Caveolin-1 in multi drug resistance and two-field lymphadenectomy for thoracic esophageal squamous cell carcinoma

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Abstract: Caveolin-1 plays a very important role in the process of tumor cell transformation. In this paper, we studied the relationship between Cav-1 and other multi drug resistance associated proteins. Moreover, the author compares outcomes according to the extent of lymphadenectomy in patients with upper and middle thoracic esophageal squamous cell carcinoma without clinical cervical metastasis. The short-term and long-term data of 842 consecutive patients who underwent esophagectomy with two-field lymphadenectomy (2FL) or three-field lymphadenectomy (3FL) between February 2005 and July 2013 were retrospectively reviewed. If postoperative infection occurred, according to susceptibility test results, patients were given sensitive antibiotics. The yield of lymph nodes harvested was higher in the 3FL group than in 2FL (P =0.000). There was less blood loss (P =0.000), shorter operative time (P =0.000) less post-operative analgesia needed (P =0.000) and earlier hospital discharge (P =0.000) in 2FL than in 3FL.Overall morbidity was similar in the two groups. However, the rate of major complications was higher after 3FL versus 2FL (P =0.015). There was no 30-day mortality in 2FL and 3FL. The 5-year survival (2FL 35% vs 3FL 38%; P =0.297) and disease-free 5-year survival (2FL 26% vs 3FL 21%; P =0.106) were comparable between the two groups. In univariate analyses, extent of lymphadenectomy was not related to overall 5-year survival. Current results indicated that 2FL may be the preferred lymphadenectomy for upper and middle thoracic esophageal squamous cell carcinoma without clinical cervical metastasis.

Keywords: Esophageal carcinoma, multidrug resistance, two-field lymphadenectomy, lymphatic metastasis.

INTRODUCTION

Caveolin-1 (Cav-1) is one of the iconic caveolar protein, mainly due to the scaffolding domain (Caveolin-1 Scaffording, Domain, CSD), a transmembrane domain and Y14 sites interact with other proteins, to regulate a variety of signaling molecules and pathways activated, thus affecting cell differentiation, proliferation, migration and apoptosis(Wang *et al.* 2015). Many studies show that *Cav-1* plays a very important role in tumor cell transformation and tumor formation process, *Cav-1* has not only abnormal expression in a variety of tumors, but also has different function and mechanism (Liu *et al.* 2017). For example, Cav-1 expression is low in breast cancer, colon cancer, ovarian cancer, may be work as tumor suppressor factor (Ghoneum *et al.* 2015). At present, Cav-1 and its regulated pathways have become one of the hot spots in cancer research (Lee *et al*. 2015). Despite the continued introduction of new chemotherapeutic drugs and chemotherapy regimens in the clinic, many tumors are still difficult to avoid recurrence after undergoing initial chemotherapy. The main reason is that the tumor cells produce multidrug resistance (MDR) to a variety of chemotherapeutic drugs resistance (Zhu *et al.* 2015).

This study intends to detect the expression of 842 cases of esophageal squamous cell carcinoma, paracancerous tissues and para carcinoma similar to normal esophageal respectively. By using immunohistochemical method in tissue Cav-1, P-gp, MRP1, BDNF, beta -cat and P53, we analyze the relationship between the expression and correlation of Cav-1 and other multi drug resistance associated protein of the protein with esophageal squamous cancer. We use SPSS21.0 software and clarify the role of Cav-1 in the occurrence and development of esophageal squamous cell carcinoma, to lay the foundation for the preliminary study on the drug resistance of follow-up.

Esophageal carcinoma is one of the most commonly diagnosed malignancies worldwide and is a leading cause of cancer-related mortality (Chen *et al.* 2015). In recent years, the incidence of esophageal carcinoma has steeply increased due in part to the development of improved diagnostic techniques and the extension of life expectancy (Lee *et al*. 2015; Wang *et al.* 2015; Zhu *et al.* 2015). Despite the introduction of multimodality therapy, chemotherapy and radiotherapy for esophageal carcinoma, its recurrence and mortality remain high (Dai *et al.* 2015). Esophageal carcinoma can be divided based on pathology into squamous cell carcinoma and adenocarcinoma, with the former accounting for approximately 95% of all esophageal carcinomas in China (Dai *et al.* 2015). Because there is no esophageal carcinoma screening system in China, most esophageal carcinomas 2have already progressed to an advanced stage at diagnosis. Patients with advanced disease are not candidates for

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endoscopic mucosal resection; thus, esophagectomy with lymphadenectomy is the primary curative method used to treat patients with operable esophageal carcinoma. However, the optimal extent of lymph node dissection remains controversial (Ghoneum *et al.* 2015). Esophageal cancer is one of the serious diseases that endanger human life and health, also, the morbidity and mortality rates of human esophageal cancer are the highest in China. Esophageal cancer has become the first place in the incidence of malignant tumor in the digestive tract. In current medical environment, esophageal cancer often has early metastasis. In the surgery, Line lymph node dissection is a consensus, but the esophagus through the neck, chest, abdomen three parts, a wide range of lymph node metastasis, and sometimes unnecessary cleaning will cause additional pain, increasing the risk of complications, Therefore, the specific methods of surgery is still controversial.

