The BJBMS publishes an "Advanced online" manuscript format as a free service to authors in order to expedite the dissemination of scientific findings to the research community as soon as possible after acceptance following peer review and corresponding modification (where appropriate). An "Advanced online" manuscript is published online prior to copyediting, formatting for publication and author proofing, but is nonetheless fully citable through its Digital Object Identifier (doi®). Nevertheless, this "Advanced online" version is NOT the final version of the manuscript. When the final version of this paper is published within a definitive issue of the journal with copyediting, full pagination, etc. the new final version will be accessible through the same doi and this "Advanced online" version of the paper will disappear.

RESEARCH ARTICLE

NEW AND EMERGING MEDICAL ENTITIES

Joanna Rogala, et al: Chromophobe renal carcinoma analysis

Small cell variant of chromophobe renal cell carcinoma: Clinicopathologic, and molecular-genetic analysis of 10 cases.

Joanna Rogala¹,², Fumiyoshi Kojima³, Reza Alaghehbandan⁴, Nikola Ptakova⁵, Ana Brave⁶, Stela Bulimbasic², Delia M. Perez Montiel⁸, Maryna Slisarenko⁹, Leila Ali¹⁰, Levente Kuthi¹¹, Kristýna Pivovarčíková¹, Květoslava Michalová¹, Boris Bartovic¹², Adriena Bartos Vesela¹³, Olga Dolejsova¹³, Michal Michal¹, Ondrej Hes¹⁰

¹Department of Pathology, Charles University in Prague, Faculty of Medicine in Plzeň, Pilsen, Czech Republic.
²Department of Pathology “Hist- Med”, Regional Specialized Hospital in Wroclaw, Poland.
³Department of Human Pathology, Wakayama Medical University, Wakayama, Japan.
⁴Department of Pathology, Faculty of Medicine, University of British Columbia, Royal Columbian Hospital, Vancouver, BC, Canada.
⁵Department of Biology and Medical Genetics, 2nd Faculty of Medicine, Charles University in Prague and Motol University Hospital, Czech Republic.
⁶Department of Pathology, General Hospital, Slovenj Gradec, Slovenia.
⁷Department of Pathology, University Hospital Centre Zagreb, Zagreb, Croatia.
⁸Department of Pathology, Institute Nacional de Cancerologia, Mexico City, Mexico.
⁹Department of Pathology, CSD Laboratory, Kiev, Ukraine.
¹⁰Department of Pathology, 'Carol Davila' University of Medicine and Pharmacy, Bucharest, Romania.
11Department of Pathology, University of Szeged, Szeged, Hungary.
12Department of Pathology, Cytopathos, Bratislava, Slovakia.
13Department of Urology, Charles University in Prague, Faculty of Medicine in Plzeň, Pilsen, Czech Republic.

*Corresponding author: Ondrej Hes, Department of Pathology, Charles University, Medical Faculty and Charles University Hospital Plzen, Alej Svobody 80, 304 60 Pilsen, Czech Republic
E-mail: hes@biopticka.cz;

DOI: https://doi.org/10.17305/bjbms.2021.6935

Submitted: 11 January 2022/Accepted: 31 January 2021/ Published online: 09 February 2022

Conflict of interests: The authors declare no conflicts of interest.

Funding: This study was supported by the Charles University Research Fund (project number Q39), by the grant of Ministry of Health of the Czech republic-Conceptual Development of Research Organization (Faculty Hospital in Plzen- FNPI 00669806), Czech Republic.

License: © The Author(s) (2022). This work is licensed under a Creative Commons Attribution 4.0 International License
ABSTRACT

The morphologic diversity of chromophobe renal cell carcinoma (ChRCC) is well-known. Aside from typical morphology, pigmented adenomatoid, multicystic and papillary patterns have been described. Ten cases of CHRCC composed of small cell population in various percentages were analysed, using morphologic parameters, immunohistochemistry and next-generation sequencing (NGS) testing. Patients were five males and five females, with age ranging from 40 to 78 years. The size of tumors ranged from 2.2 cm to 11 cm (mean 5.17 cm). Small cell component comprised 10 to 80% of the tumor volume, while the remaining was formed by cells with classic ChRCC morphology. The immunohistochemical profile of the small cell component was consistent with typical ChRCC immunophenotype, with CD117 and CK7 positivity. Neuroendocrine markers were negative. Mutations of 13 genes were found: DCIER1, FGFR3, JAK3, SUFO, FAM46C, FANCG, MET, PLCG2, APC, POLE, EPICAM, MUTYH and AR. However, only the PLCG2 mutation is considered pathogenic. The small cell variant of ChRCC further highlights and expand upon existing morphologic heterogeneity spectrum. Recognition of small cell variant of CHRCC is not problematic in tumors, where the “classic” CHRCC component is present. However, in limited material (i.e., core biopsy), this may present a diagnostic challenge. Based on the limited follow-up data available, it appears that the small cell tumor component had no impact on prognosis, since there was no aggressive behavior documented. Awareness of this unusual pattern and applying additional sections to find classic morphology of ChRCC, as well as excluding neuroendocrine nature by immunohistochemistry, may help resolve difficult cases.

