CASE SERIES

Histopathological study of carcinoma showing thymus-like differentiation (CASTLE)

Yoichiro OKUBO1*, Mai SAKAI1, Haruhiko YAMAZAKI1, Yuko SUGAWARA1, Joji SAMEJIMA2, Emi YOSHIOKA1, Masaki SUZUKI1, Kota WASHIMI1, Kae KAWACHI1, Hiroyuki HAYASHI3, Hiroyuki ITO3, Hiroyuki IWASAKI2, Tomoyuki YOKOSE1

1Department of Pathology, Kanagawa Cancer Center, 2-3-2, Nakao, Asahi-Ku, Yokohama, Kanagawa, 241-8515, Japan. 2Department of Breast and Endocrine Surgery, Kanagawa Cancer Center, 2-3-2, Nakao, Asahi-Ku, Yokohama, Kanagawa, 241-8515, Japan. 3Department of Thoracic Surgery, Kanagawa Cancer Center, 2-3-2, Nakao, Asahi-Ku, Yokohama, Kanagawa, 241-8515, Japan. and 4Department of Pathology, Yokohama Municipal Citizen’s Hospital, 56, Okazawa-cho, Hodogaya-ku Yokohama, Kanagawa, 240-0062, Japan.

Abstract

Introduction: Carcinoma showing thymus-like differentiation (CASTLE) is a rare tumour that mainly arises from the thyroid gland, or occasionally, from the head and neck. Although the 10-year survival rate of patients with CASTLE is approximately 80%, local recurrence and distant metastasis are observed in some cases. A recent systematic review for CASTLE indicated that the prognostic factors are treatment-dependent, and postoperative radiotherapy significantly improves patient survival.

Case Report: Herein, we describe and compare three cases of CASTLE, including a case with distant metastasis despite administering postoperative chemotherapy. Thus, the mechanisms underlying metastasis of CASTLE are unclear. This case study helps to elucidate the histopathological risk factors of metastasis in CASTLE.

Discussion: We found that prominent lymphovascular invasion and higher proliferative activities might be risk factors of metastasis in CASTLE. In addition, we have summarised the cytological, morphological, and immunohistochemical features of CASTLE for an accurate diagnosis.

Keywords: Carcinoma, thyroid cancer, cytology, CASTLE

INTRODUCTION

Carcinoma showing thymus-like differentiation (CASTLE) is a rare tumour that mainly arises from the thyroid gland, or occasionally, from extrathyroidal areas. This tumour is suspected to arise from the ectopic thymus tissue, remnants of the thypharyngeal duct, or branchial pouch. The first case report was published by Miyauchi et al. in 1957, in which the tumour was called an intrathyroidal epithelial thymoma. The term “CASTLE” was coined by Chan et al. in 1991, after which this term has become widely known and has been standardised. This tumour has a relatively favourable prognosis; the 5-year and 10-year survival rates of patients with CASTLE are approximately 90% and 80%, respectively. However, some cases of recurrence or metastasis have been observed after surgery. The rate of distant metastasis in CASTLE was 14%–29% and postoperative radiotherapy significantly improved patient survival.

Moreover, chemotherapy should be attempted in patients with advanced or metastatic disease. Herein, we report three cases of CASTLE including a case with distant metastasis despite administering postoperative chemoradiotherapy.

CASE PRESENTATION

CASE 1

A 68-year-old man with a 2-month history of hoarseness visited another clinic. Ultrasonography revealed a mass in the lower part of the left lobe of the thyroid, and the patient was referred to our hospital for a thorough examination. On fine needle aspiration cytology, a few clusters of tumour cells were observed, with round-to-ovoid
vesicular nuclei, relatively prominent nucleoli, and scanty cytoplasm. Papillary or follicular features were not observed (Fig. 1). Despite the relative paucity of tumour cells, the cytological examination indicated the presence of malignant tumour cells (histological type unknown). Open biopsy was not performed, and subsequently, a left hemithyroidectomy was performed as per the patient’s preferences.