Countries in Eastern Asia primarily use two main methods for establishing the extent of lymph node dissection for upper and middle thoracic esophageal squamous cell carcinomas: two-field lymphadenectomy (2FL) and three-field lymphadenectomy (3FL). The former technique involves dissection of the upper, middle, lower mediastinum, and abdominal lymph nodes potentially involved in esophageal carcinoma (Chen *et al.* 2009; Udagawa *et al.* 2012). The latter technique is defined as the dissection of the cervical, mediastinum, and abdominal lymph nodes. The extent of lymphadenectomy in esophageal cancer surgery has been controversial because of the absence of high-level evidence. In the present study, we evaluated the short- and long-term outcomes following 2FL and 3FL performed at a high-volume cancer center.

The role of neoadjuvant chemo-radiotherapy in esophageal cancer has been controversial (Abu 2017; Fang and Ruan 2017; Liu *et al.* 2017; Takahashi 2017). van Hagen has reported a multi-center, large sample, randomized controlled clinical trial concerning esophagectomy alone vs. chemo-radiotherapy followed by esophagectomy in 2012. This trial demonstrated that preoperative chemo-radiotherapy had a survival advantage over surgery alone. However, this trial was performed in the Netherlands, in which adenocarcinoma was the predominant histological type (approximately 75%). The role of neoadjuvant chemo-radiotherapy for squamous cell esophageal cancer is in doubt because of the small sample size in the squamous cell carcinoma subgroup (only 84 patients), although neo-adjuvant chemoradiotherapy has a survival advantage over surgery alone in the trial's squamous cell carcinoma subgroup. Whether neoadjuvant therapy has the survival advantage over surgery alone need to be confirmed by large sample, randomized controlled clinical trial (Abu, 2017; Fang and Ruan, 2017; Liu *et al.* 2017; Takahashi, 2017).

MATERIALS AND METHODS

We reviewed 842 consecutive patients with respectable upper and middle thoracic performed by the Department of Thoracic Surgery, Fujian Provincial Cancer Hospital from February 2005 to July 2013. All projects were approved by the ethics committee of the hospital, signed with informed consent. Ethical Approval number as 2004SDGHFF. The samples were matched with the adjacent tissues and adjacent to the normal esophagus tissues. All the specimens were fixed by 10% formalin, embedded in paraffin, and were continuously sectioned with a thickness of 4m. Histological observation was performed after HE staining. All the specimens were approved by Fujian Provincial Cancer Hospital. All patients underwent upper gastrointestinal endoscopy. Positron emission tomography- computed tomography (PET-CT) was not performed because of cost constraints. Preoperative chemotherapy or radiotherapy was not routinely administered because of the lack of high-level evidence confirming that this treatment provides a survival advantage (Qi and Fu, 2016).

At present, the treatment of esophageal cancer is still the most effective surgical resection, esophageal cancer surgery after a long period of development, from the early transesophageal hiatus esophagectomy, transthoracic resection, to the current two lymph nodes to clean esophageal cancer (Liu *et al.* 2017). In three wild lymph node dissection of esophageal cancer radical surgery, the long-term effect of surgical treatment is still less ideal. As the main reason for lymph node metastasis, esophageal cancer radical surgery lymph node dissection and surgical methods directly affect the prognosis of patients. The International esophageal disease meeting, in particular, discussed the surgical resection of esophageal cancer, according to intraoperative clearance range, will be divided into four categories, namely the standard lymph node dissection, lymph node dissection, total lymphadenectomy, three lymph node dissection The Which the standard sweep surgery should be removed under the mediastinum and upper abdomen of the lymph nodes and for the whole lymph node, and three wild lymph node dissection is on the basis of the whole sweep, the addition of double neck lymph node dissection, through the relevant literature reported that the vast majority of scholars believe that three wild lymph node dissection can greatly improve the patient Survival rate, but also increased the incidence of postoperative complications, to some extent affected the quality of life of patients, but some studies have shown that in some case-control study, three wild lymph node dissection survival rate is not greater than the expansion Two wild cleaning has a more significant increase. Therefore, in the actual work should choose what kind of surgical methods and lymph nodes in the international scope of the clean still inconclusive.

Surgery and 2FL or 3FL in esophageal carcinoma who had performed more than 300 2FL and 200 3FL procedures before 2005. In all cases, resection was performed with a curative intent. The extent of lymph node dissection (2FL or 3FL) was selected by patients and their families after the advantages and disadvantages of each approach to lymph node dissection were discussed. All patients provided written informed consent.

