Clinicopathologic Features and Histochemical Analyses of Proliferative Activity and Angiogenesis in Small Cell Carcinoma of the Esophagus

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Abstract

Background. We investigated clinicopathologic features of esophageal small cell carcinoma (SCEC) patients, and its proliferative activity and angiogenesis. **Methods.** Ten patients with SCEC from 335 esophageal carcinoma patients were clinicopathologically analyzed. For analyses of cell proliferation, apoptosis and angiogenesis of SCEC, Ki-67 immunostaining, TUNEL method, and CD 31 and CD68 immunostining were used. **Results.** Esophagectomy was performed in 9 while one with extensive SCEC was treated by repeated chemotherapy and radiotherapy. Chemotherapy was performed in 4 before and after surgery, one before surgery, and 4 after surgery. Cisplatin and etoposide (PVP) was given to 5, while irinotecan and cisplatin (CPT/P) was given to 3. Five survived over 18 month and 2 of them survived over 36 months. The microvessel count as well as the Ki-67 labeling index of SCEC was higher than those of SCC ($p=0.0033$ and $p=0.0005$, respectively). Between SCEC with and without preoperative chemotherapy, the Ki-67 labeling index was lower ($p=0.027$) and the apoptotic index was higher in the treated SCEC ($p=0.014$). Between SCEC patients who survived over or less than 18 months, the microvessel count was lower in the patients who survived over 18 months ($p=0.049$). **Conclusion.** Esophagectomy may be indicated for limited SCEC combined with chemotherapy. SCEC have high proliferative activity and rich neovascularization influences and its proliferative activity may be suppressed by chemotherapy.

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Introduction

Small cell carcinoma is a rare entitity in the esophagus; it has been reported to account for approximately 1 % of all carcinomas of the esophagus and the gastric cardia,[1,2] but the tumor is frequently observed in the esophagus rather than elsewhere in the gastrointestinal tract.[3] Small cell carcinoma of the esophagus (SCEC) has been associated with a poor prognosis, being similar to small cell-lung carcinoma (SCLC).[2,4] To improve the clinical outcome in SCEC patients, multidisciplinary treatments, including chemotherapy, may be necessary.[2,4,5] However, various diagnostic and therapeutic issues remain in SCEC patients. Regarding chemotherapy for SCLC patients, it has been reported that a combination chemotherapy consisting of irinotecan and cisplatin (CPT/P) has a better survival benefit than that consisting of cisplatin and etoposide

(PVP).[6] For SCEC patients, combination chemotherapy using PVP has been available similar to chemotherapy for SCLC patients.[7] However, both chemotherapies have not been sufficiently compared in terms of therapeutic effects for SCEC and clinical outcome of SCEC patients.

It is well known that proliferative activity of tumor cells and/or angiogenesis in tumor stroma play important rules in tumor progression and metastasis.[8,9] Although biological characteristics regarding proliferative activity of tumor cells and angiogenesis have been well investigated in squamous cell carcinoma (SCC) of the esophagus[10,11] and SCLC,[12,13] we found only a few reports investigating proliferative activity of SCEC,[14,15] and found no report investigating angiogenesis in SCEC. Ki-67 immunohistochemistry was used for evaluation of proliferative activity of tumor cells, and the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate biotin nick end labeling (TUNEL) method[16] was used for evaluation of tumor cell apoptosis.[10,11] Furthermore, microvessel density was used for evaluation of tumor angiogenesis,[17] and infiltrating macrophages were used for evaluation of angiogenesis caused by interaction between tumor and stromal cells.[18]

We retrospectively investigated clinicopathologic features in 10 patients with SCEC who were treated by surgery, chemotherapy and/or radiotherapy, and discussed therapeutic issues. Furthermore, we histochemically analyzed proliferative activity and angiogenesis in SCEC.

