General Thoracic and Cardiovascular Surgery

Title:

Biomarkers predicting the response to chemotherapy and the prognosis in patients with esophageal squamous cell carcinoma

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ABSTRACT:

Background: The prognosis of patients with esophageal squamous cell carcinoma (ESCC) has been improved by multidisciplinary therapy with chemoradiotherapy and surgery, but it remains poor. Advanced stage, malignant potential, and chemo-resistance contribute to the poor prognosis. Here, we attempted to identify predictive factors of the response to chemotherapy and the prognosis of ESCC patients.

Patients and Methods: We examined 51 ESCC patients who were treated with chemotherapy followed by radical surgery, and 23 patients who were treated with chemotherapy alone. We conducted quantitative reverse transcription polymerase chain reaction gene expression analysis using RNA extracted from 74 tumor tissue samples collected before chemotherapy and 67 tumor tissue samples collected after chemotherapy, focusing on PIK3CA, AKT-1, mTOR, 4E-BP1, p70S6K, PD-L1, and PD-L2.

Results: The proportions of patients with high expressions of AKT-1 and PD-L1 before chemotherapy were significantly higher among the non-responders than among the responders (p=0.034, p=0.020, respectively). Multivariate analyses revealed that high PD-L1 expression before chemotherapy was associated with poor response to chemotherapy (odds ratio: 2.998; 95% CI: 1.043–8.619; p=0.042) and high p70S6K expression before chemotherapy was a poor prognostic factor (hazard ratio: 2.518; 95% CI: 1.058–5.988; p=0.037). In addition, the patients with high expression of PD-L1 and PD-L2 in the tumors after chemotherapy had significantly worse survival than those with low expression of these genes (p=0.012, p=0.007, respectively).

Conclusion: These results demonstrated that PD-L1 and p70S6K in the primary ESCC tissues were related to a poor response to chemotherapy and poor prognosis, respectively.

Introduction

Esophageal squamous cell carcinoma (ESCC) is a major histological type in East Asian countries [1], and it is the 8th most common solid cancer and the 6th leading cause of cancer death worldwide [2]. In Japan, morbidity from ESCC is increasing as the aging of the population progresses, and approximately 10,000 deaths due to ESCC in Japan are confirmed yearly [3]. Although the three treatment modalities— surgery, chemotherapy and radiotherapy—have improved, the prognosis remains poor [4].

In 2015, it was reported that preoperative chemotherapy consisting of docetaxel (DTX) combined with cisplatin (CDDP) and 5-fluorouracil (5-FU) improved the prognosis of ESCC patients compared to standard FP therapy (CDDP and 5-FU) [5]. However,

chemotherapy immediately changes gene expression and induces acquired resistance [6]. Therefore, repeated chemotherapy results in acquired resistance, and the response to ESCC chemotherapy declines with repeated therapy. Overcoming this chemo-resistance is the most effective way to improve drugs' antitumor effects, and the development of novel anticancer drugs and molecular targeted agents is currently considered as the most promising approach in the field of drug therapy for ESCC.

In our animal study conducted to clarify the mechanisms underlying the acquisition of chemo-resistance, we observed that the phosphoinositide 3-kinase (PI3K) / protein kinase B (AKT) pathway was involved in a chemo-resistance related mechanism and was a possible target for overcoming chemo-resistance [7]. We speculated that this pathway was activated by signaling from various growth and proliferation factors, and that it was involved in the regulation of cell proliferation and survival [8,9]. Mammalian target of rapamycin (mTOR) is activated by upstream PI3K and AKT, and it directly phosphorylates downstream p70S6 kinase (p70S6K) and eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) to facilitate various life phenomena, such as protein translation, cell growth, ribosome biosynthesis, metabolism, proliferation, and autophagy [10,11]. The activation of the PI3K/AKT pathway in cancer cells regulates their proliferation, survival, angiogenesis, invasion, and metastasis. This activation is a common event in many types of cancer and has been associated with poor prognosis [12].

Advances in cancer immunology have also brought attention to the immune checkpoint pathway, raising hopes that this focus could lead to a promising treatment option for ESCC [13]. The importance of signal transduction between programmed death 1 (PD-1), which is expressed in T cells, and its ligand, programmed death ligand 1 (PD-L1), which is expressed in antigen-presenting cells and tumor cells, has been demonstrated [14]. In addition, it has been suggested that tumor dissemination, metastasis, and disease recurrence are caused by tumor cells bypassing the host's immune recognition through immune checkpoints [15], and that the overexpression of PD-L1 is associated with poor clinical outcomes in various solid tumors [16]. Favorable responses and survival outcomes were reported in a phase 3 study of nivolumab, an anti-PD-1 monoclonal antibody for treating a non-small cell lung cancer that is genetically similar to squamous cell carcinoma [17]. Another study demonstrated that blocking PD-L1 in cancer tissue also led to an inhibition of the PI3K/AKT pathway. Thus, further tumor suppression effects can be expected by combining molecular targeted therapies [18].