KEYWORDS: Kidney; chromophobe renal cell carcinoma; small cell variant.
INTRODUCTION

Several morphologic variations of chromophobe renal cell carcinoma (ChRCC) have been reported since Thoenes and Storkel [1, 2] first described it. Cases with morphology that differs from the typical solid-alveolar architecture seen in classic or eosinophilic ChRCC have been well-documented in the literature, including adenomatoid pigmented ChRCC, ChRCC with neuroendocrine differentiation (or with neuroendocrine-like differentiation), oncocytic ChRCC, multicystic ChRCC and ChRCC with papillary architecture [3-9]. The small cell variant of renal oncocytoma is a well-defined morphologic subtype of a common renal tumor [10-12]. Additionally, ChRCC and renal oncocytoma (RO) are thought to be closely related tumors derived from the intercalated cells. However, small cell variant of ChRCC has not been described. We selected a group of ChRCCs with a small cell component forming from 10 to 80% of the tumor volume. Clinicopathologic, morphologic, immunohistochemical and molecular-genetic analysis of 10 cases was performed.

MATERIALS AND METHODS

The database of Tumor Registry in Plzen was searched for keywords: kidney; oncocytoma; chromophobe. A total number of 2067 tumors was retrieved. All ChRCCs with “classic” morphology, as well as the eosinophilic variants, were excluded. Since no case from the RO cohort was re-classified as a small cell variant of ChRCC, all ROs were excluded. All ChRCC with so-called variant histology were re-evaluated. We particularly focused on cases with true neuroendocrine differentiation, which were excluded after the initial immunohistochemical staining for synaptophysin, chromogranin and CD56
(see later for details). Three cases with strong focal CD56 positivity in the small cell tumor component were also eliminated from the study. Out of 1092 ChRCC and 975 RO cases from the Plzen Tumor Registry, thirteen cases were found to be suitable.

For the final selection, a 10% cut-off for the small cell component was applied. Ultimately, ten cases were enrolled in the study. Each participating institution provided clinical data and follow-up information. None of the cases included in the study had ever been reported before. Tissues for microscopic examination were formalin-fixed and paraffin-embedded using standard procedure. Two to four μm thick sections were cut and stained for hematoxylin and eosin (H&E). For each case, one to thirteen paraffin blocks were available. All of the tumors were independently reviewed by three pathologists (JR, FK and OH).

**Immunohistochemistry**

The immunohistochemical study was performed using a Ventana Benchmark XT automated immunostainer (Ventana Medical System, Inc., Tucson, AZ, USA) on formalin-fixed paraffin-embedded (FFPE) tissue. The primary antibodies used were as follows: CK7 (OV-TL12/30, monoclonal, DakoCytomation, Glostrup, Denmark, 1:200), cytokeratin 20 (M7019, monoclonal; Dako; 1:100), vimentin (D9, monoclonal, NeoMarkers, Westinghouse, CA, 1:1000), CD56 (1B6, monoclonal, Leica Biosystems, Newcastle, UK, 1:100), synaptophysin (polyclonal, LabVision, Fremont, CA, 1:350), chromogranin A (monoclonal, DAK-A3, DakoCytomation, 1:600), c-kit (CD117, polyclonal, DakoCytomation, 1:300), TTF1 (monoclonal, SPT24, Ventana, 1:400), GATA3 (monoclonal, L50-823, Biocare Medical, Concord, CA, 1:100), NKX3.1 (polyclonal, Biocare Medical, 1:50), FLI 1 (monoclonal, MRQ-1, Cell Marque, Rocklin, CA, 1:50), CD99 (monoclonal, HO36-1.1, Neo Markers, Rockford, IL, 1:200), WT1 (monoclonal, 6F-
H2, DAKO, 1:50), napsin (polyclonal, Ventana, RTU), Ki-67 (monoclonal, MIB-1, DAKO, 1:400). The primary antibodies were visualized using a supersensitive streptavidin-biotin-peroxidase complex (BioGenex). Internal biotin was blocked by the standard protocol used by Ventana Benchmark XT automated stainer (hydrogen peroxide-based).

