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ORIGINAL ARTICLE

# Analysis of SPARC and TUBB3 as predictors for prognosis in esophageal squamous cell carcinoma receiving nab-paclitaxel plus cisplatin neoadjuvant chemotherapy: a prospective study

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## Abstract

**Purpose** The purpose of the study is to evaluate the predictive efficacy of secreted protein, acidic and rich in cysteine (SPARC) and the class III β-tubulin (β-tubulin III, TUBB3) in predicting therapeutic effect in patients with locally advanced esophageal squamous cell carcinoma(ESCC) who received nab-paclitaxel plus cisplatin neoadjuvant chemotherapy(CT) followed by surgery.

**Methods** Patients with stage II to III esophageal squamous cell carcinoma of different stages are recruited. The tumor biopsy tissues prior treatment from enrolled patients were examined by SPARC and TUBB3 immunohistochemistry (IHC). Correlations between SPARC/TUBB3 expression and response to chemotherapy and long-term survival in patients received surgical resection was analyzed.

**Results** A total of 35 patients with stage II to III esophageal squamous cell carcinoma were enrolled. Of the 35 enrolled patients, 30 successfully completed neoadjuvant chemotherapy and underwent R0 resection, 3 refused surgery after chemotherapy, and 2 failed to undergo radical surgery after chemotherapy. Out of patients undergoing surgery, pathological complete response (pCR) was achieved in 6 patients (6/30, 20%). The 1, 2 and 5-year disease free survival (DFS) rates were 70.0%, 36.6% and 33.3%, respectively. The 1, 2 and 5-year overall survival (OS) rates were 83.3%, 63.3% and 36.6%, respectively. SPARC and TUBB3 IHC was performed on the tumor biopsy tissues which were obtained from patients before treatment. Correlation between SPARC/TUBB3 expression and long-term survival in patients was studied. Both the median DFS and OS between SPARC negative samples and SPARC positive staining samples have no statistical difference. However, the median DFS and OS in TUBB3 negative patients was better than those in TUBB3 positive patients ($p=0.002$ for DFS, $p=0.001$ for OS). In addition, patients with pCR had longer OS and DFS time than those without pCR.COX regression analysis showed that TUBB3 prior treatment and pCR were independent prognostic factors in ESCC patients undergoing sequential surgery after preoperative chemotherapy.

**Conclusions** TUBB3 negative expression prior treatment and pCR may indicate a better prognosis for stage II and III ESCC patients after nab-paclitaxel plus cisplatin neoadjuvant chemotherapy following radical esophagectomy.

**Keywords** Esophageal carcinoma · Nab-paclitaxel · Neoadjuvant chemotherapy · Secreted protein acidic and rich in cysteine · Class III β-tubulin

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## Introduction

Esophageal cancer is generally the eighth most common cancer and the sixth most common reason of tumor-related deaths [1]. The main histological types of esophageal cancer are squamous cell carcinoma and adenocarcinoma. Squamous cell carcinoma of the middle thoracic esophagus is the mainstream in the East [2]. The therapeutic strategies of squamous cell carcinoma should be different from adenocarcinoma [3].

Both adenocarcinoma and squamous cell tumor have been treated by surgery [4]; however, multimodality treatment, which combines chemotherapy, radiotherapy or chemoradiotherapy with surgery, is essential to reduce systemic and local tumor recurrence [3, 5–8]. Particularly, therapy before surgery has been suggested for both adenocarcinoma and squamous cell esophageal cancer [9, 10].

Although preoperative chemoradiation is a standard strategy in the treatment of locally advanced esophageal cancer in USA, an updated meta-analysis shows no clear advantage of neoadjuvant chemoradiation over neoadjuvant chemotherapy alone especially in ESCC patients (65% of patients with SCC in that meta-analysis). Moreover, neoadjuvant chemotherapy seems much safer with a reduced postoperative mortality compared with preoperative chemoradiation in esophageal cancer. Therefore, the value of neoadjuvant chemotherapy in ESCC deserves further evaluation.

