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Case report

Eosinophilic cytoplasmic inclusions in type 2 papillary renal cell carcinoma

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Summary

A case of a patient with type 2 papillary renal cell carcinoma with eosinophilic cytoplasmatic inclusions is presented. About 50% of tumor cells were characterized by a well-circumscribed intra-cytoplasmatic round-to-oval or irregular inclusion/globule. Inclusions were 7-30 micron in diameter. They were glassy and pale to slightly eosinophilic in color in hematoxylin and eosin, were stained red by trichrome and were negative for periodic acid-Schiff reaction. Immunohistochemically, globules were negative for PAX8, epithelial membrane antigen, Carbonic Anhydrase IX, pan-cytokeratin (AE1/AE3), CD10, S100 protein, α-smooth-muscle actin, cytokeratin 7 and cytokeratin 34βE12. Glassy hyaline globules were not detected in any adjacent normal kidney cells. The presence of eosinophilic cytoplasmic inclusions in renal cell carcinoma, especially in papillary renal cell carcinoma, has been rarely emphasized in the literature. In this article, we review similar cases in the literature and discuss the nature of eosinophilic globules.

Key words

Ppppillary renal cell carcinoma • Eosinophilic cytoplasmatic inclusions • Sarcomatoid differentiation • Aggressive behavior

Introduction

Papillary renal cell carcinoma (PRCC) is the second most commonly encountered morphotype of renal cell carcinoma (RCC). Among adults the mean age distribution is 59-63 years [1]. Tumors are usually confined to the cortex within the renal capsule at the time of nephrectomy [2]. The cut surface of PRCC vary in color from gray to yellow to red-brown. The tumor is well circumscribed with a fibrous pseudo-capsule. It may contain intra-tumoral hemorrhage and necrosis and/or cystic degeneration. It is derived from renal tubular epithelium and has a papillary or tubulo-papillary architecture. Some tumors show a predominantly tubular architecture or a solid appearance caused by papillae tightly packed [3]. Papillae have delicate or hyalinized fibrovascular cores, that often contain psammoma bodies and foamy macrophages, covered by single layer or by pseudostratified layers of epithelial cells. PRCC, although not reported by I.S.U.P/W.H.O. 2016, has been separated in two subtypes, based on morphologic features [4 5]: a) Type 1 carcinoma have papillae covered by single layer of small epithelial cells with scanty, pale, usually basophilic cytoplasm; b) Type 2 carcinoma have papillae covered by pseudo-stratified large cells with voluminous eosinophilic cytoplasm and higher nucleolar grade. Approximately 5% of PRCCs show sarcomatoid changes [4]. Renal tumors showing papillary architecture but also features of recognized morphotypes of RCC (i.e. collecting duct carcinoma, mucinous tubular and spindle cell carcinoma, hereditary leiomyomatosis and RCC-associated RCC, and MiT family translocation RCC) should not be diagnosed as PRCC [1]. Immunohistochemical studies of PRCC show positive reactions for cytokeratin AE1/AE3, CAM5.2, high molecular weight cytokeratins, epithelial membrane antigen, AMACR, RCC antigen, vimentin, CD10, PAX8, PAX2 [3-8]. Eosinophilic inclusions are the light microscopic manifestations of aggregates of cytoplasmic products or organelles, visualized with

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hematoxylin and eosin (H&E). The presence of glassy hyaline globules (GHG) in renal carcinomas has been rarely emphasized in the literature, in particular in PRCC [9-15]. Here, we report a case of eosinophilic cytoplasmic inclusions in PRCC, with review of the literature.

