Effect of anti-PD-1 antibody, nivolumab on early gastric cancer

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Abstract Immunomodulation treatment using anti-programmed cell death protein 1 antibody is a very promising treatment for various types of advanced cancers, including melanoma, non-small cell lung cancer, and renal cell carcinoma. However, the therapeutic effects on early cancers are still unknown. We experienced 2 cases of early gastric cancers coexisting advanced melanomas. In both cases, early gastric cancers did not respond to nivolumab. Both early gastric cancers did not express programmed death ligand 1. It was speculated that immunotolerance was not fully established responsible for nivolumab in early gastric cancer, and for this reason, nivolumab could not shrink these tumors. We experienced 2 cases of early gastric cancer, where PD-L1 was negative, not responding to nivolumab.

Keywords Anti-PD-1 antibody · Nivolumab · Early gastric cancer

Introduction

Immunomodulation treatment has been proved to be effective to control advanced cancers. Anti-programmed cell death protein 1 (PD-1) antibody, a T-cell surface receptor inhibits T-cell function upon binding to its ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC). PD-L1 is expressed on variety types of cells including T cells, B cells, NK cells, dendritic cells, monocytes, macrophages, and mast cells. This molecule is also expressed in various tumor types, and contributes tumor immune escape.

A wide variety of clinical studies are being conducted globally, for anti-PD-1 antibody [1–4]. Gastric cancer is the second leading cancer death in the world. More than 700,000 peoples were dead with this cancer in 2012 [5]. PD-L1 expression was detected in 42.2% of gastric cancer ($n=102$), but not detected in normal gastric tissue [6]. Another study also indicated that PD-L1 expression was detected in 63% of gastric cancer ($n=70$) [7].

Chemotherapy is only a way to treat stage IV gastric cancer together with palliative care. 5-fluorouracil (5-FU), its oral derivatives, cisplatin, oxaliplatin, irinotecan, paclitaxel (PTX), and docetaxel are commonly used as a single or a combination. Moreover, molecular targeting agents are applied; trastuzumab is used for HER2 positive cases together with cytotoxics described above [8], and ramucirumab targeting VEGFR2, a tumor angiogenesis inhibitor is used alone or together with PTX [9]. However, a median survival time of stage IV gastric cancer is around 1 year at best by using these drugs [10, 11].

Present immunomodulation therapies such as nivolumab are toward advanced stages. It is very interesting if such kinds of treatments were conducted against early stages of cancers.

Patients and methods

Two patients of advanced melanoma with early gastric cancer as a comorbid disorder visited to our department in July, 2015 and January, 2016, respectively. These patients agreed with publication.

Immunohistochemical analyses were conducted with anti-antibodies for PD-1 [NAT105, Sigma-Aldrich (Darmstadt, Germany), 1:1], PD-L1 [SP263, Roche (Basel, Schweiz), 1:1], CD4 [SP35, Roche, 1:1], CD8 [C8/144B,

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Ajilent Technologies (Santa Clara, USA), 1:1], and CD25 [4C9, Nichirei Biosciences (Tokyo, Japan), 1:1] as previously described [12].

Case reports

Case 1

Fifty-seven-year-old male claimed lymph nodes swelling of para-aortic, common iliac, and left inguinal regions. Biopsy specimen from left inguinal region indicated that it was a metastatic melanoma (Table 1). The primary lesion was found out in his sole of left foot. This primary lesion was resected and examined histopathologically to be a primary site. He was consulted to our department. At the same time, esophagogastroduodenoscopy (EGD) indicated the

existence of early gastric cancer (moderately differentiated adenocarcinoma, tub2) with ulceration in the anterior wall near lesser curvature of pyloric zone. Gastroenterologists judged that this lesion was not suitable for endoscopic sub-mucosal dissection (ESD). We decided that metastatic melanoma was life-threatening, and chemotherapy with dacarbazine was started, but failed. Then, we treated this patient with nivolumab. His melanoma had no BRAF mutation at codon 600. After 10 cycles of treatment, para-aortic, common iliac, and left inguinal lymph node metastases reduced to 27.3, 52.6 and 50.4% in the diameters before treatment, respectively (Fig. 1a–f). However, gastric cancer stayed unchanged (Fig. 2a, b). This patient did not want further treatment with nivolumab, partially owing to the adverse event (AE) of uveitis (grade 2). He rather wanted to resect his lymph node metastases, and surgery was conducted according to the guideline, where it says that after systemic