Although cisplatin plus 5-fluorouracil scheme was often used to assess the benefits of preoperative chemotherapy, the best neoadjuvant chemotherapy regimen has not yet been established. Nab-paclitaxel is a solvent-free colloidal suspension of paclitaxel and human serum albumin. Nab-paclitaxel which has an average size of 130 nm, becomes individual albumin-paclitaxel-complexes of around 10 nm in the blood vessel. Nab-paclitaxel may take advantage of the characteristic of albumin owing to the following mechanisms: ① Paclitaxel was carried by albumin and dissociate across the blood vessel endothelium for the active gp60-receptor-mediated transcytosis of albumin; ② Nab-paclitaxel achieves intratumoral accumulation by albumin's binding to SPARC [11, 12]. The combination of Nab-paclitaxel and cisplatin had been found to be a highly effective and well-tolerated first-line treatment in metastatic ESCC.

One deficiency of preoperative chemotherapy is that surgery is put off in non-responders, increasing the possibility of disease progression or metastasis for these patients. If this happens, it may reduce the effects of preoperative therapy and increase the postoperative morbidity and mortality. Accordingly, it is urgent to find a biomarker that can predict the outcomes of neoadjuvant chemotherapy.

The matricellular glycoprotein SPARC is an albumin- and calcium-binding glycoprotein that regulates the mutual influence between cells and the extracellular matrix (ECM) [13]. SPARC is multifunctional, not only influences cell migration, invasion, tumor growth and metastasis, but also probably participates in tumor escaping immune surveillance. SPARC promotes the delivery of albumin from the ECM to tumor cells [14, 15]. Importantly, SPARC may be related to the therapeutic effect of nab-paclitaxel. For the metastatic pancreatic ductal adenocarcinoma patients receiving nab-paclitaxel plus gemcitabine, median OS of patients with a high expression of SPARC was significantly longer than the low-SPARC group [16].

Paclitaxel binds to β-tubulin, a major part of microtubule, and causes the growth arrest of cancer cells at the G2/M phase through the stabilization of microtubule [17]. Taxane resistance may be resulted from point mutation in tubulin and the selective overexpression of β-tubulin isotypes [18–20]. Some researchers found that TUBB3 restrained the assembly of β-tubulin subunits enhanced by paclitaxel. From results of preclinical studies, it has been shown that high expression levels of TUBB3 are related with paclitaxel resistance in many types of cancer cell lines. In ovarian, breast and lung cancers, TUBB3 expression is reversely correlated with paclitaxel efficacy or prognosis of patients [21–24].

How to predict the efficacy of neoadjuvant therapy is a focus in the field of locally advanced esophageal squamous cell carcinoma. We carried out a stage II clinical trial on nab-paclitaxel and cisplatin neoadjuvant chemotherapy for locally advanced ESCC in July 2011, and aimed to evaluate the role of SPARC and TUBB3 in predicting short-term efficacy and long-term survival.

This study was registered on the ClinicalTrials.gov Web site (NCT01258192).The research has been approved by Ethics Committee of Zhejiang Cancer Hospital.

## Methods

### Patients eligibility

Treated patients met the following inclusion criteria: (I) age ranges from 18 to 70 years, (II) ECOG performance status 0–1, (III) histologic diagnosis of squamous cell carcinoma of esophagus, (IV) Stage II–III assessed by endoscopic ultrasound (EUS), enhancement computed tomography of the chest and abdomen, and esophagogram. (V) tumor location with middle third and distal third of esophagus, (VI) Adequate organ function including the following: absolute neutrophil count (ANC) greater than or equal $1.5 \times 109$/L, platelets greater than or equal $80 \times 109$/L, haemoglobin greater than or equal 80 g/L, total bilirubin less than or equal to 1.5 times upper limit of normal (ULN), alanine transaminase (ALT) and aspartate transaminase (AST) less than or equal to 2.5 times ULN, calculated creatinine clearance rate $\geq 60$ ml/min, (VII) tumor can be measured according to the Response Evaluation Criteria In Solid Tumors (RECIST) criteria. (VIII) no prior invasive malignancy, (IX) no any prior anticancer therapy, and, (X) All patients provided written informed consent. Main exclusion reasons comprise: (I) carcinoma at the upper part of esophagus, (II) prior treatment for esophageal cancer, (III) active infection, (IV) pregnant or breast feeding, (V) prior invasive malignancy in previous 5 years (except for carcinoma in situ).

## Treatment

Patients enrolled received two cycles of neoadjuvant chemotherapy with nab-paclitaxel (100 mg/m$^2$, d1, d8) and cisplatin (75 mg/m$^2$, d1). Clinical reevaluation was given 3 weeks after termination of the second cycle of neoadjuvant chemotherapy. If patients were proper for curative resection, surgery was performed approximately 4 to 8 weeks after the completion of neoadjuvant chemotherapy. 2 cycles of adjuvant chemotherapy with the same regimen were given 4–6 weeks after the resection. If postoperative radiotherapy was necessary, radiotherapy should be given afte adjuvant chemotherapy. The dose and fractionation regimens were either 50.4 Gy in 28 fractions or a biologically equivalent dose. Study scheme was reported in Table 1.

