Chromophobe renal cell carcinoma with neuroendocrine differentiation/morphology: A clinicopathological and genetic study of three cases

Chisato Ohe MD a,⁎, Naoto Kuroda MD b, Keiko Matsuura MD, PhD c, Tomoki Kai MD c, Masatsugu Moriyama MD, PhD c, Shun Sugiguchi MD d, Shintaro Terahata MD d, Naoki Hosaka MD, PhD e, Ondrej Hes MD, PhD f, Michal Michal MD f, Tadashi Matsuda MD g, Yoshiko Uemura MD a

a Department of Diagnostic Pathology, Kansai Medical University, Hirakata Hospital, Osaka, 573-1191, Japan
b Department of Diagnostic Pathology, Kochi Red Cross Hospital, Kochi, 780-0062, Japan
c Department of Molecular Pathology, Faculty of Medicine, Oita University, Oita 879-5593, Japan
d Department of Pathology, Tonami General Hospital, Toyama 939-1395, Japan
e Department of Pathology, Kansai Medical University, Kori Hospital, Osaka, 572-8551, Japan
f Department of Pathology, Faculty of Medicine in Plzen, Charles University in Prague, 304 60 Plzen, Czech Republic
g Department of Urology and Andrology, Kansai Medical University, Hirakata Hospital, Osaka, 573-1191, Japan

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Abstract Chromophobe renal cell carcinoma (ChRCC) with neuroendocrine differentiation/morphology (NED/NEM) is exceedingly rare. We present three cases of ChRCC with NED/NEM, two of which showed positivity for neuroendocrine markers on immunohistochemical analysis. Patients ranged in age from 49 to 79 years (mean: 64.3 years). One of the three patients died of metastatic disease to multiple organs. Of the remaining two patients, one is currently alive without disease and the other is alive with disease. Histologically, all three tumors were composed of conventional ChRCC and NEM showed glandular and rosette formation. Immunohistochemically, tumor cells were positive for CK7, KAI1, E-cadherin, and c-kit in both ChRCC and neuroendocrine areas in three cases. CD56 and synaptophysin immunoreactivity were detected in two cases; in only the neuroendocrine area in one case and in both components in the other. Neuroendocrine granules were ultrastructurally observed at both neuroendocrine and conventional areas of ChRCC. Array comparative genomic hybridization (CGH) study indicated losses of chromosomes 1, 2, 6, 10, 17, 21, and Y in both conventional ChRCC and NED in one case. In addition, losses of chromosomes 1, 2, 4, 6, 9, 10, 13, 16p, 17, and 21 were observed in both

⁎ Corresponding author at: Department of Diagnostic Pathology, Kansai Medical University, Hirakata Hospital, 2-3-1 Shinmachi, Hirakata, Osaka, 573-1191, Japan. Tel.: +81 72 804 2794.
E-mail addresses: ohec@hirakata.kmu.ac.jp (C. Ohe).

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components of the remaining one tumor. Furthermore, loss of chromosome 5 was identified only in the neuroendocrine area in this case. We concluded that the neuroendocrine area may reflect dedifferentiation within ChRCC. It is possible that losses of chromosomes 4, 5, and 16p may be involved in the neuroendocrine differentiation or progression of ChRCC.

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1. Introduction

Chromophobe renal cell carcinoma (ChRCC) accounts for about 5% of total renal neoplasms. ChRCC is recognized as a distinct subtype of RCC with a relatively good prognosis compared to clear cell RCC. In contrast, tumors with sarcomatoid changes and perinephric invasion may show an aggressive clinical course [1,2]. Cytogenetically, losses of chromosomes 1, 2, 6, 10, 13, 17, and 21 are frequently noted in conventional ChRCC [3]. The gains of several chromosomes have been reported in sarcomatoid ChRCC [4]. Two cases of ChRCC with neuroendocrine differentiation (NED) have been reported [5,6]. It was suggested that the neuroendocrine area may occur as a result of dedifferentiation within ChRCC [5,6]. However, to our knowledge, there have been no reports regarding genetic alterations in ChRCC with NED. Here, we present two cases of ChRCC with NED and one case of ChRCC with neuroendocrine morphology (NEM), and discuss the clinicopathological findings and genetic alterations.