As noted above, various biomarkers for ESCC have been identified, but they have not yielded any results that could be linked directly to treatment. In the present study, we focused on chemo-resistance to ESCC and investigated both the PI3K/AKT and the

immune checkpoint pathways. The study was conducted with two main objectives. First, we investigated gene expressions with ESCC tissue samples before chemotherapy and identified the factors that were predictive of a response to chemotherapy and of prognosis. Second, we investigated the gene expressions in ESCC tissue samples after chemotherapy and attempted to investigate differences in the gene expression profile between before and after chemotherapy and whether the gene expression status after chemotherapy affects prognosis. Our findings will contribute to the development of individualized treatments in ESCC.

Patients and Methods

All tissue samples and related clinical data used in the present analysis were obtained from 74 ESCC patients who had undergone chemotherapy in our department during the period from 2009 to 2018. Fifty-one patients were treated with primary chemotherapy followed by radical esophagectomy, and 23 patients were treated with chemotherapy alone. All 74 ESCC tissue samples collected before chemotherapy were obtained by an upper gastrointestinal endoscopic biopsy within 2 weeks prior to the chemotherapy. The 51 ESCC tissue samples collected after primary chemotherapy followed by esophagectomy were collected from the tumor lesions immediately after resection 4 to 5 weeks after chemotherapy. Twenty-three tissue samples collected after chemotherapy were biopsied from the patients treated with chemotherapy alone within 2 weeks after chemotherapy. All tissue samples were immersed in RNA Later Stabilization Reagent (Qiagen, Hilden, Germany) just after biopsy or surgical resection and preserved by freezing at -20 °C. All samples were histopathologically confirmed as squamous cell carcinoma. The data of 7 patients who achieved a complete response (CR) were excluded from the gene expression analysis.

The protocol of this study was approved by the review board of Tokushima University Hospital, and written informed consent regarding this study was obtained from all of the patients.

Chemotherapy

All of the patients underwent primary chemotherapy with the three-agent regimen of DTX, 5-FU, and CDDP. The chemotherapy schedule consisted of the administration of DTX 25 mg/m^2/day on day 1, 5-FU 370 mg/m^2/day on days 1 to 5, and CDDP 6 mg/m^2/day on days 1 to 5. This chemotherapy regimen was repeated every week for 4 weeks, unless the patient showed disease progression or experienced an adverse event that could not be supported. The chemotherapy was performed for 4 weeks as one course

[19].

Surgery

Surgery was performed 4–5 weeks after the primary chemotherapy. The primary chemotherapy was also administered to patients with unresectable disease. The cT4 cases responding to the chemotherapy were candidates for surgery. A trans-thoracic esophagectomy and a three-field lymphadenectomy with reconstruction using the gastric tube conduit through the retrosternal or posterior mediastinal routes were performed. Radical resection was performed in all patients.

Patient demographics and follow-up

Patient demographics including age, smoking history, alcohol consumption history, alcohol flushing reaction [20], and family history were collected in a personal interview. Tumor location, size, histological differentiation, and disease stage were obtained from medical records. The staging was classified according to the 8th edition of the UICC TNM Classification. The T and N statuses of each patient were diagnosed using contrast-enhanced chest and abdomen multi-detector computed tomography (CT) and upper gastrointestinal endoscopy. A round or irregular-shaped node >10 mm or enhanced nodes >5 mm. were diagnosed as clinically positive lymph nodes. The patients' responses to chemotherapy were assessed in accordance with the Response Evaluation Criteria in Solid Tumors (RECIST) Ver.1.1, using CT images and upper gastrointestinal endoscopic findings after chemotherapy. Endoscopic evaluations of tumors were performed in the patients without measurable lesions according to the Japan Classification of Esophageal Cancer (11th edition) [21]. Patients who achieved a CR and those who obtained a partial response (PR) were defined as "responders", and those with stable disease (SD) or progressive disease (PD) were defined as "non-responders".

