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Expression of PD-1 and PD-L1 in poorly differentiated neuroendocrine carcinomas of the digestive system: A potential target for anti-PD-1/PD-L1 therapy

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Abstract

Poorly-differentiated neuroendocrine carcinoma of the digestive system has a dismal prognosis with limited treatment options. This study aimed to investigate expression of the PD-1/PD-L1 pathway in these tumors. Thirty-seven patients with a poorly-differentiated neuroendocrine carcinoma of the digestive system were identified. Their electronic medical records, pathology reports and pathology slides were reviewed for demographics, clinical history and pathologic features. Tumor sections were immunohistochemically labeled for PD-1 and PD-L1 and expression of PD-1 and PD-L1 on tumor and tumor-associated immune cells was analyzed and compared between small cell and large cell neuroendocrine carcinomas. The mean age of patients was 61 years old with 18 males and 19 females. The colorectum (n=20) was the most common primary site, other primary sites included the pancreaticobiliary system, esophagus, stomach, duodenum, and ampulla. Expression of PD-1 was detected on tumor cells (n=6, 16%) as well as on tumor-associated immune cells (n=23, 63%). The 6 cases with PD-1 expression on tumor cells also had the expression on immune cells. Expression of PD-L1 was visualized on tumor cells in 5 cases (14%), and on tumor-associated immune cells in 10 cases (27%). There was no difference in PD-1 and PD-L1 expression between small cell and large cell neuroendocrine carcinomas. In conclusion, PD-1/PD-L1 expression is a frequent occurrence in poorly-differentiated neuroendocrine carcinomas of the digestive system. Checkpoint blockade targeting the PD-1/PD-L1 pathway may have a potential role in treating patients with this disease.

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Keywords

Neuroendocrine carcinoma; Digestive system; PD-1; PD-L1; Immunotherapy

INTRODUCTION

Neuroendocrine neoplasms of the digestive system are classified into well-differentiated neuroendocrine tumor (NET) and poorly-differentiated neuroendocrine carcinoma (NEC) under the World Health Organization (WHO) grading scheme[1]. The latter includes small cell and large cell morphologies. While well-differentiated NETs are generally an indolent disease associated with a good prognosis, poorly-differentiated NECs are very aggressive and carry a dismal prognosis[2]. Reported five-year survival rates for poorly-differentiated NECs range from 6% to 11%. More than half of patients with NECs have distant disease at initial diagnosis[2]. Additionally, surgery alone is not always curative even for those with localized disease without regional lymph node and distant metastasis[3]. Most patients with localized disease recur distantly after surgery, with a median survival of only 38 months[2]. Systemic platinum-based chemotherapy is frequently used to treat patients with limited or distant disease[4]; however, the outcome still remains poor in these patients. Therefore, there is an urgent need to develop novel and effective systemic therapies.

Programmed death-1 (PD-1) and its ligand PD-L1 play an important role in T-cell activation and the host immune response to cancer[5, 6]. PD-1/PD-L1 pathway blockade is effective in treating some human cancers in first or second line settings[6, 7]. PD-L1, expressed in tumor and/or immune cells, serves as a biomarker for potential clinical response to checkpoint inhibitors[8, 9]. Interestingly, overexpression of PD-1 by tumor cells may promote cancer growth independently in melanoma. PD-1 inhibition has been shown to suppress human melanoma xenograft growth[10]. Therefore, we aimed to investigate the complex tumor-host immune milieu, through examining the expression of PD-1 and PD-L1 on immune and tumor cells, in poorly-differentiated NECs of the digestive system.

MATERIALS AND METHODS

Thirty-seven poorly-differentiated NECs of the digestive system were identified from 37 patients from our pathology archives (29 from Vanderbilt University Medical Center and 8 from University of Rochester Medical Center) between 01/01/1997 and 06/01/2016, with tumor tissue blocks available for immunohistochemical (IHC) studies. Patient's electronic medical records, pathology reports and pathology slides were reviewed for demographics, clinical history and pathologic features. This retrospective study was approved by both Vanderbilt University and University of Rochester Institutional Review Boards.