2. Materials and methods

Three cases of ChRCC with NEM were extracted for this study from 105 ChRCC cases diagnosed in Kansai Medical University Hirakata Hospital (case 1) and Kochi Red Cross Hospital, including consultation files (cases 2 and 3; case 3 originated from Tonami General Hospital) between 2006 and 2013. Among these three cases of ChRCC with NEM, two showed positivity for neuroendocrine markers on immunohistochemical analysis. Therefore, a diagnosis of ChRCC with NED was made in these two cases (cases 1 and 2), while the third was diagnosed as ChRCC with NEM (case 3). One case (case 2) was described previously [6].

2.1. Morphology and immunophenotypic studies

For all formalin-fixed and paraffin-embedded (FFPE) renal tumors from nephrectomy, sections 3 μm thick were stained with hematoxylin and eosin (H&E). Representative blocks from each case were selected for immunohistochemical studies using the Ventana Autostainer Benchmark XT (Ventana Medical Inc., Tucson, AZ). Primary antibodies to the following antigens were employed: CK7 (OV-TL 12/30, 1:100; DAKO, Glostrup, Denmark), KAI1 (G2, 1:100; Santa Cruz Biotechnology, Santa Cruz, CA), CD117 (polyclonal, prediluted; Nichirei, Tokyo, Japan), E-cadherin (NCH-38, 1:100; DAKO), CD56 (1B6, prediluted; Nichirei), synaptophysin (27G12, prediluted; Nichirei), and chromogranin A (LK2H10, prediluted; Japan Tanner, Osaka, Japan). The primary antibodies were visualized using a Ventana I-VIEW DAB Universal kit (Roche Diagnostics KK, Tokyo, Japan).

2.2. Ultrastructural studies

Materials included in paraffin blocks from two cases (cases 1 and 2) were processed for transmission electron microscopy to evaluate the presence of neuroendocrine granules. Small sections were extracted from both conventional ChRCC and neuroendocrine areas. Specimens were deparaffinized, fixed in 2% osmium tetroxide, and embedded in epoxy resin. Ultrathin sections were cut with a Reichert microtome, stained with uranyl acetate and lead citrate, and examined with an electron microscope (JEM-1400A; JEOL, Tokyo, Japan). Micrographs were taken at different magnifications (×4000–×50000).

2.3. Genomic DNA extraction and array comparative genomic hybridization

Written informed consent was obtained from two patients (cases 1 and 2) for genetic studies. Genomic DNA was extracted from FFPE tissue samples as described previously [7]. The use of tissue samples for all experiments was approved by the Oita University Ethics Committee (approval no. 700) in accordance with the Ethical Guidelines for Clinical Research of the Japanese Ministry of Health, Labour and Welfare, 2008 (http://www.mhlw.go.jp/english/). The Agilent Human Genome Array CGH microarray 44K (Agilent Technologies, Palo Alto, CA) was used for array-comparative genomic hybridization (CGH) analysis. Genomic DNA was extracted from the tumor in both conventional ChRCC and neuroendocrine areas, and the non-tumor region from the
same patients. Genomic DNA from the tumor and non-tumor region was hybridized (each sample: 2 μg), and was subjected to array-CGH in accordance with the manufacturer’s instructions. The arrays were washed, scanned with an Agilent 2565AA DNA microarray scanner (Agilent Technologies), and processed with the Agilent Feature Extraction software (version 9.5.3.1; Agilent Technologies) with linear normalization (protocol CGH-v4_95_Feb07) and the resulting data were subsequently imported into the DNA Analytics v. 4.0.81 software package (Agilent Technologies). Aberrant regions were determined by the Aberration Detection Method (ADM)-2 algorithm at a threshold of 1.0 in DNA Analytics. To detect gains and losses of chromosome regions, we set the values of parameters for aberration filters as follows: minimum number of probes in region 2, minimum absolute average log2 ratio for region 0.15, maximum number of aberrant regions 10000, and percentage penetrance per feature 0. The data obtained in array CGH analysis are available at the GEO database (http://www.ncbi.nlm.nih.gov/geo/; accession numbers GSE52641).

