CHROMOPHOBE RENAL CELL CARCINOMA
(CRCC) is a distinct subtype of renal cell carcinoma, possibly originating from the intercalated cells of collecting tubules [1, 2]. It comprises approximately 5% of all renal tumors. Birt-Hogg-Dubé syndrome and tuberous sclerosis complex (TSC) patients have an increased risk of developing CRCC, even young adults [3-6]. Microscopically, CRCC consists of large polygonal cells with a prominent cell border and cleared cytoplasm, resulting in a “plant cell appearance” [1-4]. The nuclei have wrinkled nuclear membranes and cells are frequently binuclear.

We encountered three cases of primary thyroid carcinoma exhibiting unique microscopic findings that have never been described previously to the best of our knowledge. The patients were adolescents or young
adults (AYA), and two of them had TSC. The histology was analogous to that of CRCC. Here, we report three cases of chromophobe renal cell carcinoma-like thyroid carcinoma (CRETHCA), a novel clinicopathologic entity possibly associated with TSC.

### Materials and Methods

We reviewed 12,064 primary thyroid carcinoma cases resected in Kuma Hospital from 2005 to 2015, and selected two cases (cases 1 and 2) with CRCC-like appearance. CRCC-like appearance was defined as a prominent cell border, perinuclear clear cytoplasm, a wrinkled nuclear membrane, and binucleation. One case (case 3) from Fukushima Medical University School of Medicine with similar histological findings was also included in this study. Immunohistochemical study was performed using following antibodies, thyroglobulin (polyclonal, 2 dilution, Dako, Glostrup, Denmark), TTF1 (8G7G3/1, 100 dilution, Dako Cytomation, Carpinteria, CA, USA), EMA (E29, 5 dilution, Dako, Carpinteria, CA, USA), PAX8 (polyclonal, 200 dilution, Protein Tech, Chicago, IL, USA) cytokeratin 7 (OV-TL 12/30, 50 dilution, DAKO, Carpinteria, CA, USA), cytokeratin 19 (RCK108, 250 dilution, Dako, Glostrup, Denmark) the anti-mitochondria antibody (AMA) (MTC02, 1.5 dilution, NeoMarkers, Fremont, CA, USA), calcitonin (polyclonal, 2 dilution, Dako, Glostrup, Denmark), carcinoembryonic antigen (COL1, 3 dilution, HISTOFINE, Tokyo, Japan), GATA3 (L50-823, 400 dilution, Biocare Medical, Concord, CA, USA), CD10 (56C6, 100 dilution, Novocastra, Newcastle, UK), CD117 (polyclonal, 50 dilution, Dako, Carpinteria, CA, USA), p63 (4A4, 1 dilution, HISTOFINE, Tokyo, Japan), E-cadherin (NCH38, 100 dilution, Glostrup, Denmark), MUC1 (Ma695, 100 dilution, Novocastra, Newcastle, UK), p53 (DO7, 1000 dilution, Dako, Glostrup, Denmark), and Ki-67 (MIB1, 200 dilution, Glostrup, Denmark). The mutational status of the BRAF (exon 15), NRAS, HRAS and KRAS (exons 2 and 3) genes was examined as described previously (case 1 and 2, [6]; case 3, [7]).

### Results

#### Case presentation (Table 1)

**Case 1**

The patient was a 15-year-old Japanese female with a history of TSC associated with epilepsy, a cardiac

| Table 1 Clinical and pathological findings of three chromophobe renal cell carcinoma-like thyroid carcinoma cases |
|---|---|---|
| **Age (years)** | Case 1 | Case 2 | Case 3 |
| **Gender** | Female | Male | Male |
| **Tuberous sclerosis complex** | + | − | + |
| **Location (lobe)** | Lt | Lt | Rt |
| **Size (cm)** | 4.7 | 10.0 | 1.3 |
| **Original histologic diagnosis** | FTC | PDTC | PTC, oxyphilic |
| **Treatment** | Lt Lo | TT + Lt MNLR | Rt Lo + Rt CNLR |
| **Encapsulation** | + | − | + |
| **with capsular invasion** | + | Not applicable | + |
| **Vascular invasion** | + (Extensive) | + (Extensive) | + |
| **Necrosis** | + | + | − |
| **Extrathyroidal invasion** | − | + | − |
| **Nodal metastasis (at surgery)** | − | + | + |
| **(after surgery)** | − | + | − |
| **Distant metastasis** | − | − | − |
| **Postoperative treatment** | TT + Lt MNLR | Bi MNLR + RI | None |
| **Outcome** | Alive | Alive | Alive |
| **(follow-up period)** | (13 years) | (6 years) | (15 months) |