The lymph nodes dissected in this study were as follows. The cervical lymph nodes included the cervical paraesophageal lymph nodes (No. 101 station) and the supraclavicular lymph nodes (No. 104 station). The upper mediastinal nodes included the thoracic paratracheal lymph nodes (No. 106 station) and the upper paraesophageal lymph nodes (No. 105 station). The middle mediastinal nodes included the subcarinal lymph nodes (No. 107 station), middle paraesophageal lymph nodes (No. 108 station), and hilar lymph nodes (No. 109 station). The lower mediastinal nodes (No. 110 station), and with diaphragmatic lymph nodes (No. 111 station), and posterior mediastinal lymph nodes (No. 112 station). The abdominal lymph nodes included the right cardiac lymph nodes (No. 1 station), left cardiac lymph nodes (No. 2 station), lesser curvature lymph nodes (No. 3 station), left gastric artery lymph nodes (No. 7 station), common hepatic artery lymph nodes (No. 8 station), celiac artery lymph nodes (No. 9 station), and splenic hilum lymph nodes (No. 10 station).

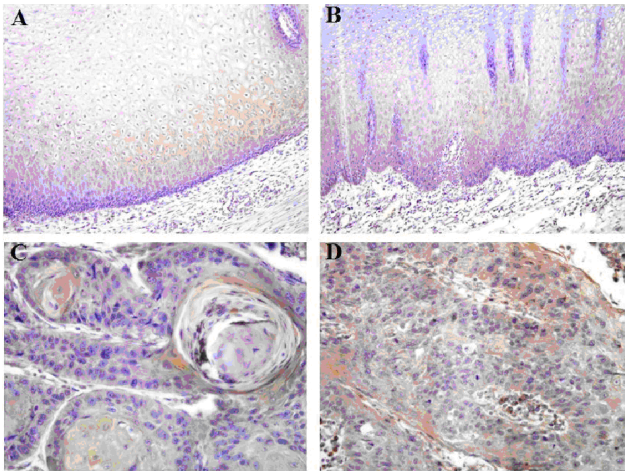

Fig. 1: Expression of Cav-1

For 2FL, an Ivor-Lewis thoractomy and an upper midline laparotomy were employed from February 2005 to January 2008. From February 2008 onward, laparoscopy-assisted 2FL consisting of an abdominal operation performed via laparoscopy and thoracic operation via Ivor-Lewis thoractomy were performed. In this procedure, the upper mediastinal and abdominal lymph nodes were dissected. For 3FL, a McKeown approach was utilized for supraclavicular lymph nodes (No. 104 station), mediastinal nodes and abdominal lymph nodes (Altorki *et al.* 2002). Operative time, postoperative complications, and hospital stay were reviewed for each patient. Postoperative complications, including both system as reported previously (Dindo *et al.* 2004). Cervical ultrasonography before discharge and before each follow-up visit, upper gastrointestinal endoscopy was performed every year. Any postoperative complication and medical condition requiring hospitalization were reviewed from the database. Disease recurrence and death were documented. Disease recurrence was defined as locoregional or distant metastasis verified by radiology or pathology. The last patient follow-up was conducted in August 2013.

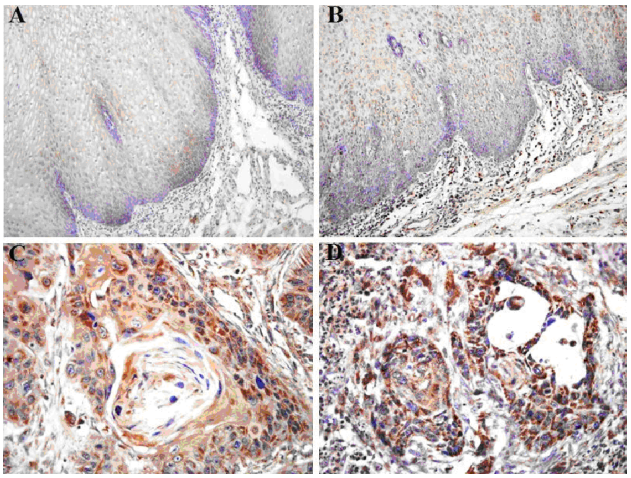

Fig. 2: Expression of P-gp

Three-field lymphadenectomy (3FL) is first carry out by Japanese scholars and strongly advocated and promoted in China has a larger range of applications.Through the promotion of three wild lymph node dissection, greatly improved the five-year survival rate of esophageal cancer patients, the recurrence rate was significantly reduced, but three field lymph node dissection surgery range, postoperative complications, due to surgical operation reasons, often more serious Complications, affecting the quality of life of patients, therefore, some scholars have suggested only for preoperative examination found cervical lymphadenopathy, high-end suspected lymph node metastasis occurred in patients with three wild lymph node dissection, but not in the preoperative examination revealed metastatic signs of patients may consider a smaller range of resection surgery. Support the three-wild cleaning point of view that esophageal cancer should be seen as systemic disease, once the tumor penetrates the submucosal layer that may occur metastasis, so even if the early esophageal cancer, should also be three wild lymph node dissection, reduce local recurrence. Multivariate studies have confirmed that the average metastatic rate of cervical lymph nodes as high as 69%, lymph node metastasis on the impact of great, positive lymph node metastasis, in addition, line three lymph node dissection in patients with 5-year survival rate than the traditional left thoracotomy Surgery compared to an

