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Mutations of *TSHR* and *TP53* Genes in an Aggressive Clear Cell Follicular Carcinoma of the Thyroid

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Abstract Clear cell follicular carcinoma is a rare type of thyroid cancer and some with aggressive biological behavior. The cytoplasmic clearing of the neoplastic cells has been attributed to the accumulation of various substances, such as glycogen, lipid, mucin, and thyroglobulin, or distension of mitochondria or endoplasmic reticulum. However, the molecular mechanisms responsible for the characteristic appearance of the cell cytoplasm and the biological behavior remain unknown. We report here a case of aggressive clear cell follicular carcinoma of the thyroid with molecular profile using targeted next generation sequencing (NGS) that presented as a metastatic tumor in a woman with a history of breast carcinoma. The NGS data revealed the coexisting of a well-characterized loss-of-function *TP53 R248Q* mutation and a putative gain-of-function mutation of *TSHR L272V*, which was suggested by the overexpression of thyroglobulin and *SLC5A5* (*NIS*) genes in this tumor. *TP53* mutations are usually related with dedifferentiation, progression, and metastasis of thyroid carcinomas. Identification of *TP53 R248Q* in this tumor correlated with its aggressive clinical behavior. Gain-of-function mutation of *TSHR* can overstimulate the thyroid follicular cells as the elevated level of TSH does and might have contributed to the development of clear cell morphology in this tumor. This report represents the first case of clear cell follicular carcinoma of the thyroid with NGS analysis and more molecular characterization is needed to elucidate the pathogenesis and provide more prognosis-relevant information for this uncommon variant of thyroid carcinomas.

Keywords Clear cell carcinoma of the thyroid · Next generation sequencing (NGS) · *TP53* · *TSHR*

Introduction

Clear cell carcinoma of the thyroid follicular cell origin is a rare tumor. Its diagnosis can be challenging, and requires exclusion of metastatic clear cell carcinoma, especially from the kidney, and tumors of thyroid C cell or parathyroid origin. Clear cell morphology may be seen in various histological types of thyroid carcinomas and is not considered to signify a separate cancer subtype but rather represent a variant of thyroid papillary, follicular, or medullary carcinomas [1–3]. The clear cytoplasm is believed to have such appearance due to accumulation of various substances, such as glycogen, lipid, mucin, and thyroglobulin, or distension of mitochondria or endoplasmic reticulum [1, 2, 4] and in some cases was suggested to be associated with overstimulation by thyroid stimulation hormone (TSH) [2, 5]. The clinical and biological behavior of clear cell carcinoma of the thyroid is not well-defined, mainly because of its rarity, although it was suggested to represent a more aggressive type of thyroid cancer [3, 6, 7]. Herein, we report a case of aggressive clear cell follicular carcinoma of the thyroid with detailed molecular mutation analysis that may shed light on the molecular mechanism for its biological behavior and distinct morphologic appearance.

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Case Report

The patient was an 89-year-old woman with history of breast carcinoma 7 years ago and was treated with lumpectomy and tamoxifen therapy. Information regarding the histological type of her breast cancer was unavailable. No evidences of recurrence were identified in the breast on the regular follow-up mammography. However, a recent bone scan and single-photon emission computed tomography (SPECT) revealed an abnormal uptake in the area adjacent to the lumbar vertebrate 3 and 4 (L3, L4), which corresponded to a large soft tissue mass in the right paraspinal and epidural area on magnetic resonance imaging (MRI). The computed tomography (CT) scan also showed a compression fracture on the L4 and hemorrhagic mass in the right psoas muscle. The lesion was fluorodeoxyglucose (FDG) avid with a maximum standardized uptake value (SUV) of 8.7 on FDG-PET/CT. There was no other FDG avid lesion in the breast, lung, liver, or other areas of the body except in the left lobe of the thyroid (SUV=6.8). A CT-guided needle biopsy of the soft tissue mass in the psoas muscle was performed. Although metastatic breast carcinoma was suspected clinically, immunohistochemistry (IHC) revealed a metastatic carcinoma of thyroid origin. The patient had no known history of thyroid diseases or thyroid nodules. The TSH, T3 (total), and T4 (free) levels were 4.33 μIU/ml, 79.5 ng/dl, and 9.9 μg/dl, respectively, and were within normal limits. The follow-up ultrasonography showed a 1.5-cm complex nodule with calcified rim in the left lobe of the thyroid, corresponding to the abnormal FDG uptake. A total thyroidectomy was performed and a thyroid carcinoma with unusual cytological features was identified. The patient did not receive radioactive iodine (RAI) ablation for the metastatic disease due to poor health condition and died 6 months after the thyroidectomy.

