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Original Research

Pattern of programmed cell death-ligand 1 expression and CD8-positive T-cell infiltration before and after chemoradiotherapy in rectal cancer

Atsushi Ogura [a,e], Takashi Akiyoshi [a,*], Noriko Yamamoto [b], Hiroshi Kawachi [b], Yuichi Ishikawa [b], Seiichi Mori [c], Koji Oba [d], Masato Nagino [e], Yosuke Fukunaga [a], Masashi Ueno [a]

[a] *Department of Gastroenterological Surgery, Cancer Institute Hospital of Japanese Foundation for Cancer Research, 3-8-31, Ariake, Koto-ku, Tokyo 135-8550, Japan*
[b] *Division of Pathology, Cancer Institute, Japanese Foundation for Cancer Research, Tokyo, Japan*
[c] *Division of Cancer Genomics, Cancer Institute, Japanese Foundation for Cancer Research, Tokyo, Japan*
[d] *Department of Biostatistics, The University of Tokyo, Tokyo, Japan*
[e] *Division of Surgical Oncology, Department of Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan*

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Synergistic effect

Abstract ***Background:*** The synergistic effect of combining immune checkpoint inhibitors with radiotherapy was reported recently, but there are few studies on programmed cell death-ligand 1 (PD-L1) expression in rectal cancer treated by preoperative chemoradiotherapy (CRT). The aim of the present study was to investigate the PD-L1 expression status before and after CRT and its association with clinicopathological characteristics and recurrence in rectal cancer.

Methods: Immunostainings of PD-L1 and CD8 were performed in 287 patients with rectal cancer treated by CRT. PD-L1 expression on the tumour cells (tPD-L1) and on the stromal immune cells (iPD-L1) was evaluated before and after CRT. CD8+ cell density in tumour area (tCD8+) before CRT and in the stromal area (sCD8+) before and after CRT was also evaluated.

Results: High tPD-L1 expression was observed in only three patients (1.0%). High iPD-L1 expression significantly increased from 31.7% before CRT to 49.2% after CRT ($P < 0.0001$). The increase in high iPD-L1 expression after CRT was only observed in patients with tumour regression grades 1 and 2. High iPD-L1 expression was associated with high

* *Corresponding author*: Fax: +81 3 3520 0141.
E-mail address: takashi.akiyoshi@jfcr.or.jp (T. Akiyoshi).

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tCD8+ cell density before CRT ($P < 0.0001$) and sCD8+ cell density after CRT ($P < 0.0001$). High tCD8+ cell density before CRT was associated with better disease-free survival (DFS) ($P = 0.0331$), but its improved effect on DFS could be observed in patients with high iPD-L1 expression ($P = 0.0081$), not in patients with low iPD-L1 expression ($P = 0.516$).
Conclusion: The present study demonstrated the significant correlations between iPD-L1 expression and CD8+ cell density both before and after CRT.

1. Introduction

Recently, some immune checkpoint inhibitors have dramatically improved long-term prognoses in several malignancies such as malignant melanoma, lung cancer and renal cell cancer which had insufficient prognosis with conventional treatment [1–3]. The programmed cell death 1 (PD-1) pathway plays a crucial role, and the programmed cell death-ligand 1 (PD-L1) is key, which is expressed on the tumour cells and inflammatory cells including T cells, B cells, dendritic cells and macrophages [4,5]. It is well known that the binding of PD-L1 on tumour cells to PD-1 receptors on T cells suppresses T-cell–mediated antitumour immune response, which is called 'immune tolerance' [4], and the blockade of this pathway gives a better prognosis in some malignancies.

In colorectal cancer, microsatellite instability (MSI) is the only potential biomarker to predict the outcome of immune checkpoint inhibitors [6,7]. Recently, several articles have suggested that irradiation has the following effects on the immune system: increasing neo-antigens; activating major histocompatibility complex (MHC) class I, which exerts a neo-antigen-specific CD8+ T-cell response; activating tumour-infiltrating lymphocytes; and enhancing the diversity of intratumoural T-cell receptors, which influence the response of immune checkpoint inhibitors [8,9]. In addition, the abscopal effect, which is a response at the distant regions out of the irradiated field, might be enhanced by combining immune checkpoint inhibitors with radiotherapy [10,11]. From these points of view, several phase II clinical trials combining immune checkpoint inhibitors with chemoradiotherapy (CRT) have been initiated in rectal cancer (NCT02586610, NCT02948348). However, there are only a few studies about PD-L1 expression in rectal cancer treated by CRT.

In the present study, we aimed to evaluate the relationship between PD-L1 expression and clinicopathological characteristics, including CD8+ cell density and tumour regression grade (TRG), in rectal cancer treated by CRT. In addition, we aimed to analyse the long-term outcomes according to the immune status focused on PD-L1 expression and CD8+ cell density before and after CRT.

2. Material and methods

2.1. Patients

This study was approved by the Scientific Review Board of our hospital and carried out in accordance with the Declaration of Helsinki. A total of 287 consecutive patients with clinical stage II/III low rectal cancer who underwent curative intent surgery after preoperative CRT between 2005 and 2014 at our hospital were included. CRT consists of oral 5-fluorouracil and a fractionated radiotherapy with a total dose of 45.0 or 50.4 Gy. The biopsy specimens before CRT were available in 281 patients, except for six without the specimens at our institution. The surgical specimens were available in all patients. If no residual tumour was detected, the center of the scar was selected for analysis.

2.2. Immunohistochemistry

Biopsy specimens before CRT and surgical specimens were immunostained with rabbit monoclonal anti-PD-L1 antibody (dilution 1:1200, ab205921; Abcam, Cambridge, UK) and mouse monoclonal anti-CD8 antibody (dilution 1:100, clone C8/144B; Nichirei, Tokyo, Japan). Four-micrometer-thick sections were cut from formalin-fixed, paraffin-embedded blocks and mounted on silane-coated glass slides, which were immunostained using the Leica Bond III automated system (Leica Biosystems Melbourne Pty Ltd, Australia).

2.3. Immunohistochemical scoring of PD-L1 expression

PD-L1 expression was evaluated by one pathologist (N.Y.). PD-L1 expression on the tumour cells was defined as tPD-L1 and that on the stromal immune cells was defined as iPD-L1. The staining intensity was evaluated as follows: 0, negative; 1, very weak expression; 2, moderate expression and 3, strong expression