Cases were divided into small cell and large cell NECs morphologically based on the 2010 WHO of classification of neuroendocrine neoplasms of the digestive tract.[1] IHC labeling for neuroendocrine markers, including synaptophysin, and chromogranin (with or without CD56) was performed on all cases. Small cell NECs were diagnosed based mostly on morphology, though all were at least focally positive for synaptophysin. All large cell NECs were positive at least for synaptophysin in the majority of tumor cells. Mixed adeno-

neuroendocrine carcinomas (defined as containing at least 25% of each component) were excluded from the study.

For IHC, 4- μ m unstained tumor sections from formalin fixed paraffin embedded (FFPE) tissue were first deparaffinized by routine methods. After antigen retrieval, the sections were stained with primary antibodies, PD-L1 [Cell Signaling: clone E1L3N, Danvers, MA; dilution: 1:200] and PD-1 [Abcam: clone NAT105, Cambridge, MA; dilution: 1:100], followed by antibody localization using the Dako Envision⁺ HRP-labeled polymer (DAKO). Staining was visualized by 5 minute incubation with diaminobenzidine.

Cell surface expression of PD-L1 and PD-1 was evaluated in tumor cells and tumor-associated immune cells, including tumor-infiltrating lymphocytes (TILs) and peritumoral inflammatory cells (lymphocytes, plasma cells and macrophages) at tumor edges. Percentage of PD-L1 and PD-1 positive tumor cells out of the total tumor cells was estimated. Positive expression was defined as membranous staining of >1% of the tumor cells expressing PD-L1 or PD-1. For tumor cells, the IHC score was 0, 1, 2, or 3 if less than 1%, from 1% to 10%, from 11% to 50%, or greater than 50% of the tumor cells were positive, respectively [11]. For immune cells, the percentage of the tumor's cross sectional area occupied by PD-1 and PD-L1 expressing immune cells was assessed. Positive expression was defined as membranous staining of 1% of the area occupied with PD-L1 or PD-1 expressing immune cells. The IHC score was 0, 1, 2, or 3 if less than 1%, between 1% and 5%, from 5% to less than 10%, or 10% and higher of the area occupied with PD-L1 expressing immune cells, respectively [11]. The IHC stains were first evaluated by two pathologists (JR and CS) separately. Discrepancies were noted in an approximately 5% of the stains. The stains with discrepancy were then jointly reviewed by the 2 pathologists under a multi-headed microscope; consensus was finally reached.

Fisher's exact test was employed to compare PD-1 and PD-L1 expression between small cell and large cell NECs.

RESULTS

General Clinicopathologic Features

The gender distribution of the thirty-seven cases was 18 males and 19 females, with a mean age of 61 years old (ranging from 32 to 80 years). There were 20 colorectal (including anorectal), 7 pancreatobiliary, 4 esophageal, 4 duodenal/ampullary, and 2 gastric primaries. Thirty-five patients were staged at initial diagnosis either by examination of resection specimens or by imaging studies; 17 (49%) patients were stage IV, 7 (20%) were stage III, 4 (11%) were stage II, and 7 (20%) were stage I (Table 1). There were 12 (32%) small cell (Figure 1A) and 25 (68%) large cell NECs (Figure 2A). Most tumors (34 of 37) were pure NECs and did not have significant components of invasive adenocarcinoma or squamous cell carcinoma. Only three tumors contained a minor component of invasive adenocarcinoma or squamous cell carcinoma, which accounted for less than 20% of the tumor. Seven patients had precursor lesions, including two colonic NECs arising from a tubular adenoma, two gallbladder NECs associated with low- and high-grade dysplasia, one anorectal NECs associated with squamous carcinoma in situ, and one esophageal primary associated with

squamous carcinoma in situ. Mean follow-up time was 23 months (ranging from 1 to 144 months). Twenty-four (65%) patients died of disease during the follow-up period, while six patients were alive with disease and seven patients alive with no disease.

