Case Report

Sporadic renal hemangioblastoma with CA9, PAX2 and PAX8 expression: diagnostic pitfall in the differential diagnosis from clear cell renal cell carcinoma

Naoto Kuroda¹, Yoshiko Agatsuma¹, Masato Tamura², Petr Martinek³, Ondrej Hes³, Michal Michal³

Departments of ¹Pathology and ²Urology, Kochi Red Cross Hospital, Kochi, Japan; ³Department of Pathology, Charles University in Prague, Faculty of Medicine in Plzen, Plzen, Czech Republic

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Abstract: To date, 13 cases of sporadic renal hemangioblastoma have been reported. In this article, we report such a case that might cause the diagnostic pitfall. A 37-year-old Japanese was found to have a renal mass by periodic medical check-up. He underwent radical nephrectomy. Macroscopically, the tumor was well-defined without fibrous capsule and the cut surface of the tumor exhibited light brown to gray-tan color without hemorrhage or necrosis. Microscopically, the tumor was made up of large polygonal to short spindle cells with eosinophilic cytoplasm with occasional vacuolization and abundant arborizing capillary network. Immunohistochemically, neoplastic cells showed diffuse positivity for inhibin-alpha, S-100 protein, vimentin, CA9, PAX2 and PAX8, but negativity for cytokeratin CAM5.2, alpha smooth muscle actin, Melanosome, Melan A, TFE3 and cathepsin K. In genetic analyses, this tumor showed no changes of VHL gene mutation, hypermethylation and loss of heterozygosity of chromosome 3p. Additionally, G-band karyotype and array comparative genomic hybridization studies showed a normal chromosome. In conclusion, the positivity for CA9, PAX2 and PAX8 in sporadic renal hemangioblastoma may cause the critical diagnostic pitfall in the differential diagnosis from clear cell renal cell carcinoma. Pathologists need to pay attention to systemic evaluation including macroscopic, microscopic and immunohistochemical findings. In some cases, molecular genetic study may be necessary.

Keywords: Hemangioblastoma, kidney, CA9, PAX2, PAX8

Introduction

Hemangioblastoma is the rare benign tumors of uncertain origin that can occur sporadically or in association with von Hippel-Lindau (VHL) disease, and arises in the central nervous system (CNS) in most cases. However, some cases of hemangioblastoma arising in extraneural sites have been reported [1, 2]. To the best of our knowledge, there are 13 cases of sporadic renal hemangioblastoma without VHL disease [3-12]. Clear cell renal cell carcinoma (RCC) with hemangioblastoma-like features has been recently described [13]. In this article, we report a case with sporadic renal hemangioblastoma with immunohistochemical expression of CA9, PAX2 and PAX8.

Materials and methods

Surgically resected renal tumor was fixed in 10% buffered formalin and embedded in paraffin, cut into 3 μm-thick sections with microtome and stained with hematoxylin and eosin, periodic acid-Schiff stain with and without diastase treatment and silver impregnation stains. For immunohistochemical stain, additional sections were cut and stained with automated immunostainer (Ventana Benchmark XT, Tucson, AZ, USA). Antibodies against cytokeratin CAM5.2 (CAM5.2, prediluted, Becton Dickinson, CA, USA), RCC Ma (PN-15, prediluted, Ventana Medical System inc, Tucson, AZ, USA), CD10 (56C6, prediluted, Novocastra Laboratories Ltd, Newcastle, UK), CA9 (D47G3, 1:200, Cell Signaling, MA, USA), inhibin-alpha (R1, 1:50, DAKO, Glostrup, Denmark), S-100 protein (polyclonal, 1:2400, DAKO, Glostrup, Denmark), vimentin (V9, 1:6400, DAKO, Glostrup, Denmark), PAX2 (polyclonal, 1:100, ZYMED Laboratories, CA, USA), PAX8 (polyclonal, 1:50, Proteintech Group, inc), Melanosome (HMB45, prediluted, DAKO, Glostrup, Denmark), Melan A
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Figure 1. Macroscopic findings. The tumor measuring 36×35×17 mm is well demarcated and the cut surface of the tumor shows light brown to gray-tan in color.

Results

A 37-year-old Japanese man was found to have a left renal tumor with an abdominal ultrasound sonography of medical check-up. However, the plain computed tomography (CT) scan disclosed no abnormalities. However, medical check-up of the following year revealed the left renal tumor and he was transferred to Department of Urology, our hospital. The dynamic CT scan disclosed the heterogeneously enhanced mass measuring about 4 cm in the upper pole of the left kidney. He had no symptoms or no family history of VHL disease. The left nephrectomy was performed on the suspicion of renal cancer.

Macroscopically, the well-demarcated tumor measuring 36×35×17 mm was observed and the cut surface of the tumor showed light brown to gray-tan in color (Figure 1). Neither necrosis nor hemorrhage was seen.

