Annals of Diagnostic Pathology 26 (2017) 23–30

Contents lists available at ScienceDirect

Annals of Diagnostic Pathology

Cystic and necrotic papillary renal cell carcinoma: prognosis, morphology, immunohistochemical, and molecular-genetic profile of 10 cases☆,☆☆

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ARTICLE INFO

Keywords:
Kidney
Papillary renal cell carcinoma
Cystic
Necrosis
Necrotics
Molecular genetics

ABSTRACT

Conflicting data have been published on the prognostic significance of tumor necrosis in papillary renal cell carcinoma (PRCC). Although the presence of necrosis is generally considered an adverse prognostic feature in PRCC, we report a cohort of 10 morphologically distinct cystic and extensively necrotic PRCC with favorable biological behavior. Ten cases of type 1 PRCC with a uniform morphologic pattern were selected from the 19 500 renal tumors, of which 1311 were PRCCs in our registry. We focused on precise morphologic diagnosis supported by immunohistochemical and molecular-genetic analysis. Patients included 8 men and 2 women with an age range of 32-85 years (mean, 62.6 years). Tumor size ranged from 6 to 14 cm (mean, 9.4 cm). Follow-up data were available in 7 patients, ranging from 0.5 to 14 years (mean, 4 years). All tumors were spherical, cystic, and circumscribed by a thick fibrous capsule, filled with hemorrhagic/necrotic contents. Limited viable neoplastic tissue was present only as a thin rim in the inner surface of the cyst wall, consistent with type 1 PRCC. All cases were positive for AMACR, OSCAR, CAM 5.2, HIF-2, and vimentin. Chromosome 7 and 17 polysomy was found in 5 of 9 analyzable cases, 2 cases demonstrated chromosome 7 and 17 disomy, and 1 case showed only chromosome 17 polysomy. Loss of chromosome Y was found in 5 cases, including 1 case with disomic chromosomes 7 and 17. No *VHL* gene abnormalities were found. Papillary renal cell carcinoma type 1 can present as a large hemorrhagic/necrotic unicystic lesion with a thick fibroleiomyomatous capsule. Most cases showed a chromosomal numerical aberration pattern characteristic of PRCC. All tumors followed a nonaggressive clinical course. Large liquefactive necrosis should not necessarily be considered an adverse prognostic feature, particularly in a subset of type 1 PRCC with unilocular cysts filled with necrotic/hemorrhagic material.

1. Introduction

Papillary renal cell carcinoma (PRCC) accounts for 15% to 20% of renal carcinomas and is a heterogeneous disease with histologic subtypes and variations in clinical behavior and outcome. It is traditionally subclassified as type 1, which is a distinct entity (morphologically, immunohistochemically, and genetically), and type 2, which is composed of more heterogeneous group of diseases [1]. Grossly, PRCCs are usually well circumscribed and may contain foci of necrosis and

☆ Disclosure of Conflict of Interest: All authors declare no conflict of interest.
☆☆ The study was supported by the Charles University Research Fund (Project No. P36), by the Ministry of Health of the Czech Republic—Conceptual Development of Research Organization (Faculty Hospital in Pilsen, 00669806), and by SVV 260283.
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http://dx.doi.org/10.1016/j.anndiagpath.2016.10.007
1092-9134/

hemorrhage. Nonetheless, unilocular cystic tumors within type 1 PRCC are rather uncommon.

We describe a cohort of PRCC, morphologically consistent with type 1 according to the Delahunt classification, which were large unilocular cystic tumors surrounded by thick-wall fibrous capsule and filled with hemorrhagic/necrotic contents, demonstrating long-term favorable clinical outcome [1,2]. The purpose of this study was to describe a unique subpopulation of type 1 PRCC with an unusual gross and histologic presentation (cystic lesion with necrotic content) to enhance our understating of the prognostic significance of tumor necrosis (TN) in these tumors.

2. Materials and methods

This study design was approved by local ethical committee (Charles University, Medical School Plzen).

Of 19 500 renal tumors and tumor-like lesions (including 1311 PRCCs) in the institutional and consultation files of the Sikl's Department of Pathology at Charles' University, Plzen, Czech Republic, 10 cases of cystic and largely necrotic type 1 PRCC were retrieved. The tissue had been fixed in neutral formalin, embedded in paraffin, 3- to 4-μm-thick sections were cut and stained with hematoxylin and eosin.

All tumors were large cystic lesions encapsulated by a thick, mostly fibrotic tissue. In 2 cases, the tumor capsule was histologically composed of so-called phenomenon inflammatory pseudotumor, for which one of them has already been reported [3]. Cysts were filled with sanguinolent necrotic material, whereas viable neoplastic structures were identified only in the inner surface of the cyst wall. Cases were further examined by immunohistochemistry and analyzed by molecular-genetic methods.

