Original Article
Small cell neuroendocrine carcinoma of the esophagus: report of 6 cases with immunohistochemical and molecular genetic analysis of KIT and PDGFRA

Tadashi Terada
Department of Pathology, Shizuoka City Shimizu Hospital, Shizuoka, Japan
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Abstract: Small cell neuroendocrine carcinoma of the esophagus (SCNECE) is a very rare, but a highly aggressive tumor. Six cases of SCNECE (0.25%) were found in the 2,438 archival pathologic specimens of esophagus in the last 20 years in our pathology laboratory. The ages ranged from 62 years to 81 years with a mean of 73 years. All cases were male. The presenting symptoms were dysphagia in 5 cases and vomiting in 1 case. The locations were lower esophagus in 4 cases and middle esophagus in 2 cases. Endoscopically, the tumor was ulcerated in 3 cases and polypoid in 3 cases. All the 6 patients were treated by chemoradiation therapy, and the survival ranged from 6 months to 25 months with a mean of 13 months. Histologically, 5 cases were pure SCNECE, 1 case showed triplicate differentiation into small cell carcinoma, adenocarcinoma and squamous cell carcinoma. Immunohistochemically, each SCNECE showed at least one of the neurocrine antigens. Cytokeratins were positive in 6/6, vimentin 0/6, synaptophysin in 4/6, CD56 4/6, neuron-specific enolase 3/6, chromogranin 0/6, p53 protein in 6/6, KIT in 6/6, and platelet-derived growth factor receptor-α (PDGFRA) in 6/6. Ki-67 labeling ranged from 56% to 100% with a mean of 79%. A retrospective genetic analysis using PCR-direct sequencing method in paraffin sections identified no mutations of KIT (exons 9, 11, 13 and 17) and PDGFRA (exons 12 and 18) genes in all the 6 cases.

Keywords: Esophagus, small cell neuroendocrine carcinoma, KIT, PDGFRA

Introduction
Small cell neuroendocrine carcinoma of the esophagus (SCNECE) is a very rare entity. Several comprehensive studies of SENECE have been reported in the English literature [1-9]. However, there have been no reports of SCNECE investigating protein expression and gene mutations of KIT and platelet-derived growth factor receptor-α (PDGFRA). The author reports herein 6 cases primary SCNECE of with an examination of protein expressions of KIT and PDGFRA and gene status of KIT and PDGFRA genes. KIT and PDGFRA genes, both mapped to 4q12, encode receptor tyrosine kinase oncoproteins called KIT (CD117) and PDGFRA, respectively [10-15]. Both molecules are transmembranous oncoproteins, and play important roles in the carcinogenesis of several tumors such as gastrointestinal stromal tumor (GIST) [10-22].

Materials and methods
The author retrospectively reviewed 2,438 esophageal pathologic specimens in the last 20 years in our pathology laboratory. As the results, six cases of SCNEC (0.25%) were found. Of the six cases, two cases had been reported [8, 9] as case reports. Many 3-μm sections were cut from each paraffin block of these 6 cases, and one of them was stained with HE. The others were subjected to immunohistochemical staining and molecular genetic analysis. The immunohistochemical analysis was performed by Dako Envision methods (Dako Corp, Glostrup, Denmark), as previously reported [23-29]. The antibodies employed were cytokeratins (AE1/3 and polyclonal, Dako, Glostrup, Denmark), synaptophysin (polyclonal, Dako), neuron-specific enolase (polyclonal, Dako), chromogranin (DAK-A3,
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Table 1. Primer sequence

<table>
<thead>
<tr>
<th>Forward Sequence</th>
<th>Reverse Sequence</th>
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<tr>
<td><strong>KIT exon 9</strong></td>
<td><strong>KIT exon 9</strong></td>
</tr>
<tr>
<td>5'-TCC TAG AGT AAG CCA GGG CTT-3'</td>
<td>5'-TGG TAG ACA GAG CCT AAA CAT CC-3'</td>
</tr>
<tr>
<td>5'-GAT CTA TTT TTC CCT TC-3'</td>
<td>5'-AGC CCC TGT TTC ATA CTG AC-3'</td>
</tr>
<tr>
<td><strong>KIT exon 11</strong></td>
<td><strong>KIT exon 11</strong></td>
</tr>
<tr>
<td>5'-GCT TGA CAT CAG TTT GCC AG-3'</td>
<td>5'-AAA GGC AGC TTG GAC ACG GCT TTA-3'</td>
</tr>
<tr>
<td><strong>KIT exon 13</strong></td>
<td><strong>KIT exon 13</strong></td>
</tr>
<tr>
<td>5'-CTC CTC CAA CCT AAT AGT GT-3'</td>
<td>5'-GTC AAG CAG AGA ATG GGT AC-3'</td>
</tr>
<tr>
<td><strong>PDGFRA exon 12</strong></td>
<td><strong>PDGFRA exon 12</strong></td>
</tr>
<tr>
<td>5'-TTG GAT ATT CAC TTA CCT GTC-3'</td>
<td>5'-CAA GGG AAA AGC TCT TGC-3'</td>
</tr>
<tr>
<td><strong>PDGFRA exon 18</strong></td>
<td><strong>PDGFRA exon 18</strong></td>
</tr>
<tr>
<td>5'-ACC ATG GAT CAG CCA GTC TT-3'</td>
<td>5'-TGA AGG AGG ATG AGC CTG ACC-3'</td>
</tr>
</tbody>
</table>