3. Results

3.1. Clinical features

The basic clinicopathological data of the patients are summarized in Table 1. Briefly, the patients ranged in age from 49 to 79 years with a mean of 64.3 years, and all patients were men. One (case 3) of three patients died of metastatic cancer to multiple organs (lung, liver, and bone) 14 months after nephrectomy. Of the remaining two patients, one (case 1) is currently alive without disease 9 months after partial nephrectomy, and the other (case 2) is alive with suspected lung and periaortic lymph node metastasis by computed tomography (CT) scan 39 months after surgery.

### Table 1  Summary of clinicopathological information of ChRCC with neuroendocrine differentiation (cases 1 and 2)/neuroendocrine morphology (case 3).

<table>
<thead>
<tr>
<th>Case</th>
<th>Age/sex</th>
<th>Clinical presentation</th>
<th>Size (cm)</th>
<th>TNM classification</th>
<th>Clinical outcome</th>
<th>Follow-up (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>49/M</td>
<td>Incidental</td>
<td>2.2 × 2.0</td>
<td>pT3aN0M0</td>
<td>No metastasis or recurrence</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>79/M</td>
<td>Significant weight loss</td>
<td>22 × 12</td>
<td>pT3bN0M0</td>
<td>Alive with disease (suspected lung and periaortic lymph node metastases by CT scan)</td>
<td>39</td>
</tr>
<tr>
<td>3</td>
<td>65/M</td>
<td>Right flank pain</td>
<td>11.5 × 10.5</td>
<td>pT3aNXM0</td>
<td>Died of disease (lung, liver, and bone metastases)</td>
<td>14</td>
</tr>
</tbody>
</table>

M: male, F: female.

Fig. 1  Representative macroscopic results. A: The tumor was well-circumscribed and beige in color (bar = 1 cm). B: On the cut surface, a tumor with cystic change was observed outside the capsule and in the perinephric fat tissue (arrow) (bar = 1 cm).
Fig. 2  Representative H&E staining of chromophobe RCC (ChRCC) with neuroendocrine differentiation (cases 1 and 2)/neuroendocrine morphology (case 3). A–C, J–L, case 1; D–F, case 2; G–I, case 3. A, D, and G: Conventional area of ChRCC. B, E, and H: The smaller neoplastic cells showed glandular and rosette formation. C, F, and I: Neuroendocrine morphology and ChRCC component showed a gradual transition with an area of smaller cells and nuclear overcrowding. J: A tubular and cystic pattern was also identified. K: The small neoplastic cells with nuclear overcrowding were observed. L: Mitotic figures were seen. (A–I, L: original magnification ×400, J–K: ×200).

Table 2  Results of immunohistochemistry.

<table>
<thead>
<tr>
<th>case</th>
<th>CK7</th>
<th>KAI1</th>
<th>E. cadherin</th>
<th>c-kit</th>
<th>CD56</th>
<th>Synaptophysin</th>
<th>Chromogranin A</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. ChRCC</td>
<td>d+</td>
<td>d+</td>
<td>d+</td>
<td>d+</td>
<td>d+</td>
<td>f+</td>
<td>−</td>
</tr>
<tr>
<td>Neu</td>
<td>d+</td>
<td>d+</td>
<td>d+</td>
<td>d+</td>
<td>d+</td>
<td>d+</td>
<td>−</td>
</tr>
<tr>
<td>2. ChRCC</td>
<td>d+</td>
<td>d+</td>
<td>d+</td>
<td>d+</td>
<td>−*</td>
<td>f+</td>
<td>−</td>
</tr>
<tr>
<td>Neu</td>
<td>d+</td>
<td>d+</td>
<td>d+</td>
<td>d+</td>
<td>d+</td>
<td>f+</td>
<td>−</td>
</tr>
<tr>
<td>3. ChRCC</td>
<td>d+</td>
<td>d+</td>
<td>d+</td>
<td>d+</td>
<td>−</td>
<td>−*</td>
<td>−</td>
</tr>
<tr>
<td>Neu</td>
<td>d+</td>
<td>f+</td>
<td>d+</td>
<td>d+</td>
<td>−</td>
<td>−*</td>
<td>−</td>
</tr>
</tbody>
</table>