Quantitative reverse transcription-polymerase chain reaction (RT-qPCR)

Total RNA was isolated from each ESCC tissue sample with an RNeasy Micro Kit (Qiagen) in accordance with the manufacturer's instructions. The extracted RNA was quantified using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Samples were excluded if RNA at a sufficient concentration could not be obtained. Next, total RNA was reverse transcribed to cDNA using PrimeScript RT Master Mix (Takara Bio, Shiga, Japan). The created cDNA was diluted 5-fold. Then, Power-Up SYBR Green Master Mix (Thermo Fisher Scientific) was added and a real-time RT-PCR analysis was performed using a Thermal Cycler Dice Real Time System II

TP900 (Takara Bio). The following conditions were used: 40 cycles of denaturing at 95 °C for 5 sec and annealing at 60 °C for 30 sec. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the housekeeping gene was used as an internal control. The following primers was used: ESM_1. The RT-PCR assay was performed three times for each sample, and mean values were used to calculate the mRNA expression levels. The relative target gene expression was obtained in a comparison with the GAPDH mRNA expression and calculated as the $2^{-\Delta\Delta Ct} \pm$ standard deviation (SD) using the ΔΔCT method.

Statistical analyses

All statistical analyses were performed using SPSS 22.0 (SPSS, Chicago, IL). The relationships between the gene expression levels and the patients' clinical characteristics and chemotherapy responses were analyzed using Fisher's exact test for categorical data or Pearson's chi-squared test. We also divided the patients into low- and high-expression groups, using the median mRNA expression level as the cutoff point. High expression status was defined as a value above the median, and low-expression status was defined as s value below the median. Continuous variables are presented as the median and interquartile range (IQR), and comparisons between two groups were performed using the nonparametric Mann-Whitney U-test. All variables were analyzed with a univariate analysis, and variables with a p-value <0.10 were entered into a multivariate analysis using the backward stepwise method; independent predictors of the chemotherapy response were then assessed. Overall survival (OS) was calculated from the date of the individual patient's treatment initiation to the date of his or her last follow-up or the date of death due to any cause. If patients were alive, they were censored at the time of their last follow-up. OS rates were analyzed using the Kaplan-Meier method and compared using the log-rank test. For the assessment of independent prognostic factors, variables with a p-value <0.10 were entered in a Cox regression analysis using the backward stepwise method. p-values <0.05 were considered significant.

Results

The expression levels of the PI3K/AKT signaling pathway and PD-L1/L2

The expression levels of PI3K, AKT-1, mTOR, 4E-BP1, p70S6K, PD-L1, and PD-L2 are shown in Table 1. The median expression level before chemotherapy was highest for 4E-BP1 (2.86; IQR: 1.35–5.77), followed by AKT-1 (1.81; IQR: 0.87–2.96). The median expression levels of the other genes were around 1.0. The median expression levels after chemotherapy were highest for 4E-BP1 (median: 1.88; IQR: 0.88–4.30), followed by AKT-1 (median: 1.86; IQR: 0.96–2.93). The AKT-1 and PD-L2 levels after chemotherapy

were higher than those before chemotherapy. The expression levels of PIK3CA (p=0.004), mTOR (p=0.001), 4E-BP1 (p=0.018) and p70S6K (p=0.005) after chemotherapy were significantly lower than those before chemotherapy.

Patient demographics and chemotherapy response

The patients' demographics are summarized in Table 2. The median age was 67 years (range 49–91 years); 64 patients were male and 10 were female. The median tumor size was 5.0 cm (range 2.0–20 cm). Distant metastases were found in 8 patients, 2 patients underwent surgery after chemotherapy, and 6 patients received chemotherapy alone. In addition, there were 4 patients with lung metastasis, 3 patients with liver metastasis, and 1 patient with skin metastasis. The number of responders was 34, and that of non-responders was 40. The responder group included 7 patients who attained a CR and 27 patients who achieved a PR. In addition, the 7 patients diagnosed as attaining a CR included 6 patients with a pathological CR and 1 patient with a clinical CR. The non-responder group included 7 patients with PD and 33 patients with SD. Compared to the responders, the non-responders included a significantly larger number of patients with a history of alcohol consumption (p=0.037) and significantly larger tumor sizes (≥5 cm) (p=0.002). No significant between-group differences were observed in the other characteristics.

Gene expressions and response to chemotherapy

The relationship between the expressions of genes before chemotherapy and the patients' responses to chemotherapy is illustrated in Fig.1, and the proportions of patients with high or low gene expression status were analyzed by Fisher's exact test as shown in Fig.1. Using the biopsied tissue samples obtained before chemotherapy, we compared the difference in the mRNA expression levels of the seven targeted genes in both the chemotherapy responder and non-responder groups. The results of our analyses demonstrated that the proportions of patients with high expression of AKT-1 and PD-L1 were significantly higher in the non-responders than the responders (p=0.034, p=0.020, respectively).

