Genomic variation associated with carcinoma showing thymus-like elements (CASTLE) in thyroid gland

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Abstract

Background: Carcinoma showing thymus-like elements (CASTLE) is a rare kind of malignant tumor of thyroid gland. The genetic mutation characteristics of CASTLE are not clear.

Methods: We retrospectively analyzed seven patients diagnosed as CASTLE tumor in our hospital, and performed whole exome sequencing (WES) in five cases to analyze the genomic variation of CASTLE in thyroid gland.

Results: The diagnosis of CASTLE was confirmed by histopathological and immunohistochemical results. Immunohistochemical staining showed that cell membranes of tumor samples in all cases were moderately to strongly positive for CD5 and CD117. WES presented a large number of single nucleotide variants (SNVs), insertions and deletions (InDel), and copy number variations (CNVs). By comparing with the TCGA database, we found novel mutations in significantly mutated genes such as FBXL16, PAQR7, LEFTY1, UBAS2, and FLNA, as well as in potential disease-related driver genes such as MLLT10, FLNA, CYLD, HLA-B, KMT2D, SFPQ, MUC16, EEF2, and KMT2C.

Conclusions: CASTLE tumors contain unique tumor driver gene mutations. The information about mutations in several novel genes obtained in this study may contribute to unraveling the molecular mechanisms responsible for the emergence of thyroid CASTLE tumors and help formulating possible in-roads for treatment.

Keywords
CASTLE, genomic variation, immunohistochemistry, prognosis, thyroid

1 | INTRODUCTION

Carcinoma showing thymus-like elements (CASTLE) is a rare type of malignant tumor that mainly occurs in the thyroid gland and, occasionally, in the soft tissue of the neck. This tumor was first described by Miyauchi et al.1 as “intrathyroidal epithelial thymoma (ITET)” in 1985 and renamed as “CASTLE” by Chan and Rosai2 in 1991. It was classified as a type of low-grade malignant thyroid cancer according to the World Health Organization Classification of Tumors3 in 2004. Its definition was updated in the latest edition of the WHO Classification of Tumors4 in 2017, and it is now referred to as “intrathyroid thymic carcinoma (ITTC).”

The majority of CASTLE tumors occur in the thyroid gland, most commonly in the lower poles. Patients frequently present with a
painless mass in the thyroid region and subsequently develop symptoms related to tracheal compression and hoarseness as the mass progressively grows.\(^3\) Cervical lymph node metastasis is frequent, whereas distant organ metastasis is seldom observed. This type of thyroid tumor has a relatively favorable prognosis compared with that of poorly differentiated thyroid carcinoma and anaplastic thyroid carcinoma.

Until recently, the majority of published studies related to CASTLE have been mainly case reports describing clinicopathological and immunohistochemical features. Only a few studies have reported on individual genetic mutations in CASTLE/ITTC. In our study, we for the first time analyzed the genomic variation and described the genetic mutation landscape in CASTLE using high-throughput sequencing. Our results may be useful for future molecular and therapeutic studies of this rare malignancy.

2 | MATERIALS AND METHODS

2.1 | Patients and treatment

We retrospectively analyzed 7 patients with thyroid CASTLE treated at our Institute between January 2010 and December 2019. Six patients were treated for the first time, and one patient was treated for the second time after disease recurrence. There were four men and three women with a median age of 52 years (range, 40–68 years). Three patients underwent radical resection and postoperative chemoradiotherapy. Another three patients underwent palliative resection and postoperative chemoradiotherapy. One patient refused surgery and received palliative chemoradiotherapy only. Tumors invading the mediastinum or common carotid artery were determined to be unresectable.

2.2 | Pathological diagnosis and immunohistochemistry

All patients were pathologically diagnosed by surgery or core needle biopsy. Specimens were fixed with 10% neutral buffered formalin, dehydrated with gradient alcohol, embedded in paraffin, cut into 3–4 μm thick sections, and then used for hematoxylin/eosin staining and optical microscopy observation. Representative wax blocks were selected for immunohistochemical staining using the EnVision two-step method. We used primary antibodies raised against the epithelial marker cytokeratin (CK), leukocyte differentiation antigens (CD5 and CD117), tumor suppressor gene P63 (P63), thyroglobulin (TG), thyroid transcription factor 1 (TTF-1), calcitonin (CT), synaptophysin (SYN), chromogranin A (CgA), proliferation-related antigen ki-67 (Ki-67), and PAX8 (Roche, Shanghai, China). Epstein–Barr virus (EBV) was detected by in situ hybridization with an RNA-specific oligonucleotide probe (Leica, Germany).

