CLINICAL SIGNIFICANCE OF PD-L1 EXPRESSION IN PARATHYROID CANCER

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Introduction. Parathyroid carcinoma accounts for 0.5% to 5% of all cases of primary hyperparathyroidism and it is an exceedingly rare endocrine malignancy first described in 1933. Most experts recommend en bloc excision at initial surgery as the only chance for its cure. Both chemotherapy and radiotherapy have not been demonstrated to be beneficial in parathyroid carcinoma. Some patients have multiple recurrences or metastases. Therefore, new therapies are urgently needed. Inhibition of the interaction between Programmed Death Receptor 1 (PD-1) and Programmed Death Receptor Ligand 1 (PD-L1) enhances T-cell responses *in vitro* and mediates clinical antitumour activity.

Aim. We analysed the expression of PD-L1 in parathyroid cancer to evaluate its potential as target for immunotherapeutic strategy.

Subjects and methods. A cohort of 18 patients were diagnosed with primary or metastatic parathyroid cancer. Immunohistochemistry was performed in 18 formalin-fixed paraffin-embedded specimens using a rabbit monoclonal antibody. A 5% cut-off value was applied for PD-L1 positivity.

Results. The anti PD-L1 antibody showed a predominantly membranous staining pattern in parathyroid cancer cells. Programmed Death Receptor Ligand-1 expression was found in 22.2% of all parathyroid carcinoma cases. There was no correlation between the expression of PD-L1 with lymph node metastasis, gender and age ($P > 0.05$).

Conclusion. This expression of PD-L1 in human parathyroid cancer suggests that patients with parathyroid cancer could profit from immunotherapeutic strategies using anti-PDL1 antibodies.

Key words: Parathyroid cancer, Programmed cell death ligand 1, PD-L1.

INTRODUCTION

Parathyroid carcinoma is an exceedingly rare endocrine malignancy first described in 1933 (1, 2). It accounts for 0.5% to 5% of all cases of primary hyperparathyroidism. Most authors recommend en

bloc excision at initial surgery as the only chance of cure. Chemotherapy has not been demonstrated to be beneficial in parathyroid carcinoma. Parathyroid carcinoma has been generally believed to be insensitive to radiotherapy for either primary or metastatic disease. Some patients of parathyroid carcinoma have multiple recurrences or metastases, underwent multiple surgeries. New therapies are urgently needed.

Immunotherapy, especially immune checkpoint inhibition therapy, is a promising therapeutic approach currently being explored for treatment-naïve or treated patients. The goal of immunotherapy is to recognize and eradicate tumors by restoring the capacity of the host's immune.

The co-inhibitory signals could be a main actor in cancer progression through the inhibition of anti-cancer immune response (3). One of the inhibitory signals is PD-1/PD-L1 axis. PD-1 is a member of the B7 receptor family and is inducibly expressed on activated T cell subsets including T follicular helper (Tfh) cells (4) and T regulatory (Treg) cells. It attenuates immune responses by negatively regulating T cell proliferation and function (5).

Its ligand, Programmed Death Receptor Ligand 1 (PD-L1) (B7-H1, CD274), is expressed on tumour cells, macrophages, T cells and certain other tissue types (6). The interaction of these two molecules negatively regulates immune responses. Of major interest is that inhibition of the interaction between PD1 and PD-L1 can enhance T-cell responses *in vitro* and mediates clinical antitumour activity (7). PD-L1 expression in tumour specimens has been described as a predictive marker for tumour response to anti-PD1 or -PD-L1 immunotherapy in various advanced tumours, including melanoma, non-small cell lung cancer, kidney cancer, colorectal cancer, castration-resistant prostate cancer and bladder cancer (7).

The aim of this study was to investigate the expression of PD-L1 in parathyroid cancer tumours.

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Table 1. Clinicopathologic data and evaluation of immunostaining for carcinoma specimens

Case no	Histo-pathological assessment of carcinoma	Gender, age (years)	Tumor location	Follow-up (months)	PD-L1 immunoreactivity
1	Definite	Female, 44	Primary	25	Diffuse loss
2	Definite	Female, 72	Primary	Unknown	Diffuse staining/intensity 2
3	Definite	Female, 62	Primary	12	Diffuse staining/intensity 2
4	Definite	Male, 60	Metastasis	Death	Diffuse staining/intensity 3
5	Definite	Male, 39	Metastasis	36	Diffuse loss
6	Definite	Female, 36	Metastasis	Death	Diffuse loss
7	Definite	Male, 34	Metastasis	16	Diffuse loss
8	Definite	Male, 60	Metastasis	Death	Diffuse loss
9	Definite	Female, 36	Metastasis	48	Diffuse loss
10	Definite	Female, 32	Metastasis	Unknown	Diffuse loss
11	Definite	Male, 65	Metastasis	Death	Diffuse loss
12	Definite	Male, 39	Metastasis	Unknown	Diffuse loss
13	Definite	Male, 41	Metastasis	Unknown	Diffuse loss
14	Definite	Female, 42	Metastasis	12	Diffuse loss
15	Definite	Male, 40	Metastasis	7	Diffuse staining/intensity 2
16	Definite	Male, 60	Metastasis	Unknown	Diffuse loss
17	Definite	Male, 73	Metastasis	Death	Diffuse loss
18	Definite	Male, 26	Metastasis	1	Diffuse loss