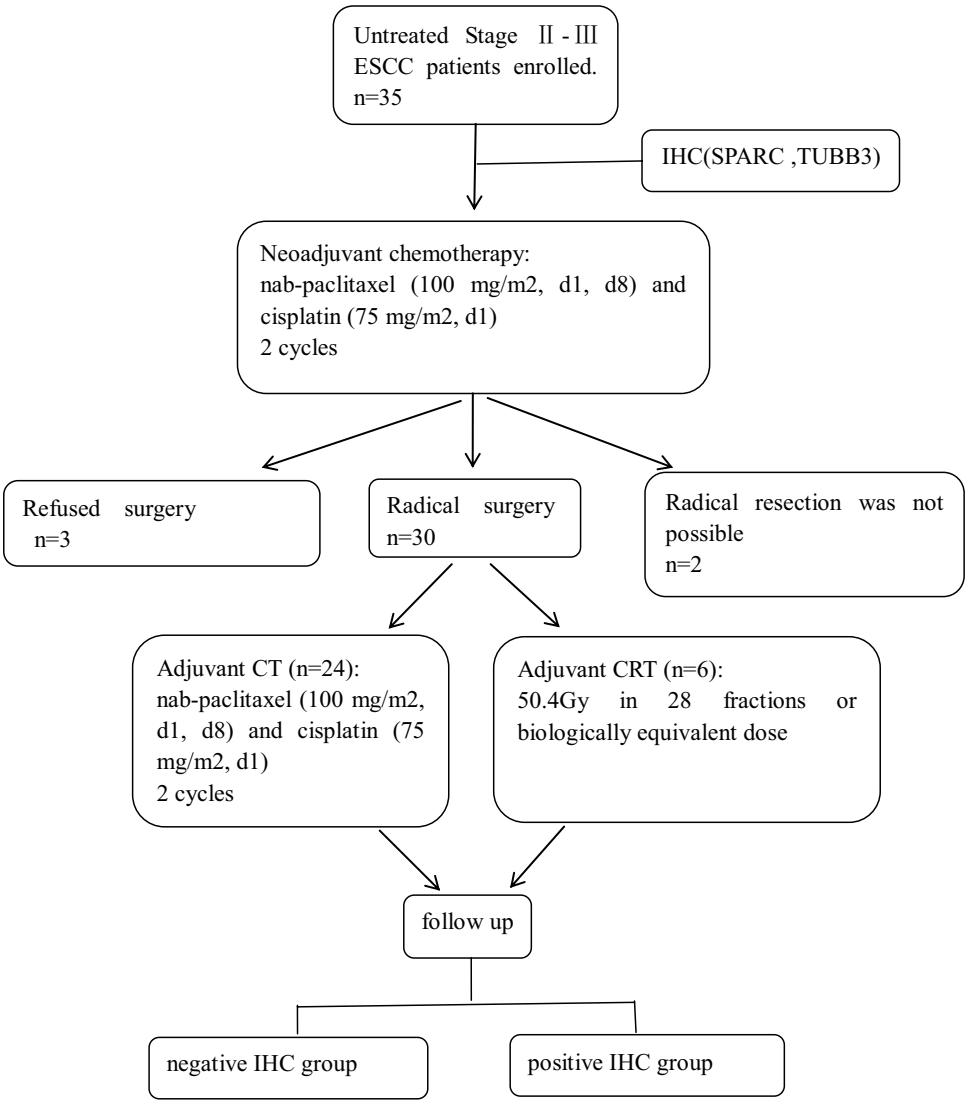
## Response evaluation

Chemotherapeutic response was evaluated 2 weeks after neoadjuvant therapy by computed tomography and EUS according to RECIST criteria version 1.1. Complete response (CR) and partial response (PR) patients were defined as responders, stable disease (SD) and progressive disease (PD) patients as non-responders. The disease-free survival (DFS) and overall survival (OS) were calculated from the day of esophagectomy to disease relapse and death from any cause, respectively.