ChRCC: conventional chromophobe renal cell carcinoma; Neu: neuroendocrine area; +: positive; -: negative, −*: basically negative, but a small number cells showed expression; d: diffuse; f: focal.
3.2. Pathological findings

The largest dimensions of the tumors ranged from 2.2 cm to 22 cm (average, 11.9 cm). All of the tumors were well-circumscribed single masses, brown to beige in color (Fig. 1A). In two cases (cases 1 and 2), cancer invasion to perinephric fat tissue was seen. In case 3, cancer invasion to renal sinus fat was recognized. In case 1, the tumor with cystic change was observed outside the capsule and in the perinephric fat tissue (Fig. 1B).

Representative H&E figures of three cases are shown in Fig. 2 (A–C, case 1; D–F, case 2; G–I, case 3). Histologically, all three tumors consisted of large cells with abundant reticular translucent cytoplasm and smaller cells with scant eosinophilic cytoplasm. The cell borders were distinct and perinuclear haloes were identified in eosinophilic cells (Fig. 2A, D, and G). The nuclei in both types showed slight pleomorphism with wrinkling of the nuclear membrane, and binucleated cells were also seen. These characteristics were consistent with typical and eosinophilic chromophobe RCC with NED/NEM.
ChRCC. In all cases, nesting growth pattern was predominant, but tubular and cystic patterns were also seen in case 1 (Fig. 2J). In two of three cases (cases 1 and 3), the eosinophilic cell component was predominant. In the eosinophilic cell proliferation area, smaller neoplastic cells were observed with nuclear overcrowding (Fig. 2K). In addition, glandular and rosette formations of small neoplastic cells were also seen (Fig. 2B, E, and H). This neuroendocrine morphology (NEM) and ChRCC component showed a gradual transition with the area of smaller neoplastic cells and nuclear overcrowding (Fig. 2C, F, and I). Sarcomatoid changes were seen in two cases, one (case 2) accounted for approximately 1% of the neoplasm, and another accounted about 15% (case 3). Calcification was also observed in all cases. In one case (case 1), mitotic figures were recognized up to 5 mitoses per 10 high power fields (HPF) (Fig. 2L), although mitosis was hardly seen in the other two cases.

3.3. Immunohistochemical findings

Immunohistochemical results are summarized in Table 2. Both conventional ChRCC area and neuroendocrine area of all three tumors showed diffuse reactivity for CK7, KAI1, E-cadherin, and c-kit (Fig. 3A–D). In the conventional ChRCC areas, one case (case 1) showed positivity for CD56 and synaptophysin (Fig. 3E–F). In neuroendocrine areas of two cases (cases 1 and 2), CD56 and synaptophysin showed reactivity (Fig. 3G–H), but one case (case 3) was negative for neuroendocrine markers. All cases were negative for chromogranin A.

3.4. Ultrastructural findings

The ultrastructure of the tumor cells in two cases showed marked formalin fixation artifacts. In both of these cases, the cytoplasm of the tumor cells contained dense-cored neuroendocrine granules measuring about 150–350 nm. These granules were observed in the cytoplasm of both the neuroendocrine area (Fig. 4A) and the conventional area of ChRCC (Fig. 4B).

