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CASE REPORT

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Genomic and immune microenvironment profiling in a case of metastatic intrathyroid thymic carcinoma

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Abstract

Metastatic intrathyroid thymic carcinoma (ITTC) is a rare cancer with no effective drugs for controlling. This case report has shown genomic and immune microenvironment profiles in metastatic ITTC and emphasized an immunosuppression via a PD-1/PD-L1 pathway, possibly strengthening the rationale for immune checkpoint blockade as a novel treatment.

KEYWORDS

immune checkpoint inhibitor, intrathyroid thymic carcinoma, mutation analysis, PD-1/PD-L1, tumor-immune microenvironment

1 | INTRODUCTION

Intrathyroid thymic carcinoma (ITTC)/carcinoma showing thymus-like differentiation (CASTLE) is a rare cancer with slow growth, which bears histological similarity with thymic carcinomas and clinically shows positive treatment responses.[1] Occasionally, ITTC metastasizes into lymph nodes and/or to distant organs, resulting in a poor prognosis.[2,3] However, no effective drugs have been developed for controlling metastatic ITTC. Herein, to explore new therapeutic strategies for a metastatic ITTC, we uncovered specific genetic mutation and immune microenvironment profiles in metastatic ITTC by next-generation sequencing (NGS), multiplex immunohistochemistry and imaging cytometry.

2 | CASE PRESENTATION

A 62-year-old woman presented with complaints of painless hoarseness and a palpable right lymph node. Laryngoscopy initially showed right recurrent nerve paralysis, and an ill-defined tumor mass was found in the right thyroid lobe using ultrasound. A computed tomography (CT) scan revealed that the tumor measured over 60 mm replacing the thyroid lobe and extended into the strap muscles compressing the trachea (Figure 1A). Multiple metastases into cervical lymph nodes and lung were also observed in the CT scan. Furthermore, after a fine needle aspiration biopsy, an anaplastic or poorly differentiated carcinoma was strongly suspected. After the diagnosis of poorly differentiated carcinoma, lenvatinib, a multi-receptor tyrosine kinase inhibitor, was used as a first-line treatment. The patient received 24 mg once daily as a starting dose. As she developed severe side effects, such as hypertension or proteinuria, the dose of lenvatinib was reduced up to 10 mg. Lenvatinib treatment exhibited a long-term antitumor response and controlled tumor progression in both the primary and metastatic sites for 15 months. During a lenvatinib administration, a liver metastasis was detected in a CT scan that gradually progressed (Figure 1B). To prevent spontaneous intra-tumoral hemorrhage in the liver metastasis and a life-threatening tracheal invasion at the primary site, both primary and metastatic lesions were surgically resected. However, it was impossible to completely remove the cervical lymph node metastasis because of carotid artery invasion. Postoperative pathology reported that CD5, p40, p63, and c-Kit were highly expressed, whereas TTF-1 and PAX8 expression was negative in the tumor tissue, concluding that the tumor was a metastatic ITTC (Figure 1C, D). The patient's treatment strategy was switched from lenvatinib administration to scheduled hypofractionated radiotherapy (45 Gy in 15 fractions) to the unresectable lesions in cervical area, followed by weekly paclitaxel administration. However, the patient discontinued paclitaxel treatment because of paclitaxel-induced severe side effects. After a failure of chemoradiotherapy, her liver metastasis re-emerged and rapidly progressed.

To explore more effective and optimal treatments for this metastatic ITTC, after obtaining the informed consent from the patient, we investigated the specific genetic mutation profile within the liver metastatic lesion using NGS-based cancer mutation analysis. The sample was classified as having a low TMB (4 mutations/Mb), and microsatellite status was stable. The specific genetic mutations in metastatic ITTC are shown in Table 1. Among those mutations, focal copy number amplifications in *CD274* and *PDCDILG2*, which are encoding PD-L1[4] and PD-L2[5] as immune checkpoint proteins, were detected. Combined positive score, which was 100, and abundant expression of PD-L1 protein were also found on most of the tumor cells by conventional immunohistochemistry (IHC) (Figure 2), suggesting that an immune checkpoint signaling pathway is associated with tumor progression in metastatic ITTC.