Appropriate positive and negative controls were employed. The slides were evaluated as follows: (-) negative; (+/-) less than 10% positive cells; (+) 10 to 25% positive cells; (++) >25 to 50% positive cells; (+++) >50 to 75% positive cells (++++) >75% of positive cells.

**Molecular genetic methods**

Mutation analysis was performed using the TruSight Oncology 500 assay (Illumina, San Diego, CA). Total nucleic acid was extracted using the FFPE DNA kit (automated on RSC 48 Instrument, Promega, Madison, Wisconsin, USA). Purified DNA was quantified using the Qubit Broad Range DNA. The quality of DNA was assessed using the FFPE QC kit (Illumina), and DNA samples having Cq<5 were used for further analysis. After the DNA enzymatic fragmentation with KAPA FragKit (KAPA Biosystems, Washington, MA), DNA libraries were generated using the TruSight Oncology 500 assay (Illumina) according to the manufacturer’s protocol.

Sequencing was performed using the NextSeq 550 sequencer (Illumina) following the manufacturer’s guidelines. Data analysis (DNA variant filtering and annotation) was performed using the OmnomicsNGS analysis software (Euformatics, Finland). The custom variant filter was set up including only non-synonymous variants with coding consequences, read depth greater than 50. Benign variants according to the ClinVar database were excluded as well [13]. The remaining subset of variants was examined visually, and any apparent artefactual variants were excluded.
**Ethical statement**

The study was performed in accordance with the Declaration of Helsinki. Ethics committee approval was not required by Charles University and University Hospital Plzen.

**RESULTS**

Table 1 summarizes the basic clinicopathologic data. The patients ranged in age from 40 to 78 years old (median 58.5 years; mean 58.5 years), with 5 males and 5 females. According to UICC 2017, four patients presented with pT1a stage, 1 with pT1b, 1 with pT2a, 1 with pT2b and 3 with pT3a. Follow-up was provided in 9 cases, ranging from 24 to 73 months (mean 50.75 months; median 48 months). Eight of the patients were alive with no evidence of disease progression. One patient was diagnosed with concurrent pancreatic carcinoma at stage pT3a and died due to widespread metastatic disease following surgery and treatment.

Tumor size spanned from 2.2 cm to 11 cm in the greatest dimension (mean 5.17 cm). Macroscopically, all lesions were well-demarcated and non-capsulated. On cut section, the tumorous parenchyma was orange-yellow to brownish in color, homogeneous, with no grossly visible necrosis. Morphologic features of the tumors are summarized in Table 2.

Microscopically, all cases had "classic chromophobe" morphology, at least focally. The extent of small cell component ranged from 10% to 80% of the tumor volume. The distribution of the small cell component was multifocal with a gradual transition from classic ChRCC to the small cell area (Fig 1 and 2). In one case (case 2), both components were sharply demarcated (Fig 3). The architecture in a majority of the cases was
predominantly solid (Fig 4), with small foci, nested, tubular or palisaded arrangement in small cell component and solid alveolar in a classic component.

The cells of the classic component were typical, large, with voluminous cytoplasm and raisinoid nuclei, accompanied by smaller, eosinophilic cells with perinuclear clearing and occasional nuclei with irregular contours (Fig 5). Cells in the small cell component showed scant cytoplasm, round to oval and frequently overlapping nuclei with non-conspicuous nucleoli (Fig 6). No nuclear grooves or coffee bean patterns were documented. There were no nuclear grooves or coffee bean patterns. In both large and small cell components, no mitotic figures were found.

In 3 cases, foci of bizarre cells with large, hyperchromatic nuclei similar to those frequently observed in oncocytoma (so-called polyploid cells) were present. None of the cases showed sarcomatoid transformation or necrosis. Results of immunohistochemical examination are summarised in Table 3A and Table 3B.

