博士学位論文

食道扁平上皮癌における
術前化学療法 DCF 療法による PD-L1 変化の検討

近畿大学大学院
医学研究科医学系専攻

三　谷　誠　一　郎

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Doctoral Dissertation

Implication of Changes in PD-L1 Expression during Neoadjuvant Chemotherapy with Docetaxel, Cisplatin, and 5-Fluorouracil (DCF) Regimen in Esophageal Squamous Cell Carcinoma

February 2023

Major in Medical Sciences
Kindai University Graduate School of Medical Sciences

Seiichiro Mitani

課博

同　意　書

2022 年　11 月　14 日

近畿大学大学院
医学研究科長　殿

共著者 安田卓司 ㊞　　共著者 ＿＿＿＿＿＿＿＿ ㊞

共著者 金村宙昌 ㊞　　共著者 ＿＿＿＿＿＿＿＿ ㊞

共著者 中川和彦 ㊞　　共著者 ＿＿＿＿＿＿＿＿ ㊞

共著者 ＿＿＿＿＿＿＿＿ ㊞　　共著者 ＿＿＿＿＿＿＿＿ ㊞

共著者 ＿＿＿＿＿＿＿＿ ㊞　　共著者 ＿＿＿＿＿＿＿＿ ㊞

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記

1．博士論文提出者氏名　　三谷誠一郎

2．専攻分野　医学系　　腫瘍病態制御学

近畿大学大学院医学研究科

課博

同　意　書

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近畿大学大学院
医学研究科長　殿

共著者　川上尚人　印　　共著者　千葉康敬　印

共著者　林秀敏　印　　共著者　鈴木英一郎　印

共著者　原谷浩司　印　　共著者　　印

共著者　白石治　印　　共著者　　印

共著者　米阪仁雄　印　　共著者　　印

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1．博士論文提出者氏名　　三谷誠一郎

2．専攻分野　　医学系　　腫瘍病態制御学

近畿大学大学院医学研究科

Esophagus
https://doi.org/10.1007/s10388-022-00976-9

ORIGINAL ARTICLE

Implication of changes in PD-L1 expression during neoadjuvant chemotherapy with docetaxel, cisplatin, and 5-fluorouracil (DCF) regimen in esophageal squamous cell carcinoma

Seiichiro Mitani[1] · Hisato Kawakami[1] · Osamu Shiraishi[2] · Hiroaki Kanemura[1] · Shinichiro Suzuki[1] · Koji Haratani[1] · Hidetoshi Hayashi[1] · Kimio Yonesaka[1] · Yasutaka Chiba[3] · Takushi Yasuda[2] · Kazuhiko Nakagawa[1]

Received: 26 September 2022 / Accepted: 28 November 2022

Abstract

Background Neoadjuvant docetaxel plus cisplatin and 5-FU (NAC-DCF) and adjuvant nivolumab monotherapy are the standard care for locally advanced resectable esophageal squamous cell carcinoma (ESCC). However, no effective biomarkers have been found in perioperative setting. We investigated how programmed death-ligand 1 (PD-L1) changes before and after NAC-DCF and how it relates to the therapeutic effect of NAC-DCF in resectable ESCC.

Methods PD-L1 expression in paired diagnostic biopsy and surgically resected tissues from ESCC patients who underwent surgical resection after receiving two or three NAC-DCF cycles was evaluated. PD-L1 positivity was defined as a combined positive score (CPS) of 10% ≤. Gene expression analysis was conducted using samples before NAC-DCF.

Results Sixty-six paired samples from 33 patients were included in PD-L1 expression analysis, and 33 Pre-NAC samples acquired by diagnostic biopsy were included in gene expression analysis. Pretreatment, 3 (9%), 13 (39%), and 17 (52%) patients harbored tumors with CPS ranges of < 1%, 1%–10%, and 10% ≤, respectively. After NAC-DCF, 5 (15%), 15 (45%), and 13 (39%) tumors presented CPS ranges of < 1%, 1%–10%, and 10% ≤, respectively. The concordance rate between Pre-and Post-NAC-DCF samples was 45%. Patients with PD-L1-negative tumors both before and after NAC-DCF ($n = 9$) had shorter survival and different gene expression profile characterized by upregulation in WNT signaling or neutrophils.

Conclusions A substantial PD-L1 expression alteration was observed, resulting in low concordance rate before and after NAC-DCF. Tumors persistently lacking PD-L1 had distinct gene expression profile with worse clinical outcomes, raising the need for further investigation.

Keywords Esophageal cancer · Neoadjuvant chemotherapy · PD-L1 · Combined positive score · WNT pathway

Introduction

Esophageal cancer is among the most fatal malignancies worldwide [1]. In Asia and Eastern Europe, squamous cell carcinoma is the most common histological type of esophageal cancer [2, 3]. Due to its aggressive nature, most patients are diagnosed in advanced stages, and even in operable stages of disease, surgery alone is insufficient. To improve clinical outcomes, multimodal treatment strategies, including surgical resection, radiotherapy, and chemotherapy, have been examined for further improvement.