Materials and Methods

Histology and Immunohistochemistry

Both the biopsy and thyroidectomy specimens were fixed in 10 % buffered formaldehyde solution (pH 7.4), dehydrated in a series of graded alcohols, and embedded in paraffin. Sections of 5 μm thickness were stained with hematoxylin-eosin (H&E). Immunohistochemical staining was performed on the paraffin-embedded tissue sections from the biopsy and total thyroidectomy specimens in a Dako autostainer with prediluted antibodies for CK7, CK20, TTF1, PAX8, thyroglobulin (TG), calcitonin, parathyroid hormone (PTH), and p53 according to the manufacture's manual.

DNA isolation and next generation gene sequencing

Tumor tissue was manually microdissected using five unstained slides from the total thyroidectomy specimen under stereomicroscopic visualization using a hematoxylin and eosin-stained section for guidance. Total nuclei acids were isolated using the DNeasy blood and tissue kit on the automated QIAcube (QIAGEN) instrument according to the manufacturer's instructions. Targeted NGS analysis was performed using ThyroSeq v2 assay as previously described [8]. Detection of point mutations and indels in 14 genes (*AKT1*, *BRAF*, *CTNNB1*, *GNAS*, *HRAS*, *KRAS*, *NRAS*, *PIK3CA*, *PTEN*, *RET*, *TP53*, *TSHR*, *TERT*, *EIF1AX*) was performed using 10 ng of DNA. The point mutations were confirmed by Sanger sequencing. The analytic sensitivity of the method was 5 % of mutant alleles. Detection of 42 gene fusion types involving the *RET*, *BRAF*, *NTRK1*, *NTRK3*, *ALK*, *PPARG*, and *IGF2BP3* genes was performed using 10 ng of RNA. The presence of more than 50 high quality reads crossing the fusion point of the transcript was required to consider the test positive for a gene fusion. Expression of the *CALCA*, *PTH*, *SLC5A5* (*NIS*), *TG*, *TTF1*, *KRT7*, and *KRT20* genes was also assessed.

Result

The CT-guided needle biopsy of the psoas mass yielded few fragments of fibrous tissue and skeletal muscle infiltrated by malignant epithelioid cells. The malignant cells were mainly in solid nests with relatively uniform ovoid nuclei and small nucleoli. There were no distinct histological or cytological features recognizable. An initial IHC panel showed the malignant epithelial cells were positive for CK7 and TTF1, and negative for CK20, CDX2, mammaglobin, GCDFP-15, and estrogen receptor. The initial findings did not support the diagnosis of metastatic breast carcinoma, but suggested a possible metastasis from a lung primary. However, imaging studies of the lung revealed multiple bilateral small ground-glass nodules (<3.5 mm) that were not FDG avid. On the other hand, a significantly increased FDG uptake was discovered in the left lobe of the thyroid. Therefore, immunostaining for TG and PAX8 was also included for further work-up, showing the tumor was diffusely positive for two markers. Thus, a diagnosis of metastatic thyroid carcinoma to the psoas/L4 was rendered.

