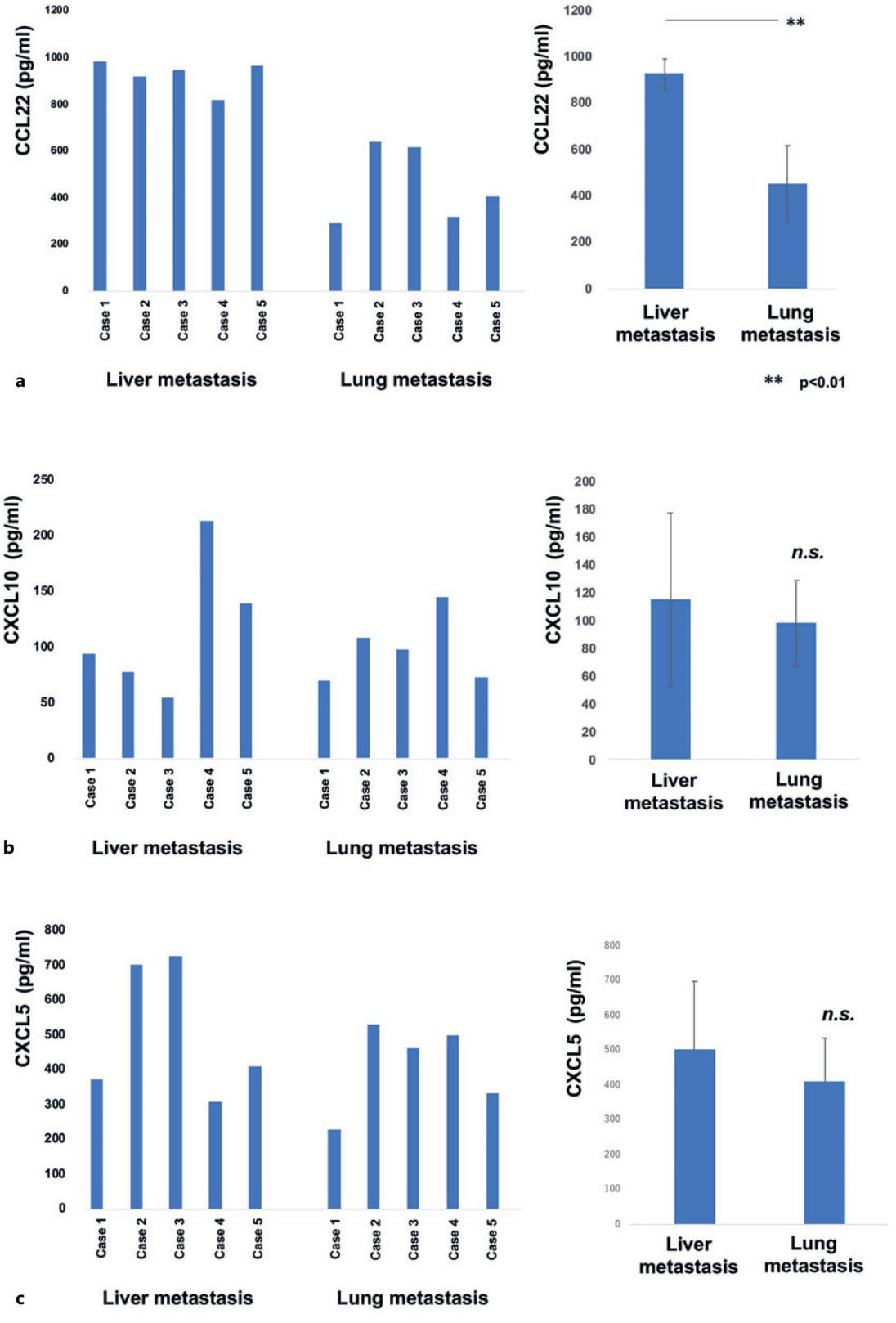

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the induction of tolerance to ICIs. Since TAMs produce various chemokines, and since several serum pro-inflammatory chemokines at baseline are useful for the prediction of the efficacy of immunomodulatory drugs [7–9, 16], we hypothesized that the baseline serum levels of inflammatory chemokines might be useful to distinguish the metastatic sites of melanoma.