We subsequently visualized the spatial distribution of PD-L1$^+$ tumor cells and PD-1$^+$CD3$^+$ T cells in the metastatic ITTC using multiplex IHC. Interestingly, the number of tumor cells expressing PD-L1 was higher in the marginal area than that in the tumor nest (Figure 3A). Intra-tumoral and stromal distribution of PD-1$^+$CD3$^+$

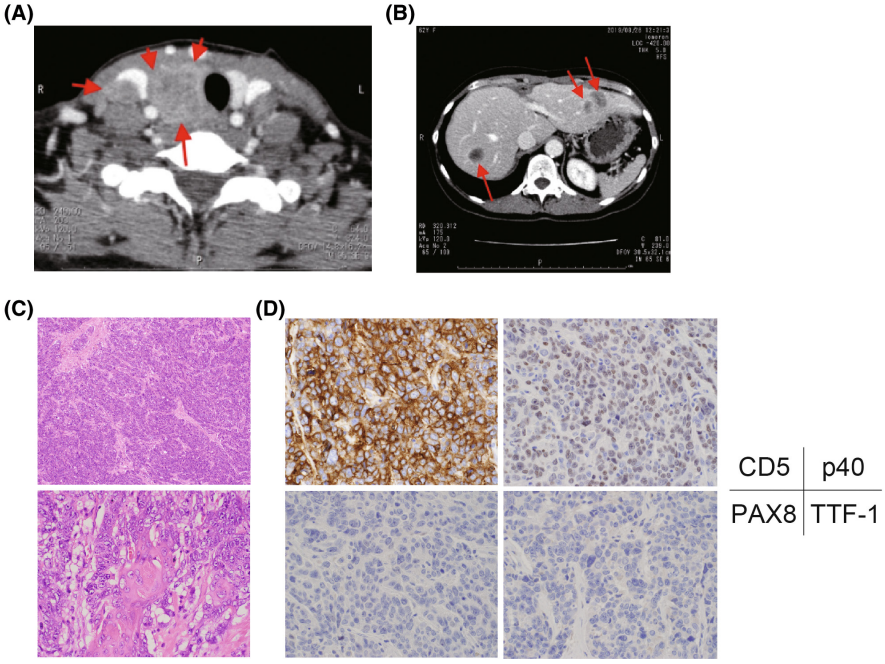

FIGURE 1 (A) Initial CT images of the primary lesion replacing the thyroid lobe. (B) Representative CT image of the liver metastasis after lenvatinib treatment. Red arrows indicate the tumor lesions. (C) Low (upper) and high (lower) magnification of HE-stained images of the metastatic ITTC. (D) Immunohistochemical staining of CD5, p40, PAX8, and TTF-1 in the metastatic ITTC

T cells was also observed in the metastatic ITTC (Figure 3A). Next, we examined whether $CD3^+CD8^+$ T cells, a cytotoxic subtype of T cells, express PD-1 in metastatic ITTC by using image cytometry and multiplex IHC. Interestingly, 41.86% $CD3^+CD8^+$T cells expressed PD-1 on their membranes (Figure 3B, C). Moreover, we evaluated PD-1 expression on $CD3^+CD8^+$ T cells in the tumor nest and in the marginal and stromal areas. The number of PD-$1^+CD3^+CD8^+$ T cells tended to be higher in the tumor nest and the marginal area than that in the stromal area (Figure 3D). These data indicated the existence of immunosuppression via a PD-1/PD-L1 pathway in the metastatic ITTC, and a PD-1/PD-L1 pathway could be therapeutically targeted.

TABLE 1 Uncoverd gene mutations in metastatic ITTC

Gene	Chromosomal region	Mutation
CD274	9p24.1	Amplification (6x)
PDCDILG2	9p24.1	Amplification (6x)
JAK2	9p24.1	Amplification (6x)
PAX5	9p13.2	Amplification (6x)
FANCG	9p13.3	Amplification (6x)
CDKN2A	9p21.3	Deletion
CDKN2B	9p21.3	Deletion
MTAP	9p21.3	Deletion
RAD51	15q15.1	Frame-shift mutation
AR	Xq12	Point mutation (c.200A > T)
BRCA1	17q21.31	Point mutation (c.1202G > A)
CSF1R	5q32	Point mutation (c.2758G > C)
GATA3	10p14	Point mutation (c.1264C > T)
MAP2K2	19p13.3	Point mutation (c.1198G > A)
NOTCH1	9q34.3	Point mutation (c.5870A > C)
RAD51B	14q24.1	Point mutation (c.757G > T)
SYK	9q22.2	Point mutation (c.1440G > A)

Given our immune data, we made the treatment decision to administer pembrolizumab to block the PD-1/PD-L1 pathway in this case. The first administration of pembrolizumab was unable to control the rapid progression of liver metastasis, leading to a deterioration of her physical condition. Pembrolizumab was then no longer an option for controlling the metastatic ITTC, and the patient died because of her liver metastasis.