## SPARC and TUBB3 expressions

Human specimens were approved to be used by the Ethics Committee of Zhejiang cancer hospital. Two step method of

**Table 1** study scheme




*ESCC* esophageal squamous cell carcinoma, *IHC* immunohistochemistry, *CT* chemotherapy, *CRT* adjuvant radiotherapy after adjuvant chemotherapy

IHC was used to detect SPARC and TUBB3 in tumor sections. Formalin-Fixed Paraffin-Embedded tissue sections with 4 μm thick were deparaffinized in xylene and hydrated in graded alcohols. After antigen retrieval in 0.01M citrate buffer (pH 6.0), sections were treated with endogenous peroxidase confining liquid (Beijing CoWin Biotech Co., Ltd., Lot. CW0117) for 10 min. Sections were rinsed and incubated with SPARC and TUBB3 (Beijing CoWin Biotech Co., Ltd.) monoclonal antibodies for 60 min, respectively. After rinsing in phosphate buffered saline (PBS), the sections were incubated with general type IgG-HRP Polymer (Beijing CoWin Biotech Co., Ltd., Lot. CW0117) for 10 min, followed by chromogenic 3,3′-Diaminobenzidine tetrahydrochloride dihydrate (DAB) for about 2–7 min. Finally, sections were conterstained with hematoxylin for 1 min followed by dehydrated in graded alcohols, cleared in xylene, and covered with coverslips. Each experiment included negative control. Sections were examined and scored by two independent professional pathologists of pathology department. Staining was graded according to the description in other researches [23]. We calculated the intensity of staining (1, weak; 2, moderate; 3, strong) and the percentage of stained cells (1, 0–10%; 2, 11–50%; 3, 51–100%). The median staining score was calculated by intensity score plus percentage score. And the expression levels of the samples were divided to be negative or positive according to the median staining score as following: positive when score $>3$, negative when score $\leq 3$.

### Statistical analysis

The primary endpoint was DFS time, which was defined as the time from the date of surgery to the date of tumor recurrence or distant metastasis. The date was limited to the time of the last tumor assessment if disease recurrence or distant metastasis did not occur.

The associations between response to neoadjuvant chemotherapy and SPARC, TUBB3 expressions were evaluated by Fisher's exact test. To analyze the relationships between biomarkers expression and survival, Kaplan–Meier curves and log-rank test were adopted. SPSS 16.0 (SPSS Inc, Chicago, Illinois, USA) was used in Statistical analysis.

The DFS and OS time were analyzed by subgroups using the Cox proportional hazards model, with variables consisting of age, gender, tumor stage, staging change, postoperative pathology, SPARC and TUBB3 expression and radiotherapy. All $P$ values were two-sided and $p<0.05$ was considered to indicate a statistically significant difference.

## Results

### Patient characteristics

Between Oct 2011 and Dec 2012, 35 patients were enrolled in this study. Their general characteristics are summarized in Table 2. 31 patients were male and 4 were female. The median age was 59 years (range 48–70 years). Stage IIA, IIB, IIIA, IIIB and IIIC disease was found in 3 (8.6%), 5 (14.3%), 10 (28.6%), 8 (22.9%) and 9 (25.7%) patients, respectively.

### Neoadjuvant chemotherapy response and postoperative pathology

All of the 35 patients received preoperative neoadjuvant chemotherapy. Chemotherapeutic response was evaluated by computed tomography and EUS according to RECIST criteria version 1.1. After preoperative chemotherapy, 23 of the 35 patients achieved objective remission (CR: 4, PR: 17), 10 were SD, and the remaining 2 were PD. 30 patients (30/35, 85.7%) underwent radical surgery, 3 refused surgery after chemotherapy, and two patients did not undergo

**Table 2** Patient- and tumor-related clinicopathological characteristics ($n=35$)

| Characteristics of patients | No. = 35 (%) |
|---|---|
| Male/female | 31 (88.6)/4 (11.4) |
| Median age (range) | 59.0 (48 ~ 70) |
| Tumor location | |
| Middle segment | 30 (85.7) |
| Lower segment | 5 (14.3) |
| ECOG performance status | |
| 0 | 25 (71.4) |
| 1 | 10 (28.6) |
| Clinical TNM stage | |
| IIA/IIB | 3 (8.6)/5 (14.3) |
| IIIA/IIIB/IIIC | 10 (28.6)/8 (22.9)/9 (25.7) |
| Patients receiving neoadjuvant chemotherapy | 35 (100) |
| Neoadjuvant chemotherapy response | |
| PR | 17 (48.6) |
| CR | 6 (17.1) |
| SD | 10 (28.6) |
| PD | 2 (5.7) |
| Surgery | 30 (85.7) |
| R0 resection | 30 (85.7) |
| pCR | 6 (17.1) |

*CR* complete response, *PR* partial response, *pCR* pathological complete response, *SD* stable disease, *PD* progression of disease

surgery because of the progression of the disease after neoadjuvant chemotherapy (Tables 1, 2).