The current standard of care in perioperative setting is nivolumab monotherapy as adjuvant treatment for resected esophageal cancer with residual pathologic lesions after chemoradiation, showing significantly longer relapse-free survival comparted to placebo in the CheckMate-577 trial [4]. Recently, the JCOG1109, a three-arm randomized phase III trial comparing preoperative cisplatin plus 5-FU (CF) versus docetaxel plus CF (DCF) versus CF-radiation, revealed that neoadjuvant DCF (NAC-DCF) prolonged survival, resulting in the change in the treatment paradigm for

✉ Hisato Kawakami
kawakami_h@med.kindai.ac.jp

[1] Department of Medical Oncology, Faculty of Medicine, Kindai University, 377-2 Onohigashi, Osaka-Sayama, Osaka 589-8511, Japan

[2] Department of Surgery, Faculty of Medicine, Kindai University, Osaka-Sayama, Japan

[3] Clinical Research Center, Kindai University Hospital, Osaka-Sayama, Japan

Published online: 09 December 2022

resectable esophageal cancer and establishment of DCF as the standard of neoadjuvant chemotherapy in Japan [5]. With the advent of these two lines of evidence, the potential of nivolumab after DCF as a way to further improve patient survival is being discussed. However, since all patients enrolled in the CheckMate-577 trial received chemoradiotherapy prior surgery, it is unclear whether nivolumab monotherapy as an adjuvant is optimal after NAC-DCF followed by surgery. Moreover, thus far, no effective biomarkers have been found to distinguish between patients not improving with NAC-DCF and, especially, those who benefit from adjuvant nivolumab for esophageal cancer.

Programmed death-ligand 1 (PD-L1) is currently the most widely validated and used biomarker when deciding whether immune checkpoint inhibitors are applicable. Based on CheckMate-648 and KEYNOTE-590 trial results, combination therapies with nivolumab or pembrolizumab has been established as the first-line treatment of advanced esophageal carcinoma [6, 7]. Simultaneously, the efficacy of anti-PD-1 antibody tended to differ according to PD-L1 expression status: in a pooled analysis, the lack of benefit in the addition of ICIs to cytotoxic chemotherapy was shown in low PD-L1-expressing tumors [8], indicating the crucial role of PD-L1 expression in metastatic settings.

In perioperative setting, however, no clear association was confirmed between PD-L1 status and nivolumab efficacy in the CheckMate-577 study, where PD-L1 was examined in the resected tumor specimen receiving chemoradiation. PD-L1 status changed over time by tumor progression or treatment modification [9–11]. For esophageal cancer, there are inconsistent results on how cytotoxic chemotherapy affects PD-L1 expression from reducing PD-L1 expression to increasing it [12, 13].

We aimed to investigate how PD-L1 changes before and after NAC-DCF and how it relates to the therapeutic effect of NAC-DCF in resectable esophageal squamous cell carcinoma (ESCC). Additionally, gene expression analysis of Pre-NAC-DCF tumor samples was performed to characterize the population with poor prognosis in NAC-DCF and to assess whether nivolumab could improve patient prognosis.

Methods

Study population

This study included patients with esophageal cancer using the following criteria: (1) histologically proven ESCC; (2) no metastatic lesion except for subclavian lymph nodes; (3) underwent NAC-DCF therapy followed by radical surgery between 2016 and 2018 at Kindai University Hospital; (4) received no adjuvant therapy; (5) Eastern Cooperative Oncology Group performance status of 0 or 1; (6) adequate organ function. Those who achieved pathological complete response by NAC-DCF were excluded, because comparisons using paired samples cannot be made. Thirty-three esophageal cancer patients were selected. All patients received two or three cycles of NAC-DCF consisting of docetaxel (day 1), cisplatin (day 1), and 5-fluorouracil (days 1–4). NAC-DCF was followed by esophagectomy with D2 or greater lymphadenectomy, and no other treatments like radiation or immunotherapy were performed preoperatively. The tumor stage was determined according to the tumor-node-metastasis classification of the American Joint Committee on Cancer, 8th edition [14]. Pathological evaluation of tumor regression was conducted according to the ratio of viable cancer cells per tumor tissue as previously reported [15]. Patient informed consent was obtained. This study was reviewed and approved by the institutional review boards of Kindai University (number: 31-234). All procedures were conducted in accordance with the principles of the Helsinki Declaration of 1964 and its later amendments.