increase of 10%-20%. And the patient recovered faster, long-term follow-up survey found that three-day lymph node dissection after surgery, the average 5-year survival rate reached 51%, while the local recurrence rate was only 9.7%. So that should be widely promoted three lymph node dissection in order to extend the survival time of patients, but no matter what point of view, are considered the highest probability of metastatic esophageal cancer in the chest, because the tumor can be transferred to the neck and abdominal lymph nodes, The paragraph should be the most three lymph nodes. Is due to three wild surgery on the operation of the higher requirements, prone to complications in our country has gradually been simpler and targeted two-way lymph node dissection and selective lymph node dissection replaced. But in some hospitals, still believe that in the case of technical conditions permit, three wild lymph node dissection is more conducive to the patient's long-term prognosis. For statistical analysis, SPSS 13.0 software was used.

RESULTS

To study the role of Cav-1 in the occurrence and development of esophageal cancer, we used immunohisto-chemistry to detect the expression of esophageal squamous cell carcinoma, adjacent tissues and adjacent normal tissues. The results showed that Cav-1 in the three tissues, and mainly located in the cytoplasm and cell membrane, as shown in fig. 1 A is similar to the normal esophageal carcinoma; B carcinoma; C differentiated ESCC tissues; D low differentiated ESCC tissues.

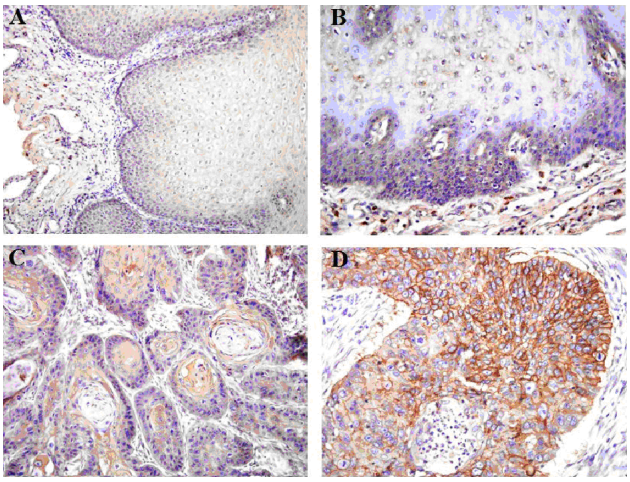

Fig. 3: Expression of MRP1

The expression of P-gp in esophageal squamous cell carcinoma, adjacent tissues and adjacent normal tissues was similar to that of Cav-1 and MRP1, mainly in the cell membrane and cytoplasm (fig. 2). The results showed that the positive rate of P-gp was significantly higher than that of esophageal squamous cell carcinoma, and the chi square test (P-gp <0.05) was significantly different (P<2). Immunohistochemistry showed that the expression of MRP1 in esophageal squamous cell carcinoma and adjacent tissues and like normal esophageal tissues, also expressed in cell membrane and cytoplasm (fig. 3). Similar to the normal esophageal tumor tissues, the positive rate of MRP1 was 17.44%. The results showed that the positive rate of MRP1 was significantly higher than that of esophageal squamous cell carcinoma, and the chi square test (MRP1 <0.05) was significantly different (P <2).

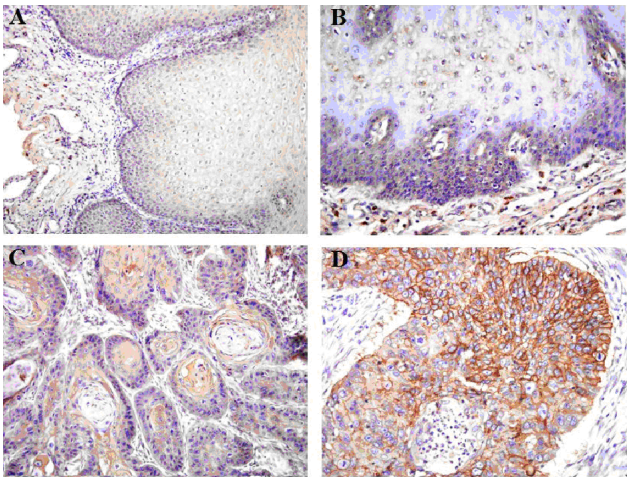

Fig. 4: Expression of P53

Immunohistochemistry showed that in addition to approximate to normal esophageal tissues, showed the expression of P53 in esophageal squamous cell carcinoma and adjacent tissues, mainly distributed in the nucleus (fig. 4). The positive rate of P53 with the development of esophageal squamous cell carcinoma was significantly increased, indicating that P53 expression up-regulated in esophageal squamous cell carcinoma is closely related to the development.