Methods

Patients

Three hundred-thirty five patients with primary carcinoma of the thoracic esophagus were treated between 1991 and 2005 in Shinshu University Hospital. Of these 335 patients, 10 patients (3.1 %) with SCEC were treated by surgery, chemotherapy and/or radiotherapy. Three of them have already been reported as case reports sporadically.[7,19] The cases of the 10 patients were retrospectively reviewed, and the following parameters were investigated: the clinicopathologic features, the preoperative diagnosis, the treatment including surgery, and chemotherapy and/or radiotherapy, and the clinical outcome after initial treatment for SCEC. Regarding chemotherapy for the SCEC patients, a combination of CPT/P was prospectively given to three recent cases before and/or after surgery because Noda et al.[6] reported that CPT/P contributed to a better prognosis than PVP in SCLC patients.

SCEC was diagnosed histopathologically using formalin-fixed and paraffin-embedded sections of biopsy specimens endoscopically taken from the tumors and/or the resected specimens after surgery, with not only hematoxylin-eosin staining, but also several other immunohistochemical staining for chromogranin A, neuron-specific enolase, synaptophysin, and/or the other neuroendocrine markers. Clinicopathologic findings of esophageal carcinomas

were described according to the TNM classification (6th edition).[20]

Histochemical analyses

SCEC tissue samples were obtained from 9 of the 10 patients who underwent esophagectomy for histochemical analyses of proliferative activity and angiogenesis, as well as the routine histopathologic examination. These tissues were fixed in 10 % formalin buffered with phosphate at pH 7.4 and embedded in paraffin. Regarding proliferative activity of SCEC, Ki-67 immunostaining was performed using an anti-Ki-67 monoclonal antibody (MIB1, diluted 1:100; Immunotech. S.A., Marseile, France) on the sections treated in a microwave oven before the staining treatment. The percentage of tumor cells with nuclei stained for Ki-67, i.e., the Ki-67 labeling index, was caluculated for each section on the basis of about 2,000 tumor cell nuclei.[10] TUNEL, used to assess apoptosis, was used according to the method of Gavrieli et al.[16] and our previous report.[10] The TUNEL reaction was visualized with 3,3`-diaminobenzidine tetrachloride (DAB), and a dark accumulation of DAB in the cancer cells (nuclei and apoptotic bodies) was judged to indicate a positive reaction to TUNEL. The rate of TUNEL-positive cells, i.e., the apoptotic index was calculated for each section on the basis of about 2,000 tumor cells.[10]

Regarding evaluation of tumor angiogenesis, immunohistochemical staining for CD31 and CD68 antigens was performed. The avidin-biotin complex method was used, and the monoclonal antibodies used in these immunostaining were: an anti-human CD31 antibody (JC70A, diluted 1:100; Genezyme/Techne, MN) and anti-human CD68 antibody (KP1, diluted 1:100; Dako A/S), respectively. Microvessels in tumor stroma were counted based on the CD31-stained sections, and the microvessel count was determined according to the method of Maeda et al.[17] Macrophages, identified by CD68 immunohistochemistry, were counted as stromal monocytes (monocytic count).[11]

For a comparison with the histochemical results mentioned above, we examined 44 cases of SCC of the esophagus who underwent curative esophagectomy with neither preoperative chemotherapy nor radiotherapy between 1995 and 2002 in Shinshu University Hospital.

Statistical analyses

The clinicopathologic features and histochemical results were analyzed by the Chi-squre test or Fisher's exact probability test, and the Mann-Whitney *U* test. Statistical significance was defined as $p < 0.05$.

Results

Clinicopathologic features

The clinicopathologic features of the 10 patients with SCEC are shown in **Tables 1** and **2**. Curative esophagectomy with two or three field-lymph node dissection was performed in nine

of the 10 patients, while only one patient (Case 10) did not undergo surgery and received chemoradiotherapy because SCEC directly invaded the aorta. Chemotherapy for SCEC was performed in all patients; four were treated both before and after surgery, one was treated only before surgery, and four were treated only after surgery. Alternative chemotherapy consisting of PVP, and cyclophosphamide, adriamycin (or epirubicin) and vincristine (CAV) was given to three and four patients before and after surgery, respectively. Chemotherapy using CPT/P was performed in the most recent three patients (Cases 8-10). External beam-radiotherapy for primary or metastatic tumors was performed in five patients. One patient who had SCEC with direct invasion to the descending aorta (T4) received PVP/CAV-therapy as well as radiotherapy. Three patients were given adjuvant radiotherapy after surgery. In Case 10 without esophagectomy, radiotherapy was performed for the primary tumor and metastatic nodes of the abdomen after CPT/P-therapy.