Immunohistochemical staining

Sixty-six paired samples from 33 patients were included in PD-L1 expression analysis. Samples before receiving NAC-DCF were obtained by diagnostic endoscopy (Pre-NAC samples), and samples after NAC-DCF were surgically resected tissues (Post-NAC samples). For Post-NAC samples, the section with enough tumor left behind to be evaluable were selected. Paraffin-embedded, formalin-fixed paraffin-embedded (FFPE) tumor tissues, with tumor cells confirmed by hematoxylin–eosin (HE) staining, were cut into 4-μm sections. Immunostaining was performed at SRL, Inc. (Tokyo, Japan) with a monoclonal antibody to PD-L1 (clone 22C3, DAKO). The stained slides were independently evaluated by expert pathologists blinded to the patients' clinical background. The combined positive score (CPS) for PD-L1 expression and PD-L1 positivity were determined as previously reported, and CPS $\geq$ 10 was referred to as positive [16].

RNA extraction and gene expression profiling

Thirty-three Pre-NAC samples acquired by diagnostic biopsy were included in the gene expression analysis. RNA was isolated using an AllPrep DNA/RNA FFPE Kit (Qiagen). RNA quality was checked by the amount of extracted RNA using a NanoDrop system (Thermo Fisher Scientific). Samples were hybridized according to the manufacturer's recommendations using PanCancer IO360 comprising 750 immune-related and 20 housekeeping genes (NanoString Technologies). Gene expression was normalized using data of the 20 housekeeping genes with the use of nSolver Analysis Software 4.0. A heatmap was constructed using Java

TreeView. We divided our ESCC patients into the following four groups according to the PD-L1 transition pattern: cohort A, PD-L1-positive both before and after NAC-DCF; B, PD-L1-positive before NAC-DCF and PD-L1-negative after NAC-DCF; C, PD-L1 negative before NAC-DCF and PD-L1-positive after NAC-DCF; and D, PD-L1-negative both before and after NAC-DCF.

Statistical analysis

The primary objective of this study was to compare PD-L1 expression between patients with ESCC before and after NAC-DCF. Survival was calculated using the Kaplan–Meier method, and significance was assessed by the log-rank test. Recurrence-free survival (RFS) was defined as the time between surgical resection and radiographic recurrence or death from any cause. Overall survival (OS) was defined as the time between surgical resection and death from any cause.

Results

Patient characteristics

The patients' baseline characteristics are summarized in Table 1. Patients' median age was 67 (range, 41–76) years. A majority of patients were male and had a smoking history. Median time interval between paired samples was 69 (range, 50–118) days. Regarding NAC's pathological effect, Grades 0, 1a, 1b, and 2 responses were observed in 3 (9%), 15 (45%), 6 (18%), and 9 (27%) cases, respectively.

Table 1 Patient characteristics

	Patients ($n=33$)
Age, years	
Median (range)	67 (41–76)
Sex	
Male	27 (82%)
Female	6 (18%)
Primary tumor location in the esophagus	
Upper thoracic esophagus	5 (15%)
Middle thoracic esophagus	14 (42%)
Lower thoracic esophagus	14 (42%)
History of smoking	
Yes	28 (85%)
No	5 (15%)
Cycles of NAC-DCF	
2	26 (79%)
3	7 (21%)
Initial T category	
cT1	1 (3%)
cT2	3 (33%)
cT3	26 (79%)
cT4	3 (33%)
Initial N category	
cN0	3 (9%)
cN1	13 (39%)
cN2	16 (48)
cN3	1 (3%)
Pathological T category	
ypT1	9 (27%)
ypT2	8 (24%)
ypT3	14 (42%)
ypT4	2 (7%)
Pathological N category	
ypN0	12 (36%)
ypN1	14 (42%)
ypN2	5 (15%)
ypN3	2 (7%)
Grading of pathological response	
0	3 (9%)
1a	15 (45%)
1b	6 (18%)
2	9 (27%)

NAC neoadjuvant chemotherapy, *DCF* docetaxel plus cisplatin and 5-FU

Changes in PD-L1 expression following neoadjuvant chemotherapy

We compared the PD-L1 expression between paired Pre-NAC and Post-NAC samples from 33 patients (Table 2, Online Resource 1). Pretreatment, 3 (9%), 13 (39%), and 17 (52%) patients harbored tumors with CPS ranges of $<1\%$, 1%–10%, and $10\% \leq$, respectively. After NAC-DCF, 5 (15%), 15 (45%), and 13 (39%) tumors presented CPS ranges of $<1\%$, 1%–10%, and $10\% \leq$, respectively. Although the PD-L1 positivity rates decreased from 52 to 39%, the difference was not statistically significant ($p=0.459$). A representative case of prominent increase in PD-L1 expression before and after NAC-DCF is presented in Online Resource 2. Noteworthy, of the 17 PD-L1-positive cases, 11 became PD-L1-negative, whereas of the 16 PD-L1-negative cases, 7 became PD-L1-positive. Hence, PD-L1 expression change after NAC-DCF was observed in 18 cases (55%); yielding the concordance rate was 45% ($p=0.346$, Wilcoxon signed-rank test). Regarding the association between the grade of NAC's pathological effect and PD-L1 status changes, a trend toward higher proportion of decreased PD-L1 expression levels (8 out of 15, 53%) was seen in responders, although this was not significant ($p=0.107$, Fisher's exact test).