Results

Clinically, the ages of patients with SENECE ranged from 62 years to 81 years with a mean of 73 years. All patients were male. The presenting symptoms were dysphagia in 5 cases and vomiting in 1 case. The locations of SCNECE were lower esophagus in 4 cases and middle esophagus in 2 cases. Endoscopically, the SCNECE was ulcerated in 3 cases and polypoid in 3 cases. All the 6 patients were treated by chemoradiation therapy, and the prognosis ranged from 6 months to 25 months with a mean of 13 months.

Histologically, 5 cases were pure SCNECE, and 1 case showed triplicate differentiation into small cell carcinoma, adenocarcinoma and squamous cell carcinoma. The pure SENECE was composed of medullary small malignant cells with hyperchromatic nuclei, molded nuclei, fine chromatin, scant cytoplasm, and absent or inconspicuous nucleoli (Figure 1). One SCNECE showed triplicate differentiations into small cell carcinoma (Figure 2A), adenocarcinoma (Figure 2B), and squamous cell carcinoma (Figure 2C). Immunohistochemically, each SCNECE showed at least one of the neuroendocrine antigens. Cytokeratins (Figure 3A) were positive in 6/6, vimentin 0/6, synaptophysin (Figure 3B) in 4/6, CD56 (Figure 3C) 4/6, neuron-specific enolase (Figure 3D) 3/6, chromogranin 0/6, p53 protein (Figure 3E) in 6/6, KIT (Figure 3F) in 6/6, and PDGFRA (Figure 3G) in 6/6. Ki-67 labeling (Figure 3H) ranged from 56% to 100% with a mean of 79%.

A retrospective genetic analysis using PCR-direct sequencing method in paraffin sections identified no mutations of KIT (exons 9, 11, 13, and 17) and PDGFRA (exons 12 and 18) gene were performed by the PCR direct sequencing method, as previously reported [16-22]. The exons of both genes were selected because they are frequent mutation sites [10-15]. The primers are shown in Table 1. In brief, genomic DNA was extracted from paraffin blocks with proteinase K digestion and phenol/chloroform extraction, and subjected to PCR for 40 cycles (94°C for one minute, 52°C for one minute, 72°C for one minute), using a thermal cycler (GeneAmp PCR system 9700, Applied Biosystems, ABI, CA). The annealing temperature was 53°C. PCR products were extracted, and subjected to a computed automatic DNA sequencer (ABI PRIZM 3100 Genetic Analyzer, Applied Biosystems, ABI, CA).
and 17) and PDGFRA (exons 12 and 18) genes in all the 6 SCNECE cases.

Discussion

In the present study, only 6 cases of SCNECE were found in the 2,438 esophageal specimens; the frequency was 0.25%, suggesting that SCNECE is a very rare tumor. Clinically, the ages of patients with SCNECE ranged from 62 years to 81 years with a mean of 73 years, and all patients were male. This suggests that SCNECE affect mainly old male. In the present series, the presenting symptoms were dysphagia in 5 cases and vomiting in 1 case, similar to esophageal squamous cell carcinoma. In the present series, the locations of SCNECS were lower esophagus in 4 cases and middle esophagus in 2 cases, suggesting that SCNEC mainly affects distal esophagus. In the present series, the survival of the patients ranged from 6 months to 25 months with a mean of 13 months, indicating that SCNEC is a very aggressive tumor.

Small cell carcinoma is defined by only HE histology [30]. According to WHO criteria [30], it is defined as a malignant epithelial tumor consisting of small cells with scant cytoplasm, ill-defined cell borders, finely granular nuclear chromatin, and absent or inconspicuous nucleoli. The tumor cells are round, oval and spindle-shaped. Nuclear molding is prominent. Necrosis is typically extensive and mitotic count is high [30]. More than 90% of small cell carcinoma has neuroendocrine features [30]. The present cases fulfill the criteria of small cell carcinoma. The present SCNECE cases had neuroendocrine features. Thus, the present cases are small cell carcinomas in strict criteria.