Clinical outcome

None of the patients had distant metastasis at the detection of this esophageal tumor; however, in nine of them, distant metastases to several organs were detected after the initial treatment including chemo- and/or radiotherapy and surgery. Only two patients are survived over 36 months; one had limited SCEC treated by surgery and postoperative chemotherapy using PVP/CAV, and the other had extensive SCEC treated repeatedly by chemotherapy using CPT/P and radiotherapy. Survival after the initial treatment ranged from 7 to 80 months, and the median survival was 13.0 months and the mean survival was 22.7 months in these patients. Five patients, including Case 10 without surgery, survived over 18 months after the initial treatment. In three of the five patients, no nodal metastasis was observed; one without preoperative chemotherapy (Case 7) had no nodal metastasis, while CR in the nodes was histologically found after surgery in the other two (Cases 3 and 4). For all the cases, the one-year and 5-year survival rates were 60.0 % and 20.0 %, respectively.

Histochemical analyses

Findings of Ki-67 immunostaining, TUNEL, CD31 and CD68 immunostining are shown in **Fig. 1**. The Ki-67 labeling index in SCEC was higher than that of SCC (56.6 ± 5.9 % vs. 48.4 ± 6.3 %; p = 0.0033; **Fig. 2-A**). As for the apoptotic index, there was no statistical difference between SCEC and SCC (1.5 ± 0.50 % vs. 1.6 ± 0.65 %; **Fig. 2-B**). The microvessel count of SCEC was higher than that of SCC (50.7 ± 18.5 vs. 27.2 ± 11.6; p = 0.0005; **Fig. 2-C**). As for the monocytic count, there was no statistical difference between SCEC and SCC (137.0 ± 79.4 vs. 188.8 ± 105.6; **Fig. 2-D**). Furthermore, these histochemical results were compared between SCEC without and with preoperative chemotherapy. The Ki-67 labeling index of SCEC with

chemotherapy was lower than that without it (52.6 ± 4.4 % vs. 61.6 ± 2.7 %; p = 0.027; **Fig. 2-E**), and the apoptotic index of SCEC with chemotherapy was higher than that without it (1.9 ± 0.32 % vs. 1.1 ± 0.21 %; p = 0.014; **Fig. 2-F**). As for the microvessel count, there was no statistical difference, although it tended to be higher in SCEC without chemotherapy than that with it (64.9 ± 20.2 vs. 39.3 ± 4.4; **Fig. 3-G**). As for the monocytic count, there was no statistical difference between SCEC without and with chemotherapy (164.2 ± 81.4 vs. 115.6 ± 71.9; **Fig. 2-H**).

Between SCEC patients who survived longer than and less than 18 months after initial treatment, the histochemical results were compared. The microvessel count in the patients who survived over 18 months was lower than that in those who survived less than 18 months (38.5 ± 5.2 vs. 60.4 ± 20.0; p = 0.049; **Fig. 2-K**), while for the Ki-67 labeling index, the apoptotic index and monocytic count, there was no statistical difference between them (53.5 ± 6.7 % vs. 59.1 ± 4.4 %; **Fig. 2-I**, 1.7 ± 0.58 % vs. 1.4 ± 0.45 %; **Fig. 2-J**, 146.8 ± 114.1 vs. 129.5 ± 51.8; **Fig. 2-L**, respectively).

Discussion

It is controversial whether surgery is necessary although chemotherapy plays a key role for treatment of SCEC patients. Casas et al.[5] reported that surgery alone was the single most powerful predictor of death in SCEC patients. There is some evidence that surgery for limited SCEC could provide long term survival; some patients with SCEC who were treated by surgical resection combined with chemotherapy were reported to have over 5-year survival.[21, 22] In our series, only one patient is alive without recurrence over 5 years after surgery. The present four SCEC patients with surgery, who survived over 18 months showed no pathologic nodal metastases in the resected specimens. Case 7 without recurrence had no nodal metastasis, which was diagnosed clinically and pathologically, and there was no nodal metastasis pathologically in the other three patients who received preoperative chemotherapy, although nodal metastases were present in the mediastinum before chemotherapy. However, in the other esophagectomized patients who died of metastatic SCEC within 18 months of the initial treatment, nodal metastases were pathologically demonstrated despite preoperative chemotherapy. Therefore, surgery may contribute to long-term survival in SCEC patients without nodal metastases like limited SCEC; however, SCEC patients with nodal metastasis have a better chance of long-term survival when nodal metastasis is controlled or cured by chemotherapy and/or radiotherapy before surgery, such as the present three patients.