Of the 30 patients undergoing surgery, pathological complete response (pCR) was achieved in 6 patients (6/30,%),and significant down-staging was observed in 19 patiens (19/30,63.3%). 24 patients (24/30,80.0%) received postoperative chemotherapy, and 6 patients (6/30,20%) received adjuvant radiotherapy after adjuvant chemotherapy (Table 1).

## Follow-up results

At a median follow-up of 30 months (range 2.5–72 months), 11/30 patients were still alive.1, 2 and 5-year disease free survival (DFS) rates were 70.0%, 36.6% and 33.3%, respectively. 1, 2 and 5-year overall survival (OS) rates were 83.3%, 63.3% and 36.6%, respectively. The median DFS time was 15.3 months (95% CI: 10.8–19.7). The median OS time was 32.0 months (95% CI: 13.2–50.8).

## The associations between TUBB3 and SPARC IHC expressions and clinical outcomes

SPARC and TUBB3 statuses in preoperative tissue were evaluated in all the 35 patients (Fig. 1). The relationship between TUBB3 and SPARC expression and neoadjuvant chemotherapy response was analyzed. No significant correlation was observed between TUBB3 and SPARC status and chemotherapeutic response (Table 3).

The median DFS (not achieved) in TUBB3 negative staining samples was longer than that in TUBB3 positive staining samples (DFS: 13.2 months, 95% CI: 11.3–15.1) ($p=0.001$. Fig. 2b). Furthermore, statistical difference of OS was observed between two groups ($p=0.002$, Fig. 2e).

However, the median DFS in SPARC negative and positive staining samples showed no statistical difference (separately 15.3 months, 15.4 months, $p=0.977$, Fig. 2a). And no statistical difference of OS was observed between SPARC negative staining and positive staining samples (separately 32.0 months, 40.0 months, $p=0.981$, Fig. 2d). Besides, statistical difference of DFS and OS was observed between pCR and non-pCR patients ($p=0.019$, $p=0.016$, respectively, Fig. 2c, f).

A series of factors as reported in Table 4 and Table 5, consisting of age, gender, tumor staging changes after neoadjuvant chemotherapy, preoperative staging, postoperative pathology, postoperative radiotherapy, SPARC and TUBB3 expression, were assessed using the univariate Cox regression analysis to evaluate the impact of the factors on the DFS and OS time of patients with ESCC. The variables found to impact DFS time in the univariate analysis were TUBB3 expression [hazard ratio (HR), 4.853; 95% CI 1.590–14.812; $p=0.006$], and postoperative pathology (pCR) (HR 0.129; 95% CI 0.017–0.973; $p=0.047$). Furthermore, it was proved in the multivariate analysis model that the predominant predictors of DFS time were TUBB3 expression (HR, 4.076; 95% CI 1.236–13.439; $p=0.021$)

**Table 3** Association between SPARC and TUBB3 IHC expression and chemotherapeutic response ($n=35$)

| | SD + PD (%) | CR + PR (%) | *P** |
|---|---|---|---|
| SPARC (%) | | | |
| + | 4 (11.4) | 14 (40) | 0.164 |
| − | 8 (22.9) | 9 (25.7) | |
| TUBB3 (%) | | | |
| + | 7 (20.0) | 11 (31.4) | 0.725 |
| − | 5 (14.3) | 12 (34.3) | |

*CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *SPARC* secreted protein, acidic and rich in cysteine, *TUBB3* β-tubulin III