Immunohistochemically, CK 7 staining pattern in small cell areas was almost identical to the staining pattern in classic ChRCC areas (Fig. 7). In 1 case (case 9), the classic component of ChRCC showed diffuse, mosaic positivity, whereas the small cell component showed a focal, oncocytoma-like pattern of staining (Fig. 8). On cell membranes, CD117 was mostly diffusely positive, with weak to moderate intensity in both components (Fig. 9). In one case (case 9), CD117 showed positive staining in the classic ChRCC component only. In both the classic and small cell components, all cases were negative for synaptophysin and chromogranin. CD56 expressed focal to patchy, very weak positivity in large cells of the classic component in 4 cases, which was considered non-specific. FLI 1 was positive in one case (case 1) in both the classic and small cell component. CK 20, GATA3, NKX 3.1, TTF1, napsin A, WT 1 and CD99 were negative in
all cases. Ki-67 positivity ranged from 2 to 20 cells per HPF in both components. Next-generation sequencing analysis was successful in 5 cases. Results are summarized in Table 4.

Mutations of 13 genes were found, namely: DCIER1, FGFR3, JAK3, SUFO, FAM46C, FANCG, MET, PLCG2, APC, POLE, EPICAM, MUTYH and AR. However, only the PLCG2 mutation is listed as pathogenic. No mutations of FLCN, VHL, SDH, TSC1, TSC2, MTOR were documented. Seven cases were suitable for TERT hot spot analysis. Two tumors carried TERT mutation in position 228 (chr5:1295228 C>T).

**DISCUSSION**

ChRCC and RO are considered tumors derived from so-called intercalated cells of collecting ducts [14, 15]. In addition to a common cell of origin, they share several morphologic features. Both tumor types are usually located within the renal cortex, both well-circumscribed, yet non-encapsulated. In the gross section, both RO and ChRCC are predominantly brown, sometimes with a scar that is centrally located in RO. However, the central scar is not specific for RO and might be found in other renal tumors as well. Histologically, both tumors are composed of oncocytic cells, although the morphologic details and immunohistochemical features may differ. Because of these similarities, several researchers hypothesized that RO might be a potential "chromophobe adenoma" that could progress to ChRCC [16-18] in a manner similar to the adenoma-adenocarcinoma sequence in the colorectal cancer [19]. However, other authors, including the authors of this research, disagree [20].

The small cell variant of RO was first described in 2001 [10,21], although the
existence of small oncocytic cells, so-called oncblasts, was documented and discussed much earlier [22]. Several papers published afterward [11, 23, 24] further defined the small cell variant of RO.

ChRCC, in its classic form, is described as a solid-alveolar tumor composed of large leaf-like cells and smaller oncocytic cells. Several morphologic variants, which differed from classic morphology, were subsequently described in the literature.

As the name indicates, the architecture of the adenomatoid microcystic pigmented variant comprises of microcystic, cribriform areas mixed with conventional ChRCC pattern. Adenomatous structures lined by small cylindrical cells with basally located nuclei constitute a second component [4].

Multicystic ChRCC is composed either of variable-sized cysts, or compressed cysts and tubules with slit-like spaces. The cellular lining is made up entirely of eosinophilic cells or a mixed population of eosinophilic and pale cells. It is likely that the two aforementioned variants represent a spectrum of one morphologic subtype in which the adenomatoid pattern progressively transforms to areas with microcystic architecture. Lipochrome pigment accumulation is constantly present in the former, whereas it was noted in less than 50% of cases in the latter group of tumors [8].

Within the ChRCC spectrum, ChRCC with papillary architecture is quite rare. However, it has been described in the literature [25]. Such a pattern was present focally. The cytologic features, on the other hand, followed a characteristic dual population of leaf-like and small eosinophilic cells. Foam cells were not present. Recently, series of CHRCC with prominent papillary architecture has been published [9]. The extent of papillary component in tumors ranged from 15-100% of the tumor volume. The cytologic characteristics were typical.
ChRCC with morphology similar to neuroendocrine tumors, namely with trabecular/palisading/cribriform pattern were also documented. Among them were the CHRCC with true neuroendocrine differentiation, confirmed by positive staining for synaptophysin, chromogranin or CD 56 [5,7].

Tumors that showed similar architecture and cytologic features, but without positive neuroendocrine immunohistochemical staining were labeled as ChRCC with neuroendocrine-like features [7].

ChRCC with neuroendocrine differentiation and neuroendocrine-like features can be remarkably similar to the small cell variant of ChRCC. To rule out cases with true neuroendocrine differentiation, we employed 3 different neuroendocrine markers (synaptophysin, chromogranin and CD56).