2.3 | Whole exome sequencing

We performed whole exome sequencing (WES) of paired tumor-affected and normal thyroid tissue samples in five of the above seven patients. We could not obtain enough high-quality DNA tissue from the other two patients because there was an insufficient sample amount (case 2) or due to DNA degradation caused by the long storage time of the tumor wax block (case 3). WES of paired samples was performed at GenePlus-Beijing Co., Ltd. (Beijing, China). Based on the sequencing data, we detected somatic variation and annotated single nucleotide variants (SNVs), insertions and deletions (InDels), and copy number variations (CNVs). Based on the primary data analysis, we conducted in-depth data mining, including analyses of tumor mutation features, significantly mutated genes (SMGs), driver gene mutations, tumor mutational burden (TMB), and microsatellite instability (MSI).

2.4 | Ethics statement

This study was approved by the independent Ethics Committee of the Cancer Hospital of the University of the Chinese Academy of Sciences.

3 | RESULTS

3.1 | Clinicopathological characteristics

All patients presented with a painless, progressively enlarging mass in the neck, located in the lower pole of the thyroid gland or paratracheal or upper mediastinum, with a hard texture and little movement. In contrast to the boundary of well-differentiated thyroid carcinoma, the CASTLE boundary is unclear, mainly with expansive growth, often invading adjacent structures, including neck muscles, trachea, esophagus, and even wrapping the common carotid artery. However, cervical lymph node metastasis is rare. Laboratory tests revealed normal levels of thyroid stimulating hormone (TSH), T3, T4, and thyroid peroxidase antibodies in our patients. The overall prognosis of patients with CASTLE is favorable. At the end of the follow-up in December 2020, six patients were alive, and one patient was lost to follow-up. The median follow-up time was 60 months (range: 22–130 months). During the observation period, one patient had cervical tumor recurrence together with distant metastasis (lung and bone), for which he received palliative systemic chemotherapy. Another patient developed lung metastasis, which was treated with the second-line systemic chemotherapy followed by immunotherapy with a PD-1 inhibitor (immunohistochemistry of the original surgically removed tissue showed high expression of PD-L1, combined positive score, CPS = 80). Table 1 summarizes the clinicopathological features and prognosis of all patients.
Immunohistochemical staining showed that cell membranes of tumor samples in all cases were moderately to strongly positive for CD5 and CD117. Partially positive signals were noted for CK, P63 and PAX8. Diffuse positive signals were observed in one case for SYN and CgA. No expression of TG, TTF-1, or CT was detected. The Ki-67 proliferation index ranged between 10% and 90%. EBV in situ hybridization produced a negative result.

### 3.2 Somatic variation analysis

Using WES, we obtained a list of all somatic variations in the five samples analyzed. A total of 8044 SNVs were detected, of which 2262 and 27 were missense and nonsense mutations, respectively. There were eight non-synonymous SNVs in the start codons, three SNVs leading to the loss of stop codons, and 16 non-synonymous SNVs in the splice-site regions. A total of 520 InDels were detected, including 15 frameshift insertion mutations, 45 frameshift deletion mutations, and 28 non-synonymous InDels located in the splice-site regions. Ninety-six CNVs were detected, of which 67 were amplifications, and 29 were deletions. Table 2 summarizes the distribution of the somatic variants in exons of patients with CASTLE.

The somatic variation information of all detected samples was summarized, and MafTools package was used to display the individual panoramic view of somatic variations. Figure 1 illustrates the top 20 mutated genes with their mutation frequencies.
After the integration of somatic SNV and InDel variations, genes with a significantly higher mutation rate than the background were defined as SMGs. Tumor sample mutations included in MuSiC2 software were used for calculating the background mutation rate, and the analysis of SMGs in our patients is shown in Table 3.

### 3.4 Driver gene mutation analysis

We compared functional mutated genes with known driver genes from the CGC723 database to identify potential disease-related driver genes. Nine significantly mutated driver genes were identified. Next, we compared these mutated driver genes with gene sets in the TCGA database for all thyroid and thymus tumor
studies. Table 4 presents the results of our driver gene mutation analysis.

