Primary small cell thyroid carcinoma combined with poorly differentiated thyroid carcinoma, evidence for a common origin: A case report

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Received January 6, 2023; Accepted March 1, 2023

DOI: 10.3892/ol.2023.13819

Abstract. Primary small cell thyroid carcinomas are extremely rare and there is still debate about their classification as a distinct disease entity. The present case report reports a small cell carcinoma (SCC) combined with poorly differentiated thyroid carcinoma (PDTC) in a 34 year old man. The tumor consisted of ~80% PDTC and ~20% SCC. The PDTC component was positive for cytokeratin and thyroid transcription factor-1 (TTF-1), and negative for calcitonin, chromogranin and synaptophysin. The SCC component was positive for synaptophysin and CD56, and negative for calcitonin, chromogranin and TTF-1. Seven months after thyroid surgery, two new lung nodules were detected. Histologically and immunohistochemically, the lung tumors were similar to the SCC component of the thyroid carcinoma. The mutational status of cancer-related genes was assessed using targeted next-generation sequencing in both the thyroid and lung, which identified similar genetic alterations. The histogenesis of SCC was evaluated through NGS analysis of the two cancer components.

Introduction

Small cell carcinoma (SCC) is a high grade malignant neuroendocrine tumor and is most commonly of pulmonary origin (1). The existence of 'real' small cell carcinoma of the thyroid gland is still debated, and in the 4th edition of the World Health Organization thyroid tumor classification (2), it was not listed as a distinct tumor type. However, intermittent reports of primary thyroid small cell carcinoma with clinical findings supporting thyroid origin and immunohistochemical results valid for SCC have been reported (3-5).

Combined small-cell carcinoma is defined as SCC combined with additional components that consist of any histological type of non-small cell carcinoma. Combined SCC is mostly reported in the lung, and head and neck, few cases of combined SCCs have been previously reported in the larynx (6,7). To the best of our knowledge, there has been no previous report of combined SCC in the thyroid. Herein, a case of primary SCC combined with PDTC in thyroid is reported.

Case report

A 34 year old male was admitted to Jeonbuk National University Hospital (Jeonju, Republic of Korea) in September 2019, because of a thyroid mass. The patient had undergone chemotherapy and bone marrow transplantation 10 years previously as treatment for T-cell lymphoblastic leukemia. Neck computed tomography showed a 4.0 cm sized mass in the left lobe of the thyroid gland (Fig. 1A). Positron emission tomography and computed tomography were performed, and metastatic lesions were not identified. A total thyroidectomy with neck lymph node dissection was performed. Macroscopically, a soft, yellowish tumor of 3.2x1.7 cm was detected. The surgical specimen was fixed in 10% neutral formaldehyde at room temperature for 24 h and the representative part of the tumor was routinely embedded in paraffin, sliced to 4-5 um thickness sections and stained with hematoxylin and eosin (H&E) for ~45 min at room temperature using a Roche VENTANA HE600 fully automated system (Roche Tissue Diagnostics; Roche Diagnostics, Ltd.) and histological characteristics were assessed using a light microscope. Microscopically, the tumor was composed of two components and had infiltrated into the extra-thyroid tissue. The main component constituted ~80% of the tumor and demonstrated typical PDTC morphology. The tumor had a mainly solid

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Key words: thyroid, small cell carcinoma, poorly differentiated carcinoma, next generation sequencing