In the current study, the small cell tumor population was uniform, showing mostly scant cytoplasm, arranged predominantly in solid, relatively compact areas or sheets. Only focal palisading or tubular structures were seen. Such patterns were located in transition zones between small cell and classic ChRCC components, always comprising less than 5% of the small cell component volume.

There is another parallel between the small cell oncocytoma and the small cell variant of ChRCC. In ChRCC with an adenomatoid pattern, groups of small cells are located on the edges of adenomatoid structures or on the edges of fibrotic, scar-like foci. A similar phenomenon has been well-documented in a small cell variant of RO [11]. In classic RO, however, groups of small oncocytes are frequently found in identical location.

Pseudorosettes or ribbon-like patterns were not seen in the small cell ChRCC variant. The presence of such structures is an interesting phenomenon in the context of differential diagnosis. In a series of small cell variants of RO [11], pseudorosettes with a PAS-positive central core were described. However, we do not believe such structures can
be used as a differential diagnostic feature.

Immunohistochemical profiles of our cases were compatible with the classic variant of ChRCC, mostly showing strong, diffuse or focal positivity for CK 7, along with diffuse or focal, weak to moderate positivity for CD117 in both tumor components. However, one case (case 9) was exceptional: the small cell component expressed an oncocytoma-like CK7 staining pattern with diffuse, mosaic positivity in the classic part, whereas CD117 was positive solely in the classic part.

Focal weak positivity for CD56 was considered non-specific, and other neuroendocrine markers were negative. Interestingly, there was a strong positive immunohistochemical reaction for FLI1, which was present in both components in case 1. Unfortunately, the case was not suitable for molecular genetic analysis due to the low quality of DNA/RNA, but the morphology supported the diagnosis of ChRCC.

In cases with overlapping features between ChRCC, as well as cases with worrisome clinical features, association with the syndromic disease should be considered. According to clinical reports, we have no evidence of syndromic disease within our cohort.

Furthermore, next-generation sequencing was used to screen molecular profiles of our cases. Only 5 tumors were suitable for a complete NGS analysis. We were unable to document any genetic alteration linked to syndromic diseases. FLCN, VHL and/or SDH gene mutations were not detected. The significance of the only pathogenic mutation of PLCG2 gene found in our cohort remains unclear.

Renal tumors with mTOR pathway abnormalities were documented recently. Some of these newly recognized subtypes are characterized by an eosinophilic/oncocytic or chromophobe-like morphology [26]. Among them are eosinophilic solid and cystic RCC (ESC RCC), eosinophilic vacuolated tumor (EVT) and a low-grade oncocytic tumor (LOT), which can have eosinophilic/oncocytic or chromophobe-like morphology. Therefore, these
tumors should be considered in the differential diagnosis of small cell ChRCC variant.

There were no morphologic features of the above-mentioned entities in our cases, and no mutations in the mTOR pathway genes were detected. However, one of our cases showed overlapping immunophenotype with EVT with positivity for CD117 and CK7 negative/focally positive.

The grading and biological behavior of ChRCC are notoriously inconsistent. Fuhrman’s grading system, classic ISUP/WHO modification of Fuhrman’s system [12], and even grading system proposed by Paner et al. are all practically not applicable [27,28].

Sarcomatoid transformation and/or necrosis were the only morphologic factors significantly associated with poor prognosis in a multi-institutional study recently published by Ohashi et al. [29]. There was no necrosis or sarcomatoid change in any of our cases.

Based on the limited available follow-up data, it is difficult to speculate about the potential impact of the presence of the small cell tumor component on prognosis. In no case was the aggressive behavior documented. However, the follow-up period is relatively short, with a median of 48 months.

Several neoplastic entities should be considered in differential diagnosis, especially with limited material in core biopsy, where the diagnosis may be challenging compared to the more straightforward diagnostic process in resections.

In differential diagnosis, the presence of small-cell differentiation, tubular or palisading pattern, raises the question of potential neuroendocrine differentiation (either primary or metastatic).

Primary neuroendocrine tumors of the kidney are exceedingly rare. According to the WHO Classification (2016), they are subdivided into two groups: I) well differentiated neuroendocrine tumor (carcinoid and atypical carcinoid), and II) poorly differentiated
neuroendocrine carcinoma including small cell and large cell variants [12]. Morphologically, carcinoids display similar features as their counterparts in other anatomical sites. Their neuroendocrine nature is confirmed by immunohistochemistry with positive staining for neuroendocrine markers.