2.1. Immunohistochemistry

The immunohistochemical study was performed using a Ventana Benchmark XT automated stainer (Ventana Medical System, Inc, Tucson, AZ). The following primary antibodies were used: cytokeratin AE1/3 VM (AE1/AE3/PCK26, monoclonal; Ventana-Roche, Mannheim, Germany, RTU), wide-spectrum keratin (OSCAR, monoclonal, 1:2000; Covance, Princetown, NJ), cytokeratin (CAM 5,2 monoclonal, 1:200; Becton-Dickinson, San Jose, CA), racemase/AMACR (P504S, monoclonal, 1:50; Zeta, Sierra Madre, CA), vimentin (D9, monoclonal, 1:1000; Neomarkers, Westinghouse, CA), carbonic anhydrase IX (rhCA9, monoclonal, 1:100; RD Systems, Abingdon, GB), CD31 (JC70A, monoclonal, 1:50; DakoCytomation, Glostrup, Denmark), CD34 (QBEnd-10, monoclonal, 1:100; DakoCytomation), c-kit (CD 117, polyclonal; DakoCytomation, RTU), cathepsin K (monoclonal, 3F9, 1:100; Abcam, Cambridge, UK), PAX-8 (polyclonal rabbit, 1:25; Cell Marque–Medac/RNDr. A. Manthey, Rocklin, CA), TFE3 (polyclonal, 1:100; Abcam), HIF-1α (ESEE122, 0.5:150; Abcam), HIF-2α (ep190b, 1:30; Abcam), and phospho-mTOR (Ser2448, 1:80; Cell Signaling Technology, Danvers, MA). Appropriate positive and negative controls were used.

2.2. Molecular-genetic study

2.2.1. Fluorescence in situ hybridization methods

Four-micrometer-thick section was placed onto a positively charged slide. Hematoxylin and eosin–stained slide was examined for the cell counting area determination.

The unstained slide was routinely deparaffinized and incubated in the 1× Target Retrieval Solution Citrate pH 6 (Dako, Glostrup, Denmark) for 40 minutes at 95°C and subsequently cooled for 20 minutes at room temperature in the same solution. The slide was washed in deionized water for 5 minutes and digested in protease solution with Pepsin (0.5 mg/mL; Sigma-Aldrich, St Louis, MO) in 0.01 M HCl at 37°C for 20 minutes. The slide was then placed into deionized water for 5 minutes, dehydrated in a series of ethanol solution (70%, 85%, and 96% for 2 minutes each), and air-dried. Probes for aneuploidy detection of chromosomes 7 and 17 (Vysis/Abbott Molecular, Des Plaines, IL; see Table 1) were mixed with water and LSI/WCP (Locus-Specific Identifier/Whole Chromosome Painting) Hybridization buffer (Vysis) in a 1:2:7 ratio. An appropriate amount of probe mix was applied on the specimen, covered with a glass coverslip and sealed with a rubber cement. The slide was incubated in the ThermoBrite instrument (StatSpin/Iris Sample Processing, Westwood, MA) with co-denaturation parameters 85°C for 8 minutes and hybridization parameters 37°C for 16 hours. Rubber-cemented coverslip was then removed and the slide was placed in a posthybridization wash solution (2× SSC/0.3% NP-40) at 72°C for 2 minutes. The slide was air-dried in the dark, counterstained with DAPI (Vysis), coverslipped and immediately examined.

2.2.2. Fluorescence in situ hybridization interpretation

The section was examined with an Olympus BX51 fluorescence microscope (Olympus Corporation, Tokyo, Japan) using a ×100 objective and filter sets Triple Band Pass (DAPI/SpectrumGreen/SpectrumOrange) and Single Band Pass (SpectrumGreen/SpectrumOrange). Scoring of aneuploidy was performed by counting the number of fluorescent signals in 100 randomly selected nonoverlapping tumor cell nuclei. The slide was independently enumerated by 2 observers (OH and PG). Monosomy and polysomy for studied chromosomes were defined as the presence of one signal per cell in greater than 45% and 3 and more signals in greater than 10% (mean + 3 SD in normal nonneoplastic control tissues), respectively.

2.2.3. DNA extraction and bisulfite DNA conversion

DNA for molecular-genetic investigation was extracted from formalin-fixed, paraffin-embedded tissue. Several 5-μm-thick sections were placed on the slides. Hematoxylin and eosin–stained slides were examined for identification of neoplastic tissue. Subsequently, neoplastic tumor and nonneoplastic tissue from unstained slides were scraped and DNA was isolated by the NucleoSpin Tissue Kit (Macherey-Nagel, Düren, Germany).

Bisulfite conversion of DNA was carried out using EZ DNA Methylation–Gold Kit (DNA input 500 ng; Zymo Research, Orange, CA).

All procedures were performed according to the manufacturer's protocols.

2.3. VHL gene analysis

Mutation analysis of exons 1, 2, and 3 of the *VHL* gene was performed using polymerase chain reaction (PCR) and direct sequencing. Polymerase chain reaction was carried out using primers shown in Table 2. The reaction conditions were as follows: 12.5 μL of HotStar Taq PCR Master Mix (Qiagen, Hilden, Germany), 10 pmol of each primer, 100 ng of template DNA, and distilled water up to 25 μL. The amplification program consisted of denaturation at 95°C for 15 minutes and then 40 cycles of denaturation at 95°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 1.5 minute for all amplicons. The program was finished by 72°C incubation for 7 minutes.

The PCR products were checked on 2% agarose gel electrophoresis.

Successfully amplified PCR products were purified with magnetic particles Agencourt AMPure (Agencourt Bioscince Corporation, A Beckman Coulter Company, Beverly, MA), both side sequenced using Big Dye Terminator Sequencing kit (Applied Biosystems, Foster City, CA) and purified with magnetic particles Agencourt CleanSEQ (Agencourt Bioscince Corporation) all according to the manufacturer's protocol, and subsequently run on an automated sequencer ABI Prism 3130xl (Applied Biosystems) at a constant voltage of 13.2 kV for 20 minutes. All samples were analyzed in duplicates. Analyses of positive samples were repeated.