PD-1 expression on tumor and tumor-associated immune cells

PD-1 expression was observed on tumor cells and/or tumor-associated immune cells. PD-1 expression on tumor cells was seen in 6 of 37 (16%) cases (Table 1). The expression was focal (IHC score 1) in 5 of the 6 cases, all of which were large cell NECs. In the sixth case, 50% of tumor cells expressed PD-1 (IHC score 3; Figure 1B). This particular patient had small cell NEC in the esophagus, associated with focal invasive squamous cell carcinoma (approximately 15% of the tumor area identified by p40 labeling) and squamous cell carcinoma in situ. The rate of PD-1 expression did not differ among the small cell NECs (1/12, 8%) and large cell NECs (5/25, 20%; $p=0.64$; Table 2).

PD-1 expression on tumor-associated immune cells was observed in 23 of 37 (62%) cases. Immune cells with PD-1 expression included peri-tumoral inflammatory cells at tumor edges (Figure 1C, 2B, and 3B) and tumor infiltrating lymphocytes (Figure 3B). IHC scores of 1, 2 and 3 were observed in 17, 1, and 15 cases, respectively. Of the 23 cases, 7 (7/12, 58%) were small cell and 15 (16/25, 65%) large cell NECs ($p=1.00$, Table 2). All six tumors with tumor cell PD-1 expression also expressed PD-1 on tumor-associated immune cells. Overall, 23 of 37 (62%) cases displayed PD-1 expression on tumor cells and/or immune cells; seven were in small cell (7/12, 58%) and 16 in large cell NECs (16/25, 64%) ($p=1.00$).

PD-L1 expression on tumor and immune cells

PD-L1 expression on tumor cells was observed in 5 of 37 (14%) cases, including two (2/12, 17%) small cell and three (3/25, 12%) large cell NECs (Figure 2C), with IHC scores of 1 ($n=3$), 2 ($n=1$), and 3 ($n=1$). PD-L1 expression on tumor-associated immune cells was seen in 10 of 37 (27%) NECs (Figures 1D and 3C), including five (5/12, 42%) small cell and five (5/25, 20%) large cell NECs ($p=0.24$; Table 2), with IHC scores of 1 ($n=6$) and 3 ($n=4$). The PD-L1 expressing immune cells were peri-tumoral inflammatory cells, located at tumor edges, and included histiocytes, plasma cells and lymphocytes (Figure 1D). Overall twelve out of 37 (32%) NECs were positive for PD-L1 expression on tumor and/or tumor-associated immune cells, including 6 of 12 (50%) small cell NECs and 6 of 25 (24%) large cell NECs ($p=0.15$).

In total, 24 of 37 (65%) NECs expressed PD-1 and/or PD-L1 on tumor and/or tumor-associated immune cells; 11 (46%) expressed both PD-1 and PD-L1, 12 (50%) only expressed PD-1, and 1 (4%) only expressed PD-L1. Thirteen of 20 (65%) patients with colorectal primaries expressed one or both markers on tumor and/or immune cells, which was not different from the overall population of digestive system NECs ($p=1.00$).

DISCUSSION

We have shown that PD-1/PD-L1 expression is a frequent phenomenon in poorly differentiated NECs of the digestive system. We detected PD-L1 expression on tumor cells and tumor-associated immune cells in these tumors. PD-L1 expression was more likely to be

seen on peri-tumoral immune cells at tumor edges (n=10) than on tumor cells (n=5). Like other tumor types, poorly-differentiated NECs in our study frequently expressed PD-1 on immune cells, including tumor infiltrating lymphocytes and peritumoral inflammatory cells at tumor edges[11–14]. Unexpectedly, we also found that tumor cells in 6 of 37 cases expressed PD-1 at least focally.