The gene expression levels among the non-responders (n=40) before and after chemotherapy are provided in Fig.2. After chemotherapy, the expression of p70S6K was significantly lower than before chemotherapy (p=0.027), and the expressions of PIK3CA, AKT-1, mTOR, 4E-BP1, and PD-L1 were also lower after than before chemotherapy, albeit not significantly (p=0.076, p=0.062, p=0.485, p=0.216 and p=0.259, respectively); only the expression of PD-L2 was higher after than before chemotherapy (p=0.276).

In a univariate analysis of 20 factors including the patients' demographics and gene expressions before chemotherapy, we observed that the patients' alcohol history, tumor size, and expressions of PIK3CA, AKT-1, 4E-BP1, PD-L1, and p70S6K were all factors predictive of the response to chemotherapy. In the multivariate analysis including these seven factors, two independent predictive factors of a poor response to chemotherapy were tumor size ≥5cm (odds ratio [OR]: 6.408; 95% CI: 1.714–23.95; p=0.006) and a high expression of PD-L1 before chemotherapy (OR: 2.998; 95% CI: 1.043–8.619; p=0.042) (Table 3).

Gene expression and prognosis

The relationship between prognosis and genes expressions was analyzed. The median length of follow-up for the censored cases was 4.0 years (range; 0.7-9.2 years) from the date of primary chemotherapy. The OS curves according to the gene expressions before chemotherapy are presented in Fig.3. The patients with high expressions of AKT-1, mTOR, and p70S6K before chemotherapy were significantly more likely to have a poor prognosis compared to those with low expressions of these genes (p=0.026, p=0.021, and p=0.017, respectively). High expressions of PIK3CA, 4E-BP1, PD-L1, and PD-L2 before chemotherapy were not related to poor prognosis (p=0.324, p=0.058, p=0.275, and p=0.770, respectively). The OS of the patients with high expressions of PD-L1 and PD-L2 after chemotherapy was significantly worse than that of the patients with low expressions of these genes after chemotherapy (p=0.012 and, p=0.007, respectively), as shown in Fig.4.

In the univariate analysis including all 20 factors, poor differentiation (p=0.001), distant metastasis (p<0.001), and high expressions of AKT-1, mTOR, p70S6K, and 4E-BP1 before chemotherapy were revealed as poor prognostic factors. The Cox regression analysis including these six factors showed that poor differentiation (hazard ratio [HR]: 4.115; 95% CI: 1.733–9.803; p=0.001), distant metastasis (HR: 7.194; 95% CI: 2.538–20.40; P<0.001), and high expression of p70S6K (HR: 2.518; 95% CI: 1.058–5.988; p=0.037) were independent poor-prognosis factors before chemotherapy (Table 4).

Discussion

We used ESCC tissue samples from 74 patients who underwent chemotherapy to investigate biomarkers that may be related to the patients' response to chemotherapy and prognoses, and we focused on the PI3K / AKT pathway and the immune checkpoints PD-L1 and PD-L2. High expression of PD-L1 in the tumor before chemotherapy was revealed as a predictive factor of poor response to chemotherapy.

These results are consistent with previous reports in which the overexpression of PD-L1 was associated with poor clinical outcomes in patients with various solid tumors [4,12,16]. Thus, our present findings revealed that PD-L1 and p70S6K were strongly associated with the response and prognosis of patients treated with 5-FU+CDDP+DTX combined chemotherapy, respectively.

Because chemotherapy immediately changes gene expression and induces acquired resistance [6], we hypothesized that the genes related to chemo-resistance would be upregulated after just one course of chemotherapy compared to before chemotherapy. However, the results were not consistent with this hypothesis: there were no genes upregulated after chemotherapy in comparison with before chemotherapy. AKT-1, PD-L1 and PD-L2 were identified as the genes whose median values were higher after chemotherapy. Our findings suggested that a further examination of chemo-resistance focusing on AKT-1, PD-L1 and PD-L2 was needed.

Activation of the PI3K/AKT pathway has been reported to be associated with poor prognosis and reduced survival in many cancers [22, 23]. Our present findings identified p70S6K as a factor related to poor prognosis. p70S6K plays an important role in downstream signaling in the PI3K/AKT pathway, an important role in translational regulation, and central roles in the regulation of cancer cell proliferation and survival [24]. Activation of the PI3K/AKT pathway has thus been considered to be associated with tumor stage, size, differentiation, lymph node metastasis, distant metastasis, and various clinical parameters in gastrointestinal carcinomas such as gastric cancer and colon cancer [25, 26]. The chemo-resistance of cancer cells can lead to poor prognosis, and complex changes in signals as well as microenvironments in tumors are associated with acquired resistance. *In vitro* studies have shown that inhibitors of the PI3K/AKT pathway were effective as chemotherapeutic agents against various cancer cells [27, 28], but no specific inhibitors of PI3K/AKT have been reported with regard to chemo-resistance in esophageal cancer.