Lt, Left; Rt, Right; FTC, follicular thyroid carcinoma; PDTC, poorly differentiated thyroid carcinoma; PTC, Papillary thyroid carcinoma; Lo, lobectomy; TT, total thyroidectomy; MNLR, modified neck lymph node resection; CNLR, central neck lymph node resection; Bi, Bilateral; RI, Radioactive iodine therapy.
The tumors were solid and whitish tan in color. In case 1 and 3, the tumors were mostly encapsulated and had focally infiltrated into the surrounding thyroid tissue; however, they were limited to the thyroid (Fig. 1). The tumor in case 2 occupied the left lobe, was non-encapsulated, and showed extensive infiltrative growth. The cut surface was markedly lobulated (Fig. 2).

**Pathological findings**

The patients were a 19-year-old Japanese male. A nodule, measuring 10.0 × 5.7 × 3.5 cm, was detected in his left thyroid. The nodule was visualized as a hot area by gallium scintigraphy. Serum thyroglobulin, T4, and TSH levels were 46.8 ng/mL, 0.98 ng/dL, and 1.664 µIU/mL, respectively. FNAC revealed suspected poorly differentiated thyroid carcinoma (PDTC). The patient underwent a total thyroidectomy with modified left lymph node dissection. The resulting pathological diagnosis was PDTC associated with nodal metastasis. Second and third lymph node dissections were performed when metastases appeared 2 and 4 years after the initial operation, respectively. Subsequently, the patient underwent radioactive iodine therapy and no metastatic foci were detected by the 6-years follow-up.

**Case 2**

The patient was a 19-year-old Japanese male. A nodule, measuring 10.0 × 5.7 × 3.5 cm, was detected in his left thyroid. The nodule was visualized as a hot area by gallium scintigraphy. Serum thyroglobulin, T4, and TSH levels were 46.8 ng/mL, 0.98 ng/dL, and 1.664 µIU/mL, respectively. FNAC revealed suspected poorly differentiated thyroid carcinoma (PDTC). The patient underwent a total thyroidectomy with modified left lymph node dissection. The resulting pathological diagnosis was PDTC associated with nodal metastasis. Second and third lymph node dissections were performed when metastases appeared 2 and 4 years after the initial operation, respectively. Subsequently, the patient underwent radioactive iodine therapy and no metastatic foci were detected by the 6-years follow-up.

**Case 3**

The patient was a 21-year-old Japanese male. He had a history of TSC associated with epilepsy, bilateral renal cysts, and renal angiomyolipoma. His serum thyroglobulin, T4, and TSH levels were 11.4 ng/mL, 1.06 ng/dL, and 2.150 µIU/mL, respectively. A nodule measuring 16.9 mm in the greatest dimension was found by ultrasonographic examination of the right lobe of the thyroid. The tumor was irregular, heterogeneous, and low echoic, and diagnosed as oxyphilic variant of papillary thyroid carcinoma (PTC) based on FNAC findings. The patient underwent a right lobectomy with central neck lymph node resection. The histologic diagnosis was same as that from FNAC. Neither regional recurrences nor distant metastases were found during the 15-month follow-up.
Microscopic findings of the carcinoma cells seen in the three cases were mostly similar. The tumors were composed predominantly of large polygonal carcinoma cells showing a trabecular pattern (Fig. 3). Focally, alveolar (Fig. 4) and microfollicular patterns were seen. The tumor nests were surrounded by thin vascular stroma. The lumen of the microfollicles displayed inspissated colloid. The cell membrane of the carcinoma cells was well defined. The cytoplasm was abundant, finely granular, and eosinophilic. The nuclei were round to irregular and had prominent nucleoli (Fig. 5). The chromatin was pale and vesicular.