The expression of BDNF in the adjacent tissues of the adjacent tissues and the adjacent tissues of the normal esophagus was almost not expressed. The positive signal in the esophageal squamous cell carcinoma was mainly located in the cytoplasm (fig. 5). Statistical analysis showed that the positive rate of BDNF was slightly increased with the development of esophageal squamous cell carcinoma, but there was no significant difference (P >0.05).

Immunohistochemical staining showed that the expression of β-catenin was mainly distributed on the cell membrane, which was similar to that of normal esophagus, while the expression of cytoplasm and nucleus in esophageal squamous cell carcinoma and adjacent tissues (fig. 6). It is suggested that the localization ofβ-catenin from the cell membrane to the nucleus and cytoplasm may be closely related to the occurrence of esophageal cancer.

Table 1: Demographic data

Variables	2FL (n=482)	3FL (n=360)	P value
Age (y)	60.41±10.62	59.81±11.28	0.426
Gender (Male: Female)	363:119	259: 101	0.271
Comorbidity			0.786
Chronic obstructive pulmonary disease	12	7	
Hypertension	51	20	
Diabetes Mellitus	25	8	
Atrial fibrillation	22	12	
Earlier myocardial infarction	3	2	
Clinical T stage			1.000
T1	16	15	
T2	123	103	
T3	343	242	
Clinical N stage			1.000
N0	199	132	
N1	72	49	
N2	211	179	
Clinical M stage			-
M0	482	360	
ASA score			0.329
I	183	145	
II	273	203	
III	26	12	

Table 2: Surgical and pathological data

Variables	2FL (n=482)	3FL (n=360)	P value
Operative time (min)	241.71±36.56	281.93±32.33	0.000
Blood loss (ml)	390.00 (250-600)	485.00 (250-650)	0.000
Pathological T stage			0.136
T1	6	10	
T2	101	91	
T3	232	154	
T4a	143	105	
Pathological N stage			0.467
N0	152	103	
N1	65	59	
N2	173	137	
N3	92	61	
Pathological M stage			-
M0	482	360	
Differentiation grade			0.216
G1 (good)	95	79	
G2 (moderate)	226	147	
G3 (poor)	161	134	
Residual tumor (R0/R1/R2)	474/8/0	356/4/0	0.507
Number of harvested lymph nodes	25.00 (16-42)	29.00 (19-44)	0.000
Cervical lymph nodes dissected	-	5.00 (2-8)	-
Mediastinal lymph nodes dissected	12.00 (6-20)	12.00 (6-20)	0.519
Abdominal lymph nodes dissected	13.00 (7-22)	13.00 (7-22)	0.835
Post-operative analgesia (d)	3.00 (2-5)	3.00 (2-6)	0.000
Hospital stay (d)	18.00 (15-30)	19.00 (15-31)	0.000

Table 3: Univariate analyses of variables associated with 5-year overall survival

Variables	Number of patients	Five-year survival (%)	P value
Extent of lymphadenectomy			0.297
2FL	482	35%	
3FL	360	38%	
Age			0.269
≤60	380	39%	
>60	462	36%	
Gender			0.365
Male	622	36%	
Female	220	40%	
ASA score			0.987
I	328	40%	
II	476	3/%	
III	38	36%	
Pathological T stage			0.000
T1	31	62%	
T2	192	51%	
T3	386	32%	
T4	248	13%	
Pathological N stage			0.000
N0	255	59%	
N1	124	37%	
N2	310	19%	
N3	153	6%	
Lymph node ratio			0.000
≤0.05	263	54%	
>0.05	579	21%	
Differentiation grade			0.369
G1 (good)	174	39%	
G2 (moderate)	373	36%	
G3 (poor)	295	34%	

Lymph node ratio= number of positive nodes/total number of dissected nodes

Patient demographic data are summarized in table 1.During this study, 482 esophagectomies with 2FL and 360 esophagectomies with 3FL were performed successfully. In the 2FL group, 212 procedures performed via laparoscopically assisted 2FL and 270 cases utilized open Ivor-Lewis esophagectomy. There were no significant differences in age, gender, comorbidity, clinical stage, or American Society of Anesthesiologists score between surgical groups (P>0.05). 43% of patients who underwent 2FL had their procedure performed via laparoscopy-assisted Ivor-Lewis esophagectomy. Laparoscopy-assisted surgery has the natural advantages of a shorter incision, minor trauma, and faster recovery over open surgery (Liu et al. 2016). The equivalence of lymph node dissection by laparoscopy vs. open surgery has been confirmed randomized clinical trials. KLASS trial, the largest of these randomized clinical trials, showed that dissection during gastrectomy using laparoscopy was similar to the occurrence during open surgery. In the present study, there were no significant differences in abdominal lymph node dissection between laparoscopy-assisted surgery and laparotomy (data not shown). Dissection of the abdominal lymph node was assisted by Dr. Minggang Ying, a general surgeon with proven expertise in gastric cancer employed at our cancer center. Dr. Ying has performed more than 200 laparoscopy D2 lymphadenectomies and the first 50 laparoscopy-assisted Ivor-Lewis esophagectomies.