PD-L1 expression by tumors can induce T-cell inactivation through interaction with PD-1 on T cells, consequently leading to cancer immune evasion[5]. PD-L1 can be expressed by both tumor cells and tumor-associated immune cells as shown by our current study. Expression of PD-L1 by tumor cells detected by IHC has been shown to correlate with response to PD-1 inhibitors in some tumor types (e.g. lung cancer)[15], but is more difficult to predict in other tumors, such as melanoma[8, 16]. Multiple studies have shown that PD-L1 expression on tumor-associated immune cells can be higher than on tumor cells in some tumors. In addition, PD-L1 expression on tumor-associated immune cells by IHC, especially PD-L1 positive cells at tumor edges, can be predictive of response to PD-1 inhibitors in these tumors[8, 16, 17]. Of note, although our sample size is limited, our data suggests that small cell NECs perhaps were more likely to express PD-L1 than their large cell counterparts. Overall our data showed that approximately one third of the digestive NECs examined expressed PD-L1 on tumor and/or immune cells; intuitively this suggests checkpoint inhibition may play a role in the treatment of these tumors.

Multiple antibodies have been employed to detect PD-L1 expression. In this study, we used the clone E1L3N from Cell Signaling which targets the intracellular domain of PD-L1. Mahoney KM et al demonstrated that antibodies directed against the intracellular domain of PDL1 can clearly delineate the membrane of PD-L1–positive cells in formalin-fixed paraffin-embedded tissue[18]. Additionally, a recent study suggests that IHC using E1L3N was able to discriminate between responders and non-responders in melanoma patients by evaluating PD-L1 expression on tumor-associated immune cells; PD-L1 expression on non-tumor inflammatory cells are more predictive of response to PD-1 inhibitors than expression on tumor cells[8].

Expression of PD-1 by tumor cells has been reported in one melanoma study[10]. The study proposed stimulation of PD-1 on melanoma cells can promote tumor cell proliferation and thus inhibition of it could be another means for blocking potential tumor growth. This, however, remains hypothetical as PD-1 expression on tumor cells has not been demonstrated in other tumor types in existing literature. Another hypothesis would be that tumor expression of PD-1 could help upregulate PD-L1 tumor expression, aiding in evasion of the host immune response. Approximately 15% of poorly-differentiated NECs of the digestive system express PD-1 on tumor cells. Although the significance is unknown at this point, it is possible PD-1 on tumor cells could be another target for PD-1 inhibitors.

In conclusion, expression of PD-1/PD-L1 is frequently detected in poorly-differentiated NECs of the digestive system. This suggests that PD-1 or PD-L1 inhibitors should be studied in poorly differentiated NECs.

Acknowledgments

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- PD-1 and PD-L1 expression in digestive neuroendocrine carcinomas is examined.
- PD-1 and PD-L1 expression is a frequent occurrence in these carcinomas.
- PD-1 or PD-L1 inhibitors may have a potential role in treating these carcinomas.

Figure 1.

Expression of PD-1 and PD-L1 in a representative small cell neuroendocrine carcinoma (original magnification 200X). A. A representative hematoxylin and eosin section of small cell neuroendocrine carcinoma; B. Expression of PD-1 by tumor cells; C. Expression of PD-1 by tumor-associated immune cells (Note: clusters of tumor cells strongly expressing PD-1 at the right-upper corner); D. Expression of PD-L1 by peri-tumoral immune cells.

Figure 2.

Expression of PD-1 and PD-L1 in a representative large cell neuroendocrine carcinoma (original magnification 200X). A. A representative hematoxylin and eosin section of large cell neuroendocrine carcinoma; B. Expression of PD-1 by tumor-associated immune cells at the tumor edges; C. Expression of PD-L1 by tumor cells.

Figure 3.