3 | DISCUSSION

In this study, we uncovered a *CD274* amplification, encoding for PD-L1, in a metastatic lesion of ITTC using NGS-based cancer mutation analysis. This amplification was also concordant with abundant PD-L1 expression on tumor cells, denoting a copy-number-dependent mechanism of PD-L1 overexpression in the metastatic ITTC. Only clinical case of metastatic ITTC has reported that pathological expression of PD-L1 was observed in 60% of tumor cells within a metastatic lesion of CASTLE localized to the parotid gland, and pembrolizumab was effective in controlling tumor progression without severe adverse events.[3] Tahara et al.[6] also investigated PD-L1 expression on tumor cells in nine primary lesions of ITTC and confirmed PD-L1 expression in all samples. In our case, when the decision was taken to administer pembrolizumab, it was too late to improve the patient's condition or prognosis. However, this accumulating evidence associated with *CD274* copy number alterations and concordant expression of PD-L1 encouraged us to consider using an immune checkpoint inhibitor (ICI) such as pembrolizumab as a good systemic therapeutic option for treating metastatic ITTC.

Multiplex IHC and image flow cytometry have been employed for the pathological evaluation of tumor-immune microenvironment, yielding information on the types of immune cells that localize in tumor tissue.[7] As shown in Figure 3D, cytotoxic T cells infiltrating into the tumor nest or marginal area frequently expressed PD-1. These results indicate that the patient's metastatic

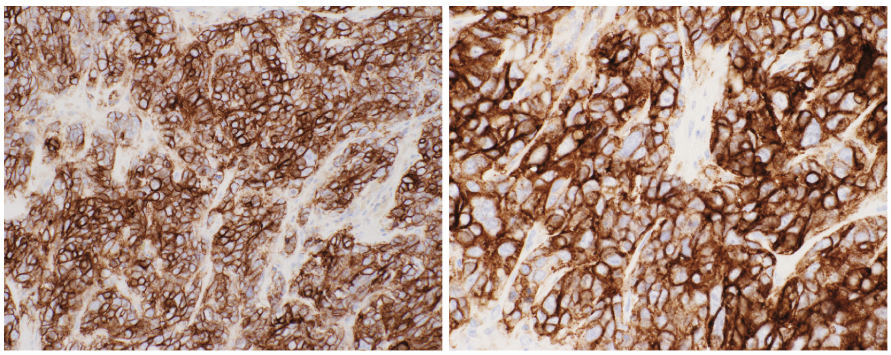

FIGURE 2 Low (left) and high (right) magnification images of PD-L1 immunohistochemical staining on tumor cells within the metastatic ITTC