The role of neoadjuvant chemo-radiotherapy in esophageal cancer has been controversial (Abu 2017; Fang and Ruan 2017; Liu *et al.* 2017; Takahashi 2017). van Hagen has reported a multi-center, large sample, randomized controlled clinical trial concerning esophagectomy alone vs. chemo-radiotherapy followed by esophagectomy in 2012. This trial demonstrated that preoperative chemo-radiotherapy had a survival advantage over surgery alone. However, this trial was performed in the Netherlands, in which adenocarcinoma was the predominant histological type (approximately 75%). The role of neoadjuvant chemo-radiotherapy for squamous cell esophageal cancer is in doubt because of the small sample size in the squamous cell carcinoma subgroup (only 84 patients), although neo-adjuvant

chemoradiotherapy has a survival advantage over surgery alone in the trial's squamous cell carcinoma subgroup. Whether neoadjuvant therapy has the survival advantage over surgery alone need to be confirmed by large sample, randomized controlled clinical trial (Abu 2017; Fang and Ruan 2017; Liu *et al.* 2017; Takahashi 2017).

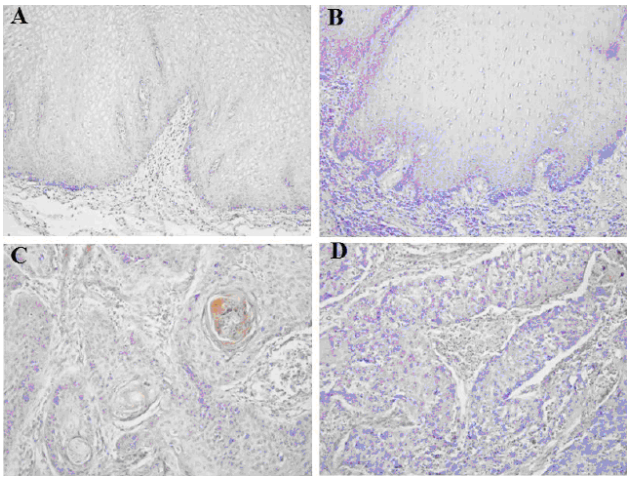

Fig. 5: Expression of BDNF

Patient surgical outcomes and pathological outcomes are summarized in tables 2 and 3, respectively. No cases of laparoscopically assisted esophagectomy required conversion to open laparotomy. No intraoperative or in-hospital deaths occurred in this study. Blood loss (P< 0.05) and operative time (P<0.05) were significantly lower in 2FL than in 3FL. There were no significant group differences in pathological stage and residual tumor (P>0.05). lymph nodes was higher in 3FL than in 2FL (P <0.05). Patients in the 2FL group enjoyed a significantly faster recovery, required less postoperative analgesia (P< 0.05).

Positive lymph nodes were found in 68.4% (330/482) of 2FL and 71.3% (257/360) of 3FL patients. Among the 842 cases, the most frequent region with metastasis was the upper mediastinal nodes (33.7%), followed by the middle mediastinal nodes (17.3%), lower mediastinal nodes (9.2%), abdominal nodes (8.4%), and cervical nodes (0.9%).

The overall postoperative morbidity was similar in both groups (2FL 29.7% vs. 3FL 32.2%; P = 0.146). However, when the severity of surgical complications was compared, more patients that underwent 3FL experienced major complications (2FL 4.3% vs. 3FL 8.3%; P =0.015). Vocal cord paralysis and respiratory insufficiency occurred more often in the 3FL group, and reoperation was required more frequently by patients who underwent 3FL (2FL 0.8% vs. 3FL 2.7%; P = 0.029).

The median follow-up duration for all patients was 60 months (range, 2-103 months), with a similar median follow-up duration for each surgical group. For the whole cohort, overall 5-year survival and disease-free 5-year survival rates were 37% and 22%, respectively. Disease recurrence was noted for 253 patients (52.5%) in the 2FL group and 191 patients (53.1%) in the 3FL group (P= 0.871). Type of recurrence was also comparable: 34.9% with locoregional recurrence and 17.6% with distant metastases in the 2FL group vs. 35.8% and 17.2%, respectively, in the 3FL group (table 5). Overall 5-year survival (2FL 35% vs. 3FL 38%; P=0.297) and 5-year disease-free survival (2FL 26% vs. 3FL 21%; P=0.106) were not different between the two groups.