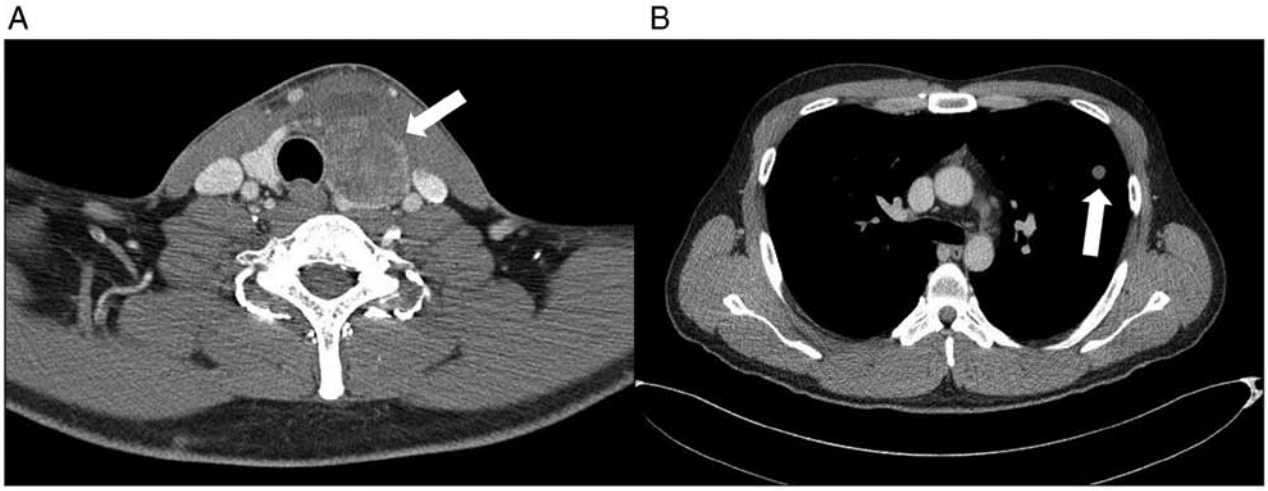

Figure 1. (A) Computed tomography scan which showed a 4.0 cm sized tumor in the left lobe of the thyroid gland. The border of tumor indicated infiltration into adjacent tissue. (B) Computed tomography scan which showed a lung nodule in the upper, left lobe.

growth pattern and certain parts showed follicular growth with frequent mitoses (Fig. 2A and B). The remaining 20% of the tumor demonstrated SCC morphology. The tumor cells demonstrated hyperchromatic oval to spindle-shaped nuclei without prominent nucleoli, had scant cytoplasm, showed occasional nuclear molding and mitoses were frequent (Fig. 2C and D). Tissue sections were processed according to the aforementioned method for hematoxylin and eosin staining and used for immunohistochemistry. The sections were stained on a Benchmark ULTRA automated immunohistochemistry stainer (Roche Tissue Diagnostics; Roche Diagnostics, Ltd.) using an OptiView DAB IHC Detection Kit (Roche Tissue Diagnostics; Roche Diagnostics, Ltd.) according to the following procedure. Heat induced epitope retrieval was performed using ULTRA cell conditioning solution (ULTRA CC1; Roche Tissue Diagnostics; Roche Diagnostics, Ltd.) for 32 min at 100˚C. Sections were incubated with Optiview Peroxidase Inhibitor (3% hydrogen peroxide solution) for 4 min at room temperature and with primary antibodies for calcitonin (1:100; cat. no. 760-2611; Roche Diagnostics), ready to use CD56 (cat. no. 760-4596; Roche Diagnostics), ready to use CEA (cat. no. 760-4594; Roche Diagnostics), ready to use chromogranin (cat. no. 780-4422; Roche Diagnostics), ready to use cytokeratin (cat. no. 790-4555; Roche Diagnostics) and ready to use synaptophysin (cat. no. 760-4595; Roche Diagnostics) for 12 min at 37˚C, followed by sequential incubation with the OptiView DAB IHC Detection Kit (Optiview HQ Universal Linker for 8 min, Optiview HRP Multimer for 8 min, Optiview DAB and Optiview H_2O_2 for 8 min, Optiview Copper for 4 min), at room temperature. The OptiView HQ Universal Linker kit (cat. no. 760-770; Roche Diagnostics) contained a cocktail of HQ-labeled secondary antibodies (goat anti-mouse IgG, goat anti-mouse IgM and goat anti-rabbit) at an unspecific concentration <50 μg/ml in buffer and OptiView HRP Multimer contained a mouse monoclonal anti-HQ labeled HRP antibody at an unspecific concentration <40 μg/ml in buffer. Ten slides were removed from the stainer and counterstained using