Immunotherapy based on inhibition of the immune checkpoint pathway has advanced remarkably in recent years, and it has been applied to various types of carcinomas. Although immunotherapy is expected to be effective against chemo-resistance, immunity-related adverse events often make it difficult to continue immunotherapy-based treatment [29]. Personalized immunotherapies based on biomarkers are thus desired [16, 30]. The high expression of PD-L1 in the tumors before chemotherapy was independently correlated with poor responses to chemotherapy in our study. In contrast, gene expression analyses of the tumors treated with chemotherapy revealed that patients with high expressions of PD-L1 and PD-L2 had significantly worse OS than patients

with low expressions of these genes. Moreover, PD-L1 expression in the tumor was increased after platinum-based chemotherapy, and PD-L1 expression in non-small cell lung cancer has been reported to indicate poor clinical outcome [31]. Interestingly, PD-L1 and PD-L2 were identified as prognostic factors after chemotherapy consisting of DTX, 5-FU, CDDP in this study, and high expression of PD-L1 was related to poor response to chemotherapy and high expression of PD-L1 after chemotherapy was one of the poor prognostic factors. These findings suggested that the chemo-resistance due to high expression of PD-L1 was induced by chemotherapy and resulted in poor survival rates. The immune checkpoints thesis suggests that PD-L1 and PD-L2 are important biomarkers of the prognosis or response to ESCC treatment. These biomarkers could serve a useful role in individualized treatment. Targeted therapies that inhibit these genes are expected to counteract chemo-resistance and improve the poor prognosis of patients with ESCC. Our present results support the development of new combination treatments using conventional cytotoxic drugs, PI3K/AKT signal inhibitors, and immune checkpoint inhibitors.

In conclusion, the present study demonstrated that the expressions of PD-L1 and p70S6K in the primary ESCC tissues were related to a poor response to chemotherapy and a poor prognosis, respectively, and these genes could thus be attractive targets in the treatment of ESCC by improving the intracellular environment.

Study limitations: The assessments were performed on a relatively small number of patients from a single center, and the follow-up periods were as short as 1–2 years in some patients. The tissue samples were collected from patients at various stages, and included surgical and biopsy samples. A multicenter study with a larger population of ESCC patients and more samples is necessary to validate our results.

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Compliance with ethical standards

Human rights statement and informed consent

All procedures were in accordance with the ethical standards of the responsible institutional committee on human experimentation and with the Helsinki Declaration of 1964 and later versions. All patients provided informed consent for the use of their data and materials.

Conflict of Interest Statement

All authors have no conflicts of interest associated with this study.

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Figure:

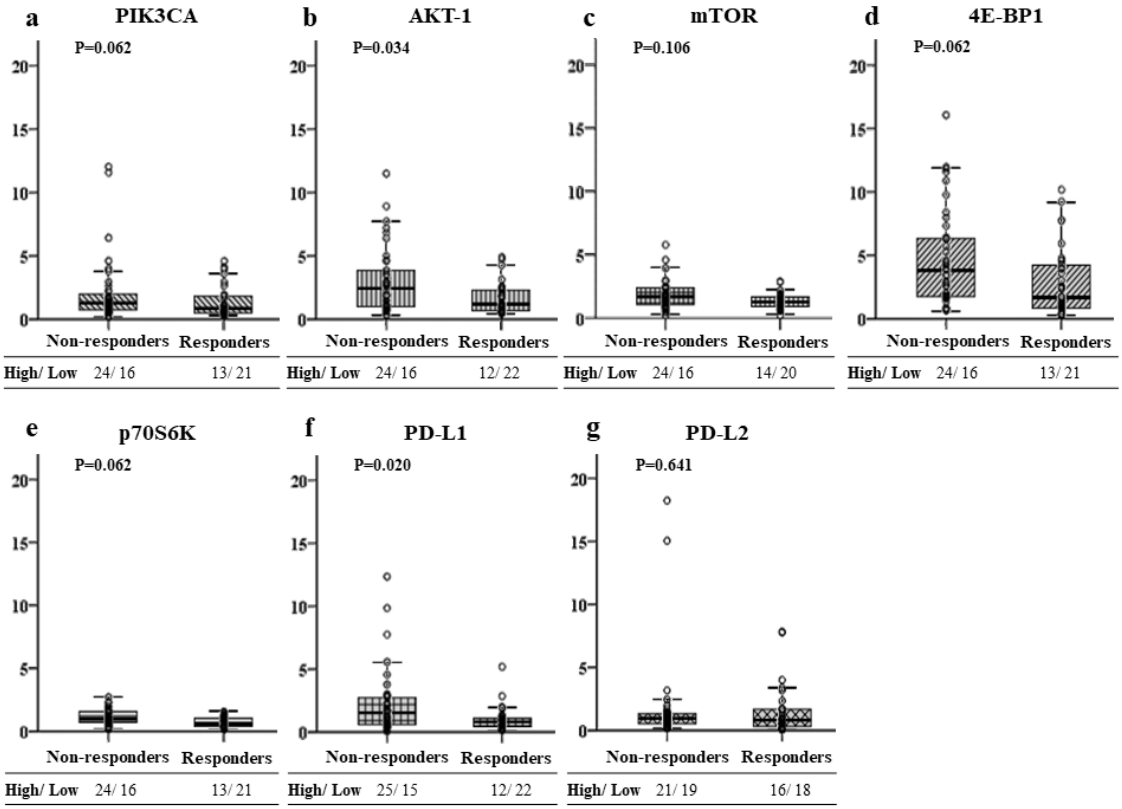

Fig. 1. The relationships between gene expressions before chemotherapy and the chemotherapy response

The mRNA expression levels of the seven targeted genes in the biopsied tissue samples before chemotherapy in the non-responder and responder groups are shown, and the numbers of patients with high/low gene expression status in each gene is shown below. The median values for each gene were as follows: (a) PIK3CA, 1.08; (b) AKT-1, 1.81; (c) mTOR, 1.40; (d) 4E-BP1, 2.86; (e) p70S6K, 0.83; (f) PD-L1, 0.97; and (g) PD-L2, 0.86. The median values were adopted as the cutoff points; high expression status = above the median.

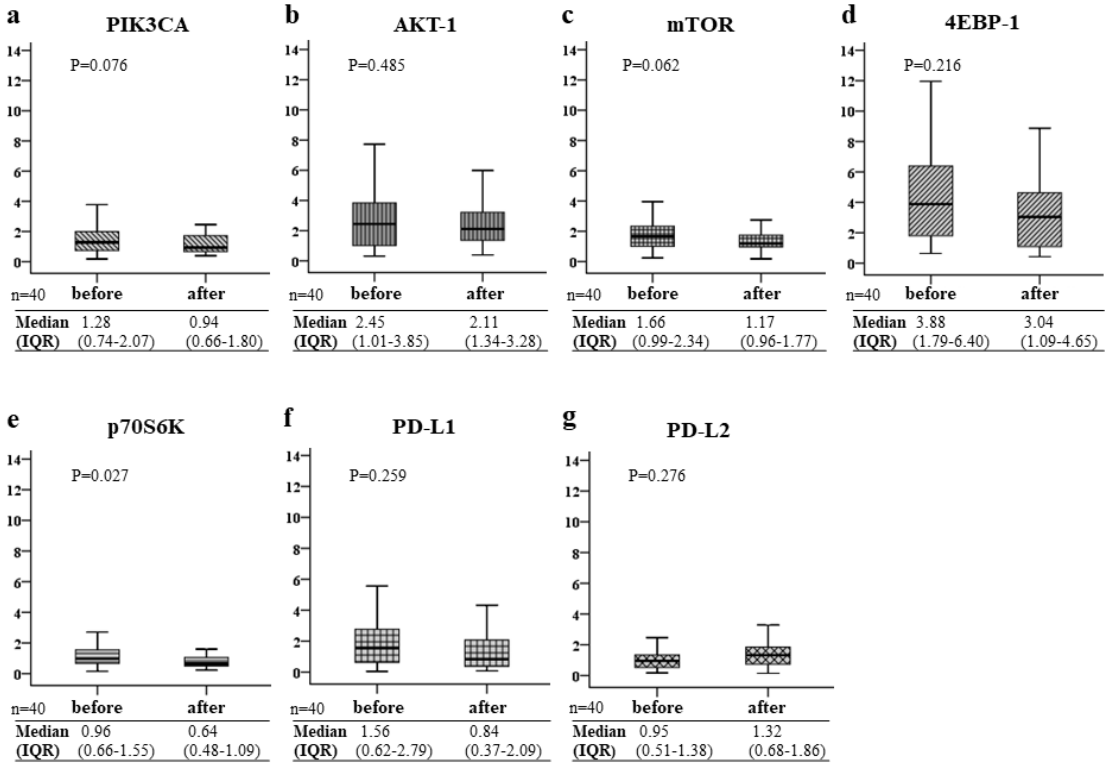

Fig. 2. Comparisons of the gene expressions before and after chemotherapy in non-responders

(a) Comparisons of the expressions of PIK3CA, (b) AKT-1, (c) mTOR, (d) 4E-BP1, (e) p70S6K, (f) PD-L1, and (g) PD-L2. Comparisons between pairs of groups were assessed using the nonparametric Mann-Whitney U-test. IQR: interquartile range.