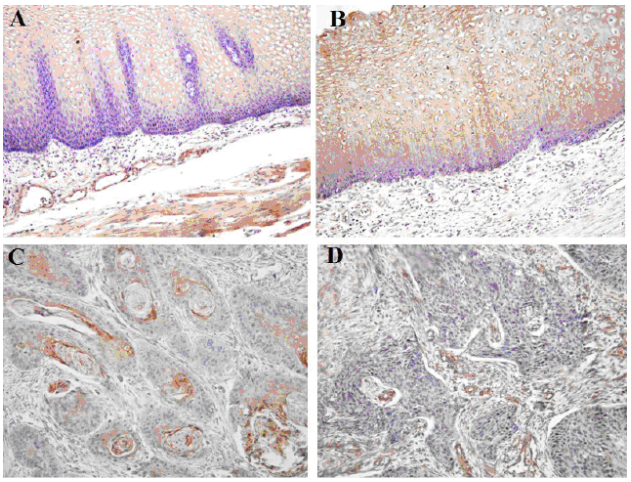

Fig. 6: β-catenin

Variables that were associated with patient overall survival in the univariate analyses are presented in table 3. Pathological T stage, pathological N stage, and lymph node ratio were all related to patient survival. However, the extent of lymphadenectomy was not related to survival. In the multivariate analysis, pathological T stage (entered as a categorical variable, with T1 as the reference category) and pathological N stage (entered as a categorical variable, with N0 as the reference category) were each independent prognostic variables predictive of 5-year overall survival [pathological T stage: T2, Hazard ratio (HR): 2.6, 95% confidence interval (CI): 0.9-4.9; T3, HR: 3.3, 95% CI: 1.6-5.5; T4, HR: 4.6, 95% CI: 2.0-6.3, P =0.009; pathological N stage: N1, HR: 2.1, 95% CI: 1.6-4.6; N2, HR: 3.3, 95% CI: 1.6- 4.8; N3, HR: 4.1, 95% CI: 2.3-6.2, P=0.010]. The lymph node ratio appeared marginal as an independent prognostic factor (HR: 3.6, 95% CI: 1.3-5.5, P = 0.072).

DISCUSSION

Cav-1 is one of the marker proteins of the cell membrane, which is expressed in the cells, such as endothelial cells, fat cells and epithelial cells (Lee *et al.* 2015). The expression of Cav-1 is closely related to the tumor, and it is found that Cav-1 is expressed in many kinds of tumors, but its expression level is different in different tumors. As

in the malignant tumor of kidney, prostate cancer, bladder cancer cells, Cav-1 expression; in ovarian cancer, breast cancer, colon cancer and other tumors, the expression of Cav-1 was decreased (Wang *et al.* 2015; Zhu *et al.* 2015). Moreover, follicular thyroid carcinoma has almost undetectable expression. Therefore, it is believed that Cav-1 plays a dual role in the process of tumorigenesis, which can not only act as a tumor suppressor gene, but also play a carcinogenic effect. Our results show that the expression of Cav-1 in esophageal squamous cell carcinoma was significantly higher than that in adjacent tissues and normal epithelial tissue, so Cav-1 in esophageal squamous cell carcinoma occurred in a cancer promoting effects, results of the results agree with previous results (Abu 2017). The primary drug resistance of esophageal squamous cell carcinoma (ESCC) is increased, P-gp and MRP1 belong to the ATP binding membrane (ABC) transporter family, which is thought to be the major protein mediating multidrug resistance (MDR). The two groups were mainly located on the cell membrane, which was similar to Cav-1, and P-gp had different expression patterns in different tumors. For example, in ovarian cancer and lung cancer, the expression decreased, but the expression was up-regulated in colon cancer and adrenal carcinoma. MRP1 is also expressed in many tumor tissues, such as gastric cancer, breast cancer and cervical cancer. In esophageal squamous cell carcinoma, we found that the expression of two genes was up-regulated, which was consistent with the results of previous studies (Liu *et al.* 2017; Takahashi 2017). These results indicate that the esophageal squamous cell carcinoma has a high primary resistance.

3FL is commonly performed on patients with operable upper and middle thoracic esophageal carcinoma in China, Japan, and Korea. Advocates of 3FL theorize that 3FL has the advantages of achieving more accurate staging and better long-term outcomes, although 3FL is also associated with increased morbidity and mortality (Altorki *et al.* 2002; Altorki *et al.* 2016; Qian *et al.* 2016). However, this conclusion continues to be debated because of the lack of multi-center, large sample, randomized, controlled clinical trials directly comparing 2FL and 3FL at a high evidence-based medicine level. To our knowledge, there are a few reports that directly compare 2FL and 3FL. Controversy exists regarding the best extent of lymphadenectomy with esophageal cancer. Some studies have failed to show a survival benefit with 3FL, whereas others have reported that 3FL produces the survival advantage over 2FL in these patients (Tsiaras *et al.* 2016). However, these studies were limited by their small study cohort, retrospective nature, and comparison with historical controls. Conversely, a randomized clinical trial performed by Nishihira et al. showed that 2FL and 3FL produced similar 5-year overall survival (2FL: 48.0%; 3FL: 66.2%; P = 0.192). This trial was limited in that the sample was very small, with only 30 patients in the 2FL group and 32 patients in the 3FL group. A retrospective study from Korea evaluating 57 and 34 patients that underwent 3FL and 2FL, respectively, also reported that 3FL provided no survival benefit with specialized cases (5-year overall survival: 2 FL, 52%, 3FL, 44%, P = 0.65; disease-free 5-year survival: 2FL, 39%, 3FL, 38%, P = 0.97). However, the sample in that study was small. As described for our retrospective case series, 5-year overall survival and disease-free survival were similar in both surgical groups, and the long-term outcomes were similar to those in published reports (Nishihira *et al.* 1998; Shim *et al.* 2010). To our knowledge, our study is the second largest series comparing 2FL and 3FL, evaluating 482 patients who underwent 2FL and 360 patients who underwent 3FL. Based on current published evidence, some surgeons have hypothesized that 3FL survival benefits may be due to stage migration and not therapeutic benefit.