An elective total thyroidectomy was performed 3 months later. Grossly, the left lobe of the thyroid was slightly enlarged and measures 5×4×1.5 cm and the right lobe and isthmus grossly were unremarkable. Sectioning revealed an approximately 3.6×2.5×1.2 cm ill-defined tan, firm area with nodular appearance and hemorrhagic and necrotic changes in the left lobe of the thyroid. Histological sections showed the left lobe

and the isthmus were infiltrated by variably sized and shaped solid nodules with intertwining normal thyroid tissue. The nodules were filled with solid nests consisted of neoplastic cells with distinct clear cytoplasm. The neoplastic cells had uniform round or oval nuclei with small nucleoli, occasional nuclear grooves, and scattered mitotic figures. No significant nuclear pleomorphism, nuclear clearing, intranuclear cytoplasmic inclusions, or papillary structures were identified. The tumor extended beyond the thyroid capsule and invaded into the perithyroid soft tissue with multiple foci of lymphovascular invasion (Fig. 1a–d). The tumor was positive for TTF-1, PAX8, and TG (Fig. 1e), and negative for calcitonin and PTH, confirming a malignant tumor of thyroid follicular cell origin. The tumor was almost entirely composed of neoplastic cells with clear cytoplasm and lacked of cytological and histological features of papillary or anaplastic thyroid carcinomas; thus, a diagnosis of clear cell variant of thyroid follicular carcinoma was rendered.

The DNA extracted from representative formalin-fixed and paraffin-embedded tissue sections was subjected to mutational analysis with targeted NGS, which identified a previously characterized c.743G>A (R248Q) *TP53* mutation and a c.81C>G (L272V) *TSHR* mutation. Strong nuclear staining for p53 was present in almost all tumor nuclei (Fig. 1f). In addition, the molecular analysis also revealed significantly increased expression of sodium/iodide symporter (*NIS*) and *TG* genes. No *BRAF*, *RAS*, *RET/PTC*, *PPARG/PAX8*, or other mutations commonly found in thyroid carcinomas were identified.

Discussion

Here, we describe a case of aggressive thyroid carcinoma with clear cell morphology and distinct molecular alterations consisted of coexisting *TP53* and *TSHR* mutations accompanied by overexpression of the *TG* and *NIS* gene, but no clinical evidence of hyperthyroidism.

Clear cell follicular carcinoma of the thyroid is very uncommon, representing less than 1 % of thyroid cancers [9, 10]. These tumors may be composed entirely or predominately of clear neoplastic cells. In the routine H&E-stained sections, the cytoplasm can be watery clear or has fine, pale eosinophilic granularity. The diagnosis requires exclusion of metastatic clear cell carcinoma, especially from the kidney, benign or malignant tumors of thyroid C-cells, and parathyroid tumors