Microscopically, the tumor consisted of sheets or nests of large polygonal to short spindle cells and ample arborizing capillary network (Figure 2A). The tumor cells generally showed eosinophilic, clear to vacuolated cytoplasm, and lipoblast-like cells (Figure 2B) or rhabdoid cells (Figure 2C) showing eccentric nuclei were also identified. Eosinophilic globules resembling hyaline globules (Figure 2D) and intranuclear cytoplasmic pseudo-inclusions (Figure 2E) were occasionally seen. Nuclei of neoplastic cells were oval to round with focal marked atypical features, but mitotic activity was absent. Hemangiopericytomatous pattern was focally observed (Figure 2F). The stroma contained myxoid area, fibrosis with hyalinization and smooth muscle cells (Figure 2G). Perivascular hyalinization was also focally present (Figure 2H). There was no infiltration to renal pelvis, sinus or extrarenal fat tissue and renal vein. Eosinophilic globules showed the positivity for periodic acid-Schiff stain with diastase pre-treatment (Figure 2I). Glycogen particles were also identified in the cytoplasm of neoplastic cells. In silver impregnation stain, reticular fibers generally surrounded individual tumor cells.

Immunohistochemically, tumor cells showed diffuse expression for inhibin-alpha (Figure 3A), S-100 protein (Figure 3B), CA9 (Figure 3C), PAX2 (Figure 3D), PAX8 (Figure 3E) and vimentin, but no expression for cytokeratin CAM5.2 (Figure 3F), RCC Ma, CD10, Melanosome, Melan A, TFE3, Cathepsin K, ALK, alpha smooth muscle actin and brachyury.

No mutations of EGFR gene were identified. G-band karyotype of the renal tumor showed 46, XY, inv (9) (p13q22) [1]/46, XY [19]. The array CGH showed no chromosomal changes. This tumor showed absence of VHL gene mutation, hypermethylation and 3p LOH.

Discussion

In the differential diagnosis of hemangioblastoma arising in central nervous system and
metastasis from clear cell RCC, application of some available markers have been tried. Inhibin-alpha [16-20], brachyury [21] and aquaporin 1 [18] has been studied as a positive marker, and RCC Ma [22], CD10 [17], PAX2 [19, 20], PAX8 [20] and AE1/AE3 [18] has employed as a negative marker. Among them, we suppose that inhibin-alpha may be available marker in identifying hemangioblastoma. We agree to Doyle’s opinion that brachyury is not available positive maker for hemangioblastoma [12]. However, the application of these markers in the differential diagnosis between sporadic renal hemangioblastoma and clear cell RCC cannot be simply interpreted and availably used because extraneural hemangioblastoma often show the organ-specific immunophenotype. Previously, 13 cases of sporadic renal hemangioblastoma have been reported [3-12] and these case are summarized in Table 1. Among them, some cases of sporadic renal hemangioblastoma showing positivity for CA9 [5, 9], CD10 [7, 9], PAX2 [9] and PAX8 [11] have been actually described. In the present case, tumor cells also showed the immunolabeling to CA9, PAX2 and PAX8. Additionally, two cases of clear cell RCC with hemangioblastoma-like features have been recently reported Pathologists should pay attention that hemangioblastoma-like component in clear cell RCC may demonstrate the positivity to inhibin-alpha [13]. We would like to emphasize that macroscopic and microscopic findings are most important in the distinction of renal hemangioblastoma from clear cell RCC. The distinction of these diseases is clinically and pathologically critical, because renal hemangioblastoma behaves in a benign fashion, whereas clear cell RCC is actually a malignant neoplasm. Namely, the cut surface of renal hemangioblastoma shows brown-
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...ish, grayish white, gray to yellowish, brownish white, gray to brown or red-brown to gray-yellow color [4-12]. This color in sporadic renal hemangioblastoma is entirely different from yellow color in clear cell RCC. Microscopically, the tumor is composed of sheets of polygonal cells and prominent capillary network. Cytoplasmic vacuoles are often observed [3-12]. Sporadic renal hemangioblastoma seems to have no relationship to VHL gene mutation [6, 9] hypermethylation or loss of heterozygosity (LOH) of chromosome 3p [9]. In my opinion, the molecular genetic study should be performed for the definite diagnosis of difficult cases in discriminating renal hemangioblastoma from clear cell RCC at the routine histological and immunohistochemical procedures. Although the immunohistochemical expression of EGFR was...

Figure 3. Immunohistochemical findings. A. Inhibin-alpha. B. S-100 protein. C. CA9. D. PAX2. E. PAX8. F. cytokeratin CAM5.2. Neoplastic cells are diffusely positive for inhibin-alpha, S-100 protein, CA9, PAX2 and PAX8, but negative for cytokeratin CAM5.2.
Table 1. Summary of previously reported 13 cases of sporadic renal hemangioblastoma in English literature

<table>
<thead>
<tr>
<th>Case number</th>
<th>Age</th>
<th>Sex</th>
<th>Size (cm)</th>
<th>CA9</th>
<th>PAX2</th>
<th>PAX8</th>
<th>VHL mut.</th>
<th>3p LOH</th>
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<td>1</td>
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<tr>
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<td>3.5</td>
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<tr>
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<td>3.2</td>
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<td>61</td>
<td>M</td>
<td>5.3</td>
<td>NP</td>
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<td>NP</td>
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<tr>
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<td>F</td>
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<tr>
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<td>Wang</td>
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<td>10</td>
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<td>F</td>
<td>5.5</td>
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<td>d+</td>
<td>NP</td>
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<tr>
<td>11-13</td>
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<td>?</td>
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<td>NP</td>
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<td>+ (1/3)</td>
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<tr>
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<td>3.6</td>
<td>d+</td>
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<td>-</td>
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</table>

CA, carbonic anhydrase; PAX, paired box; VHL, von Hippel-Lindau; mut, mutation; LOH, loss of heterozygosity; M, male; F, female; NP, not performed; d, diffuse; f, focal; +, positive; -, negative.

observed in renal hemangioblastoma, no EGFR gene mutations are detected in the present study [7, 9].