### Table 4: Preliminary screening result of driver genes.

| Gene    | Sample affected | Sample percent % | Role in cancer | Tumor type       | Comprehensive299 |
|---------|-----------------|------------------|----------------|------------------|------------------|
| MLLT10  | 2               | 40               | Oncogene, fusion | AL               | /                |
| FLNA    | 2               | 40               | –              | Phyllodes tumor of the breast | 3.24%          |
| CYLD    | 2               | 40               | TSG            | Cylindroma       | 0.98%            |
| HLA-B   | 2               | 40               | –              | –                | 0.91%            |
| KMT2D   | 2               | 40               | oncogene, TSG  | Medulloblastoma, renal | 8.33%          |
| SFPQ    | 2               | 40               | TSG, fusion    | Papillary renal  | /                |
| MUC16   | 2               | 40               | Oncogene       | HNSCC, melanoma  | /                |
| EEF2    | 2               | 40               | –              | –                | 1.07%            |
| KMT2C   | 2               | 40               | TSG            | Medulloblastoma  | 8.11%            |

Abbreviations: AL, acute leukemia; Comprehensive299, mutation frequency in this database; HNSCC, head and neck squamous cell cancer; Sample Affected, number of samples having that gene mutations; Sample Percent %, the proportion of sample; TSG, tumor suppressor gene.

### Table 5: The result of tumor mutational burden (TMB).

| Sample | Functional mutation | InDel burden | TMB value | Grade   |
|--------|---------------------|--------------|-----------|---------|
| Case 1 | 18                  | 0            | 0.54      | TMB-Low |
| Case 4 | 33                  | 4            | 0.99      | TMB-Low |
| Case 5 | 49                  | 2            | 1.47      | TMB-Low |
| Case 6 | 43                  | 8            | 1.29      | TMB-Low |
| Case 7 | 1612                | 17           | 48.28     | TMB-High|

Abbreviations: Functional mutation, number of functional non-synonymous mutations; InDel burden, number of InDels caused frame shift mutations.

### Table 6: The result of microsatellite instability (MSI).

| Sample | MSI score | Status |
|--------|-----------|--------|
| Case 1 | 0.003000  | MSS    |
| Case 4 | 0.007600  | MSS    |
| Case 5 | 0.008700  | MSS    |
| Case 6 | 0.005000  | MSS    |
| Case 7 | 0.206900  | MSI-High |

Note: Status: there were three types of microsatellite instability, including MSS, MSI-high, and MSI-low. Patients with MSI-high might be more sensitive to immune checkpoint inhibitor drugs.

In this study, we use MSI sensor software to detect the microsatellite region, and then records the proportion of unstable microsatellite loci as the score of MSI. Table 6 presents the results of microsatellite instability analysis.

### 3.5 | TMB analysis

Tumor mutational burden is defined as the number of non-synonymous somatic mutations per Mb (mut/Mb) of coding interval. It is a biomarker which could classify tumor patients into TMB-high, TMB-medium, and TMB-low grades. Table 5 presents the results of our tumor mutational burden analysis.

### 3.6 | MSI analysis

Microsatellite instability is a variation in the length of microsatellite repeats which due to the defect of DNA mismatch repair mechanism. CASTLE is a low-grade malignant cancer of the thyroid gland with morphological resemblance to thymic epithelial tumors. CASTLE probably arises from thymic remnants in the thyroid gland, as evidenced by the presence of ectopic thymic tissue in the vicinity of the tumor in some cases. Patients frequently present with a painless mass in the thyroid region. Enlarged tumor usually invades the recurrent laryngeal nerve or trachea, leading to symptoms such as cough, hoarseness, and even poor breathing. Tsutsui et al. described six patients with thyroid CASTLE that invaded the trachea. Further, Tran et al. reported a case of CASTLE involving nine rings of the trachea.

Except for the usual position in the lower pole of the thyroid, the imaging performance of CASTLE has no specificity compared to that of other thyroid tumors. Yamamoto et al. reported that sonographic images of CASTLE showed heterogeneously solid tumors without cystic components or calcification. Wu et al. described CASTLE tumors as ill-defined nodular masses of uniform density on plain CT scans with heterogeneous enhancement after the injection of a contrast medium. On MRI, CASTLE tumors presented with homogeneous isointensity on the T1-weighted images and slight hyperintensity on
the T2-weighted images. Most tumors showed heterogeneous enhancement on post-contrast images, whereas some were markedly enhanced. All of these imaging features are insufficient for the differential diagnosis of CASTLE.