Table 2 Comparison of PD-L1 expression between the Pre- and Post-NAC samples

	Post-NAC samples	
	PD-L1 positive	PD-L1 negative
Pre-NAC samples		
PD-L1 positive	6 (18%)	11 (33%)
PD-L1 negative	7 (21%)	9 (27%)

Association between PD-L1 expression and survival outcomes

The patient characteristics by cohort A, B, C, and D are summarized in Online Resource 3. Recurrence after radical surgery occurred in 11 patients (33%). The median follow-up time after the initiation of NAC and surgical resection was 40.3 months (range, 7.9–66.4) and 38.1 months (range, 5.7–64.5), respectively. The recurrence rates were 17%, 36%, 14%, and 56% in cohorts A, B, C, and D, respectively, showing a trend toward higher recurrence rates in cohort D. Consequently, survival outcomes were worse in cohort D (Fig. 1a, b). Although the difference was nonsignificant, cohort D showed a worse trend in RFS (median, not reached in cohorts A, B, and C vs. 10.4 months in cohort D; $p = 0.068$) and a significantly shorter OS (median, not reached in cohort A, B, and C vs. 39.6 months in cohort D; $p = 0.013$) than the other cohorts. Among 11 patients with recurrence, three underwent palliative chemotherapy with immune checkpoint inhibitors, with one case each from cohorts A and B, achieving partial response and one case from cohort C not responding to immunotherapy.

Gene expression profile

We performed immune-related gene expression profiling (irGEP) to investigate the relationship between PD-L1 expression changes by NAC-DCF and gene expression in the tumor pretreatment. By visualizing, cohort D had a distinct gene expression profile compared with the other cohorts (Fig. 2). Among the genes, genes related to WNT signaling, including *SOX11, WNT2, WNT4,* and *WNT5B,* were expressed at higher levels in cohort D. Contrarily, genes related to antigen presentation, including *TAP1, TAPBP, HLA-A, B, C, F, DRA,* and *DMA,* were expressed at lower levels in cohort D. Volcano plot analysis by RNA-sequence data further extracted the genes that were significantly differentially expressed between cohort D and others (Online Resource 4; list of the top 5 genes shown in Table 3). We confirmed that the expression of *WNT2* that is related to WNT signaling and *CXCR1* that is related to cytokine and chemokine signaling were significantly different at a single *gene* level.

Next, we further focused on the pathway signature differences between cohort D and others. WNT, costimulatory, and cytokine and chemokine signaling were clearly enriched in cohort D (Fig. 3a). Although, decreased

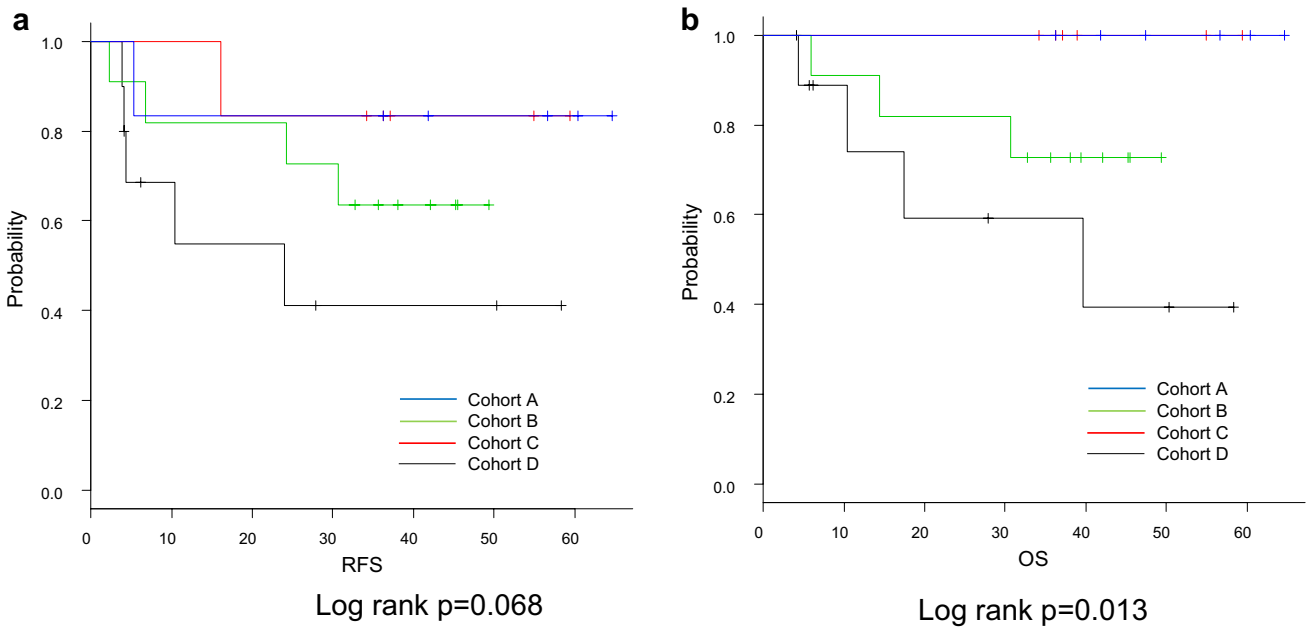

Fig. 1 Survival analyses according to PD-L1 status before and after neoadjuvant chemotherapy. **a** Recurrence-free survival. **b** Overall survival