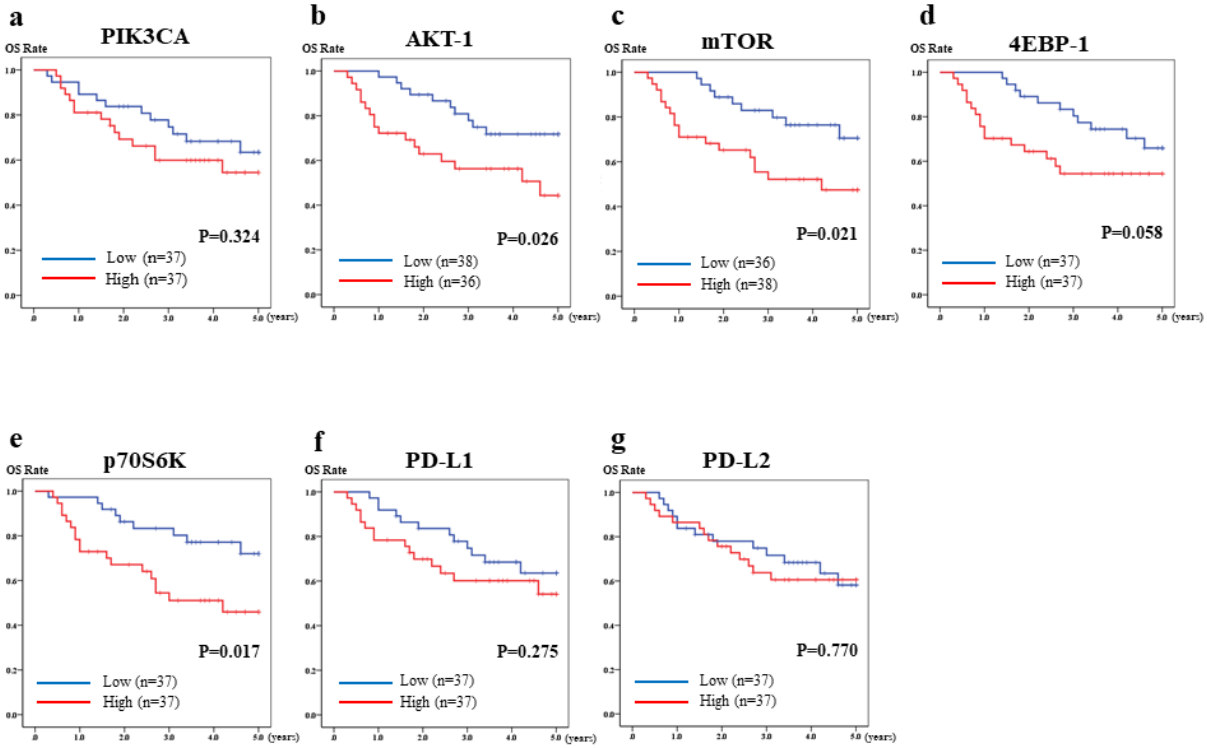

Fig. 3. The prognoses of ESCC patients according to mRNA expression levels before chemotherapy

Kaplan-Meier curves generated by the log-rank test for overall survival (OS) are shown for the patient groups with high- and low-mRNA expression of the following genes in biopsy samples before chemotherapy: PIK3CA (a), AKT-1 (b), mTOR (c), 4E-BP1 (d), p70S6K (e), PD-L1 (f), and PD-L2 (g).