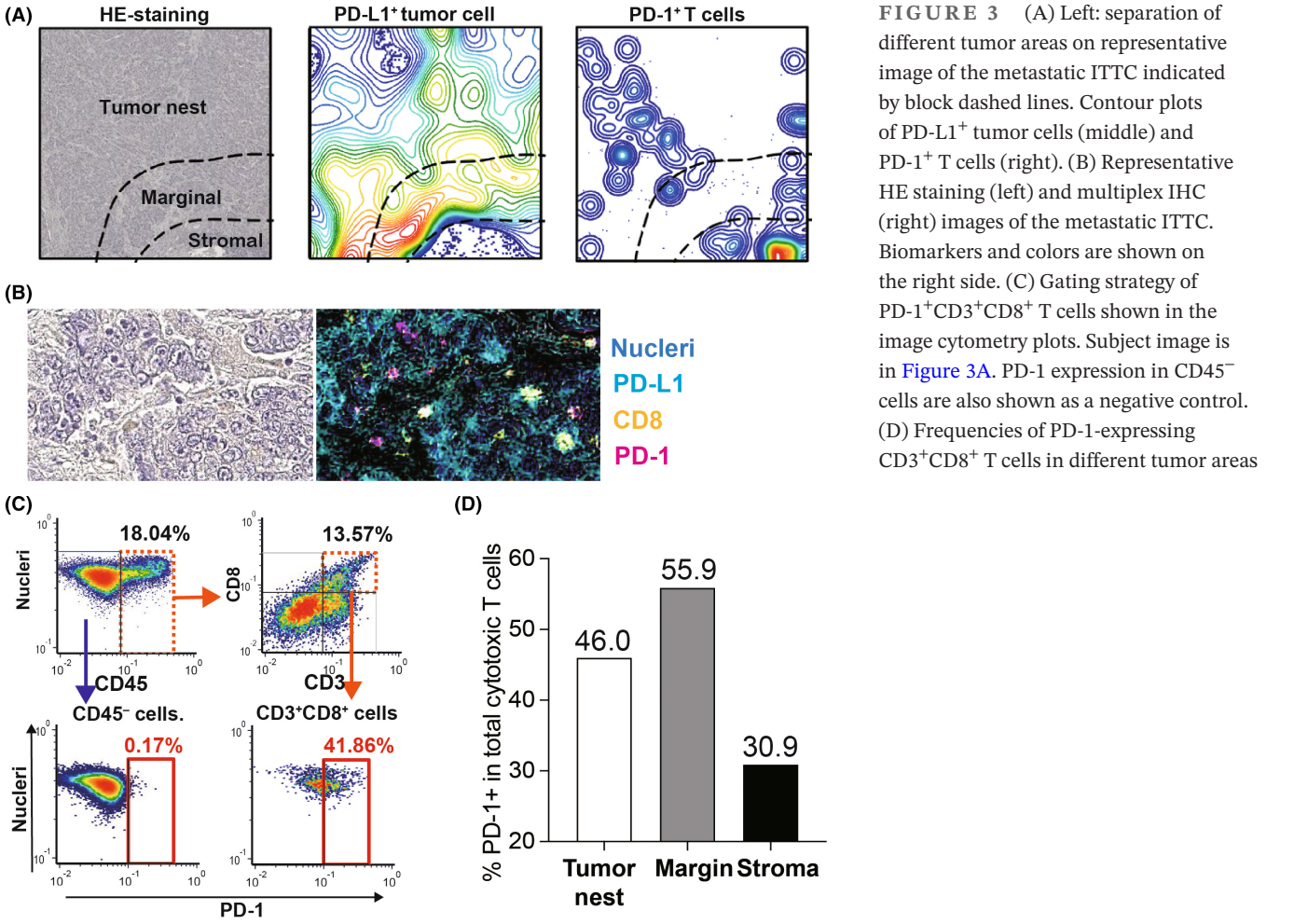

FIGURE 3 (A) Left: separation of different tumor areas on representative image of the metastatic ITTC indicated by block dashed lines. Contour plots of PD-L1$^+$ tumor cells (middle) and PD-1$^+$ T cells (right). (B) Representative HE staining (left) and multiplex IHC (right) images of the metastatic ITTC. Biomarkers and colors are shown on the right side. (C) Gating strategy of PD-1$^+$CD3$^+$CD8$^+$ T cells shown in the image cytometry plots. Subject image is in Figure 3A. PD-1 expression in CD45$^-$ cells are also shown as a negative control. (D) Frequencies of PD-1-expressing CD3$^+$CD8$^+$ T cells in different tumor areas

ITTC was characterized by high T cell infiltration and categorized as an immune-inflamed tumor that generally shows a better response to ICIs. However, when the first administration of pembrolizumab was performed, liver metastasis was in progressive, and it might be too late to control metastatic ITTC and improve her physical condition.

4 | CONCLUSION

Our study revealed several chromosomal mutations and the existence of immunosuppression via a PD-1/PD-L1 pathway in the metastatic ITTC. Further clinical studies with larger numbers of patients with metastatic ITTC will be needed to obtain the robust evidence required to recommend the use of an ICI for metastatic ITTC treatment.

AUTHOR CONTRIBUTIONS

The authors confirm contribution to the manuscript as follows: H. Ishii, T. Tsujikawa, and D. Sakurai contributed to study conception and design. A. Kinouchi and K. Sakamoto contributed to clinical data collection. H. Ishii, N. Oishi, and T. Kondo contributed to pathological evaluation and NGS analysis. T. Tsujikawa, J. Mitsuda, H. Ogi, and K. Itoh contributed to multiplex IHC and image analyses. S. Hirano and D. Sakurai contributed to the critical review of this manuscript. H. Ishii, T. Tsujikawa, and D. Sakurai contributed to drafting and editing the manuscript. All authors approved the final version of the manuscript.

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CONFLICT OF INTEREST

The authors have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data on this current study are available from the corresponding author on a reasonable request.

ETHICAL APPROVAL

This study was approved by the Ethics Committee of University of Yamanashi. These pathological and genomic analyses of the patient data in this case report were performed as a part of a routine diagnosis.

CONSENT

Written informed consent was obtained from the patient's next of kin to publish this report in accordance with the journal's patient consent policy.

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