Ewing sarcoma/Peripheral neuroendocrine tumor (PNET) must be considered in cases composed of small, round, densely packed blue cells, especially on limited material and in a young patient. PNET shows features of a highly malignant neoplasm, with numerous mitotic figures and necrosis. PNET is characterized by diffuse positivity for vimentin, CD99 and FLI-1 in immunohistochemistry. In certain cases, neuroendocrine markers may be positive [30].

In none of our cases we found mitoses. However, in case 2, PNET was a differential diagnosis on core biopsy. On a final resection specimen, 80% of the tumor was composed of a small cell component with PNET-like morphology, solid architecture and densely packed cells with oval, overlapping nuclei, as well as areas with typical ChRCC morphology, haphazardly present throughout the tumor mass.

Immunohistochemical examination revealed negative staining with vimentin (typical pattern characteristic for oncocytoma), whereas FLI 1 and CD99 were negative. The morphologic characteristics of cases positive for CD99 or FLI-1 were distinct from PNET, and staining was interpreted as non-specific. None of the analyzable cases showed mutation/translocation in the EWSR gene. However, cases with non-specific FLI-1 and CD99 staining were not analyzable by NGS.

Wilms tumor (WT), blastemal-rich variant, is another example of a tumor within the spectrum of small round blue cell renal tumors. Blastema-rich WT is composed of primitive cells with sticking, highly malignant morphology showing diffuse immunoreactivity for
vimentin and WT1 [12]. None of our cases showed neither such morphology nor positive staining for WT1 and/or vimentin.

The tendency of urothelial carcinoma (UC) to mimic primary renal cell carcinomas, particularly in high-grade forms, is well-known. In this regard, a macroscopic examination can give many clues for differential diagnosis. In UC, renal pelvis involvement and infiltrative growth pattern with desmoplastic response are common, whereas in ChRCC, pushing border and expansile growth pattern are more common. The infiltrative growth pattern was not reported in our study. In addition, the immunohistochemical profile of our cases differed from that of typical UC.

The final situation in differential diagnosis that should be considered is that sarcomatoid differentiation within ChRCC is relatively common. Some authors suggest that sarcomatoid dedifferentiation is more prevalent in ChRCC than in any other RCC subtype [31]. The great majority of the sarcomatoid component, on the other hand, is present in the form of a high-grade, spindle-cell, mesenchymal-looking neoplastic cell population. Necrosis is common and mitotic activity is usually brisk [32].

We were not able to identify any spindling or conspicuous mitotic figures within small cell areas, as well as necrosis. Our cases also lacked the infiltrative pattern of small cells, which would be expected in sarcomatoid dedifferentiation. The architecture and cytology of small cell component were clearly epithelial and monotonous. All of the aforementioned characteristics argue against sarcomatoid differentiation.

**CONCLUSION**
We herein present a group of 10 ChRCCs with a small cell component that constitutes up to 80% of the tumor volume. Awareness of this unusual pattern and applying additional sections to find classic morphology of ChRCC, as well as excluding neuroendocrine nature by immunohistochemistry, may help resolve difficult cases.

However, a small cell morphology does not present major diagnostic problem in resected tumors, on limited material namely as a core biopsy such morphology may create diagnostic challenge.
REFERENCES


tumour 'oncocytoma-chromophobe renal cell carcinoma' of the kidney: a report of
seven sporadic cases. BJU international. 2009;103(10):1381-4.

classic chromophobe renal cell carcinomas have similar frequent losses of multiple
chromosomes from among chromosomes 1, 2, 6, 10, and 17, and this pattern of genetic
abnormality is not present in renal oncocytoma. Modern pathology : an official journal

oncocytoma with intracytoplasmic lumina: a case report with ultrastructural findings of

22. Eble JN, Hull MT. Morphologic features of renal oncocytoma: a light and electron

23. Magro G, Gardiman MP, Lopes MR, Michal M. Small-cell variant of renal
oncocytoma with dominating solid growth pattern: a potential diagnostic pitfall.


with focal papillary configuration, nuclear basaloid arrangement and stromal osseous