Fig. 1 Therapeutic effects of nivolumab for advanced melanomas. **a** para-aortic lymph node metastasis of case 1, before treatment, **b** pelvic lymph node metastasis of case 1, before treatment, **c** left inguinal lymph node metastasis of case 1, before treatment, **d** para-aortic lymph node metastasis of case 1, after treatment, **e** pelvic lymph node metastasis of case 1, after treatment, **f** left inguinal lymph node

metastasis of case 1, after treatment, **g** Esophageal melanoma of case 2, before treatment, **h** Liver metastasis of case 2, before treatment, **i** EGD view of esophageal melanoma of case 2, before treatment, **j** esophageal melanoma of case 2, after treatment, **k** liver metastasis of case 2, after treatment, **l** EGD view of esophageal melanoma of case 2, after treatment. The red arrows indicate target lesions

Fig. 2 Therapeutic effects of nivolumab for early gastric cancer. Endoscopic views of early gastric cancer of case 1 before (a) and after (b) nivolumab. Endoscopic views of case 2 before (c) and after (d) nivolumab

therapy, if the other diseases are negative, it should be considered to resect the diseases [13]. At the same time, laparoscopy-assisted distal gastrectomy was also conducted.

Case 2

Seventy-seven year-old male was advanced esophageal melanoma with multiple liver metastases (Table 1). EGD indicated esophageal melanoma of type 1 tumor expanded from 35 cm from the maxillary central incisor to the esophagocardial junction. This melanoma had no BRAF mutation at codon 600. EGD also indicated the existence of Ip type of early gastric cancer with submucosal invasion at best (well differentiated adenocarcinoma, tub1) in the posterior wall near lesser curvature of pyloric zone. Priority of the treatment was judged to be against melanoma, and nivolumab treatment was conducted after failure of dacarbazine treatment. After 3 cycles of treatment, no changes were observed in melanoma lesions (Fig. 1g, h, j, k). After additional 2 cycles, esophageal stenosis was worsened (Fig. 1i, l). Furthermore, activities of daily living, performance status, and cognitive capacities were also affected at that time. We decided to finish nivolumab treatment, and offered best supportive care. Fatigue and appetite loss of this patient were considered to be partial symptoms of AE of nivolumab. During nivolumab treatment, his gastric cancer was stable (Fig. 2c, d).

Immunohistochemical analyses

We carried out immunohistochemical analyses on melanoma lesions as well as gastric cancer specimens (Figs. 3, 4). The para-aortic and common iliac lymph node metastases of case 1 expressed high levels of PD-L1 and the inguinal lymph node metastasis modestly (Fig. 3i, o). However, early gastric cancer of case 1 did not express PD-L1 (Fig. 4c). In case 2, primary esophageal melanoma did not express PD-L1 (Fig. 3u). The early gastric cancer of case 2 also did not express PD-L1 (Fig. 4i).

All data from cases 1 and 2 were comparative to the treatment outcomes of nivolumab. CD4/CD8 positive T lymphocyte infiltration was apparent in the para-aortic and common iliac lymph node metastases of case 1 (Fig. 3j, k, p, q). These data might represent reactivation of cytotoxic T lymphocyte. On the other hand, CD25 positive T lymphocyte was observed in the para-aortic lymph node metastasis (Fig. 3l). That might indicate that activities of regulatory T cell contributing immune-tolerance were still remaining. In case 2, CD4/CD8 positive T lymphocyte infiltration was absent in the primary esophageal melanoma (Fig. 3v, w).

The primary melanoma of case 1, which had been already resected before nivolumab treatment did not express PD-L1 (Fig. 3c). Primary esophageal melanoma of case 2 also did not express PD-L1. Similarly, both of early gastric cancer did not express PD-L1. Different

Fig. 3 Immunohistochemical analyses of metastatic melanomas. The primary site in the left foot sole (FS) of case 1 is analyzed with hematoxylin and eosin staining (HE), anti-PD-1, anti-PD-L1, anti-CD4, anti-CD8, anti-CD25 antibodies, respectively (**a–f**). Para-aortic lymph node metastasis (PALN) of case 1 is analyzed with HE, anti-PD-1, anti-PD-L1, anti-CD4, anti-CD8, anti-CD25 antibodies,

respectively (**g–l**). Pelvic lymph node metastasis (PELN) of case 1 is analyzed with HE, anti-PD-1, anti-PD-L1, anti-CD4, anti-CD8, anti-CD25 antibodies, respectively (**m–r**). Esophageal melanoma (ESO) of case 2 is analyzed with HE, anti-PD-1, anti-PD-L1, anti-CD4, anti-CD8, anti-CD25 antibodies, respectively (**s–x**)