Mayer's hematoxylin (ScyTek Laboratories, Inc.) for 2 min at room temperature manually. Immunohistochemical staining was evaluated using a light microscope. The PDTC component was positive for cytokeratin and thyroid transcription factor-1 (TTF-1), and negative for calcitonin, chromogranin and synaptophysin (8). The SCC component was positive for synaptophysin and CD56, and negative for calcitonin, chromogranin, CEA and TTF-1 (Fig. 2E and F) (9). Conventional nuclear features of papillary thyroid carcinoma, such as nuclear clearing, intranuclear groove and nuclear pseudo-inclusions, were not identified (10). Seven months after thyroid surgery, two lung nodules were detected (Fig. 1B). A wedge resection of the left upper lobe was performed. Histologically, the lung tumor was similar to the SCC component of the thyroid tumor (Fig. 3A and B). The lung tumor was positive for synaptophysin and CD56, and negative for TTF-1, chromogranin and calcitonin (Fig. 3C and D). At the time of diagnosis of the patient's thyroid tumor, no lung lesions suggestive of metastasis were observed on the chest computed tomography. Next generation sequencing (NGS) was performed to investigate the relationship between the small cell component and PDTC of thyroid cancer, and to evaluate the relationship between the lung cancer and thyroid cancer. Targeted NGS was performed on both thyroid and lung lesions. Formalin-fixed paraffin-embedded (FFPE) tumor tissue was used. Hematoxylin and eosin-stained slides were reviewed, and tumor areas with sufficient viable tumor cells were marked and used as a guide for macro-dissection. Areas with >50% tumor volume were used for examination. Briefly, total nucleic acid was isolated from tumor tissue using a Recover All Total Nucleic Acid Isolation Kit for FFPE (Ambion; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocols. After extracting DNA and RNA from FFPE specimens, the quality and concentration of DNA and RNA were assessed using a Qubit 3.0 Fluorometer with the Qubit™ dsDNA HS Assay Kit (cat. no. Q32854; Thermo Fisher Scientific, Inc) and Qubit™ RNS HS Assay Kit (cat. no. Q32852; Thermo Fisher Scientific, Inc). Library preparation for an Oncomine™ Comprehensive Assay v3 (IonTorrent; Thermo Fisher Scientific, Inc.) was performed. For library preparation, the multiplex PCR-based Ion Torrent AmpliSeq™ technology (Thermo Fisher Scientific, Inc.) with Oncomine™ Comprehensive Assay v3M Kit (cat. no. A36111;