### TABLE 1. Basic clinicopathologic data of ChRCC with small cell morphology.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex</th>
<th>Age</th>
<th>Tumor size (cm)</th>
<th>Stage</th>
<th>Follow up (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>65</td>
<td>7.5 x 5.4 x 4</td>
<td>pT3a</td>
<td>DUD*</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>78</td>
<td>diam. 3.2</td>
<td>pT1a</td>
<td>24 AW</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>61</td>
<td>2.1 x 2.2 x 1.5</td>
<td>pT2b</td>
<td>70 AW</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>52</td>
<td>diam. 4.3</td>
<td>pT1a</td>
<td>36 AW</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>71</td>
<td>diam. 2.6</td>
<td>pT1a</td>
<td>36 AW</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>45</td>
<td>9 x 8 x 5</td>
<td>pT2a</td>
<td>73 AW</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>56</td>
<td>3.1x2.2x1.9</td>
<td>pT3a</td>
<td>48 AW</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>58</td>
<td>2.8 x 2.8 x 2.1</td>
<td>pT1a</td>
<td>48 AW</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>59</td>
<td>10 x 11 x 8</td>
<td>pT3a</td>
<td>NA</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>40</td>
<td>6 x 4.8 x 4.5</td>
<td>pT1b</td>
<td>71 AW</td>
</tr>
</tbody>
</table>

Abbreviations: M: male; F: female; AW: alive without evidence of disease; diam: diameter; NA: not available; DUD: death of unrelated disease; *Simultaneously diagnosed with pancreatic adenocarcinoma pT3a, after surgery and treatment patient died of Metastatic pancreatic adenocarcinoma.
TABLE 2. Morphologic parameters.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>% of small cell area</th>
<th>Architecture of small cell component</th>
<th>Bizarre cells &lt; 5%</th>
<th>Transition between classic and small cell necrosis</th>
<th>MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80</td>
<td>Alveolar</td>
<td>+</td>
<td>gradual</td>
<td>0/HPF</td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td>Solid <em>/</em>*</td>
<td>-</td>
<td>abrupt</td>
<td>0/HPF</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>Solid <em>/</em>*</td>
<td>-</td>
<td>gradual</td>
<td>0/HPF</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>Solid **</td>
<td>-</td>
<td>gradual</td>
<td>0/HPF</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>Solid</td>
<td>+</td>
<td>gradual</td>
<td>0/HPF</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>Solid **</td>
<td>-</td>
<td>gradual</td>
<td>0/HPF</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
<td>Solid *</td>
<td>-</td>
<td>gradual</td>
<td>0/HPF</td>
</tr>
<tr>
<td>8</td>
<td>30</td>
<td>Solid *</td>
<td>-</td>
<td>gradual</td>
<td>0/HPF</td>
</tr>
<tr>
<td>9</td>
<td>20</td>
<td>Solid *</td>
<td>-</td>
<td>gradual</td>
<td>0/HPF</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>Solid,**</td>
<td>+</td>
<td>gradual</td>
<td>0/HPF</td>
</tr>
</tbody>
</table>

Abbreviations: (-) absent; (+) present; *focal palisading <5% of the tumor; **focal tubulary pattern <5% of the tumor; MI: mitotic index.
TABLE 3A. Morphologic parameters.

<table>
<thead>
<tr>
<th>Case No</th>
<th>Syn.</th>
<th>Chrom.</th>
<th>CD5 6</th>
<th>TTF1</th>
<th>Napsin A</th>
<th>CK7</th>
<th>CD11 7</th>
<th>CK 20</th>
<th>GA TA3</th>
<th>NK X3.1</th>
<th>FL 11</th>
<th>CD 99</th>
<th>V imm</th>
<th>W T 1</th>
<th>Ki67</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>++++</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>-#</td>
<td>+/-</td>
<td>-</td>
<td>2-10/hp f</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>++++</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>-#</td>
<td>+/-</td>
<td>-</td>
<td>15-20/hp f</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-#</td>
<td>+/-</td>
<td>+/-</td>
<td>-</td>
<td>8-15/hp f</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>++++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-#</td>
<td>+/-</td>
<td>+/-</td>
<td>-</td>
<td>4-8/hp f</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>++++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-#</td>
<td>+/-</td>
<td>+/-</td>
<td>-</td>
<td>5-10/hp f</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>++++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-#</td>
<td>+/-</td>
<td>+/-</td>
<td>-</td>
<td>1-8/hp f</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>++++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-#</td>
<td>+/-</td>
<td>+/-</td>
<td>-</td>
<td>1-4/hp f</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-#</td>
<td>+/-</td>
<td>+/-</td>
<td>-</td>
<td>5-10/hp f</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-#</td>
<td>+/-</td>
<td>+/-</td>
<td>-</td>
<td>1-10/hp f</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>++++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-#</td>
<td>+/-</td>
<td>+/-</td>
<td>-</td>
<td>10-15/hp f</td>
</tr>
</tbody>
</table>