The preoperative diagnosis of CASTLE is extremely difficult. Fine-needle aspiration biopsy does not have much value in the diagnosis of CASTLE owing to the limited tissue amount. Collins et al. analyzed 10 cases of CASTLE and demonstrated general cytomorphological characteristics, such as syncytial fragments of malignant cells with pleomorphic large nuclei, vesicular chromatin, and prominent nucleoli in the background of lymphocytes. However, CASTLE can only be definitively diagnosed using postoperative histopathological examination and immunohistochemical staining. Okubo et al. elucidated the histopathological risk factors for CASTLE tumors. CASTLE morphology shows a superficial resemblance to the lobulation seen in thymomas and thymic carcinomas, and the tumor islands are commonly penetrated by delicate vessels. The nuclei are oval, pale to vesicular, and have small distinct nucleoli. In some cases, tumor cells appear spindle-shaped or display variable degrees of squamous-like differentiation in the form of distinct cell borders.

Immunohistochemical positivity for CD5 and CD117, sometimes accompanied by the positivity for p63 and CK, is helpful in the diagnosis of CASTLE. TG, TTF-1, or calcitonin expression has almost never been observed. Owing to the extremely low incidence rate, there is little evidence for the possibility of molecular diagnosis of CASTLE, which will require further studies. Veits et al. previously described chromosomal imbalances in CASTLE, similar to those found in thymomas and thymic carcinomas. Therefore, they thought it reasonable to assume that the treatment of advanced CASTLE should follow the guidelines for thymic cancer. However, Hirokawa et al. found some specific immunohistochemical signals in thymic lymphoepithelioma-like carcinoma (LELC), which were essentially similar to those in ITET/CASTLE: both tumors were positive for CD5, P63, and c-KIT, for example. However, there were fewer immunopositive cells in LELC than in ITET/CASTLE. In addition, LELC was characterized by more invasive growth, so the authors recommended to consider ITET/CASTLE and thymic LELC as different tumors.

Only a few recent studies have reported genetic mutations in CASTLE/ITTC. Veits et al. described mutations in the KRAS, EGFR, PDGFRA, and KIT genes in CASTLE tumors. Rajeshwari et al. presented a rare case of CASTLE that occurred in association with Hashimoto thyroiditis and was characterized by low-level expression of the T790M somatic mutation in EGFR. Wong et al. reported a case of CASTLE of the parotid gland and described alterations in the PPARG, BRCA2, and NOTCH1 genes revealed by massive parallel sequencing analysis. Tahara et al. investigated the mutation status of the KIT, EGFR, BRAF genes, and TERT promoter in ITTC and thymic carcinoma. The authors suggested that recurrent TERT promoter mutations may be a key event related to tumor progression in ITTC. However, there is too little literature to obtain enough genetic information of CASTLE/ITTC.

In our study, we for the first time used WES to detect the genomic variation of CASTLE tumors and found novel mutations in SMGs such as FBXL16, PAQR7, LEFTY1, UBA52, and FLNA, as well as in potential disease-related driver genes such as MLLT10, FLNA, CYLD, HLA-B, KMT2D, SFPQ, MUC16, EEF2, and KMT2C. Notably, mutations in the MLLT10 and HLA-B genes have not been found previously in the TCGA database of common thyroid or thymus tumors, which may indicate that CASTLE is different from thyroid and thymic carcinomas. MLLT10 encodes a transcription factor, and it had been identified as a partner gene involved in several chromosomal rearrangements resulting in leukemia. HLA-B (major histocompatibility complex, class I, class B) is a protein-coding gene. Diseases associated with HLA-B mutations include severe cutaneous adverse reaction and spondyloarthropathy. We also noted a mutation in the FLNA gene. The loss-of-function mutations in the FLNA gene may contribute to the development of cancer, facilitating the spreading and migration of tumor cells, and give rise to a more aggressive disease. Due to the limited number of cases of such disease, we have not been able to verify the potential role of these gene mutations in CASTLE tumor progression.

Tumor heterogeneity is an important feature of malignant tumors. It is prominent in different types of cancer, even among different individuals with the same tumor type. In this study, we found that there were significant differences in genomic variation between different samples, including the number and somatic mutations, TMB, and MSI. Case 7 had nearly 10 times the number of mutations than the other. The prognosis of case 7 (22 months) was significantly worse than that of the other six cases (39–130 months). Is there a correlation between the two? However, owing to the limited sample size, it is very difficult to conduct in-depth mechanism research and draw exact conclusion. We could not explore in detail the relationship between genomic variation and CASTLE tumorigenesis.