Materials and methods

Fixation of tissue is carried out in 10% neutral buffered formalin for 24 hours. Once tissue is embedded in paraffin, a 3-4 micron tissue section is cut onto charged glass slides. Positive and negative controls were present into the tissue or added on the glass.
The detection system for immunostaining is BOND Polymer Refine Detection on staining platform LEICA BOND III. 30 min at 100°C in Bond Epitope Retrieval Solution 1 used to antigen retrieval for antibodies: α-smooth-muscle actin (clone AB1-1A4; Dako; 1:1000), Epithelial Membrane Antigen (clone E29; Dako; 1:500), Immunoglobulin light chains λ (polyclonal; Dako; 1:100.000), CD10 (clone 56C6; Leica; 1:50); 20 min for antibody PAX8 (clone Mrq50; Menarini; 1:800) and Immunoglobulin light chains κ (polyclonal; Dako; 1:50.000); 15 min for antibody Carbonic Anhydrase IX (clone TH22; Leica; 1.50); 40 min for antibody Cytokeratin 34βE12 (clone 34βE12; Menarini; 1:100); 30 min at 100°C in Bond Epitope Retrieval Solution 2 for antibody alpha-methylacyl-CoA racemase (clone 13H4; Dako; 1:1000); 10 min at 37°C with Bond Epitope Retrieval Enzyme for antibodies Cytokeratin 7 (clone OV-TL 12/30; Menarini; 1:500) and pan-cytokeratin AE1/AE3 (clone AE1/AE3; Cell Marque; 1:1000); nothing retrieval for S100 protein (polyclonal; Leica; 1:1000).

Case report

In July 2019 a 71-year-old man with a past history of prostatic hyperplasia came to our hospital for hematuria and flank pain. Computer tomography (CT) detected a renal mass. Extensive clinical examination revealed no signs of metastasis. The patient underwent right radical nephrectomy. The patient's post-operative course was without complications. The specimen obtained by nephrectomy weighed 1200 g, with a 12 x 10 x 8 cm well circumscribed mass in the kidney. The cut surface varied from light gray to red-brown, with intratumoral hemorrhage and necrosis (80% of tumor). The tumor showed extension to peri-renal tissues, without extension to fascia of Gerota (distance 1 mm), renal vein, vena cava and lymph nodes (pT3a, A.J.C.C. 8th Ed.) [16]. Histologically the tumor was circumscribed by a fibrous pseudo-capsule (Fig. 1A). The pseudo-capsule was incomplete, with tumor tissue focally intermixed with renal parenchyma. The tumor showed a predominantly tubule-papillary architecture with occasional solid areas (Fig. 1B). Papillae showed delicate or hyalinized fibrovascular cores, that sometimes contained foamy macrophages. No psammoma bodies were detected. Papillae were covered by a single layer or pseudo-stratified layers of large epithelial cells with voluminous eosinophilic cytoplasm, with large and spherical or light irregular nuclei, with prominent nucleoli (grade 3; I.S.U.P./W.H.O. 2016) [1] (Fig. 1C). Approximately 5% of tumor showed sarcomatoid change (grade 4; I.S.U.P./W.H.O. 2016) [1] (Fig. 1D). About 50% of tumor cells were characterized by a well-circumscribed intracytoplasmatic round-to-oval or irregular inclusion/globule with a halo. Inclusions were 7-30 micron in diameter. They were glassy and pale to slightly eosinophilic in color in H&E ("glassy hyaline globules"). Glassy hyaline globules were stained red by trichrome staining, but stained negatively with periodic acid-Schiff (PAS) with or without diastase treatment (Fig. 3), silver and Hale's colloidal iron. Glassy hyaline globules were not detected in any adjacent normal kidney cells. A moderate number of small lymphocytes and plasma cells were interspersed throughout the tumor. Immunohistochemical studies showed diffuse expression of PAX8, alpha-methylacyl-CoA racemase and epithelial membrane antigen, only focal reaction for Carbonic Anhydrase IX and pan-cytokeratin (AE1/ AE3), and negative staining for immunoglobulin light chains κ and λ, CD10, S100 protein, α-smooth-muscle actin, cytokeratin 7 and cytokeratin 34βE12 (Fig. 2). Immunohistochemistry indicated that the eosinophilic inclusions were negative for all antibodies studied. We did not perform electron microscopy evaluation of eosinophilic inclusions. At the time of nephrectomy total body nuclear magnetic resonance was negative. Four months later nuclear magnetic resonance showed bilateral pleural effusions, subcapsular hepatic nodule, suspected for metastasis, and multiple bone metastasis. A bone biopsy of iliac crest showed epithelial atypical cells organized in cellular cords with "renal phenotype" by immunohistochemistry: expression of cytokeratin (AE1/AE3), CD10, PAX8, Carbonic Anhydrase IX, and Vimentin (Fig. 3).