Regarding patient short-term outcomes, a well-known disadvantage of 3FL is the degree of surgical invasiveness, which can increase postoperative morbidity. In our series, overall morbidity was comparable for both groups, but patients that underwent 3FL experienced more major complications. This difference was mainly due to a higher rate of postoperative respiratory insufficiency, which is in line with published findings(Shim *et al.* 2010). In our study, patients who underwent 2FL enjoyed less trauma such as blood loss and a shorter operative time required less postoperative analgesia and shorter hospitalization. This difference was mainly due to the decreased invasiveness of laparoscopy-assisted abdominal surgery, as previously reported.

The pattern and rate of lymph node metastases and disease recurrence was highly variable depending on the sample size, tumor histological type and TNM stage, and the extent of lymphadenectomy. Several studies have reported a low cervical lymph node recurrence rate. In our series, cervical lymph node metastases and recurrence rates were 2.2% and 4.5% respectively. The recurrence rate for mediastinal lymph nodes, abdominal lymph nodes, and distant organs limit the relevance of cervical lymph node dissection.

In our series, 43% of patients who underwent 2FL had their procedure performed via laparoscopy-assisted Ivor-Lewis esophagectomy. Laparoscopy-assisted surgery has the natural advantages of a shorter incision, minor trauma, and faster recovery over open surgery (Liu et al. 2016). The equivalence of lymph node dissection by laparoscopy vs. open surgery has been confirmed randomized clinical trials. KLASS trial, the largest of these randomized clinical trials, showed that dissection during gastrectomy using laparoscopy was similar to the occurrence during open surgery. In the present study, there were no significant differences in abdominal lymph node

dissection between laparoscopy-assisted surgery and laparotomy (data not shown). Dissection of the abdominal lymph node was assisted by Dr. Minggang Ying, a general surgeon with proven expertise in gastric cancer employed at our cancer center. Dr. Ying has performed more than 200 laparoscopy D2 lymphadenectomies and the first 50 laparoscopy-assisted Ivor-Lewis esophagectomies.

The role of neoadjuvant chemo-radiotherapy in esophageal cancer has been controversial (Abu 2017; Fang and Ruan 2017; Liu *et al.* 2017; Takahashi 2017). van Hagen has reported a multi-center, large sample, randomized controlled clinical trial concerning esophagectomy alone vs. chemo-radiotherapy followed by esophagectomy in 2012. This trial demonstrated that preoperative chemo-radiotherapy had a survival advantage over surgery alone. However, this trial was performed in the Netherlands, in which adenocarcinoma was the predominant histological type (approximately 75%). The role of neoadjuvant chemo-radiotherapy for squamous cell esophageal cancer is in doubt because of the small sample size in the squamous cell carcinoma subgroup (only 84 patients), although neo-adjuvant chemoradiotherapy has a survival advantage over surgery alone in the trial's squamous cell carcinoma subgroup. Whether neoadjuvant therapy has the survival advantage over surgery alone need to be confirmed by large sample, randomized controlled clinical trial (Abu 2017; Fang and Ruan 2017; Liu *et al.* 2017; Takahashi 2017).

CONCLUSION

This study had several limitations. It evaluated single-center and not multiple-center patient data and was based on a retrospective non-randomized analysis with no prospective randomized analysis. Thus, we cannot exclude bias in surgeon selection of patients and surgical approaches. This limitation should be taken into account when interpreting the study's results. Prospective randomized trials comparing 2FL and 3FL are not easy to execute because 3FL is technologically difficult to grasp and there is currently no consensus about 2FL.

In conclusion, this study demonstrates that 2FL should be accepted as a valuable procedure for lymphadenectomy to treat middle and lower esophageal squamous cell carcinoma without clinical cervical metastasis given that it provides a similar overall survival and incidence of recurrence compared to 3FL. Further prospective randomized multi-center trials are warranted before incorporating 2FL into specialized patients.

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