Intra-tumoral monocytes, such as TAMs, produce various chemokines stimulated by cancer-stromal factors [17]. TAMs could produce characteristic chemokines, including CCL22, CXCL5, and CXCL10 [17], which are correlated with the development of melanoma [18–20]. For example, CCL22 recruits CCR4+ Tregs at the tumor site, leading to the induction of peripheral immune tolerance to developing melanoma [18]. CXCL5 increases PD-L1 expression on cancer fibroblasts to suppress the antitumor immune response against melanoma [20]. Interestingly, baseline levels of serum CXCL5 are also reported as a prognostic marker to predict the efficacy of anti-PD1 Abs monotherapy for unresectable melanoma [7], and they could also be a biomarker for the prediction of irAEs caused by anti-PD1 Abs [9]. In addition, serum CXCL10 levels are significantly increased in advanced melanoma patients with signs of progression compared to patients with stable disease [17]. These reports suggested that baseline serum levels of these chemokines might be increased in patients with metastatic melanoma to the liver who are resistant to anti-PD1 Abs monotherapy.

From the above findings, the serum levels of CCL22, CXCL5, and CXCL10 at baseline were evaluated in melanoma patients, 5 with liver metastases and 5 with lung metastases, treated with anti-PD1 Abs, and their levels were compared with those of historical controls of these chemokines in unresectable melanoma cases [7]. Of these chemokines, the serum levels of CCL22, but not CXCL5 and CXCL10, in patients with liver metastases were increased compared to patients with lung metastases or historical controls. Interestingly, the serum level of CCL22 is a biomarker for disease activity of systemic malignancies such as cutaneous T-cell lymphomas [16]. Since CCL22 promotes the recruitment of Tregs at the tumor site [18], and since Tregs maintain an immunosuppressive tumor microenvironment in skin cancers together with other immunosuppressive cells [17], the increased levels of CCL22 might correlate with the decrease in the systemic antitumor immune response against melanoma. Indeed, Tregs express various immune checkpoints, including PD1 and CTCL4, and suppress the cytotoxic function and proliferation of conventional effector T cells to maintain an immunosuppressive tumor microenvironment [21]. Taken together, the presence of elevated serum CCL22 levels might be a biomarker for liver metastases, as well as a poor prognosis, in melanoma patients, and blockade of CCL22 might enhance the antitumor effects of ICIs in melanoma patients.

Limitation

Since the number of cases in the present study was limited, further cases are needed to confirm this observation.

Statement of Ethics

The protocol for this human study was approved by the Ethics Committee of Tohoku University Graduate School of Medicine, Sendai, Japan (permit no. 2020-1-759). All methods were performed in accordance with the relevant guidelines and regulations. All patients provided written informed consent prior to enrollment in the study. All patients provided

Fig. 1. Serum levels of CCL22, CXCL5, and CXCL10 in each case. Serum levels of CCL22 (**a**), CXCL5 (**b**), and CXCL10 (**c**) were examined by ELISA in patients with liver metastases (n = 5) and with lung metastases (n = 5) at baseline.

written informed consent for publication of the details of their medical case and any accompanying images.

Conflict of Interest Statement

The authors have no conflicts of interests to declare.

Funding Sources

This study was supported in part by the Japan Agency for Medical Research and Development (21ym0126041h0001).

Author Contributions

Taku Fujimura designed the research study. Kentaro Ohuchi, Ryo Amagai, and Taku Fujimura collected and analyzed the ELISA data. Kentaro Ohuchi, Ryo Amagai, Yumi Kambayashi, Yoshihide Asano, and Taku Fujimura treated the patients and collected the clinical data and samples. Taku Fujimura wrote the manuscript. Yoshihide Asano and Taku Fujimura supervised the study.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its online supplementary material. Further inquiries can be directed to the corresponding author.

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