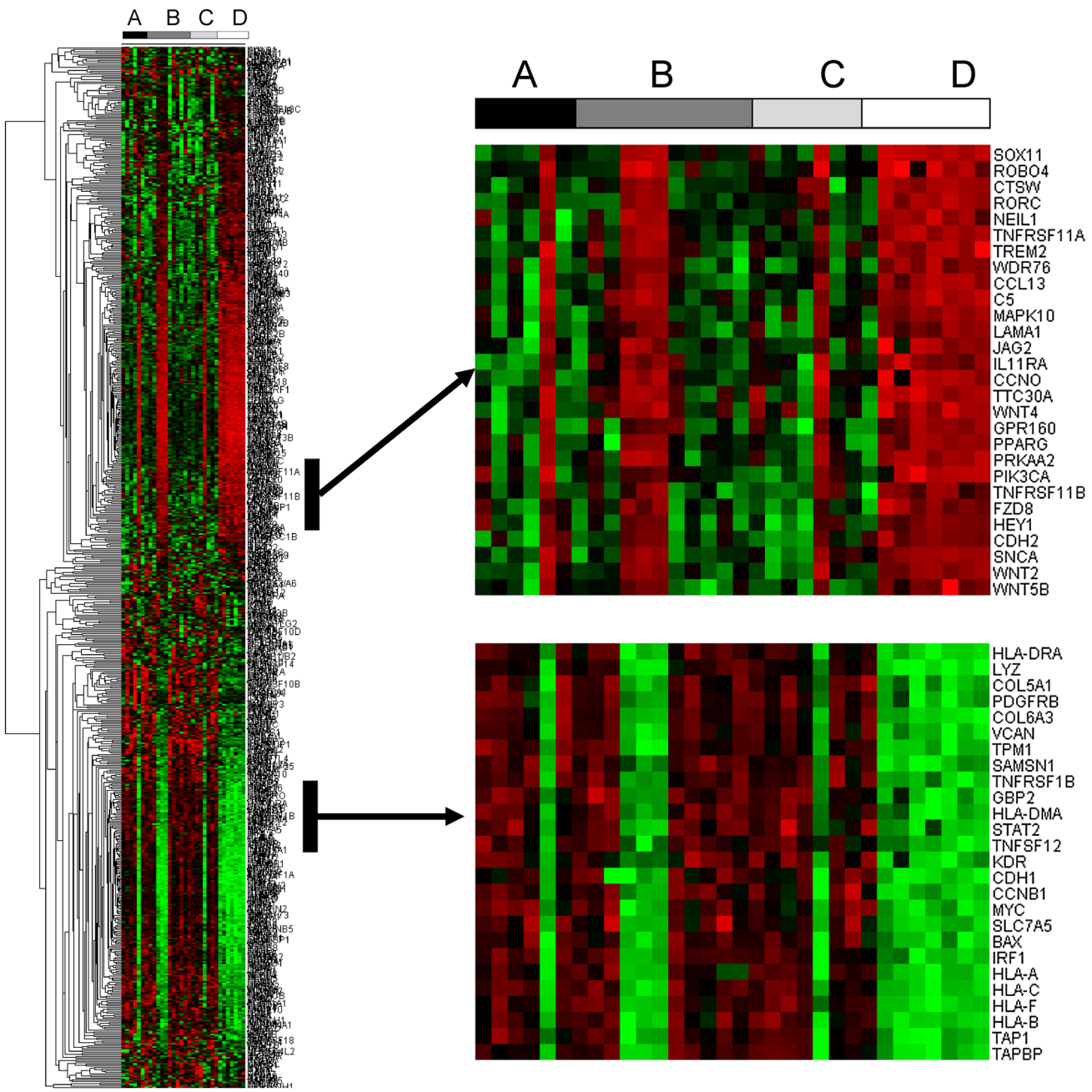

Fig. 2 Heatmap of immune-related gene expression according to PD-L1 expression before and after neoadjuvant chemotherapy

pathway signature levels indicative of antigen presentation, cytotoxicity, and TGF-beta signaling were seen in cohort D (Fig. 3a). Additionally, gene signature analyses related to each immune cell type revealed that signatures, such as B cell, T cells, CD8 T cell, dendritic cells, regulatory T cells (Treg), and neutrophils, were significantly elevated in cohort D than in the other cohorts (Fig. 3b).