3.5. Genetic findings

In case 2, chromosomes 1, 2, 6, 10, 17, 21, and Y were lost in both the conventional ChRCC area and neuroendocrine area (Fig. 5A:a–b). In case 1, losses of chromosomes 1, 2, 4, 6, 9, 10, 13, 16p, 17, and 21 were observed in both components of the tumor (Fig. 5A:c–d), whereas loss of chromosome 5 was identified only in the neuroendocrine area (Fig. 5A:d). As shown in Fig. 5A-c, A-d, and B, deletion at 16p including 16p13 contained a small area of copy number loss. No chromosomal gains were observed in the two tumors examined.
Fig. 5  A: Array CGH profiles of chromophobe RCC (ChRCC) with neuroendocrine differentiation (NED) cases. Whole genomic profiles of case 1 (a, b) and case 2 (c, d) are shown. Horizontal lines: oligonucleotide probes are shown in order from chromosomes 1–22. Vertical lines above the center represent regions of gain, and those below the center represent regions of loss. The shaded areas indicate regions of copy number aberrations according to the ADM-2 algorithm. a: Typical ChRCC area of case 1. b: ChRCC with NED area of case 1. In both areas, losses of chromosomes 1, 2, 6, 10, 17, 21, and Y were seen. c: Typical ChRCC area of case 2. d: ChRCC with NED area of case 2. In both components, losses of chromosomes 1, 2, 4, 6, 9, 10, 13, 16p, 17, and 21 were observed (the red arrow indicates loss of chromosome 16p). Loss of chromosome 5 was also noted only in the neuroendocrine area (blue arrow). B: Detailed genomic profiles of chromosome 16 indicated by a red arrow in A-c and d are shown. Horizontal lines above the center represent regions of gain, and those below the center represent regions of loss. The deletion at 16p including 16p13 contained a small area of copy number loss.
4. Discussion

Some primary renal tumors showing NED, including carcinoid tumor, small cell carcinoma, and large cell neuroendocrine carcinoma, have been reported [8–12]. In the kidney, it is rare that neuroendocrine components are admixed with RCC components. There have been only a few reports of mucinous tubular spindle cell carcinoma of the kidney with NED [13,14], and ChRCC with NED [5,6]. This phenomenon should be strictly distinguished from primary neuroendocrine tumors of the kidney [8–12]. A case of combined ChRCC and carcinoid tumor without overlap of these components has been described [15]. On the other hand, a case of tubular ChRCC resembling neuroendocrine tumor has also been noted [16]. These cases were also different from ChRCC with NED. All three cases had no past history of neuroendocrine tumor, therefore a renal metastasis from another site would be excluded.

Parada and Pena described the first case of ChRCC with NED in a 56-year-old man [5]. The authors reported that the tumor showed a mixture of typical and cosinophilic patterns of ChRCC with neuroendocrine areas. Immunohistochemically, both ChRCC and neuroendocrine areas showed positivity for CK7 and c-kit. The neuroendocrine areas were positive for chromogranin A and CD56, while there was no evidence of neuroendocrine expression in the conventional ChRCC areas.

In this study, histological and immunohistochemical features were consistent with the diagnosis of ChRCC. In addition, NEM including glandular and rosette formations was recognized on light microscopy. In two of three cases, we diagnosed ChRCC with NED because the area of NEM was immunohistochemically positive for neuroendocrine markers. Notwithstanding, one case was diagnosed as ChRCC with NEM, as NEM showed no reaction for neuroendocrine markers. Both ChRCC and NEM areas showed the same immunohistochemical pattern of CK7, KA11, E-cadherin, and c-kit, known as chRCC markers [1,2]. Interestingly, in this study, neuroendocrine areas as well as conventional ChRCC areas in one case were immunohistochemically positive for neuroendocrine markers and neuroendocrine granules were also observed on ultrastructural examination. These immunohistochemical and ultrastructural results suggested that ChRCC and NEM area may share the same origin.

With regard to the pathogenesis of primary neuroendocrine tumor, it has been suggested that neuroendocrine tumors may originate from i) misplaced or entrapped neural crest, ii) neuroendocrine differentiation of primitive totipotent stem cells, or iii) preexisting neuroendocrine cell hyperplasia from metaplastic/teratomatous epithelium, because neuroendocrine cells are basically absent from the normal kidney [11]. In the case of ChRCC with NED/NEM, we suggest that differentiation toward neuroendocrine cells may show dedifferentiation similar to the sarcomatoid change or rhabdoid differentiation.