Discussion

In the literature, the presence of glassy hyaline globules in renal carcinomas has been rarely discussed, in particular in PRCC [9-15]. Eosinophilic inclusions can be positive or negative in PAS with or without diastase treatment [9 13 15] and are usually stained red with tri-

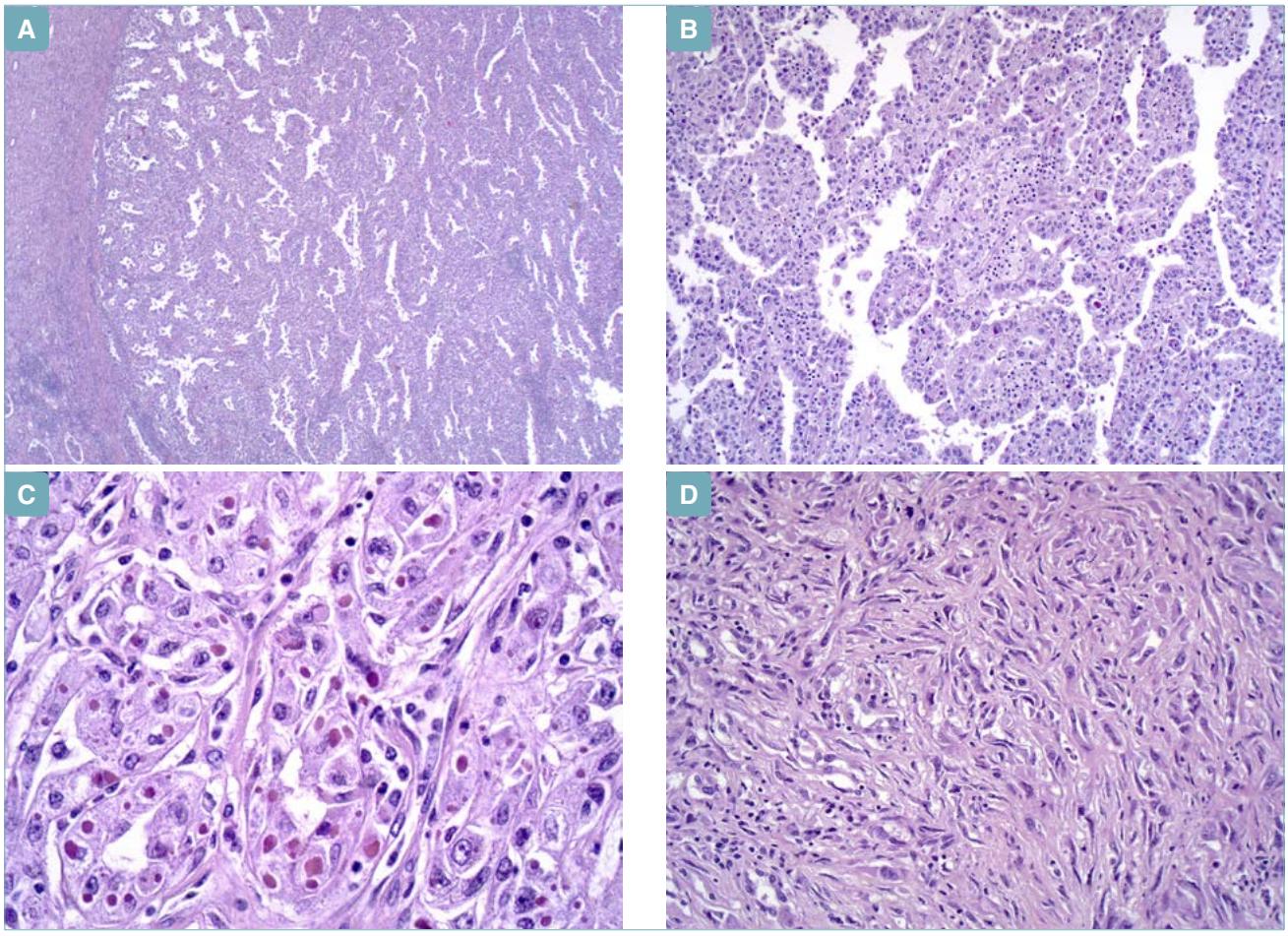

Fig. 1. Hematoxylin and eosin. (A) The tumor was well circumscribed with a fibrous pseudo-capsule. (B) Tubule-papillary architecture; papillae showed delicate or hyalinized fibrovascular cores, with inflammatory cells and occasional foamy macrophages. (C) Papillae were covered by atypical epithelial cells characterized by a well-circumscribed intra-cytoplasmatic round-to-oval or irregular inclusion with a halo. (D) Approximately 5% of tumor showed sarcomatoid changes.

chrome [9 13 15]. The eosinophilic cytoplasmic inclusions in the present case were PAS negative but stained red with trichrome. It has been suggested GHG may be aggregations of intermediate filaments (i.e. Mallory bodies), but no antigenic expression has been detected in immunohistochemistry. Ultrastructurally, intracytoplasmic eosinophilic inclusions consist of a central dense granular layer surrounded by membrane-bound oval organelles containing dense substances, and an outermost layer of clear space between the inclusion and cytoplasmic organelles [11 17]. They may consist of accumulation of an amorphous secretion of stellate shape inside of the cisternae of the rough endoplasmic reticulum [9 10]. Eosinophilic cytoplasmic inclusions were detected in 49 of 64 clear cell RCCs and in 5 of 33 PRCCs, but no GHG were found in 22 cases of chromophobe cell carcinomas and 26 renal oncocytomas [9]. In clear cell RCC, Paneth cell-like granules, which are closely packed, variably sized eosinophilic cytoplasmic inclusions, have been detected. They are confined to the apical portion of the cytoplasm, positive with PAS with and without diastase digestion and negative with trichrome stain. Ultrastructurally, these inclusions consist of an electron-dense single membrane-bound structure, which is consistent with lysosomes [16]. Also, in RCC and oncocytoma PAS-positive spherical globules, which are accumulations of basement membrane material, have been detected in extracellular location [19]. Several types of inclusions have been identified in renal neoplasms. GHG are a characteristic feature of clear cell RCC and in a small minority of papillary renal cell carcinomas, but not in chromophobe RCC and oncocytoma. Thus, the presence of GHG in a renal cell tumor may be useful for

