Annals of Diagnostic Pathology 19 (2015) 261–268

Contents lists available at ScienceDirect

Annals of Diagnostic Pathology

Chromophobe renal cell carcinoma with neuroendocrine and neuroendocrine-like features. Morphologic, immunohistochemical, ultrastructural, and array comparative genomic hybridization analysis of 18 cases and review of the literature ☆

Kvetoslava Peckova, MD [a], Petr Martinek, MSc [a], Chisato Ohe, MD [b], Naoto Kuroda, MD [c], Stela Bulimbasic, MD, PhD [d], Enric Condom Mundo, MD [e], Delia Perez Montiel, MD [f], Jose I. Lopez, MD [g], Ondrej Daum, MD, PhD [a], Pavla Rotterova, MD, PhD [a], Bohuslava Kokoskova, MD [a], Magdalena Dubova, MD [a], Kristyna Pivovarcikova, MD [a], Kevin Bauleth, MD [a], Petr Grossmann, PhD [a], Milan Hora, MD [h], Kristyna Kalusova, MD [h], Whitney Davidson, MD [i], David Slouka, MD, PhD [j], Sulc Miroslav, MD [k], Petr Buzrla, MD [l], Mirka Hynek, MD, PhD [m], Michal Michal, MD [a], Ondrej Hes, MD, PhD [a,n,*]

[a] Department of Pathology, Charles University, Medical Faculty and Charles University Hospital Plzen, Plzen, Czech Republic
[b] Department of Diagnostic Pathology, Kansai Medical University, Hirakata Hospital, Osaka, Japan
[c] Department of Diagnostic Pathology, Kochi Red Cross Hospital, Kochi, Japan
[d] Department of Pathology, University Hospital Dubrava, Zagreb, Croatia
[e] Department of Pathology, Bellvitge University Hospital, Barcelona, Spain
[f] Department of Pathology, Instituto Nacional de Cancerologia, Mexico City, Mexico
[g] Department of Pathology, Cruces University Hospital, Barakaldo, Spain
[h] Department of Urology, Charles University, Medical Faculty and Charles University Hospital Plzen, Plzen, Czech Republic
[i] Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS
[j] Department of Otorhinolaryngology, Charles University, Medical Faculty and Charles University Hospital Plzen, Plzen, Czech Republic
[k] Bioptical Laboratory Chomutov, Chomutov, Czech Republic
[l] Department of Pathology, University Hospital Ostrava, Ostrava, Czech Republic
[m] Department of Radiology, Medical Faculty and Charles University Hospital Plzen, Plzen, Czech Republic
[n] Biomedical Centre, Charles University, Medical Faculty and Charles University Hospital Plzen, Plzen, Czech Republic

ARTICLE INFO

Keywords:
Kidney
Chromophobe renal cell carcinoma
Neuroendocrine differentiation
Immunohistochemistry
Chromosomal numerical aberrations
aCGH

ABSTRACT

Chromophobe renal cell carcinoma (CRCC) with neuroendocrine differentiation (CRCCND) has only recently been described. Eighteen cases of CRCC with morphologic features suggestive of neuroendocrine differentiation were selected from among 624 CRCCs in our registry. The tissues were fixed in neutral formalin, embedded in paraffin, cut into 4- to 5-μm-thick sections, and stained with hematoxylin and eosin. As CRCC with neuroendocrine features, tumors with following morphology were suggested: (1) trabecular/palisading/ribbon-like, gyriform, insular, glandular, and solid pattern; (2) uniform polygonal cells formed in small islets; and (3) cribriform pattern in combination with palisading. Selected cases were further analyzed using immunohistochemistry, electron microscopy, array comparative genomic hybridization, and fluorescence in situ hybridization. Cases were classified as CRCCND or CRCC with neuroendocrine-like features (CRCCND-L) based on the immunohistochemical expression of neuroendocrine markers: CRCCND, 4 cases, age range 49 to 79 years, size ranged from 2.2 to 22 cm, and CRCCND-L, 14 cases, age range 34 to 74 years, size range 3.8 to 16.5 cm. Follow-up information was available for 11 of 18 patients aged 0.5 to 12 years. Two of 4 CRCCNDs showed aggressive clinical course with metastatic spreading. Chromophobe renal cell carcinomas with neuroendocrine differentiation were focally positive for CD56 (4/4), synaptophysin (4/4), chromogranin A (1/4), and neuron-specific enolase (3/4). All 14 CRCCND-Ls were mostly negative or very weakly focally positive for some of the aforementioned markers. All 18 tumors were positive for cytokeratin 7 and CD117. Ultrastructural analysis showed poorly preserved neuroendocrine granules only in 2 of 4 analyzed CRCCNDs. Losses

☆ The study was supported by the Charles University Research Fund (project no. P36) and by the project CZ.1.05/2.1.00/03.0076 from European Regional Development Fund.
* Corresponding author at: Department of Pathology, Charles University, Medical Faculty and Charles University Hospital Plzen, Alej Svobody 80, 304 60 Plzen, Czech Republic.
E-mail address: hes@medima.cz (O. Hes).

http://dx.doi.org/10.1016/j.anndiagpath.2015.05.001
1092-9134/

of chromosomes 1, 2, 6, and 10 were found in all analyzable CRCCNDs, whereas multiple losses (chromosomes 1, 2, 6, 10, 13, 17, and 21) and gains (chromosomes 4, 11, 12, 14, 15, 16, 19, and 20) were found in CRCCND-L.