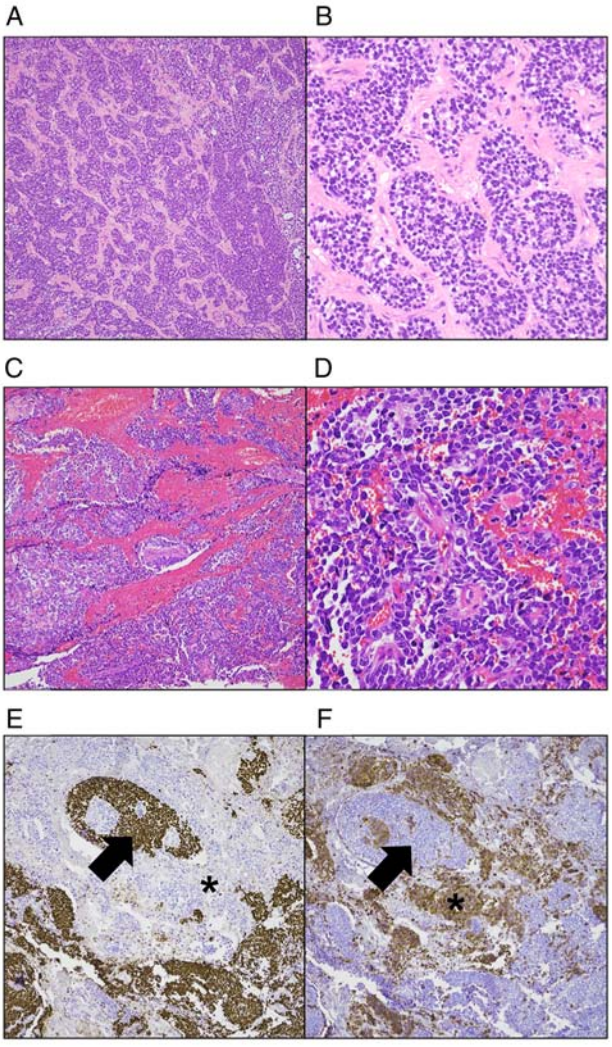

Figure 2. Histopathologic features of the thyroid tumor. The tumor was composed of two components; the main component was a PDTC and the secondary component was a SCC. (A) The PDTC component demonstrated a solid growth pattern (H&E staining; original magnification, x200). (B) The high-power field of view the tumor cells of the PDTC component demonstrated scant cytoplasm with round to oval nuclei and mild pleomorphism (H&E staining; original magnification, x400). (C) The SCC component demonstrated a solid growth pattern (H&E staining; original magnification, x200). (D) In the high-power field of view of the SCC component, the tumor cells demonstrated minimal cytoplasm with hyperchromatic oval to spindle-shaped pleomorphic nuclei and were without prominent nucleoli. Numerous mitoses were also seen (H&E staining; original magnification, x400). (E) Immunohistochemical staining for TTF-1 showed positivity in the PDTC component (arrow). Whereas, the SCC component was negative for TTF-1 (asterisk) (original magnification, x200). (F) Immunohistochemical staining for synaptophysin. The tumor cells of the PDTC component did not show immunoreactivity to synaptophysin (arrow). Whereas, the SCC component was positive for synaptophysin (asterisk) (original magnification, x200). PDTC, poorly differentiated thyroid carcinoma; SCC, small cell carcinoma; H&E, hematoxylin and eosin; TTF-1, thyroid transcription factor-1.

Thermo Fisher Scientific, Inc) was used. The individual libraries were diluted to a final concentration of 50 pM and samples were pooled and processed to library amplification on Ion Spheres using an Ion 550™ Kit (cat. no. A34538; Thermo Fisher Scientific, Inc.) and library enrichment on the Ion Chef (Thermo Fisher Scientific, Inc.). An IonTorrent S5 XL platform was used for sequencing according to the manufacturer's protocols. The direction of sequencing was single end type

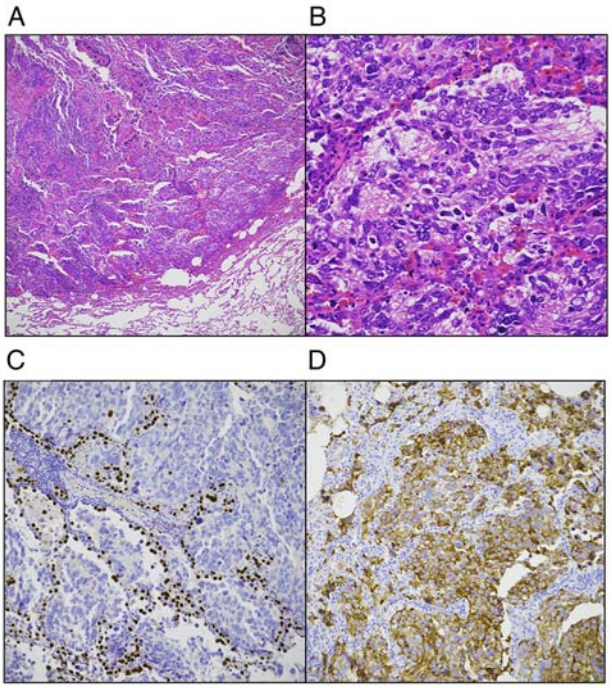

Figure 3. Histopathologic features of the lung nodule. (A) The tumor demonstrated high cellularity with a solid growth pattern (H&E staining; original magnification, x40). (B) The tumor cells had minimal cytoplasm with round to oval pleomorphic nuclei. Abundant mitoses were also present (H&E staining; original magnification, x400). The tumor cells were (C) negative for thyroid transcription factor-1 (original magnification, x200) and (D) positive for synaptophysin (original magnification, x200). H&E, hematoxylin and eosin.

and 200 bp for length. Alignment to the hg19 human reference genome and variant calling were performed using Torrent Suite version 5.12.1 (Thermo Fisher Scientific, Inc.) and Ion Reporter software version 5.18 (Thermo Fisher Scientific, Inc.). Only the PDTC area was included in the examination for the thyroid lesion. Alterations of *ATRX* (c.6793G>T, p.Glu2265*), *TP53* (c.377A>G, p.Tyr126Cys) and *MYCL* (c.332G>T, p.Gly111Val) were demonstrated in both lesions. In the lung SCC lesions, *KIT* and *PDGFRA* amplification and *CDK2* (c.783C>A, p.Ser261Arg) mutations were also demonstrated. The patient was scheduled to receive 6 cycles of paclitaxel, cisplatin and etoposide-based chemotherapy, according to the normal procedures of Jeonbuk National University Hospital. However, the response was poor and after receiving 4 cycles of chemotherapy the patient died due to progression of the disease. The patient's death occurred 12 months after the total thyroidectomy.