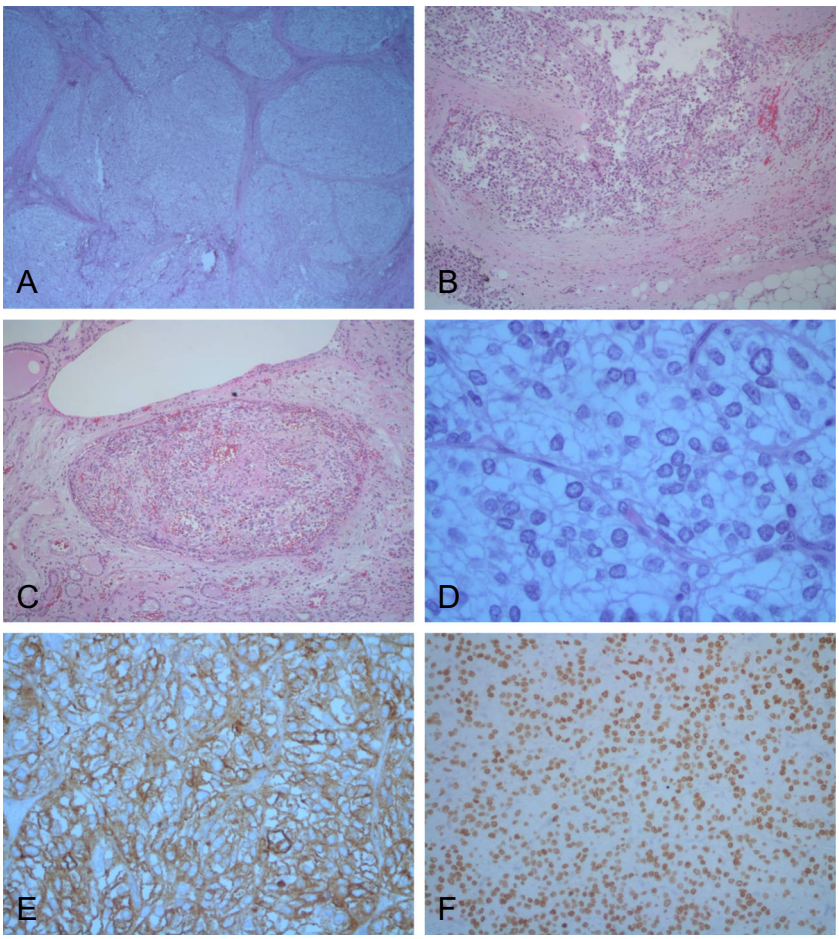

Fig. 1 Clear cell carcinoma of the thyroid. **a** Variably sized nodules with neoplastic clear cells (H&E, ×100); **b** the tumor extending beyond the thyroid capsule and invading into the perithyroid soft tissue (H&E, ×200); **c** vascular invasion (H&E, ×200); **d** clear cell morphology of the neoplastic cells (H&E, ×400); **e** TG (IHC, ×400); **f** p53 (IHC, ×400)

[1–3]. Distinguishing between these tumors by histological examination only may not be possible, ancillary tests, especially IHC, are necessary for confirmation. IHC should include markers for thyroid follicular epithelial cells, calcitonin for thyroid C-cells, and PTH for parathyroid tissue. TTF1, PAX8, and TG in combination represent a reliable IHC panel for thyroid follicular cells. TG is the most specific marker for thyroid follicular cells and its detection by IHC is diagnostic for metastatic thyroid follicular cell-derived carcinomas outside of the thyroid gland. However, poorly differentiated or anaplastic thyroid carcinomas and clear cell carcinoma of the thyroid might be negative for this marker [1, 11]. In addition, interpretation of TG IHC staining within the thyroid gland may be problematic because of its diffusion and nonspecific sequestration by adjacent cells, including metastatic tumor cells, may result in misinterpretation of this stain [12]. TTF1 and PAX8, embryonic developmental transcription factors for thyroid follicular cells, are typically retained in poorly differentiated thyroid carcinomas. Both have distinct nuclear staining that may minimize false positive interpretation. TTF-1 and PAX8 are also detected in most of lung adenocarcinomas and renal cell carcinomas, respectively. A dual positive staining for TTF-1 and PAX8 virtually is also diagnostic for carcinomas of thyroid follicular cell origin and permits their differentiation from metastatic renal cell carcinoma and adenocarcinomas of the lung [13].

The clearing of cytoplasm, which gives the characteristic microscopic appearance to the cells composing these tumors, has been attributed to accumulation of various substances, including glycogen, lipid, mucin, and thyroglobulin, or distended mitochondria or endoplasmic reticulum [1, 2, 4]. TSH-overstimulation has been proposed as one of underlying mechanism for this process [14]. In this case, the patient's serum levels of T3, T4, and TSH were within normal ranges before the thyroid surgery; however, a mutant *TSHR* gene (*TSHR L272V*) was identified. Mutations of *TSHR* genes can cause either inactivation (loss-of-function) [15] or activation (gain-of-function) of the receptors [16]. The gain-of-function mutations of *TSHR* are frequently associated with autonomous (or toxic) thyroid adenomas and more than 50 gain-of-mutations of *TSHR* have been identified [16]. *TSHR L272V* mutation has not been reported before and its functional consequences are unknown. However, a concomitant overexpression of the *TG* and *NIS* genes in this tumor, usually decreased in thyroid carcinomas [17, 18], suggests that the *TSHR L272V* may be a gain-of-function mutation. Gain-of-function mutants of *TSHR* can overstimulate thyroid follicular cells by the cAMP signaling cascade as the elevated level of TSH does. Thus, we suggest that gain-of-function mutation of *TSHR* might be an alterative mechanism for the development of clear cell morphology in thyroid neoplasms.