Abbreviations: ( - ) negative; (+/- ) less than 10% positive cells; (+) 10 to 25% positive cells; (+++) >25 to 50% positive cells; (++++) >50 to 75% positive cells; (++++) >75% positive cells; #cytoplasmatic positivity; *high background staining, difficult to interpret; hpf: high-power field; Syn: synaptophysin; Chrom: chromogranin; Vim: vimentin.
### TABLE 3B. Results of immunohistochemical examination of classic ChRCC component.

<table>
<thead>
<tr>
<th>Case No</th>
<th>Syn</th>
<th>Chrom</th>
<th>CD 56</th>
<th>TT</th>
<th>Nap sin A</th>
<th>CD 7</th>
<th>CD 117</th>
<th>CD 20</th>
<th>GAT A3</th>
<th>NKX 3.1</th>
<th>FL I1</th>
<th>CD 99</th>
<th>Vi m</th>
<th>W T1</th>
<th>Ki6 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>#</td>
<td>+/-</td>
<td>-</td>
<td>2-10/hpf</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>#</td>
<td>-</td>
<td>-</td>
<td>5-15/hpf</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>#</td>
<td>-</td>
<td>-</td>
<td>8-15/hpf</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>#</td>
<td>-</td>
<td>-</td>
<td>2-8/hpf</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>#</td>
<td>-</td>
<td>-</td>
<td>5-10/hpf</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>#</td>
<td>-</td>
<td>-</td>
<td>1-5/hpf</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>#</td>
<td>-</td>
<td>-</td>
<td>1-4/hpf</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>#</td>
<td>-</td>
<td>-</td>
<td>5-10/hpf</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>#</td>
<td>-</td>
<td>-</td>
<td>5-10/hpf</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>#</td>
<td>-</td>
<td>-</td>
<td>10-15/hpf</td>
</tr>
</tbody>
</table>

Abbreviations: ( - ) negative; (+/- ) less than 10% positive cells; (+) 10 to 25% positive cells; (++) >25 to 50% positive cells; (+++) >50 to 75% positive cells (++++) >75% positive cells; #cytoplasmatic positivity; *high background staining, difficult to interpret; hpf: high-power field; Syn: synaptophysin; Chrom: chromogranin; Vim: vimentin.
### TABLE 4. Results of Next Generation Sequencing.

<table>
<thead>
<tr>
<th>CASE NR</th>
<th>DIC</th>
<th>ER1</th>
<th>TE</th>
<th>RT</th>
<th>FGF</th>
<th>R3</th>
<th>JA</th>
<th>K3</th>
<th>SU</th>
<th>FU</th>
<th>FAM</th>
<th>46C</th>
<th>FAN</th>
<th>CG</th>
<th>M ET</th>
<th>PLC</th>
<th>G2</th>
<th>A</th>
<th>PC</th>
<th>PO</th>
<th>LE</th>
<th>EPC</th>
<th>AM</th>
<th>MUT</th>
<th>YH</th>
<th>A</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>10</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

**Coding effect**
- Missence
- Nonsence
- TERT

**NA**-not analyzable
FIGURE 1. The distribution of the small cell component was multifocal, with a gradual transition from classic ChRCC to the small cell area in the majority of cases.
FIGURE 2. Classic chromophobe cells were intermingled among a dense population of small cell component.
FIGURE 3. Case where the both components were sharply demarcated without transitional zone between both cell types.
FIGURE 4. The architecture was solid in some cases.
FIGURE 5. (a-f) Effect of TDF and TDF-AgNPs on the prefrontal cortex pyramidal cel The cells of classic component were typical with voluminous cytoplasm and raisinoid nuclei.
FIGURE 6. The cells in the small cell component showed scant cytoplasm and round to oval nuclei. Mitotic activity was absent.
FIGURE 7. Tumors were CK7 positive in both components.
FIGURE 8. In case 9, small cell component displayed a patchy pattern of reactivity with CK 7, superficially resembled reactivity of renal oncocytoma.
FIGURE 9. CD117 was positive in the vast majority of cases in diffuse membranous pattern.