Discussion

Primary SCCs have been intermittently reported previously, but most of them have presented as low-grade lymphoma or PDTC following immunohistochemical evaluation. Approximately 6-7 cases of primary small cell thyroid carcinomas (SCTCs) meeting the diagnostic criteria for small cell carcinoma have been reported in previous years (3,4,5,9,11). Eusebi *et al* (5) reported two cases of SCC that met the morphologic and immunohistochemical criteria for SCC. Since then, there have been intermittent reports of SCC of the thyroid gland,

but it is very rare and has not yet been accepted as a distinct disease entity. Much like its pulmonary counterparts, primary SCTC demonstrated an aggressive clinical course and poor outcome (5).

The present case report reported a thyroid cancer composed of two different components. The main component demonstrated typical morphologic and immunohistochemical features of PDTC. Whereas the second component demonstrated morphologic features similar to pulmonary SCC including hyperchromatic oval to spindle-shaped nucleus, no nucleoli, scant cytoplasm, occasional nuclear molding, and frequent mitoses. The second component was positive for synaptophysin and CD56, and negative for calcitonin, chromogranin, CEA and TTF-1. There were no amyloid depositions in the tumor. In the 4th edition of WHO classification of thyroid tumors, high-grade neuroendocrine carcinomas are not listed as a distinctive type (2) and the fact that this tumor exhibited neuroendocrine differentiation, meant that calcitonin-negative medullary thyroid carcinoma (MTC) needed to be considered in the differential diagnosis. However, the tumor had no histological characteristics for MTC, and the morphological characteristics, such as minimal cytoplasm, nuclear molding, high mitotic figure, and immunohistochemical features such as being positive for CD56 and synaptophysin were similar to those of lung SCC (9). Sporadic MTCs are well-differentiated and locally aggressive tumors (12). However, in this tumor, metastasis to the lung occurred rapidly and was observed just 7 months post-surgery. The patient's rapid lung metastasis was also consistent with the biological behavior of SCC (13). For proper treatment and accurate prediction of prognosis, it was considered more appropriate to diagnose the second component of this tumor as SCC rather than as a variant of medullary carcinoma.

NGS tests were performed on lung lesions and PDTC regions in the thyroid lesions. Three identical mutations were observed in the thyroid and lung lesions, which confirmed that the lung lesions had metastasized from the thyroid gland. Furthermore, as the same genetic mutations were observed in PDTC and SCC, it was possible to infer that the origin of the two components were the same. In the SCC lesion, KIT and PDGFRA amplification were additionally observed. Previous studies have reported that *TP53* mutation and, *KIT* and *PDGFRA* amplification are rare in well-differentiated thyroid cancer and frequently observed in anaplastic thyroid carcinomas (14,15). The genetic alterations demonstrated in both lesions suggested that both tumors were of the same cell origin and that SCC may arise through the acquisition of additional genetic alterations. It is generally accepted that ATC and PDTC usually develop from the dedifferentiation of differentiated thyroid cancer (16). The concept of combined small cell lung cancer (CSCLC) is well-established in the lung, and the prevalence of CSCLC is reported to be 2-28% of all small cell lung cancer cases (17); however, the cellular origin of CSCLC remains unclear. Mangum *et al* reported that the cellular origin of CSCLC showed that different components (small cell vs non-small cell) of CSCLC shared nearly 75% common mutations (17). Based on these results, it was reported that one component of CSCLC arose separately from the other component at a relatively late time point in the presence of a different microenvironment (17). These previous reports support the hypothesis of the present case report that the patient's PDTC and SCC developed from a common precursor.

Acknowledgements

Not applicable.

Funding

This case report was supported by The Fund of Biomedical Research Institute, Jeonbuk National University Hospital.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YTH performed the surgery and provided treatment for the patient. ARA, KMK and MJC analyzed the NGS sequencing data. MJC and KMK evaluated the histopathological images and prepared the figures. MJC wrote the manuscript. KMK and MJC confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The report of this study was approved by the Institutional Review Board of the Jeonbuk National University Hospital with a waiver of informed consent for publication (IRB no. 2022-09-055).

Consent for publication

Written informed consent was obtained from the patient for the publication of this case report.

Competing interests

The authors declare that they have no competing interests.

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