It is intrigue to notice that the current tumor was not hyperfunctioning as shown by normal serum levels of T3, T4, and TSH before the thyroid surgery. We hypothesize that this functional disparity might be due to additional impairments in the multiple steps of thyroid hormone synthesis and/or secretion of the neoplastic cells as a result of malignant transformation. These impairments resulted in a non-functioning thyroid tumor even in the presence of gain-of-function mutation of *TSHR* and contributed to its insidious growth and delayed manifestation.

Significant progress has been achieved in our understanding of molecular pathogenesis of thyroid carcinomas over recent years [19–22]. The recent effort of the Cancer Genome Atlas (TCGA) has identified the putative tumor-initiating genetic alteration in as much as 98.8 % of papillary thyroid carcinomas [19]. Common molecular alterations in thyroid cancer include point mutations in the *BRAF*, *NRAS*, and *KRAS* genes and *RET/PTC*, *PPARG/PAX8*, or *NTRK* fusions [19–22]. All of these genetic changes were studied using targeted NGS, but not identified in this case [8]. In addition to *TSHR L272V* mutation, the other significant finding is the identification of *TP53 R248Q*, a well-characterized loss-of-function mutation of this tumor suppressor gene [23]. The roles of *TP53* mutations are intrigue in thyroid carcinoma. They are rarely detected in differentiated thyroid carcinomas, but frequently present in poorly differentiated or anaplastic thyroid carcinomas, indicating *TP53* inactivations are more associated with dedifferentiation, progression, and metastasis, hence poorer prognosis, than initiation of thyroid carcinomas [24] [25–27]. Detection of *TP53 R248Q* in this tumor had correlated well with its aggressive pathologic and clinical features.

It is still debatable regarding the significance of clear cell morphology in thyroid carcinomas. Some have suggested clear cell carcinomas were more aggressive, frequently with extrathyroidal extension and distant metastases, and resulted in patients' death [3], whereas others considered that clear cell morphology did not change biological or clinical features of thyroid carcinomas and the clinical outcomes of clear cell follicular carcinomas of the thyroid are determined by the underlying histological types [1, 2]. The discrepancy might be due to the rarity of these tumors and inconsistence in morphological diagnoses. More and more evidences indicate that genetic alterations might be more relevant with pathologic and clinic features of thyroid carcinomas [19]. This report represents the first case of clear cell follicular carcinoma of the thyroid with molecular characterization utilizing NGS. Similar analysis on more cases is needed to elucidate the molecular pathogenesis and to define the clinical significance of this unique variant of thyroid carcinomas.