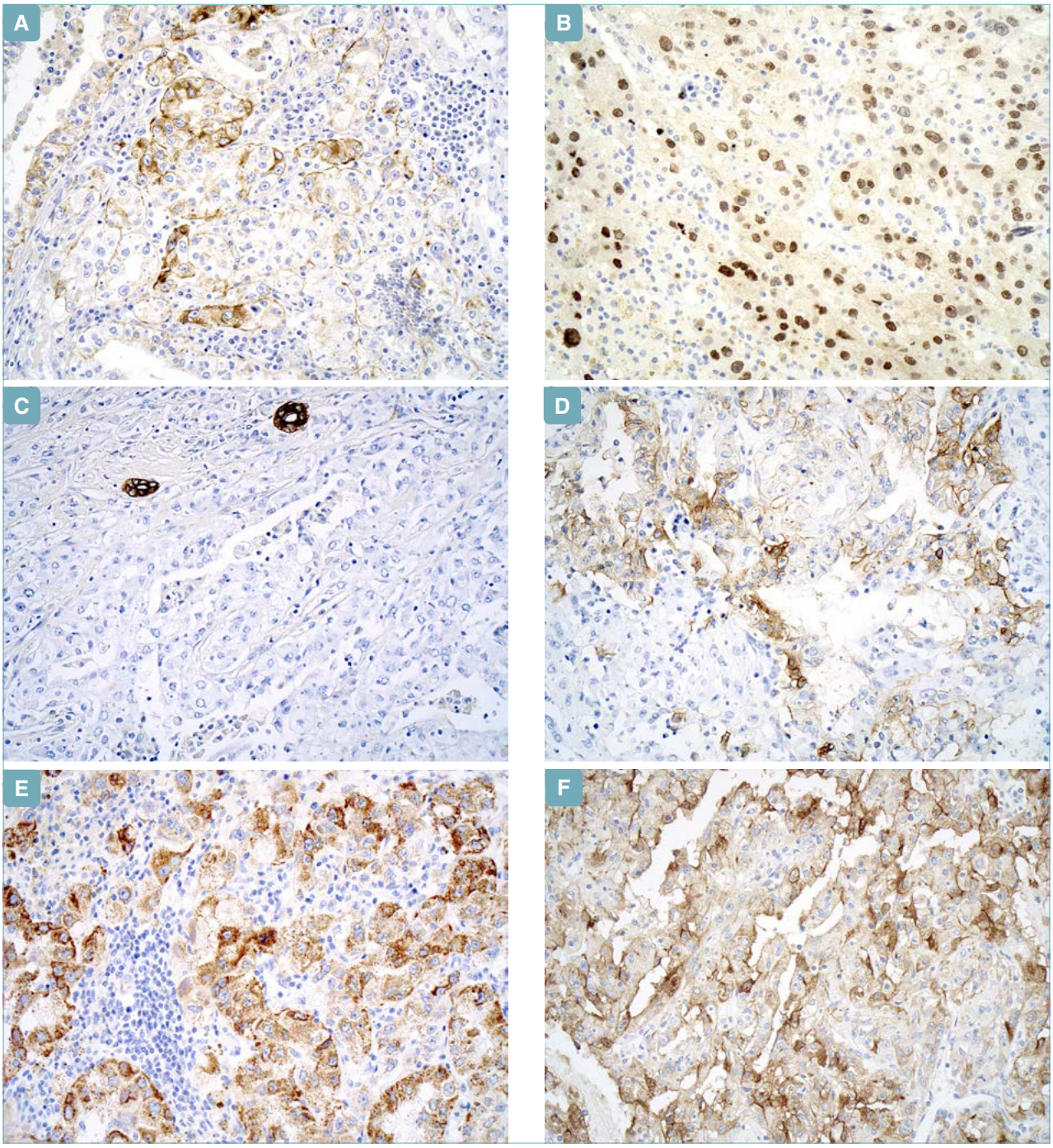

Fig. 2. Immunohistochemistry reactions. (A) Focal expression of pan-cytokeratin AE1/AE3. (B) Diffuse expression of PAX 8. (C) Negative staining for cytokeratin 7 (two normal tubules positive). (D) Focal expression of carbonic anhydrase IX. (E) Diffuse expression of alpha-methylacyl-CoA racemase, with negative eosinophilic cytoplasmic inclusions. (F) Diffuse expression of epithelial membrane antigen, with negative eosinophilic cytoplasmic inclusions.

excluding a diagnosis of chromophobe cell carcinoma or oncocytoma.

In conclusion, we present a rare case of papillary type 2 carcinoma with intra-cytoplasmic glassy hyaline globules, focal sarcomatoid differentiation, and aggressive behavior.