Interestingly, we observed some usual histologic features including rhabdoid cells, eosinophilic globules resembling hyaline globules, intranuclear pseudoinclusion, perivascular hyalinization, smooth muscle stroma and hemangiopericytomatous pattern. Rhabdoid cells may appear as one of dedifferentiation phenomenon in clear cell RCC. The combination of rhabdoid cells and focal marked atypical nuclei may lead pathologist to the misdiagnosis of clear cell RCC with rhabdoid features [23]. However, rhabdoid phenotype has been described in sporadic renal hemangioblastoma [7]. Hence, pathologist should be careful in these settings. Previously, the presence of eosinophilic globules has been reported in sporadic renal hemangioblastoma [4]. However, eosinophilic globules have been noted in clear cell RC, papillary RCC, chromophobe RCC, renal oncocytoma and RCC associated with tuberous sclerosis complex, and therefore, this finding is of no use in the differential diagnosis [24-28]. Previously, we have found that d-PAS-positive hyaline-globule-like structures in carcinoma or sarcoma component may be related to secondary lysosomes [29, 30]. The similarity and difference of eosinophilic globules in hemangioblastoma and other renal tumors should be clarified by the future large scale study. Intranuclear pseudoinclusions may be observed in usual ductal hyperplasia of the breast, papillary thyroid carcinoma, thyroid hyalinizing trabecular tumor, meningioma and pulmonary adenocarcinoma [31, 32]. Additionally, intranuclear pseudoinclusions have been previously observed in sporadic renal hemangioblastoma [7] and chromophobe RCC [33]. This morphologic finding does not seem to be helpful in the differential diagnosis of renal benign and malignant tumors. Intranuclear pseudoinclusions in sporadic renal hemangioblastoma may reflect the deep invagination of cytoplasmic component into nucleus. In addition, perivascular hyalinization can be often observed in renal epitheloid angiomyolipoma (eAML) [34]. Pathologists can distinguish renal hemangioblastoma from eAML because of routine histological features and immunohistochemical panel including Melanosome and Melan A. We do not know whether smooth muscle stroma is a neoplastic component or not in the present case, but pathologists should distinguish sporadic renal hemangioblastoma from RCC with smooth muscle stroma [35]. The accumulation of similar cases is needed in order to elucidate the significance of smooth muscle stroma in sporadic renal hemangioblastoma. Although hemangiopericytomatous pattern is often described in sporadic renal hemangioblastoma [5, 11, 12], the distinction from solitary fibrous tumor is important [36]. In this setting, the immunohistochemical positivity of STAT6 in solitary fibrous tumors is decisive.

In comparative genomic hybridization (CGH) analysis of 10 sporadic cerebellar hemangioblastomas without VHL disease, losses of chromosomes 3 (70%), 6 (50%), 9 (30%), and 18q (30%) and a gain of chromosome 19 (30%) have been detected [37]. In another CGH study of 22 CNS hemangioblastomas (18 cerebellum and 4 medulla) with and without VHL disease, chromosomal abnormalities have been identified in 6 cases (27%) of cases. They included 5 case of chromosome 6q loss, 3 case chromosome 3 loss and 1 chromosome 8 loss [38].
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Accordingly, tumor suppressor genes containing chromosomes 3 and 6q may be involved in the early phase of tumorigenesis of CNS hemangioblastoma [38]. As we were very interested whether sporadic renal hemangioblastoma are identical to CNS hemangioblastoma, we investigated chromosomal change in the present study using G-band karyotype and array CGH. However, this tumor did not show chromosomal alterations.

In conclusion, pathologists should be aware that sporadic renal hemangioblastoma share some immunophenotypes to clear cell RCC including CA9, PAX2 and PAX8. We recommend the positive immunohistochemical panel of inhibin-alpha and S-100 protein for sporadic renal hemangioblastoma, because NSE is non-specific marker and S-100 protein is generally negative for clear cell RCC [39]. These similar immunohistochemical findings may suggest that sporadic renal hemangioblastoma has a close relationship to clear cell RCC. Further examination in a large scale cohort will be required in order to clarify the difference and relationship between these two entities.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Naoto Kuroda, Department of Diagnostic Pathology, Kochi Red Cross Hospital, Shin-Honnachi 2-13-51, Kochi City, Kochi 780-8562, Japan. Tel: +81-88-822-1201; Fax: +81-88-822-1056; E-mail: kurochankochi@yahoo.co.jp

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