Fig. 4 Immunohistochemical analyses of early gastric cancer. The early gastric cancer of case 1 is analyzed with HE staining, anti-PD-1, anti-PD-L1, anti-CD4, anti-CD8, anti-CD25 antibodies, respectively

(**a–f**), and case 2 (**g–l**). The immunoreactivity for each target is representatively shown in **m–r** as a reference

from advanced gastric cancer, early gastric cancer might not respond to nivolumab maybe due to absence of PD-L1 expression. PD-L1 expression might be lacking in the early or primary lesion, and induced in the

advanced lesions, such as metastatic lesions. For these reasons, nivolumab might be ineffective to early gastric cancers.

Table 1 Clinical presentation of the cases

	Melanoma					Gastric Cancer		
		Site	Therapeutic effect	BRAF mut	After care	Site, type	Hist	After care
Case 1	Primary	Lt sole	(resected)	(–)	ND	Py, AW, Lc, Iic	Tub 2	Resected
	Meta	Para Ao LN	PR		Resected			
		Pelvic LN	PR		Resected			
		Lt inguinal LN	PR		Resected			
Case 2	Primary	Esophagus	SD	(–)	BSC	Py, PW, Lc, Ip	Tub 1	BSC
	Meta	Liver	SD	ND				

Meta metastasis, *Ao LN* aortic lymph node, *PR* partial response, *ND* not done, *Py* pylorus, *AW* anterior wall, *Lc* lesser curvature, *tub2* moderately differentiated, *SD* stable disease, *BSC* best supportive care, *PW* posterior wall, *tub1* well differentiated

Discussion

Before treatment, we thought early gastric cancers could be eliminated with nivolumab. In case 1, PD-L1 expression was negative in the early gastric cancer specimen as well as the primary melanoma. However, PD-L1 expression was positive in the metastatic lymph nodes of melanoma. It seems that PD-L1 expression was induced during progression in this melanoma case.

In case 2, PD-L1 expression was not detected in any lesions. The expression levels of PD-L1 were correlated to the outcomes of nivolumab.

The Cancer Genome Atlas (TCGA) gastric cancer analysis has provided gastric adenocarcinoma was divided into four molecular subtypes [14]. The EBV-positive gastric cancer subgroup demonstrated high levels of PD-L1/L2 overexpression [14].

In the ESMO conference preliminary data from the phase IB anti-PD-1 antibody pembrolizumab trial (KEY-NOTE-012) in advanced gastric cancer, patients with PD-L1 positive advanced gastric adenocarcinoma were treated with pembrolizumab. A total of 39 patients enrolled after screening 162 samples were positive for PD-L1 (65%) [15]. An updated analysis of this trial has suggested an objective response rate of 22% and a median response duration of 24 weeks [15]. There was a positive correlation with PD-L1 positivity and PFS ($P=0.032$).

However, little is known concerning immunogenicity or immunotolerance of the early cancers. In the literature, it was reported that PD-L1 is highly expressed in only 6.8% of the stage I non-small cell lung carcinoma [16]. However, high levels of PD-L1 expression were observed in 55.5% of the smaller sized breast cancer less than 2 cm [17]. In the stage II colorectal cancer, the high levels of PD-L1 expression were observed in 48.8% [18]. Very recently, it was reported that PD-L1 expression was associated with tumor size and lymph node status by a meta-analysis of 1,901 gastric cancer patients [19]. However, there are no correlations between PD-L1 and depth

of invasion or tumor stage in this analysis. It is still controversial whether PD-L1 expression might correlate to gastric cancer progression or not. In our cases, both early gastric cancers were negative for PD-L1 expression. In early gastric cancer, immunogenicity might be developed insufficiently. Otherwise, immunotolerance might not be fully developed. In this report, we experienced 2 cases of early gastric cancer, where PD-L1 was negative, not responding to nivolumab. However, further observation is warranted for final conclusion.

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