Expression of PD-1 and PD-L1 in a representative small cell neuroendocrine carcinoma. A. A representative hematoxylin and eosin stain section of small cell neuroendocrine carcinoma (original magnification 100X); B. Expression of PD-1 by tumor-infiltrating lymphocytes and peri-tumoral immune cells (original magnification 200X); C. Expression of PD-L1 by peri-tumoral immune cells.

clinicopathologic features and PD-1/PD-L1 pathway expression

Table 1

Case #	Site	age	gender	stage	DOD	follow-up (month)	Type	PD-1 tumor cells	PD-1 immune cells	PDL-1 tumor cells	PDL-1 immune cells
1	ampulla	59	M	II	DOD	9	small cell	0	0	0	0
2	ampulla	32	F	IIB	DOD	7	large cell	0	1	0	0
3	gallbladder	68	F	III	AWD	46	large cell	0	0	0	0
4	colon	30	M	I	AWD	78	small cell	0	0	0	0
5	colon	64	F	III	DOD	48	large cell	1	1	0	1
6	colon	59	F	IV	DOD	18	small cell	0	0	0	0
7	colon	62	F	IV	DOD	8	large cell	0	0	0	0
8	colon	60	F	IV	DOD	3	small cell	0	1	0	0
9	colon	64	F	III	DOD	28	large cell	1	1	1	1
10	pancreas	73	M	IV	DOD	8	large cell	0	0	0	0
11	pancreas	61	M	II	DOD	64	large cell	0	1	0	0
12	pancreas	45	M	III	DOD	4	large cell	0	0	0	0
13	colon	62	F	IV	DOD	25	large cell	0	1	0	0
14	colon	63	F	IV	DOD	14	large cell	0	0	0	0
15	colon	70	F	IV	DOD	1	small cell	0	0	0	0
16	gastric	51	F	IV	DOD	8	large cell	0	0	0	0
17	Rectum	77	F	III	DOD	6	large cell	1	1	0	1
18	colon	46	M	I	AWNED	35	large cell	1	1	0	0
19	colon	71	M	IV	DOD	4	large cell	0	0	0	0
20	colon	65	M	IV	AWD	25	large cell	0	1	0	0
21	anorectum	48	F	I	DOD	30	small cell	0	0	0	1
22	gallbladder	67	F	I	AWD	15	small cell	0	3	0	1
23	colon	80	M	III	AWNED	30	large cell	0	1	3	0
24	gallbladder	59	F	IV	AWD	11	small cell	0	1	1	0
25	esophagus	60	M	I	ANED	16	small cell	3	3	1	3
26	esophagus	44	M	IV	AWD	27	large cell	0	0	0	0

Case #	Site	age	gender	stage	DOD	follow-up (month)	Type	PD-1 tumor cells	PD-1 immune cells	PDL-1 tumor cells	PDL-1 immune cells
27	esophagus	73	M	IV	DOD	3	small cell	0	3	0	3
28	Rectum	48	M	IV	DOD	8	large cell	0	1	0	0
29	anorectum	60	F	N/A	AWNED	21	small cell	0	2	0	0
30	stomach	74	M	IV	AWNED	144	large cell	0	3	0	3
31	cecum	51	M	IV	DOD	1	large cell	0	0	0	0
32	duodenum	77	F	IV	DOD	11	large cell	0	1	0	0
33	rectum	80	F	II	DOD	21	small cell	0	1	0	1
34	gallbladder	74	M	N/A	DOD	35	large cell	0	1	0	0
35	rectum	57	F	I	AWNED	28	large cell	1	1	0	0
36	small bowel	57	M	I	AWNED	20	large cell	0	3	2	3
37	GE junction	77	M	III	DOD	8	large cell	0	1	0	0

Table 2

Tumor type	PD-1				PD-L1			
	Tumor cells only	Immune cells only	Both tumor and immune cells	Total	Tumor cells only	Immune cells only	Both tumor and immune cells	Total
Small cell NEC (n=12)	0	6	1	7	1	4	1	6
Large cell NEC (n=25)	0	11	5	16	1	3	2	6
Total (n=37)	0	17	6	23	2	7	3	12