Surgical resection is the first-choice treatment for CASTLE. Options for surgery include lobectomy, subtotal thyroidectomy, total thyroidectomy, and neck dissection. Patients with tracheal involvement usually undergo partial tracheal resection and synchronous reconstruction. Although Dong et al. demonstrated a favorable outcome merely by radical surgery, postoperative radiotherapy or chemoradiotherapy is often recommended, especially for patients with extracapsular invasion, positive surgical margins, or regional lymph node metastasis.

A meta-analysis of 82 cases of CASTLE reported a recurrence rate of 18.57% and the longest follow-up time of 26 years. Salvage surgery combined with adjuvant radiotherapy is the most frequently used treatment for local and regional recurrence. There is insufficient evidence regarding the efficacy of chemotherapy in cases of distant metastasis because of the rarity of CASTLE. Hanamura et al. reported a case of CASTLE with lung metastasis that showed a good response to first-line (cisplatin, doxorubicin, vincristine, and cyclophosphamide) and second-line (carboplatin and paclitaxel) chemotherapy regimens. With the development of immunotherapy, immune checkpoint inhibitors have proven to be effective in some solid tumors. Lorenz et al. reported a patient with parotid CASTLE tumor and pleural metastasis who achieved partial remission after treatment with the PD-1 inhibitor pembrolizumab. In our study, case 2 developed lung metastasis 2 years after the initial treatment and received platinum-based combined chemotherapy followed by the PD-1 inhibitor immunotherapy for 1 year. The patient’s lung metastases were controlled, and no significant new lesions were found.
5 | CONCLUSION

Thyroid CASTLE is rare and difficult to diagnose preoperatively. Clinicians should pay extra attention to tumors growing in the lower pole of the thyroid as well as in the paratracheal and upper mediastinum. Generally, CASTLE has a favorable prognosis. Radical resection is considered the best treatment. Adjuvant radiotherapy or chemoradiotherapy is recommended for locally advanced cases or with lymph node metastasis. For patients with recurrent or distant metastatic CASTLE, platinum-based combined chemotherapy might be effective, and immunotherapy could be considered.

CASTLE tumors contain unique tumor driver gene mutations. We suggest that at the molecular level, CASTLE differs from thyroid and thymic carcinomas. Due to the limited number of samples, this study could not establish a clear relationship between the discovered gene mutations and tumorigenesis. The information about mutations in several novel genes obtained in this study may contribute to unraveling the molecular mechanisms responsible for the emergence of thyroid CASTLE tumors and help formulating possible in-roads for treatment.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES

1. Miyauchi A, Kuma K, Matsuzuka F, et al. Intra-thyroidal epithelial thymoma: an entity distinct from squamous cell carcinoma of thyroid. World J Surg. 1985;9:128-135.
2. Chan JK, Rosai J. Tumors of the neck showing thymic or related branchial pouch differentiation: a unifying concept. Hum Pathol. 1991;22:349-367.
3. DeLellis RA, Lloyd RV, Heitz PU, Eng C. World Health Organization Classification of Tumours: Pathology and Genetics of Tumours of Endocrine Organs. IARC Press; 2004:96-97.
4. Lloyd RV, Osamura Ry, Kloppel G, Rosai J. WHO Classification of Tumours of Endocrine Organs. 4th ed. IARC Press; 2017:125-126.
5. Tsutsui H, Hoshi M, Kubota M, et al. Management of thyroid carcinoma showing thymus-like differentiation (CASTLE) invading the trachea. Surg Today. 2013;43:1261-1268.
6. Tran J, Zafereo M. Segmental tracheal resection (nine rings) and reconstruction for carcinoma showing thymus-like differentiation (CASTLE) of the thyroid. Head Neck. 2019;41:3478-3481.
7. Yamamoto Y, Yamada K, Motoi N, et al. Sonographic findings in three cases of carcinoma showing thymus-like differentiation. J Clin Ultrason. 2013;41:574-578.
8. Wu B, Sun TQ, Gu YJ, et al. CT and MR imaging of thyroid carcinoma showing thymus-like differentiation (CASTLE): a report of ten cases. Br J Radiol. 2016;89:20150726.
9. Collins JA, Ping B, Bishop JA, Ali SZ. Carcinoma showing thymus-like differentiation (CASTLE): cytopathological features and differential diagnosis. Acta Cytol. 2016;60:421-428.
10. Okubo Y, Sakai M, Yamazaki H, et al. Histopathological study of carcinoma showing thymus-like differentiation (CASTLE). Malays Pathol. 2020;42:259-265.