*Tested by Fisher's Exact Test

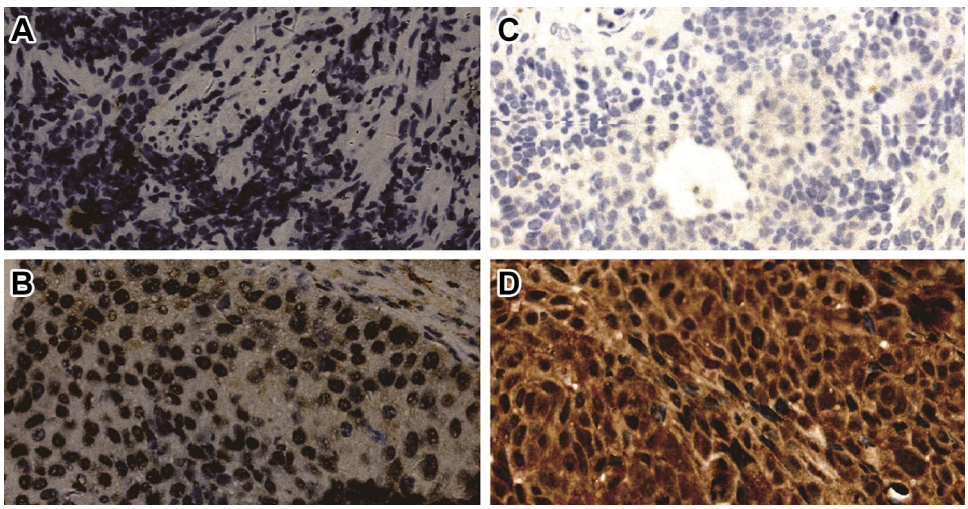



**Fig. 1** Representative images of TUBB3 and SPARC immunohistochemical expressions (magnification, ×400). **a** TUBB3 negative; **b** TUBB3 positive; **c** SPARC negative; **d** SPARC positive