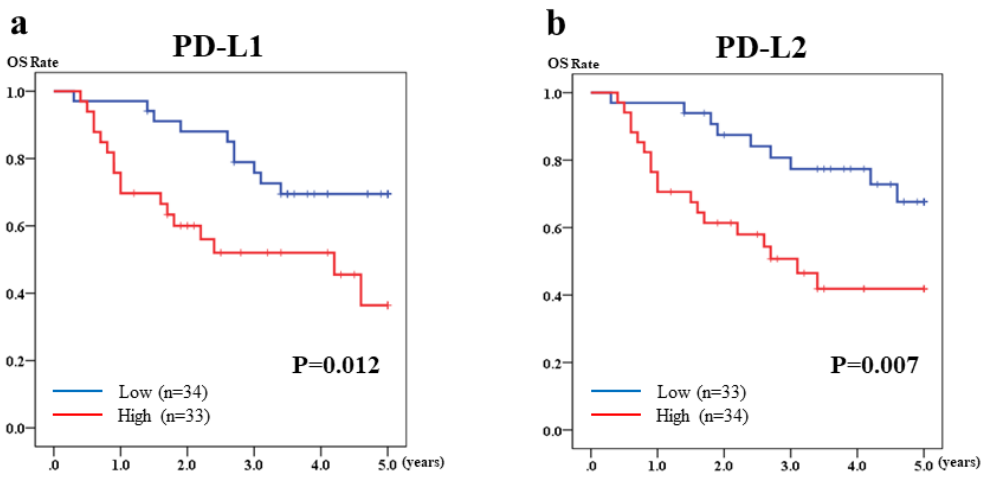

Fig. 4. The prognoses of the ESCC patients according to mRNA expression levels after chemotherapy

Kaplan-Meier curves generated by the log-rank test for overall survival (OS) are shown for the patient groups with high and low expression levels of the following genes in biopsy samples after chemotherapy: (a) PD-L1, (b) PD-L2.

Table:

	mRNA expression levels [median (IQR)]		
Genes	**Before treatment (N=74)**	**After treatment (N=67)**	**P-value**
PIK3CA	1.08 (0.61-1.84)	0.79 (0.55-1.29)	0.004
AKT-1	1.81 (0.87-2.96)	1.86 (0.96-2.93)	0.175
mTOR	1.40 (0.95-1.89)	1.12 (0.70-1.39)	0.001
4E-BP1	2.86 (1.35-5.77)	1.88 (0.88-4.30)	0.018
p70S6K	0.83 (0.41-1.23)	0.59 (0.40-0.82)	0.005
PD-L1	0.97 (0.52-1.94)	0.64 (0.28-1.83)	0.277
PD-L2	0.86 (0.48-1.46)	1.05 (0.52-1.78)	0.449

Table 1. mRNA expression levels before and after chemotherapy

IQR: interquartile range.

Characteristics	Status	Non-responders (n=40)	Responders (n=34)	P-value
Sex	Male / Female	37 / 3	27 / 7	0.171
Age	<65y / ≧65	10 / 30	14 / 20	0.138
Smoking index	<400 / ≧400	9 / 31	10 / 24	0.498
Alcohol history	No / Yes	2 / 38	8 / 26	0.037
Alcohol flush reaction	No / Yes	16 / 24	12 / 22	0.677
Cancer family	No / Yes	26 / 14	20 / 14	0.585
Tumor size	<5cm / ≧5cm	4 / 36	14 / 20	0.002
Differentiation	Well・mod / Poor	32 / 8	30 / 4	0.338
Tumor location	Ut・Mt / Lt・Ae	6・19 / 15・0	2・18 / 12・2	0.747
Depth of invasion	T1・T2 / T3・T4	2・4 / 27・7	1・4 / 16・13	0.972
LN metastasis	N0・N1 / N2・N3	2・13 / 14・11	5・9 / 16・4	0.747
Metastasis	M0 / M1	34 / 6	32 / 2	0.275
TNM stage	I・II / III・IV	2・3 / 21・14	0・5 / 15・14	0.523
Chemotherapy response	CR / PR / SD / PD	0 / 0 / 33 / 7	7 / 27 / 0 / 0	—

Table 2. Relationships between the patients' demographics and the responses to chemotherapy

LN: lymph node; CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease.

			Response		
Factors	**Status (n)**	**Cut off**	**OR**	**95%CI**	**P-value**
Tumor size	<5cm (18) / ≧5cm (56)	≧5cm	6.408	1.714-23.95	0.006
Alcohol history	No (10) / Yes (64)	YES	4.625	0.801-26.68	0.087
PD-L1	High (37) / Low (37)	High	2.998	1.043-8.619	0.042

Table 3. Multivariate analysis of predictive factors of the response to chemotherapy

OR: odds ratio; CI: confidence interval.

			Overall survival (OS)		
Factors	**Status (n)**	**Cut off**	**HR**	**95%CI**	**P-value**
Differentiation	well・mod (62) / poor (12)	poor	4.115	1.733-9.803	0.001
Metastasis	M0 (66) / M1 (8)	M1	7.194	2.538-20.40	<0.001
AKT-1	High (36) / Low (38)	High	2.145	0.923-4.975	0.076
p70S6K	High (37) / Low (37)	High	2.518	1.058-5.988	0.037

Table 4. Multivariate analysis of prognostic factors before chemotherapy

HR: hazard ratio; CI: confidence interval.