On gross examination, the tumour measured 30 × 21 mm and was ill-defined and solid with whitish cross sections in the middle area of the left lobe of the thyroid gland (Fig. 2A-B).

Histological examination showed a fibrous...
background of cells with nested squamoid tumour cells and lymphocytes in the thyroid (Fig. 2C). Tumour cells showed prominent vascular invasion (13 vascular invasive foci were observed per 100 high-powered fields; HPFs) and invasion of the laryngeal nerve (Fig. 2D-E). Tumour cells had a round-to-oval-shaped nucleus with a conspicuous nucleolus (Fig. 2F), with 2 mitotic figures per 10 HPFs. Based on all these findings, the tumour was diagnosed as an anaplastic carcinoma of the thyroid gland, and adjuvant chemoradiotherapy (carboplatin-paclitaxel-based combination chemotherapy, up to a total of eight courses and radiation with cumulative doses of 60 Gy) was administered. The clinicopathological findings of the case are summarised in Table 1.

Seven years after the initial surgery, follow-up computed tomography (CT) revealed a growing nodule in the anterior mediastinum. As a metastatic lesion was suspected, the nodule was resected (Fig. 3A-B). Histological findings of the lesion in the anterior mediastinum were similar to those of the first thyroid lesion (Fig. 3C-D). Therefore, the thyroid lesion was re-examined, and tumour cells showed focal positivity for CD5 and c-kit but were negative for TTF-1 and thyroglobulin, similar to the tumour cells from the lesion in the anterior mediastinum. The Ki-67 labelling index (hot spot counting) of the thyroid lesion was 30%.

Finally, the initial thyroid lesion was diagnosed as CASTLE, and the lesion from the anterior mediastinum was identified to be a metastasis of the CASTLE. No recurrence was observed 3 years after the second surgical intervention.

**CASE 2**

A 56-year-old man with a one-month history of hoarseness visited another hospital. Physical examination revealed a palpable mass in the thyroid gland, and subsequent fine needle aspiration cytology suggested a poorly differentiated carcinoma; therefore, the patient was referred to our hospital for surgery.

On gross examination, the tumour measured 25 × 22 mm and was ill-defined and solid with whitish cross sections in the lower to the middle area of the left lobe of the thyroid gland (Fig. 4). Histological examination showed invasive solid islands of squamoid cells with fibrous and inflammatory cells in the background. Tumour cells invaded the laryngeal nerve, and a few instances of vascular invasion were observed (1 vascular invasive focus was observed per 100 HPFs). Tumour cells had a round, large nucleus with a prominent nucleolus, but did not have a mitotically active area (≤1 mitotic figure per 10 HPFs). Neither papillary nor follicular structures

| TABLE 1. Clinicopathological findings of all three cases |
|----------------|----------------|----------------|
|               | Case 1         | Case 2         | Case 3         |
| Age (years)   | 68             | 56             | 52             |
| Sex           | Male           | Male           | Female         |
| Tumour size (mm) | 30 × 21     | 25 × 22        | 12 × 10        |
| Site          | Intrathyroidal tumour | Intrathyroidal tumour | Extrathyroidal tumour |
| Mitotic counts | 2 mitotic figures per 10 high-powered fields | Less than 1 mitotic figure per 10 high-powered fields | Less than 1 mitotic figure per 10 high-powered fields |
| Ki-67 labelling index (hot spot counting) | 30% | 10%-20% | 10%-20% |
| Vascular invasion | 13 vascular invasive foci were observed per 100 high-powered fields | 1 vascular invasive focus was observed per 100 high-powered fields | 3 vascular invasive foci were observed per 100 high-powered fields |
| Lymph node metastasis | Not dissected | Absent (0/7) | Absent (0/1) |
| Distant metastasis | Present (7 years after surgery) | None | None |
| Chemoradiotherapy | Administered | Not administered | Not administered |
FIG. 3: Macroscopic and histopathological findings of the recurrent lesion in the anterior mediastinum in Case 1. (A-B) The tumour in the anterior mediastinum was partially resected. A ~10-mm solid nodular tumour with a whitish cut surface and irregular margins can be observed. (C) Tumour cells invaded the connective tissue with erosive solid nests having unclear margins (Victoria blue-HE staining, x100). (D) Similar to the lesion in the thyroid, some nests showed squamous differentiation with lymphocyte infiltration (Victoria blue-HE staining, x200).