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11. Wang YF, Liu B, Fan XS, et al. Thyroid carcinoma showing thymus-like elements: a clinicopathological, immunohistochemical, ultrastructural, and molecular analysis. Am J Clin Pathol. 2015;143:223-233.
12. Cui XJ, Zhang CY, Su P, et al. Clinicopathological characteristics of head and neck carcinoma showing thymus-like element [Chinese]. Zhonghua Bing Li Xue Za Zhi. 2017;46:155-159.
13. Veits L, Mechtersheimer G, Steger C, et al. Chromosomal imbalances in carcinoma showing thymus-like elements (CASTLE). Virchows Arch. 2011;459:221-226.
14. Hirokawa M, Miyauchi A, Minato H, Yokoyama S, Kuma S, Kojima M. Intra-thyroidal epithelial thymoma/carcinoma showing thymus-like differentiation: comparison with thymic lymphoepithelioma-like carcinoma: a possibility of development from a multipotent stem cell. APMIS. 2013;121:523-530.
15. Veits L, Schupfner R, Hufnagel P, et al. KRAS, EGFR, PDGF-A, KIT and COX-2 status in carcinoma showing thymus-like elements (CASTLE). Diagn Pathol. 2014;9:116.
16. Rajeshwari M, Singh V, Nambirajan A, Mridha AR, Jain D. Carcinoma showing thymus-like elements: report of a case with EGFR T790M mutation. Diagn Cytopathol. 2018;46:413-418.
17. Wong EH, Tetter N, Tzankov A, Muller L. CASTLE tumor of the parotid: first documented case, literature review, and genetic analysis of the head. Head Neck. 2018;40:E1-E4.
18. Tahara I, Oishi N, Mochizuki K, et al. Identification of recurrent TERT promoter mutations in intra-thyroidic thymic carcinomas. Endocr Pathol. 2020;31:274-282.
19. Li C, Xin W, Sy MS. Binding of pro-prion to filament A by design or an unfortunate blunder. Oncogene. 2010;29:5329-5345.
20. Gao R, Jia X, Ji T, Feng YT, Yang AM, Zhang GJ. Management and prognostic factors for thyroid carcinoma showing thymus-like elements (CASTLE): a case series study. Front Oncol. 2018;8:477.
21. Fung AC, Tsang JS, Lang BH. Thyroid carcinoma showing thymus-like differentiation (CASTLE) with tracheal invasion: a case report. Am J Case Rep. 2019;20:1845-1851.
22. Dong WW, Zhang P, Li J, et al. Outcome of thyroid carcinoma showing thymus-like differentiation in patients undergoing radical resection. World J Surg. 2018;42:1754-1761.
23. Kong FF, Ying HM, Zhai RP, et al. Clinical outcome of intensity modulated radiotherapy for carcinoma showing thymus-like differentiation. Oncotarget. 2016;7:81199-81205.
24. Noh JM, Ha SY, Ahn YC, et al. Potential role of adjuvant radiation therapy in cervical thymic neoplasm involving thyroid gland or neck. Cancer Res Treat. 2015;47:426-440.
25. Ge W, Yao YZ, Chen G, Ding YT. Clinical analysis of 82 cases of carcinoma showing thymus-like differentiation of the thyroid. Oncol Lett. 2016;11:1321-1326.
26. Attene F, Pisano IP, Pala C, Marrouso A, Bozzo C, Scognamiglio F. Surgical management of primary and recurrent carcinoma showing thymus-like elements (CASTLE). Ann Ital Chir. 2017;88:247-252.
27. Hanamura T, Ito K, Uehara T, Fukushima T, Sasaki S, Sasaki K, Koizumi T. Chemosensitivity in carcinoma showing thymus-like differentiation: a case report and review of the literature. Thyroid. 2015;25:969-972.
28. Lorenz L, von Rappard J, Arnold W, et al. Pembrolizumab in a patient with a metastatic CASTLE tumor of the parotid. Front Oncol. 2019;9:734.

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