Regarding chemotherapy for SCEC, several authors reported that it is needed for improvement of survival in SCEC patients.[3, 5] Recently, combination chemotherapy with CPT/P

for SCEC has been sporadically reported,[23] while previously the combination with PVP has been frequently used.[7] Because no standard regimen has been established for SCEC, several regimens have been used based on SCLC regimens, which has been well researched in many SCLC patients. Although the PVP-regimen was frequently used in the present patients treated before 2002 (Cases 1-7), we prospectively changed the chemotherapeutic regimen from PVP-therapy to CPT/P-therapy based on a report that CPT/P contributed to a better prognosis than PVP in SCLC patients.[6] The fact that Case 10 with extensive SCEC, who was repeatedly treated with chemotherapy using CPT/P, showed a long-term survival is very interesting. A large study to assess whether this regimen could play a key role in terms of survival benefit in SCEC patients is necessary.

Radiation frequently induces a clinical response of SCEC regression.[24, 25] Radiotherapy has been adapted for extensive SCEC, while radiotherapy has contributed to an improvement in prognosis in limited SCEC.[25] In our series, T4-SCEC, such as Case 1, was down-staged in T2 by extra-beam radiotherapy, and SCEC was surgically removed. In extensive SCEC such as Case 10, with locally advanced SCEC and widespread node metastases, radiotherapy for local foci and nodal metastasis may control tumor progression when surgery is not indicated. Although radiotherapy may locally contribute to induce regression of SCEC, systemic chemotherapy should be combined in order to improve the survival.

The prognosis for SCEC patients is usually poor. In previous reports, the median survival of patients with SCEC ranged from three to 7.5 months.[1, 2, 4] In recent reports, survival has been slightly prolonged, but most of the patients with SCEC, especially extensive SCEC, still have a poor prognosis.[26] In the present study, although the median survival was 13 months, five patients survived over 18 months after the initial treatment and two patients survived over 36 months. There are few published survival analyses[22, 25] and the 5-year survival rate was about 20 %. Similar data was found in our series. To improve the poor prognosis in the SCEC patients mentioned above, we should precisely diagnose SCEC by esophagogastroscopy, and use multimodality treatment including surgery, chemotherapy and/or radiotherapy.

With regard to the proliferative activity of tumor cells, high proliferative activity of SCLC has been demonstrated, as immunohistochemically evaluated by the Ki-67 labeling index.[12] The Ki-67 labeling index of SCEC was observed in a previous report by Lam et al.,[15] and their data was similar to the present results in that the mean value of the Ki-67 labeling index was 56 % in SCEC. In the present study, the Ki-67 labeling index of SCEC was higher than that of esophageal SCC; the aggressive nature of SCEC is evidenced by these data. Furthermore, chemotherapy may suppress SCEC progression, because the Ki-67 labeling index of SCEC

treated with chemotherapy was lower than that without it. On the other hand, apoptosis plays an essential role in tumor progression, including esophageal carcinomas.[10] Although a few reports regarding p53 overexpression[15] and Bcl-2 positivity[27] in SCEC were observed, there are no reports regarding the SCEC apoptotic index. In the present study, the SCEC apoptotic index was lower than that of esophageal SCC. Furthermore, the SCEC apoptotic index treated with chemotherapy was higher than that without it. Therefore, considering these data on the Ki-67 labeling index and apoptotic index, chemotherapy plays an important role in SCEC treatment, although its proliferative activity is very high.