FIG. 4: Macroscopic and histopathological findings of Case 2. (A) The tumour measured 25x22 mm and was ill-defined and solid with whitish cross-sections in the lower to the middle area of the left lobe of the thyroid gland. (B) Invasive solid islands of squamoid cells with fibrous and inflammatory cells in the background (Victoria blue-HE staining, x40). (C) Tumour cells invaded the laryngeal nerve (Victoria blue-HE staining, x400).
were present. Tumour cells were positive for CD5 and c-kit but negative for TTF-1 and thyroglobulin. The Ki-67 labelling index (hot spot counting) was 10%–20%. The tumour was diagnosed as CASTLE, and no recurrence was observed 5 years after surgery. The clinicopathological findings of the case are summarised in Table 1.

CASE 3
A 52-year-old woman showed an intrathyroidal mass on ultrasonography performed as part of a routine health check-up. Subsequent fine needle aspiration cytology revealed adenomatous goitre. Although the patient was asymptomatic after 2 years of follow-up, ultrasonography revealed a rapidly growing mass between the lower part of the left lobe of the thyroid and the paratracheal lymph node area. Fine needle aspiration cytology of the area indicated metastatic carcinoma, suggestive of squamous cell carcinoma; therefore, the patient was referred to our hospital for surgical treatment. Although no primary site could be determined despite careful work-up at our hospital, minimal primary thyroid tumour was suspected, and left hemithyroidectomy was performed after obtaining the patient’s consent.

On gross examination, the tumour measured 12x10 mm and was relatively sharply demarcated and solid with whitish cross-sections. Moreover, this tumour was adjacent to the lower part of the left lobe of the thyroid (Fig. 5A).

Histological examination revealed that the tumour had invasive solid nests with a fibrous background in the extrathyroidal area (below the lower part of the left lobe of the thyroid; Fig. 5B) and there was no lymph node tissue around the tumour lesion. A few instances of the vascular invasion were observed (3 vascular invasive foci were observed per 100 HPFs). Tumour cells had a round-to-oval-shaped nucleus with a conspicuous nucleolus, with ≤1 mitotic figure per 10 HPFs; squamous cell differentiation was unclear (Fig. 5C). Tumour cells were positive for CD5, c-kit, p40, synaptophysin, and chromogranin A and negative for TTF-1, thyroglobulin, and PAX8. The Ki-67 labelling index (hot spot counting) was approximately 10%–20% (Fig. 6). The tumour was diagnosed as CASTLE, and no recurrence was observed 8 months after surgery (without the need for chemoradiotherapy). The clinicopathological findings of the case are summarised in Table 1.

DISCUSSION
CASTLE is associated with a better prognosis than are thyroid squamous cell carcinoma, anaplastic carcinoma, and neuroendocrine carcinoma. However, patients have shown distant recurrence after surgery, and one of our patients (Case 1) showed distant recurrence. Although Case 1 showed relatively low mitotic rates (2 mitotic figures per 10 HPFs), this activity was higher than that observed in the other two cases. Furthermore, Case 1 showed prominent lymphovascular invasion and a higher Ki-67 labelling index. These findings indicated that the proliferative activities (mitotic activity and Ki-67 labelling index) and the presence of prominent lymphovascular invasion might be risk factors of metastasis in CASTLE.