Table 3 List of the top 5 genes that were elevated in patients with PD-L1-negativity before and after NAC-DCF

Gene	Log2 fold change	*Q* value	Gene sets
FCRL2	3.88	0.0000337	
WNT2	3.32	0.000361	Hedgehog signaling WNT signaling
EGF	3.62	0.000361	Hypoxia MAPK PI3K-Akt
CX3CR1	2.88	0.00148	Cytokine and chemokine signaling
GZMH	2.76	0.00376	Cytotoxicity Lymphoid compartment

Discussion

This is the first study to evaluate immunological changes induced by cytotoxic chemotherapy using paired ESCC samples before and after NAC-DCF, the new standard of neoadjuvant chemotherapy. Comparison of tumor samples obtained before and after NAC-DCF revealed varying PD-L1 statuses, including those that did not change, those that turned negative to positive, those that turned positive to negative, or those that were consistently positive, yielding a change from baseline in 55% of cases. It is remarkable that these dramatic PD-L1 changes occurred at a median time of 69 days, corresponding to the intervals of two to three NAC-DCF cycles.

Thus far, several reports have examined the effect of cytotoxic chemotherapy on PD-L1 status in ESCC, but results have been inconsistent. While there have been many reports of PD-L1 expression being increased by platinum-based anticancer drugs, there were also reports of PD-L1 expression levels being increased by neoadjuvant chemoradiotherapy and PD-L1 positivity rates being decreased post-neoadjuvant chemotherapy [17]. The lack of uniformity in chemotherapy regimens and treatment modalities adopted in the previous studies may have likely prevented a correct determination of whether PD-L1 changes are consistent. In our study, we utilized the unified regimen DCF and demonstrated that PD-L1 can be altered during short cycles of chemotherapy in ESCC, which was consistent with the finding of a previous study on gastroesophageal adenocarcinomas showing a similar concordance rate of 63% [18]. We found that patients with unchanged PD-L1-negative expression during NAC-DCF (cohort D) had the worst survival compared to the other patients.

To explore the potential reason for cohort D's poor outcome, we performed gene expression analysis using Pre-NAC samples and found that cohort D had a distinct irGEP compared to others. Of note, significant differences were observed in the pathways, especially for the WNT signaling pathway. Aberrant WNT signal is also known to be associated with stemness of tumor cells leading epithelial-to-mesenchymal transition phenotype and, hence, resistance to cytotoxic chemotherapies [19].

A dysregulated activation of WNT pathways is also involved in cancer-mediated inflammation via inflammatory cytokine production [20]. Our irGEP analysis indeed showed that cohort D had a significantly higher expression of cytokines/chemokines and associated inflammatory cells including neutrophil. Importantly, tumor infiltrating neutrophils are considered a negative predictive factor for the response to NAC in ESCC [21]. The reasons for the poor prognosis despite the high infiltration of anti-tumor immune cells, such as CD8 + T cell, are unclear, but it is possible that the negative effects of WNT and associated inflammation, pro-tumoral Treg and neutrophil infiltration, and reduced antigen-presenting capacity are very significant. Activating the WNT pathways and neutrophil infiltration are generally associated with negative outcomes by immunotherapy [22, 23]. Considering these factors were linked to persistently negative PD-L1 expression in patient who did not gain treatment effects of NAC-DCF, nivolumab adjuvant therapy may not be the best choice to improve ESCC patient survival. The reason for the persistently negative PD-L1 in cohort D is unclear from our current analysis. However, this may be due to suppression of antigen-presentation cells by β-catenin, a key factor in the WNT signaling pathway [24]. Further studies are strongly needed, as PD-L1 expression is nowadays an important biomarker for treatment selection.

This study has some limitations. First, this is an exploratory study with a small number of cases without adjusting the clinicopathological factors; therefore, the results of our survival analyses should be interpreted cautiously. Although PD-L1 positivity in ESCC has been suggested as predictive of a better prognosis, many reports including meta-analysis have shown that high PD-L1 expression is associated with poor prognosis, which is inconsistent with our results [25]. Second, irGEP was performed using only Pre-NAC samples; thus, biological etiology should be further studied. Third, although PD-L1 positivity was defined based on a previous report, the optimal cut-off value of PD-L1 positivity remains to be determined. Furthermore, although spatial heterogeneity in PD-L1 expression has been reported previously [18, 26], the heterogeneity within tumors has not been evaluated in the present study. The low concordance rate of the Pre-NAC and Post-NAC samples may be due to intra-tumor heterogeneity. Finally, the mechanisms for the persistently negative PD-L1 in cohort D remain unclear, which warrants further study. However, the present study is the first to perform a direct comparison of PD-L1 status before and after the new standard neoadjuvant DCF regimen and to explore