In this study, one patient died of the tumor 14 months after the pathological diagnosis, and lung and lymph node metastases were suspected by CT scan in one patient. With regard to the prognostic parameters for ChRCC, sarcomatoid change and higher pT stage (pT3a) were reported to be correlated with aggressive behavior [2,17]. In the present study, two of three cases had a huge mass with focal sarcomatoid growth. ChRCC with sarcomatoid differentiation is known to its aggressive clinical behavior. In one of the largest series to date, the mean percentage of sarcomatoid differentiation was 67% [18]. However, in our cases, the areas showing sarcomatoid change represented a low proportion of each tumor. Therefore, we estimated that the sarcomatoid change in our case had a relatively low impact on patient’s prognosis. On the other hand, one case was at high pT stage (pT3a) regardless of the small renal mass without sarcomatoid lesions. Accordingly, pathologists should be aware that NED/NEM in ChRCC may also reflect a poor prognosis. Recently, Paner et al. proposed chromophobe tumor grading based on the assessment of geographic nuclear crowding and anaplasia, because it is controversial whether the Fuhrman nuclear grade of ChRCC has prognostic utility or not [19]. In the present study, the ChRCC showed a gradual transition to NEM with an area of smaller neoplastic cells and nuclear overcrowding. Consequently, the geographic nuclear crowding proposed by Paner et al. may be the essential first step for ChRCC with NEM. In terms of prognosis, it is important to distinguish ChRCC with NEM from renal small cell oncocytoma with pseudorosettes [20]. Pathologists should recognize this tumor entity, because these two tumors were different from benign or malignant.

Here, we presented the first report of NED in ChRCC with genetic abnormalities. Losses of chromosomes 1, 2, 6, 10, 13, 17, and 21 are frequently observed in ChRCC [3]. In addition, gains of chromosomes 4, 7, 15, 19, and 20 are often identified irrespective of Paner grade [21]. In the present study, however, no chromosomal gain was observed in two cases of ChRCC examined. Chromosomal aberrations of one case were almost the same as the results reported previously in ChRCC, but the other case showed additional copy number aberrations, such as losses of chromosomes 4, 6, and 16p. Furthermore, loss of chromosome 5 was selectively identified in the neuroendocrine area. To our knowledge, loss of chromosome 16p in ChRCC has not been reported previously [22]. In addition, loss of chromosome 4 seems to be less frequent in ChRCC and losses of chromosomes 3, 5, and 9 were observed in 23%–40% of ChRCC cases [22]. Losses of chromosome 4, 4p, or 4q have been noted in some neuroendocrine tumors at other anatomical sites, including the thyroid gland, digestive tract, thymus, and skin [23–26]. Loss of chromosome 5q was detected in 10% of thymus neuroendocrine tumors, and loss of heterozygosity (LOH) in chromosome 5q occurred in all tumors of pulmonary large cell...
neuroendocrine carcinoma \[25,27\]. LOH of chromosome 16p13 was identified in a case of malignant islet cell tumor associated with tuberous sclerosis, consisted with the present findings \[28\]. Similar to clear cell RCC, losses of chromosomes 4p and 9p may be associated with poor prognosis \[29\]. Taking all of these results into consideration, it is possible that losses of chromosomes 4, 5, and 16p may be involved in the neuroendocrine differentiation or progression of ChRCC. Further large-scale studies are needed to identify the genes responsible for neuroendocrine differentiation in ChRCC.

5. Conclusions

We reported two cases of ChRCC with NED and one case of ChRCC with NEM. The NED/NEM may reflect dedifferentiation within ChRCC. It is possible that losses of chromosomes 4, 5, and 16p are involved in the neuroendocrine differentiation or progression of ChRCC. We suggest that ChRCC with NED/NEM may show an aggressive clinical course.

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References