On histopathological examination, CASTLE typically shows a solid tumour nest with islands of infiltrating cells at the periphery. Tumour cells show large, round, oval, polygonal, or spindle nuclei with prominent nucleoli. Moreover, lymphocyte infiltration in the tumour nest is often observed. Some carcinomas should be considered as differential diagnoses for CASTLE, including neuroendocrine carcinoma, poorly differentiated carcinoma of the thyroid, squamous cell carcinoma, and anaplastic carcinoma. Confirming immunoreactivity for CD5 and c-kit can assist in differentiating CASTLE from other carcinomas; however, unfortunately, CASTLE does not always show immunoreactivity for both. Therefore, when the tumour is negative for CD5 and c-kit, morphological features (relatively lower mitotic rate and background of abundant lymphocytes) and confirmation of immunoreactivity for PAX8 are important for diagnosis.

Thus, although CASTLE is occasionally immunoreactive for neuroendocrine markers, low mitotic rates and the presence of background lymphocytes are the features that distinguish this from neuroendocrine carcinoma. Moreover, low mitotic rates and negativity for PAX8 are helpful for differentiating between CASTLE and poorly differentiated carcinoma of the thyroid, squamous cell carcinoma, and anaplastic carcinoma.

Herein, we also discuss the usefulness of cytology for diagnosing CASTLE. Although fine-needle aspiration cytology is the primary diagnostic tool for thyroid neoplasms, it is often difficult to diagnose CASTLE based on cytology alone. Common cytological
features of this tumour are clusters and sheets of round tumour cells with a high nucleus to cytoplasm ratios, large nuclei with prominent nucleoli, amphophilic cytoplasm, and lymphocytic background. However, it is often difficult to obtain sufficient tumour cells and lymphocytes, and thyroid squamous cell carcinomas and anaplastic carcinoma with squamous cell differentiation are frequently confused with CASTLE on cytology. These factors make it difficult to diagnose CASTLE using cytology alone. Therefore, the possibility of CASTLE should be considered if tumour cells lack papillary features (nuclear grooves, ground glass nuclei, and intranuclear cytoplasmic inclusions) and follicular features (abundant follicular epithelial cells, microfollicular structures, and lack of colloid material), especially because papillary and follicular thyroid carcinomas account for more than 90% of all thyroid malignant tumours. Moreover, CASTLE often arises from the lower part of the thyroid gland, and this tumour grows slower than thyroid squamous cell carcinoma and anaplastic carcinomas.

FIG. 5: Macroscopic and histopathological findings of the thyroid lesion in Case 3. (A) The tumour measured 12x10 mm and was relatively sharply demarcated and solid with whitish cross sections. This tumour was adjacent to the thyroid gland. (B) An ill-defined solid nest proliferated near the thyroid gland, but the tumour cells were slightly distant from the thyroid gland itself (Victoria blue-HE staining, x40). (C) Sheets of atypical epithelial cells with lymphocyte infiltration can be observed. Morphologically, squamous cell differentiation was not well defined (Victoria blue-HE staining, x200).

FIG. 6: Representative immunohistochemistry findings of Case 3. (A-B) Tumour cells show positive immunoreactivity for CD5 and c-kit (original magnification, x100). (C) The Ki-67 labeling index (hot spot counting) is ~10%-20% (original magnification, x100). (D-E) Tumour cells showed positive immunoreactivity for p40 and negative immunoreactivity for TTF-1 (original magnification, x100). (F) Tumour cells showed focal positive immunoreactivity for synaptophysin (original magnification, x100).
carcinoma. Therefore, if preoperative biopsy cannot be performed, oncologists should make the diagnosis on the basis of the primary site findings, clinical findings, and cytological findings.

In conclusion, high proliferative activities and the presence of prominent lymphovascular invasion might be risk factors of metastasis in CASTLE. Confirmation of immunoreactivity for CD5 and c-kit may be helpful in differentiating CASTLE from other carcinomas, but some CASTLE cases are negative for these markers. Therefore, morphological features (a relatively low mitotic activity) and other immunohistochemical features are also essential for accurate diagnosis of CASTLE. Furthermore, although cytology is the primary diagnostic tool for thyroid neoplasms, it is often difficult to diagnose CASTLE based on cytology alone. Therefore, oncologists should consider the possibility of CASTLE if thyroid tumour cells lack papillary and follicular features and the clinical symptoms/signs and imaging findings are indicative of thyroid neoplasms.

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