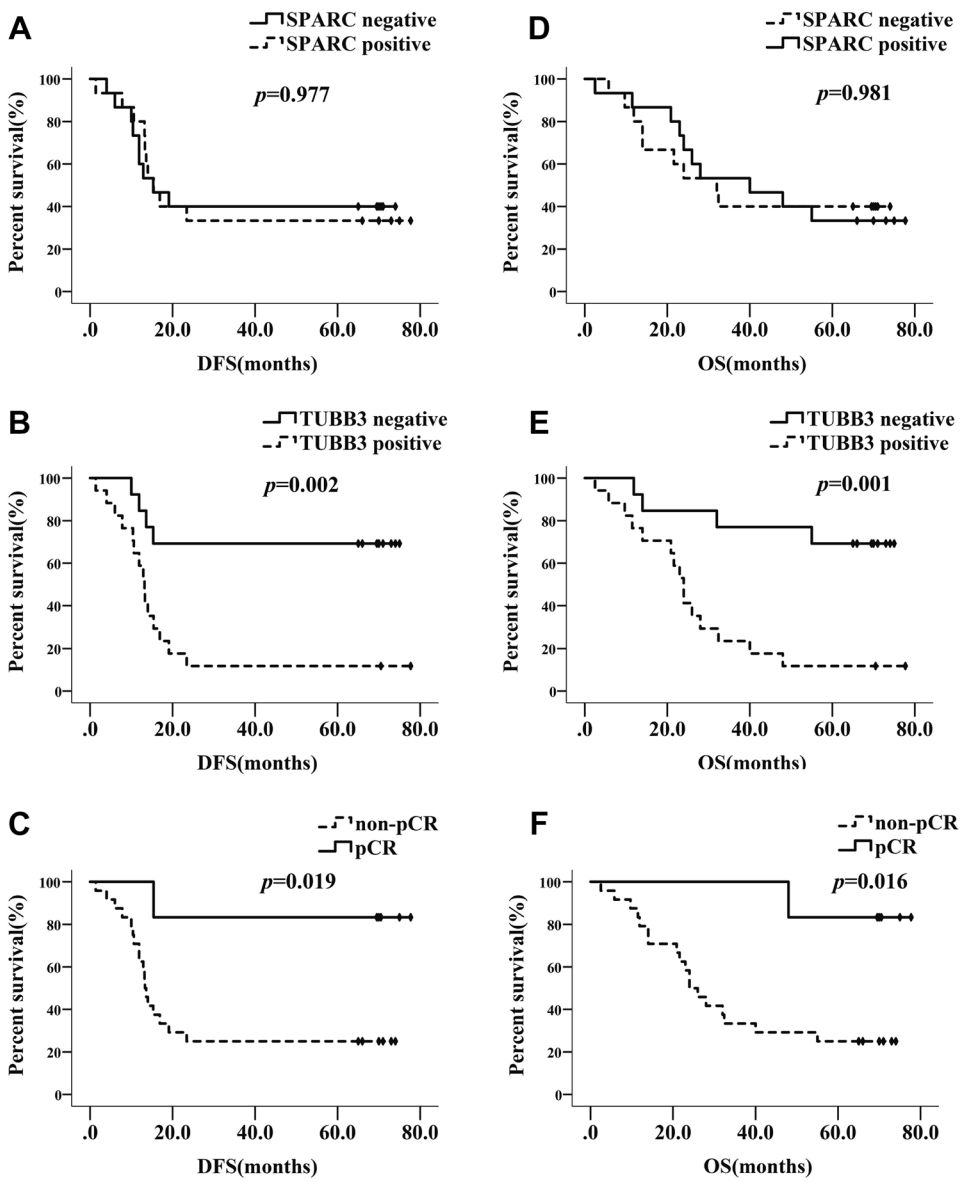



**Fig. 2** Kaplan–Meier curves of estimated survival classified by expression of SPARC and TUBB3 and postoperative pathology. **a** Disease free survival (DFS) and **d** overall survival (OS) classified by expression of SPARC. **b** DFS and **e** OS classified by expression of TUBB3. **c** DFS and **f** OS curves classified by postoperative pathology [pathological complete response(pCR) or non-pathological complete response (non-pCR)]

**Table 4** Univariate analysis and multivariate analysis for presictors of disease-free survival ($n=30$)

| Variables | Univariable analysis | | | Multivariable analysis | | |
|---|---|---|---|---|---|---|
| | *p* | HR | 95% CI | *p* | HR | 95% CI |
| Age (≥ 60 vs.<60 years) | 0.273 | 1.665 | 0.669–4.149 | | | |
| Sex (female ve male) | 0.884 | 0.860 | 0.115–6.461 | | | |
| Stage (stage III vs. II) | 0.056 | 4.204 | 0.967–18.289 | 0.261 | 2.462 | 0.512–11.845 |
| Descent stage (yes vs. no) | 0.303 | 0.619 | 0.248–1.544 | | | |
| pCR (yes vs. no) | 0.047 | 0.129 | 0.017–0.973 | 0.036 | 0.114 | 0.015–0.869 |
| RT (yes vs. no) | 0.740 | 0.811 | 0.236–2.792 | | | |
| SPARC (positive vs. negative) | 0.977 | 1.014 | 0.411–2.499 | | | |
| TUBB3 (positive vs. negative) | 0.006 | 4.853 | 1.590-14.812 | 0.021 | 4.076 | 1.236–13.439 |

*CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *HR* hazard ratio, *SPARC* secreted protein, acidic and rich in cysteine, *TUBB3* β-tubulin III

and postoperative pathology (pCR) (HR, 0.114; 95% CI 0.015–0.869; $p=0.036$. Table 4). Similarly, it was revealed that TUBB3 and pCR expression were independent prognostic factors for OS when univariate and multivariate analyses were used to assess the impact of these indicators on OS, as reported in Table 5.

### Adverse events

Adverse events (AEs) were graded using the Common Terminology Criteria for Adverse Events Version 4.0. The most common grade 3 and 4 hematologic toxicities during preoperative neoadjuvant chemotherapy were neutropenia, anemia, thrombocytopenia and vomiting, and were reported in 11.4, 8.6, 5.7, and 14.3% of patients, respectively. There were no treatment-related deaths in the study. Operative complications included a case of anastomotic leakage. During postoperative adjuvant chemotherapy, grade 3 and 4 hematologic AEs were observed in 20.8% patients.

## Discussion

ESCC is still difficult to be treated [1]. ESCC patients can benefit from preoperative therapies in multiple mechanisms. First, preoperative therapies may reduce the size of tumor, and facilitate complete resection. Second, they may eradicate potential micro-metastases, and decrease recurrence after operation. Third, drug can be transported to the tumor tissue more efficiently through uninjured blood vessels [10]. A successful neoadjuvant chemotherapy should have a good treatment effect to avoid patients possibly curable by operation alone evolving to an inoperable status during preoperative chemotherapy. So far standard neoadjuvant chemotherapy regimen for ESCC has not been established [10]. It is hard to consider that the nab-paclitaxel plus cisplatin regimen is better than other regimens based on our results, because only 30 patients accepted surgery and lack of control arm. However, this regimen deserves further research as neoadjuvant chemotherapy for ESCC. In our results, the response rate was 65.7%, which is similar to those of other studies [5, 10]. At a median follow-up of more than 60 months, 11 of the 30 patients accept surgeries were still alive. The 1, 2 and 5-year disease free survival (DFS) rates were 70.0%, 36.6% and 33.3%, respectively. That means more than half of patients suffered from ESCC recurrence in 5 years and died because of disease. So it is urgent to discover some predictive biomarkers to guide the treatment and improve the response and survival.