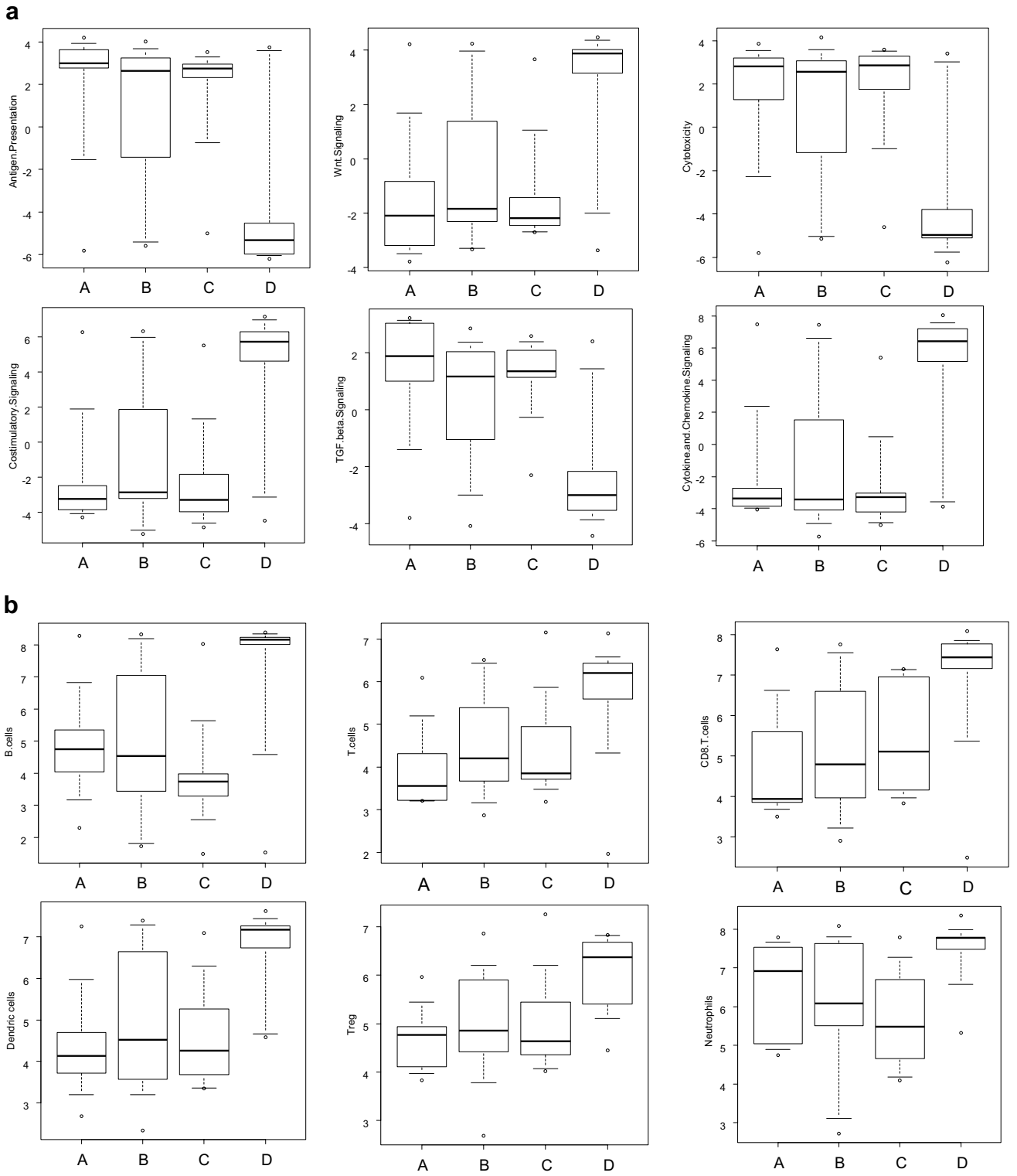

Fig. 3 Box plots for gene expression signatures. **a** Box plots for gene expression signatures according to the pathway analysis. **b** Box plots for gene expression signatures according to the immune cell types

the underlying mechanisms of PD-L1 changes using irGEP in ESCC patients.

In summary, a substantial PD-L1 expression alteration was observed during a relatively short course of cytotoxic NAC-DCF. Persistent low PD-L1 expression during NAC-DCF was associated with poor outcomes, likely due to the aberrant WNT signal. Further research is needed to confirm these results and reveal precise biology.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10388-022-00976-9.

Author contributions All authors contributed to the study conception and design. Material preparation and data collection were performed by SM, HK, OS, HK, SS, KH, HH, KY, TY, and KN. Data analysis was conducted by YC. The first draft of the manuscript was written by SM and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This study was supported by 2022 Kindai University Research Enhancement Grant (SR17).

Declarations

Ethical Statement This study was approved by the institutional review boards of Kindai University (number: 31–234).