Angiogenesis also plays an important rule in tumor progression and metastasis.[8] In the present study, angiogenesis in SCEC was assessed by the microvessel count and monocytic count. In esophageal SCC, we previously reported that not only angiogenesis assessed by the microvessel count, but also angiogenic promotion assessed by the monocytic count, and the status of macrophage infiltration into tumor stroma are necessary for tumor progression and metastasis.[10, 11] Angiogenesis in SCLC has been well analyzed,[13] but no report has described neovascularization and macrophage infiltration in the stroma of SCEC. Angiogenesis, as evaluated by microvessel count in SCEC, may be more vigorous than that of esophageal SCC although macrophage infiltration, which may induce angiogenic promotion by releasing several cytokines and angiogenic growth factors, is not higher than that of esophageal SCC. This high neovascularization may influence aggressive behavior, including distant metastases in SCEC. SCEC with chemotherapy or with long-term survival had a lower microvessel count. In this situation, it is considered that chemotherapy is an important treatment for SCEC.

In conclusion, surgery may be indicated for limited SCEC combined with chemotherapy, and may be additionally indicated for SCEC when nodal metastasis is controlled or cured by chemotherapy. SCEC had high proliferative activity and rich neovascularization, and the biologic features may influence its clinicopathologic aggressiveness. The proliferative activity is suppressed by chemotherapy, so chemotherapy may improve their outcomes.

References

1. Nichols GL, Kelsen DP. Small Cell carcinoma of the esophagus. The Memorial Hospital experience 1970 to 1987. *Cancer* 1989; **64**: 1531-3.
2. Law SY, Fok M, Lam KY, Loke SL, Ma LT, Wong J. Small cell carcinoma of the esophagus. *Cancer* 1994; **73**: 2894-9.
3. Brenner B, Tang LH, Klimstra DS, Kelsen DP. Small-cell carcinoma of the gastrointestinal tract: a review. *J Clin Oncol* 2004; **22**: 2730-9.

4. Huncharek M, Muscat J. Small cell carcinoma of the esophagus. The Massachusetts General Hospital experience, 1978 to 1993. *Chest* 1995; **107**: 179-81.
5. Casas F, Ferrer F, Farrus B, Casals J, Biete A. Primary small cell carcinoma of the esophagus: a review of the literature with emphasis on therapy and prognosis. *Cancer* 1997; **80**: 1366-72.
6. Noda K, Nishikawa Y, Kawahara M, et al; Japan Clinical Oncology Group. Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. *N Engl J Med* 2002; **346**: 85-91.
7. Koide N, Hiraguri M, Kishimoto K, et al. Small cell carcinoma of the esophagus with reference to alternating multiagent chemotherapy: report of two cases. *Surg Today* 2003; **33**:294-8.
8. Folkman J. What is the evidence that tumors are angiogenesis dependent? *J Natl Cancer Inst* 1990; **82**: 4-6.
9. Carson DA, Ribeiro JM. Apoptosis and disease. *Lancet* 1993; **341**: 1251-4.
10. Koide N, Nishio A, Hiraguri M, Hanazaki K, Adachi W, Amano J. Coexpression of vascular endothelial growth factor and p53 protein in squamous cell carcinoma of the esophagus. *Am J Gastroenterol* 2001; **96**: 1733-40.
11. Koide N, Nishio A, Sato T, Sugiyama A, Miyagawa S. Significance of macrophage chemoattractant protein-1 expression and macrophage infiltration in squamous cell carcinoma of the esophagus. *Am J Gastroenterol* 2004; **99**: 1667-74.
12. Bohm J, Koch S, Gais P, Jutting U, Prauer HW, Hofler H. Prognostic value of MIB-1 in neuroendocrine tumours of the lung. *J Pathol* 1996; **178**:402-9.
13. Lucchi M, Mussi A, Fontanini G, Faviana P, Ribechini A, Angeletti CA. Small cell lung carcinoma (SCLC): the angiogenic phenomenon. *Eur J Cardiothorac Surg* 2002; **21**: 1105-10.
14. Okudera K, Ito K, Kameda Y, Nakamura N, Kitamura H. Immunohistochemical analysis for cell proliferation-related protein expression in small cell carcinoma of the esophagus; a comparative study with small cell carcinoma of the lung and squamous cell carcinoma of the esophagus. *Histol Histopathol* 1999; **14**: 479-85.
15. Lam KY, Law S, Tung PH, Wong J. Esophageal small cell carcinomas: clinicopathologic parameters, p53 overexpression, proliferation marker, and their impact on pathogenesis. *Arch Pathol Lab Med* 2000; **124**: 228-33.
16. Gavrieli Y, Sherman Y, Ben-Sasson SA. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. *J Cell Biol* 1992; **119**: 493-501.