Binuclear carcinoma cells were frequently observed (Fig. 5). Markedly irregular nuclei with perinuclear clearing mimicked koilocytosis (Fig. 6).

In case 1, carcinoma cells with abundant eosinophilic cytoplasm were focally present (Fig. 7), and the colloid within the microfollicles was occasionally associated with calcification mimicking psammoma bodies. Vascular invasion was present in all cases, and extensive in case 1 and 2 (Fig. 8). Necrotic areas were focally observed in case 1 and 2. Case 2 showed extrathyroidal invasion. In case 1 and 3, the carcinoma was limited to the thyroid. Case 2 and 3 revealed nodal metastasis.

Fig. 3 The tumor is composed of large polygonal carcinoma cells with a trabecular pattern (Case 2).

Fig. 4 Carcinoma cells showing alveolar growth. The nests are surrounded by thin vascular stroma (Case 3).

Fig. 5 The cytoplasm is abundant, finely granular, and eosinophilic. Binuclear carcinoma cells are frequently observed (Case 3).

Fig. 6 Carcinoma cells with markedly irregular shaped nuclei and perinuclear clearing mimicking koilocytosis (Case 2).
Chromophobe RCC-like thyroid carcinoma

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Cytoplasm (eosinophilic variant) may predominate [1, 2]. Histopathology of the three cases we studied showed infiltrative growth, a trabecular pattern, rich vascular stroma, well-defined cell membranes, markedly irregular nuclei, binucleation, a koilocytotic appearance, and

Histochemical and immunohistochemical findings

Histochemical and immunohistochemical results were essentially similar for the three cases and are shown in Table 2. Hale’s colloidal iron staining was focally and weakly positive in case 2 (Fig. 9), but negative in case 1 and 3. Immunohistochemically, the tumor cells were positive for thyroglobulin (Fig. 10a), TTF1 (Fig. 10b), and EMA, but the staining was not intensive. PAX8 (Fig. 10c) was strongly immunoreactive. Cytokeratin 7 and cytokeratin 19 were detected at the periphery of the cytoplasm. Similarly, AMA was expressed at the periphery (Fig. 10d). On the other hand, the carcinoma cells tested negative for calcitonin, carcinoembryonic antigen, GATA3, CD10, CD117, and p63. E-cadherin was detected in the cell membrane. The apical cell membrane of carcinoma cells was focally positive for MUC1, while p53 staining was weakly positive. Ki-67 labeling indices for case 1, 2, and 3 were 10.5%, 8.2%, and 3.5%, respectively.

Table 2  Histochemical, immunohistochemical and mutational findings for three chromophobe renal cell carcinom-like thyroid carcinoma cases

<table>
<thead>
<tr>
<th>Test for</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
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<tr>
<td>Hale’s colloidal iron</td>
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<tr>
<td>Thyroglobulin</td>
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<tr>
<td>KRAS exons 2 and 3</td>
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−, negative staining; +, focally and weakly positive staining; ++, diffusely and moderately positive staining; ++++, diffusely and strongly positive staining; wt, wild-type.

Mutational analysis

BRAF, NRAS, HRAS, and KRAS mutations were not detected in any of the three cases.

Discussion

Microscopically, CRCC is characterized by large polygonal cells with prominent cell borders, perinuclear halos, wrinkled nuclear membranes, and binucleation, resulting in a “plant cell appearance” or koilocytosis [1-4, 8]. Carcinoma cells with granular and eosinophilic cytoplasm (eosinophilic variant) may predominate [1, 2]. Histopathology of the three cases we studied showed infiltrative growth, a trabecular pattern, rich vascular stroma, well-defined cell membranes, markedly irregular nuclei, binucleation, a koilocytotic appearance, and
Fig. 9  Hale’s colloidal iron staining is focally and weakly positive (Case 2).