References

1. Carcangiu ML, Sibley RK, Rosai J. Clear cell change in primary thyroid tumors. A study of 38 cases. Am J Surg Pathol 1985;9:705–22.
2. Civantos F, Albores-Saavedra J, Nadji M, Morales AR. Clear cell variant of thyroid carcinoma. Am J Surg Pathol 1984;8:187–92.
3. Schroder S, Bocker W. Clear-cell carcinomas of thyroid gland: a clinicopathological study of 13 cases. Histopathology 1986;10:75–89.
4. Ropp BG, Solomides C, Palazzo J, Bibbo M. Follicular carcinoma of the thyroid with extensive clear-cell differentiation: a potential diagnostic pitfall. Diagn Cytopathol 2000;23:222–3.
5. Andrews GA, Kniseley RM. Transformation of thyroidal carcinoma to clear-cell type. Am J Clin Pathol 1956;26:1427–38.
6. Jayaram G. Cytology of clear cell carcinoma of the thyroid. Acta Cytol 1989;33:135–6.
7. Woolner LB, Beahrs OH, Black BM, Mc CW, Keating FR, Jr. Classification and prognosis of thyroid carcinoma. A study of 885 cases observed in a thirty year period. Am J Surg 1961;102:354–87.
8. Nikiforov YE, Carty SE, Chiosea SI, *et al.* Highly accurate diagnosis of cancer in thyroid nodules with follicular neoplasm/suspicious for a follicular neoplasm cytology by ThyroSeq v2 next-generation sequencing assay. Cancer 2014;120:3627–34.
9. Peter I, Besznyak I, Szanto J, *et al.* Clear cell thyroid cancer—undifferentiated type—an immunohistochemical and electron microscopical study. Arch Geschwulstforsch 1989;59:121–8.
10. Koike A, Naruse T, Kanemitsu T, *et al.* Clear cell carcinoma of the thyroid. A case report. Jpn J Surg 1989;19:237–40.
11. Harach HR, Franssila KO. Thyroglobulin immunostaining in follicular thyroid carcinoma: relationship to the degree of differentiation and cell type. Histopathology 1988;13:43–54.
12. Dayal Y, Ucci AA, Safaii H, Pohl D. Thyroglobulin and clear-cell tumors. Am J Surg Pathol 1986;10:70–2.
13. Cimino-Mathews A, Sharma R, Netto GJ. Diagnostic use of PAX8, CAIX, TTF-1, and TGB in metastatic renal cell carcinoma of the thyroid. Am J Surg Pathol 2011;35:757–61.
14. Diaz-Arias AA, Bickel JT, Loy TS, *et al.* Follicular carcinoma with clear cell change arising in lingual thyroid. Oral Surg Oral Med Oral Pathol 1992;74:206–11.
15. Cassio A, Nicoletti A, Rizzello A, *et al.* Current loss-of-function mutations in the thyrotropin receptor gene: when to investigate, clinical effects, and treatment. J Clin Res Pediatr Endocrinol 2013;5 Suppl 1:29–39.
16. Kleinau G, Biebermann H. Constitutive activities in the thyrotropin receptor: regulation and significance. Adv Pharmacol 2014;70:81–119.
17. Latif R, Ali MR, Ma R, *et al.* New small molecule agonists to the thyrotropin receptor. Thyroid 2015;25:51–62.
18. Ringel MD, Anderson J, Souza SL, *et al.* Expression of the sodium iodide symporter and thyroglobulin genes are reduced in papillary thyroid cancer. Mod Pathol 2001;14:289–96.
19. Cancer Genome Atlas Research Network. Integrated genomic characterization of papillary thyroid carcinoma. Cell 2014;159:676–90.
20. Fagin JA, Mitsiades N. Molecular pathology of thyroid cancer: diagnostic and clinical implications. Best Pract Res Clin Endocrinol Metab 2008;22:955–69.
21. Nikiforov YE. Thyroid carcinoma: molecular pathways and therapeutic targets. Mod Pathol 2008;21 Suppl 2:S37-43.
22. Xing M, Haugen BR, Schlumberger M. Progress in molecular-based management of differentiated thyroid cancer. Lancet 2013;381:1058–69.
23. Yoshikawa K, Hamada J, Tada M, *et al.* Mutant p53 R248Q but not R248W enhances in vitro invasiveness of human lung cancer NCI-H1299 cells. Biomed Res 2010;31:401–11.
24. Shahedian B, Shi Y, Zou M, Farid NR. Thyroid carcinoma is characterized by genomic instability: evidence from p53 mutations. Mol Genet Metab 2001;72:155–63.
25. Donghi R, Longoni A, Pilotti S, *et al.* Gene p53 mutations are restricted to poorly differentiated and undifferentiated carcinomas of the thyroid gland. J Clin Invest 1993;91:1753–60.
26. Fagin JA, Matsuo K, Karmakar A, *et al.* High prevalence of mutations of the p53 gene in poorly differentiated human thyroid carcinomas. J Clin Invest 1993;91:179–84.
27. Ito T, Seyama T, Mizuno T, *et al.* Unique association of p53 mutations with undifferentiated but not with differentiated carcinomas of the thyroid gland. Cancer Res 1992;52:1369–71.