17. Maeda K, Chung YS, Takatsuka S, et al. Tumor angiogenesis as apredictor of recurrence in gastric carcinoma. *J Clin Oncol* 1995; **13**: 477-81.
18. Leek RD, Lewis CE, Whitehouse R, Greenall M, Clarke J, Harris AL. Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma. *Cancer Res* 1996; **56**: 4625-9.
19. Nimura Y, Koide N, Nishio A, et al. Effective treatment with chemotherapy and surgical resection for small cell carcinoma of the esophagus: report of a case. *Hepatogastroenterology* 1999; **46**: 1778-81.
20. Sobin LH, Wittekind CH, eds. International union against cancer. TNM classification of malignant tumors, 6th ed. New York: Wiley-Liss, 2002.
21. Yachida S, Matsushita K, Usuki H, Wanibuchi H, Maeba T, Maeta H. Long-term survival after resection for small cell carcinoma of the esophagus. *Ann Thorac Surg* 2001; **72**: 596-7.
22. Nishimaki T, Suzuki T, Nakagawa S, Watanabe K, Aizawa K, Hatakeyama K. Tumor spread and outcome of treatment in primary esophageal small cell carcinoma. *J Surg Oncol* 1997; **64**: 130-4.
23. Endo K, Kohnoe S, Toh Y, et al. A case of esophageal small cell carcinoma with multiple liver metastases responding to chemotherapy with irinotecan plus cisplatin. *J Exp Clin Cancer Res* 2005; **24**: 647-50.
24. Jereczek-Fossa B, Airoldi M, Vasario E, Redda MG, Valente G, Orecchia R. Small cell carcinoma of the esophagus: a case report and review of the literature. *Tumori* 2000; **86**: 174-7.
25. Nemoto K, Zhao HJ, Goto T, et al. Radiation therapy for limited-staged small-cell esophageal cancer. *Am J Clin Oncol* 2002; **25**: 404-7.
26. Wu Z, Ma JY, Yang JJ, Zhao YF, Zhang SF. Primary small cell carcinoma of esophagus: report of 9 cases and review of literature. *World J Gastroenterol* 2004; **10**: 3680-2.
27. Takubo K, Nakamura K, Sawabe M, Arai T, Esaki Y, Miyashita M, Mafune K, Tanaka Y, Sasajima K. Primary undifferentiated small cell carcinoma of the esophagus. *Hum Pathol* 1999; **30**: 216-21.

Figure Legends (Small cell carcinoma of the esophagus/ Koide N et al.)

Fig. 1: Histochemical findings of SCEC.

A. **Ki-67 immunostaining.** A Ki-67-positive reaction is observed in the nuclei of the tumor cells.

B. **TUNEL.** A TUNEL-positive reaction is observed in the condensed nuclei of the tumor cells (arrows).

C. **CD31 immunostaining.** A positive reaction for the CD 31 antigen is observed in the microvessels in the tumor stroma.

D. **CD68 immunostaining**. Stromal cells infiltrated in SCEC mainly show a positive reaction for CD68.

Fig. 2: Histochemical analyses of the Ki-67 labeling index, apoptotic index, microvessel count, and monocytic count.

SCEC, small cell carcinoma of the esophagus; SCC, squamous cell carcinoma; Cx, preoperative chemotherapy; > 18, survival over 18 months after initial treatment for SCEC; < 18, dead within 18 months after initial treatment for SCEC.

A-D, The histochemical results were compared between SCEC and SCC. **E-H**, The histochemical results were compared between SCEC with and without preoperative chemotherapy. **I-L**, The histochemical results were compared between SCEC patients who survived over and less than 18 months after initial treatment.

A, E and I: Ki-67 labeling index. **B, F and J**: apoptotic index. **C, G and K**: microvessel count. **D, H and L**: monocytic count.