In the present study, 5 cases were pure SCNECE, 1 case showed triplicate differentiation into small cell carcinoma, adenocarcinoma and squamous cell carcinoma. As to the latter one case, some reports revealed that small cell carcinoma of the esophagus coexisted with squamous cell carcinoma and adenocarcinoma [6, 31]. It has been considered that small cell carcinoma may arise from totipotential stem cell with triple differentiations [31]. In the present cases, SCNECE may arise from such totipotential stem cells.
In the present cases, immunohistochemically, cytokeratins were positive in 6/6, vimentin 0/6, synaptophysin in 4/6, CD56 4/6, neuron-specific enolase 3/6, chromogranin 0/6, p53 protein in 6/6, KIT in 6/6, and PDGFRA in 6/6. Ki-67 labeling ranged from 56% to 100% with a mean of 79%. The positivity of cytokeratin and negativity of vimentin indicate the epithelial nature of the present SCNECE. Among the neuroendocrine markers, synaptophysin and CD56 was most sensitive followed by neuron-specific enolase. In the present series, no chromogranin-positive SCNECE was present. The expression of p53 indicates p53 gene mutation. The high Ki-67 labeling shows highly proliferative activity of the present SCNECE cases.

The novel findings in the present study are that immunoreactive KIT and PDGFRA were present in all cases. The present study is the first report of SCNECE that examined KIT and PDGFRA proteins and KIT and PDGFRA genes. KIT and PDGFRA proteins and KIT and PDGFRA genes have rarely been investigated in extrapulmonary small cell carcinoma [32-45], while several comprehensive reports are present in small cell lung carcinoma. KIT has been reported to be expressed in 30-80% of the small cell lung carcinoma [43, 46, 47]. The present case shows that esophageal SCNECE also expresses KIT protein. Only one study of PDGFRA protein has been reported in small cell lung carcinoma [43], and only two studies of KIT and PDGFRA in SCNECE have been reported [8, 9]. The present study showed PDGFRA expression, suggesting that SCNECE expresses this oncoprotein.

The present cases did not identify mutations of KIT and PDGFRA genes. Most reports of small cell lung carcinoma have shown no mutations
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in KIT genes [43, 46], except for Boldrini et al. [47] who found five mutations in 60 small cell lung carcinomas. On the other hand, Terada [43] and Sihto et al. [46] identified no KIT mutations in many cases of small cell lung carcinomas. More studies of KIT mutations remain to be performed in the SCNECE. With regard to PDFGRA mutations, Terada [43] and Sihto et al. [46] found no mutations in many cases of small cell lung carcinomas. Sihto et al. [46] insisted that KIT expression in small cell lung carcinoma is due not to KIT gene mutations but to KIT gene amplification.

Among many KIT-positive tumors, GIST is representative [10-15]. It is thought that GIST arises from interstitial cell of Cajal, a pacemaker neuronal cell that normally expresses KIT protein [10-15]. In contrast, SCNECE is an undifferentiated carcinoma with neuroendocrine phenotypes. The original cell of SCNECE is unknown. Recently, Blumming et al. [48] found that GIST expresses synaptic vesicle proteins, and suggested that GIST has endocrine features. Therefore, it is suggested that there may be an association between GIST and SCNECE in that the both entities have neuroendocrine features.

Several studies of GIST have revealed that there are minute subclinical microGISTs or “GIST tumorlets” in the gastrointestinal tract [49-51]. The incidence of these is about 20%, and these are considered as GIST precursors. Frequent KIT mutations (about 46%) and occasional PDGFRA mutations (about 4%) are present in these “GIST tumorlets” [49-51]. However, these “GIST tumorlets” do not always develop into clinical GIST. Other genetic events are necessary for the development of clinical GIST. In contrast, little is known about the precursor lesions in SENECE.

Recently, the phosphorylation (activation) status of KIT and PDGFRA has been studied [52, 53]. This is particularly important in KIT mutation-negative tumors as in the present case. KIT kinase activation and downstream signaling proteins leading to tumorigenesis have been studied, but little is known as yet. Protein kinase C-theta and PI3-kinase/AKT are activated in imatinib-resistant GIST [52, 53], and analyses of these KIT signaling molecules may be important in the treatment of GIST. Such studies are not performed in SCNECE. In the present study, the author could not investigate these molecules, because no relevant antibodies were available. KIT tyrosine kinase activity and KIT signaling abnormalities in SCNECE remain to be elucidated.

In summary, the author reported 6 very rare cases of SCNECE with KIT and PDGFRA expressions but without KIT and PDGFRA mutations.

Conflict of interest statement
The author declares no conflict of interest.

Acknowledgements
This work was approved by Ethics Committee of our hospital.

Address correspondence to: Tadashi Terada, Department of Pathology, Shizuoka City Shimizu Hospital, Miyakami 1231 Shimizu-Ku, Shizuoka 424-8636, Japan. Tel: 81-54-336-1111; Fax: 81-54-336-1315; E-mail: piyo0111jp@yahoo.co.jp

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