Table 2. PDL1 expression in Parathyroid carcinoma and patient characteristics

Characteristic	Case No.	PD-L1+(no/%)	P-value
Lymphatic metastasis			
YES	6	1/6	0.746
NO	12	3/12	
Gender			
Female	7	2/7	0.748
Male	11	2/11	
Age			
≤50	11	1/11	0.189
>50	7	3/7	

MATERIALS AND METHODS

Patient selection

This retrospective study was conducted in a cohort of 18 patients diagnosed with primary or metastatic parathyroid cancer from Beijing Shijitan Hospital of Capital Medical University from 2010 to 2016 (Table 1).

The patient age ranged from 26 to 73 with a median of 47.8 years. This study was approved by the ethical committee of our hospital.

Immunohistochemistry

Four-micron sections from formalin-fixed paraffin-embedded tumour specimens were used throughout this study. To detect the PD-L1 protein, we used the monoclonal rabbit Antibody PD-L1/274 (CLONE SP142, Spring Bioscience, USA). A dilution of 1:70 resulted in a strong and distinct membranous signal without unspecific background staining in positive controls (PD-L1-positive lung cancer cases). Programmed

Death Receptor Ligand-1-negative lung cancer cases were used as negative controls. Two well-experienced pathologists examined the immunohistochemical slides without any prior information on the clinicopathological features of the patient samples.

Percentages of PD-L1 positive tumour cells and staining intensity were evaluated for each sample. Staining intensity was scored considering 0 as negative or trace, 1 as weak, 2 as moderate and 3 as high. In absence of any standardized scoring system, all cases with staining intensity ≥ 2 in more than 5% of tumour cells were considered as positive, similarly to previous studies (8-10). Moreover, a semi-quantitative approach was used to generate a score for each tissue core. The percentage of stained cells (0–100%) was multiplied by the dominant intensity pattern of staining ranging from 0 to 3. Therefore, the overall semiquantitative score ranged from 0 to 300.

Statistical analyses

Clinical characteristics and associations with biomarkers were examined comparing the differences by χ^2 -test. The significance level for all analyses was set at $P < 0.05$.

RESULTS

Atypia of parathyroid carcinoma cells was not obvious.

Vascular invasion (Fig. 1A), penetration envelope, and/ or metastasis in the adjacent tissues were observed. Three cases had metastasis to lung (Fig. 1B). One case had lymph node metastasis. One

case had anterior pericardial metastasis. Parathyroid hormone of parathyroid carcinoma cells was positive staining (Fig. 1C).

PD-L1 was successfully evaluated in 18 specimens. PD-L1 expression was located in parathyroid carcinoma cells membrane (Fig. 1D). PD-L1 expression was found in 22.2% (4/18) parathyroid cancer. There was no correlation between the expression of PD-L1 with lymph node metastasis, gender and age ($P > 0.05$, Table 2).

DISCUSSION

In this study, we observed PD-L1 expression in tumor tissue from parathyroid cancer. The expression of PD-L1 in this cohort is comparable with other genitourinary cancer like bladder cancer (20%) (11) or

non-genitourinary cancers like breast cancer (23.4%) (12) or colorectal cancer (36%) (13) or testicular seminomas (73%)(14) or oral cavity (73%) (15).

Malignant tumors can escape the host's immune system by a process termed "immunoediting", turning the immune microenvironment into an immunosuppressive state. An important pathway in the immune anti-tumor response is the PD-1/PD-L1 axis (16-18). PD-L1 expression in tumor cells is thought to be predictive for tumor response to immunomodulatory therapies targeting the PD-1/PD-L1 pathway (19).

Using immunohistochemistry and a cut-off value for positivity defined as at least 5% tumor cells displaying membranous PD-L1 staining, we found PD-L1 expression was 22.2% (4/18) in parathyroid cancer. To date, we have not found any report on PD-L1 expression in parathyroid cancer in English literature.

Figure 1A. Parathyroid carcinoma infiltrating into vessel, 200X.

Figure 1B. Parathyroid carcinoma with metastasis to lung, 100X.

Figure 1C. PTH staining in parathyroid carcinoma, 200X.

Figure 1D. PD-L1 staining in parathyroid carcinoma, 200X.

In conclusion, the expression of PD-L1 in parathyroid cancer indicates that immune checkpoint blockade, which could be a new promising therapy approach in parathyroid cancer and further investigation in clinical trials is needed if any.

Conflict of interest

The authors declared no conflict of interest.

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