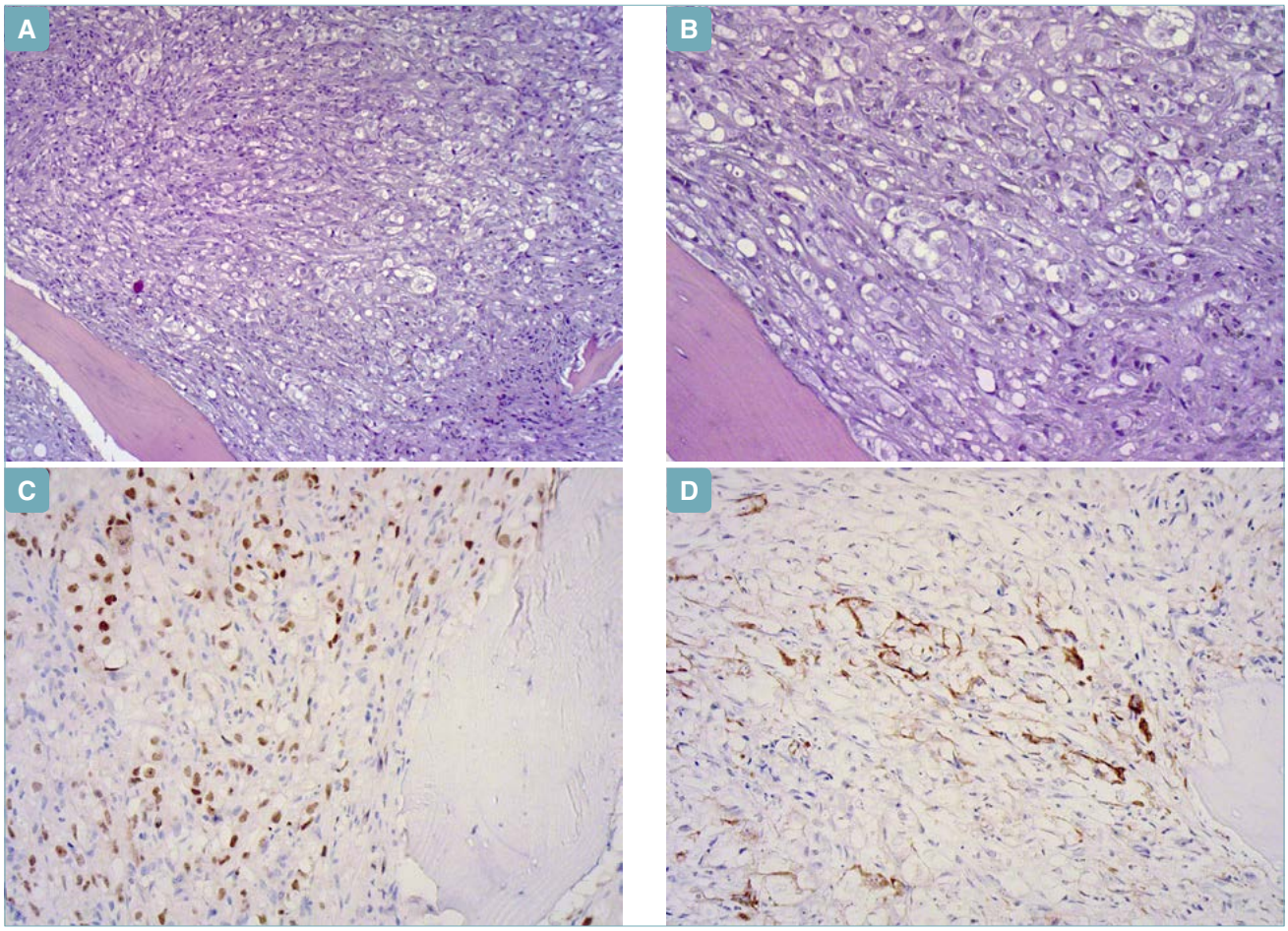

Fig. 3. (A) Bone localization of epithelial atypical cells organized in cellular cords (hematoxylin and eosin). (B) Large atypical cells with irregular nucleus and clear, abundant cytoplasm (hematoxylin and eosin). (C) Diffuse expression of PAX 8. (D) Focal expression of Carbonic Anhydrase IX.

Conflict of interest statement

None declared.

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