SPARC is an albumin-binding protein. SPARC always indicating poor survival is overexpressed and secreted in a number of cancers [14]. Paclitaxel was carried by albumin and dissociate across the blood vessel endothelium for the active gp60-receptor-mediated transcytosis of albumin and then the nab-paclitaxel achieves intratumoral accumulation by albumin's binding to SPARC. So compared with traditional paclitaxel, paclitaxel accumulation in tumor was much more easier for nab-paclitaxel [11, 12]. In our analysis, the response rates were 52.9% (9/17) and 77.7% (14/18) for SPARC negative and positive patients, respectively. The response rate was a little higher in SPARC positive patients compared with SPARC negative patients with no statistifically significance. Moreover, the median DFS time was almost the same between two groups. Thus, SPARC expression may not be a good predictor of nab-paclitaxel efficacy.

Paclitaxel acts on microtubule, promotes microtubule stabilization and affect tumor cells divisions [17]. It has been revealed that TUBB3 was highly expressed in tumor cells which tended to resist paclitaxel. After transfected with TUBB3 cDNA, the mammalian cells with TUBB3 expression developed resistance to paclitaxel. In many types of cancer, for example ovary, breast, head and neck cancers, TUBB3 expression was correlated with response or survival of patients with paclitaxel treatment [20–23]. In our study, TUBB3 was evaluated on tissue samples collected before treatment. In 35 samples treated with nab-paclitaxel

**Table 5** Univariate analysis and multivariate analysis for presictors of over survival ($n=30$)

| Variables | Univariable analysis | | | Multivariable analysis | | |
|---|---|---|---|---|---|---|
| | *p* | HR | 95%CI | *p* | HR | 95%CI |
| Age (≥60 vs. <60 years) | 0.160 | 1.93 | 0.771–4.834 | | | |
| Sex (female vs. male) | 0.885 | 1.160 | 0.154–8.714 | | | |
| Stage (stage III vs. II) | 0.059 | 4.139 | 0.950–18.033 | 0.387 | 2.042 | 0.405–10.287 |
| Descent stage (yes vs. no) | 0.391 | 0.671 | 0.269–1.670 | | | |
| pCR (yes vs. no) | 0.043 | 0.123 | 0.016–0.932 | 0.016 | 0.070 | 0.008–0.604 |
| RT (yes vs. no) | 0.655 | 0.754 | 0.219–2.601 | | | |
| SPARC (positive vs. negative) | 0.981 | 0.089 | 0.401–2.437 | | | |
| TUBB3 (positive vs. negative) | 0.003 | 5.482 | 1.765–17.029 | 0.006 | 7.546 | 1.798–31.672 |

*PD* progressive disease, *HR* hazard ratio, *SPARC* secreted protein, acidic and rich in cysteine, *TUBB3* β-tubulin III, *CR* complete response, *PR* partial response, *SD* stable disease

plus cisplatin as neoadjuvant chemotherapy, the response rate for TUBB3 negative patients was higher than TUBB3 positive patients, 70.6% (12/17), and 61.1% (11/18), respectively, although the difference was not statistically significant. Besides, the median DFS and OS time was longer in TUBB3 negative patients after radical ESCC operation than that of positive patients. Furthermore, it was revealed in the multivariate analysis model that TUBB3 expression was one of the predominant predictors of DFS and OS time. Therefore, TUBB3 expression prior treatment may be a predictor for nab-paclitaxel efficacy as neoadujvant chemotherapy for stage IIand III ESCC patients.

We also found that patients with pathological CR had longer DFS and OS than patients without pCR. It was revealed in the multivariate analysis model that pCR was another predominant predictors of DFS and OS time. This result is similar to that of other studies. For example, the RTOG trial 8911 showed that pCR patients after preoperative therapy had an improved DFS when compared with non-pCR patients [25].

In conclusion, our findings demonstrate that TUBB3 negative expression prior treatment and pCR may indicate a better prognosis for stage II to III ESCC patients after nab-paclitaxel plus cisplatin neoadjuvant chemotherapy following radical esophagectomy. Considering the small sample size of the study, the findings need to be proved by further large sample studies.

**Acknowledgements** We wish to thank all the patients who volunteered for this study.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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