1. Introduction

Chromophobe renal cell carcinoma (CRCC) represents approximately 5% of renal carcinomas. Microscopically, these tumors are described as mostly solid or solid alveolar; however, the morphologic spectrum has expanded to include microcystic, adenomatoid, and focal papillary arrangements [1-4].

Chromophobe renal cell carcinoma with true neuroendocrine differentiation (CRCCND) and CRCC with a neuroendocrine-like pattern (CRCCND-L) have only recently been described; a limited number of cases have been reported [5-7].

In this study, we attempt to distinguish and compare true neuroendocrine differentiation in CRCC to CRCC with a neuroendocrine-like pattern and to evaluate the biological nature of both forms.

2. Materials and methods

The tissues were fixed in neutral formalin, embedded in paraffin, cut into 4- to 5-μm-thick sections, and stained with hematoxylin and eosin.

We selected 18 cases of CRCC with morphologic features suggestive of neuroendocrine differentiation from among 624 CRCCs in our files. As CRCCs with neuroendocrine features, tumors with following morphology were suggested: (1) palisading/ribbon-like, gyriform patterns; (2) insular pattern; and (3) cribriform/pseudorosettoid pattern or small cell islets in combination with palisading.

Selected cases were further analyzed using immunohistochemistry (IHC), electron microscopy, array comparative genomic hybridization (aCGH), and fluorescence in situ hybridization (FISH).

3. Immunohistochemistry

The immunohistochemical study was performed using a Ventana Benchmark XT automated stainer (Ventana Medical System, Inc, Tucson, AZ) on formalin-fixed, paraffin-embedded (FFPE) tissue. The following primary antibodies were used: cytokeratin 7 (CK7) (OV-TL12/30, monoclonal, 1:200; DakoCytomation, Glostrup, Denmark), c-kit (CD 117, polyclonal, RTU; DakoCytomation), CD56 (1B6, monoclonal, 1:100; Leica Biosystems, Newcastle, UK), Ki-67 (MIB1, monoclonal, 1:1000; Dako, Glostrup, Denmark), synaptophysin (polyclonal, 1:350; LabVision, Fremont, CA), chromogranin A (monoclonal, DAK-A3, 1:600; DakoCytomation), CD99 (O13, monoclonal, 1:200; Ventana, Mannheim, Germany), cytokeratin 20 (CK20) (Ks20.8, monoclonal, 1:250; DakoCytomation). The primary antibodies were visualized using the supersensitive streptavidin-biotin-peroxidase complex (Biogenex, Fremont, CA). Appropriate positive and negative controls were used.

4. Ultrastructure

Electron microscopy evaluation was performed on 7 cases. Small pieces of FFPE from 3 cases of CRCCND and 4 cases of CRCCND-L were deparaffinized and further routinely processed for ultrastructural analysis. Semithin sections of epoxy-embedded tissue were stained with toluidine blue and examined by light microscopy. Ultrathin sections from representative areas were cut, stained with uranyl acetate and lead citrate, and examined with a Jeol (Tokyo, Japan) JEM 1400 Transmission Electronic Microscope.

Table 1
Clinicopathologic features

Case	Age	Sex	Site	Size (cm)	Color	Pattern	Follow up (y)
CRCCND							
1.[a]	79	M	Right	22 × 12 × 10	Brownish	SCI	3.5 AWD (CT scan, lymph nodes mediastinum)
2.	66	F	Left	12 cm	Yellow	SCI	Metastatic spreading in time of diagnosis, 0.5 AWD (local recurrence and bone meta)
3.	67	M	Right	5.6	Yellowish	PSC	LE
4.[a]	49	M	Left	Diam. 2.2	Beige	SCI	1 AW after partial nephrectomy
CRCCND-L							
1.	70	M	Right	2.6 × 3 × 2.3	Brown	P	AW 8/2014
2.	69	F	NA	12 × 5	Brown	PSC	NA
3.	74	F	Right	16.5	Yellow hemorrhagic, atrophic kidney	PSC	LE
4.	47	F	NA	3.8	NA	PSC	NA
5.	67	F	Left	7.3 × 6.8	Grayish	SC PR	LE
6.	46	M	Left	10 × 8 × 2	Grayish	SCI	3 y AW, then LE
7.	51	M	Left	8 × 7 × 6	Brownish	PSC	LE
8.	72	M	NA	NA	NA	SCPR	LE
9.	72	F	left	6.4	Pink to tan	PSC	AW 8/2014
10.	51	M	Left	Diam. 5	Yellow	PSC	AW 8/2014
11.	34	F	Left	14 × 11 × 10.5	Yellow	P	AW 8/14
12.	70	M	Right	13 × 10 × 9	Brown	P	AW 8/2014
13.	63	M	Left	Diam. 5.5	Brown	PSC	AW 2010, then LE
14.	49	F	NA	Diam. 13	NA	P	AW 9/14

Abbreviations: M, male; F, female; SCI, small cell islets; PSC, palisading and small cell areas; P, palisading; SCPR, small cells and pseudorosettes; AWD, alive with disease; CT, computed tomography; meta, metastasis; LE, loss of evidence; Diam., diameter; AW, alive and well; NA, not available.

[a] Cases have been published previously.