Fig. 10  Carcinoma cells are weakly positive for thyroglobulin (a: Case 1) and TTF-1 (b: Case 3). PAX8 is strongly positive (c: Case 1). Anti-mitochondria antibody is expressed at the periphery of the cytoplasm (d: Case 3).
associated vascular invasion. The histologic findings of
the carcinoma cells in our three cases and CRCC were
similar. Metastatic renal cell carcinoma (RCC) can
coccur as a solitary lesion and even before a diagnosis of
RCC is established [9, 10]. Therefore, we should distin-
guish our cases from metastatic RCC. However, there
is no doubt that they primarily occurred in the thyroid,
because they were immunohistochemically positive for
thyroglobulin and TTF1, and negative for CD10, which
is expressed in RCC.

Our cases (1, 2, and 3) had been originally diagnosed
as FTC, PDTC, or oncocytic variant of PTC, respect-
ively. However, FTC was excluded by the predom-
inant trabecular growth pattern and marked nuclear
irregularity of the tumor cells, which are features of
PDTC [11-13]. Indeed, the predominant trabecular
growth pattern, cellular atypism, necrosis, and vascular
invasion indicated PDTC. However, compared to usual
cases of PDTC, the outcome was extremely favorable
and the patients were younger [11-13]. The trabecular
growth pattern and abundant eosinophilic cytoplasm
may point to a diagnosis of poorly differentiated onco-
cytic follicular carcinoma (PODOFC) [14]. However,
oncocytic carcinoma cells were a minor component in
our cases. In addition, perinuclear halos and wrinkled
nuclear membranes, which were observed in our cases,
are not features of PODOFC. In addition, the mutational
analysis that we performed did not identify the genetic
alterations that were usually detected in PTC and FTC.

To the best of our knowledge, CRETHCA has not been
described previously.

Interestingly, CRETHCA and CRCC share common
clinical findings. Two of the three cases we studied were
TSC patients. TSC is a genetic disorder that causes
tumors in many different organs, primarily in the brain,
eyes, heart, kidney, skin, and lungs [3, 4]. The incidence
of RCC in TSC patients is reported to be 2% to 4%,
which is higher than that in the general population [15].
CRCC comprises 5% to 10% of RCC cases, but RCCs
in TSC patients are predominantly classified as CRCC
[3, 16-18]. CRCC is diagnosed mainly in the sixth
decade of life, but TSC-associated CRCC is diagnosed
more frequently at a younger age, and our cases were
also in AYA. According to a report by Guo et al. [3],
there was no evidence of metastatic disease after surgery
in TSC-associated RCC. Our cases included vascular
invasion and a growth pattern consistent with PDC, but
nevertheless, the outcome was favorable.

Patients with TSC present with multiple tumor types
involving multiple organs. There are several reports
of thyroid lesions in patients with TSC. Auladell et al.
retrospectively reviewed chest computed tomography
scans of 93 patients with TSC [19], and found thyroid
abnormalities in 19 (20.4%) patients. However, a his-
tological examination was performed only in two cases
(one follicular adenoma, one papillary carcinoma).
Dicorato et al. reported a case of medullary thyroid
carcinoma associated with TSC [20]. Thus far, how-
ever, the relationship between thyroid tumors and TSC
has not been established beyond doubt.

As two CRETHCA cases were associated with TSC,
we think that the etiopathogenesis of CRETHCA could
link to the genetic abnormality of TSC. The occur-
cence of CRETHCA in AYA and favorable prognosis
suggests this possibility. The case of CRETHCA not
associated with TSC in this report may be a sporadic
form. This is similar to the cribriform variant of PTC,
which involves familial polyposis coli-associated and
sporadic cases, occurrence in AYA, and favorable prog-
nosis regardless of the presence of solid growth and tall
cell components [21, 22].

In conclusion, we report three CRETHCA cases
with unique microscopic findings that have not been
described previously. They involve AYA who had a
favorable prognosis regardless of vascular invasion
and growth patterns resembling PDTC. In two cases,
the TSC genetic abnormality seemed to be related to
the etiopathogenesis of CRETHCA. Further genetic
studies are necessary to confirm the hypothesis.

Disclosure

The authors have no conflicts of interest to declare
regarding grant support or financial relationships.

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