Conflict of interest Seiichiro Mitani reports grants from Taiho Pharmaceutical Co., payment or honoraria from Taiho Pharmaceutical Co., and participation on advisory board of Chugai Pharmaceutical Co. Ltd. Hisato Kawakami reports consulting fees from Daiichi-Sankyo Co. Ltd.; honoraria from Bristol-Myers Squibb Co. Ltd., Eli Lilly Japan K.K., MSD K.K., Ono Pharmaceutical Co. Ltd., Chugai Pharmaceutical Co. Ltd., Daiichi Sankyo Co. Ltd., Merck Biopharma Co., Ltd., Takeda Pharmaceutical Co. Ltd., Teijin Pharma Ltd., Incyte Biosciences Japan., Yakult Pharmaceutical Industry., and Taiho Pharmaceutical Co. Ltd.; lecture fees from Glaxo Smith Kline K.K., and Otsuka Pharmaceutical Co., Ltd.; and research funding from Chugai Pharmaceutical Co. Ltd., Taiho Pharmaceutical Co. Ltd, Kobayashi Pharmaceutical. Co., Ltd., and Eisai Co. Ltd. Koji Haratani reports grants from AstraZeneca K.K. and MSD K.K. and payment or honoraria from AS ONE Corporation, Bristol-Myers Squibb Co. Ltd., MSD K.K., and Ono Pharmaceutical Co. Ltd. Kimio Yonesaka reports grants from Daiichi Sankyo Co., Ltd. and payment or honoraria from Chugai Pharmaceutical Co. Ltd., AstraZeneca K.K., Eli Lilly Japan K.K., Takeda Pharmaceutical Co. Ltd., MSD K.K., and Nippon Kayaku. Hayashi reports grants from Chugai Pharmaceutical Co. Ltd., AstraZeneca K.K., Astellas Pharma Inc., Merck Sharp & Dohme K.K., Ono Pharmaceutical Co. Ltd., Nippon Boehringer Ingelheim Co. Ltd., Novartis Pharma K.K., Pfizer Japan Inc., Bristol-Myers Squibb Co. Ltd., Eli Lilly Japan K.K., Daiichi-Sankyo Co. Ltd., Merck Serono Co. Ltd./Merck Biopharma Co. Ltd., Takeda Pharmaceutical Co. Ltd., Taiho Pharmaceutical Co. Ltd., SymBio Pharmaceuticals Limited, AbbVie Inc., inVentiv Health Japan, ICON Japan K.K., Gritstone Oncology Inc., Parexel International Corp., Kissei Pharmaceutical Co. Ltd., EPS Corporation, Syneos Health, Pfizer R&D Japan G.K., A2 Healthcare Corp., Quintiles Inc./IQVIA Services Japan K.K., EP-CRSU Co. Ltd., Linical Co. Ltd., Eisai Co. Ltd., CMIC Shift Zero K.K., Kyowa Hakko Kirin Co. Ltd., Bayer Yakuhin Ltd., EPS International Co. Ltd., and Otsuka Pharmaceutical Co. Ltd., payment or honoraria from AstraZeneca K.K., Boehringer Ingelheim Japan Inc., Bristol-Myers Squibb Co. Ltd., Chugai Pharmaceutical Co. Ltd., Eli Lilly Japan K.K., Kyorin Pharmaceutical Co. Ltd., Merck Biopharma Co. Ltd., Merck Sharp & Dohme K.K., Novartis Pharmaceuticals K.K., Ono Pharmaceutical Co. Ltd., Taiho Pharmaceutical Co. Ltd., and Takeda Pharmaceutical Co. Ltd., and consulting fees from AstraZeneca K.K., Boehringer Ingelheim Japan Inc., Bristol-Myers Squibb Co. Ltd., Chugai Pharmaceutical Co. Ltd., Eli Lilly Japan K.K., Pfizer Japan Inc., Shanghai Haihe Biopharma, Takeda Pharmaceutical Co. Ltd., and Merck Biopharma Co. Ltd. Kazuhiko Nakagawa reports grants from MSD K.K., Daiichi Sankyo Co. Ltd., Taiho Pharmaceutical Co. Ltd., Chugai Pharmaceutical Co. Ltd., AstraZeneca K.K., SymBio Pharmaceuticals Limited., Novartis Pharma K.K., IQVIA Services JAPAN K.K., Covance Japan Inc. AbbVie Inc., Medical Research Support, Nippon Boehringer Ingelheim Co. Ltd., SYNEOS HEALTH CLINICAL K.K., Pfizer R & D Japan G.K., Eisai Co. Ltd. Takeda Pharmaceutical Co. Ltd., Sanofi K.K., EPS Corporation, Pfizer Japan Inc. Sysmex Corporation, Ono Pharmaceutical Co. Ltd., Eli Lilly Japan K.K., Bristol Myers Squibb Company, A2 Healthcare Corp., PAREXEL International Corp., Japan Clinical Research Operations, GlaxoSmithKline K.K., and payment or honoraria from Eli Lilly Japan K.K., Chugai Pharmaceutical Co., Ltd., KYORIN Pharmaceutical Co. Ltd., Ono Pharmaceutical Co. Ltd., Pfizer Japan Inc., MSD K.K., Merck Biopharma Co. Ltd. All remaining authors declare no conflicts of interest.

Data availability The data will be made available, on reasonable request, starting from the time of publication.

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