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Renal cell carcinoma with leiomyomatous stroma—further immunohistochemical and molecular genetic characteristics of unusual entity

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ABSTRACT

Renal cell carcinoma (RCC) with leiomyomatous stroma (RCCLS) is a recently recognized entity with indolent biological behavior. The diagnostic implication of absence/presence of *VHL* gene mutation, *VHL* hypermethylation, or/and loss of heterozygosity of chromosome 3p (LOH 3p) is widely discussed. Criteria for establishing a diagnosis of RCCLS are still lacking. Fifteen RCCLSs were retrieved from our registry. The cases were studied with consideration to the morphology, immunohistochemistry, and molecular genetics. All cases were composed of low-grade epithelial cells with clear cytoplasm arranged in nests intermingled with abundant leiomyomatous stroma. Age range of the patients was 33 to 78 years. The tumor size ranged from 1.5 to 11 cm. Six of the patients were males, and 9, females. Of the 15 tumors sent for molecular genetic testing, only 12 cases were analyzable. All cases were analyzable immunohistochemically. Of 12 of these cases, 5 showed complete absence of *VHL* gene mutation, *VHL* hypermethylation, and LOH 3p. Of these 5 cases, 3 were positive for cytokeratin 7 (CK 7). All of the 5 cases were positive for carbonic anhydrase 9, vimentin, and CD10. The remaining 7 of 12 genetically analyzable cases were found to have had *VHL* hypermethylation, LOH 3p, *VHL* gene mutation, or a combination of the former 2 characteristics. These 7 cases were positive for vimentin. Variable reactivity was found for CK 7, carbonic anhydrase 9, α-methylacyl-CoA racemase, and CD10. In 1 of these 7 cases, gains on chromosomes 7 and 17 as well as hypermethylation of *VHL* gene were found. This case was considered as clear cell RCC with aberrant status of chromosomes 7 and 17. Conclusions: (1) Leiomyomatous stroma is not specific for the so called RCCLS. It can be seen also in otherwise typical clear cell RCCs. (2) There are no characteristic morphological/immunohistochemical features unique for "RCCLS." (3) Our results indicate that only tumors with the absence of the *VHL* gene mutation, hypermethylation, and LOH 3p can be diagnosed as RCCLS. (4) Relation of RCCs with a prominent smooth muscle stroma to the renal angiomyoadenomatous tumor/clear cell papillary (tubopapillary) RCC is not clearly evident from our study and has to be further analyzed on larger cohort of the patients.

1. Introduction

Renal cell carcinomas (RCCs) with a prominent smooth muscle stroma (RCCSMSs) are rare neoplasms, which were first described by Canzonieri et al in 1993 [1] and subsequently documented by Kuhn et al in 2006 [2] and other investigators [3-5]. Microscopically, RCCSMSs are composed of an intimate intermixture of 2 distinct components: epithelial and stromal. The epithelial component is represented by clear epithelial cells with mild nuclear atypia (mostly Fuhrman grade 2) arranged in adenomatous structures with predominantly nested or tubular pattern associated with focal papillary and solid areas.

Exact diagnostic criteria are not established. Morphology and immunohistochemical features (namely, cytokeratin 7 [CK 7] positivity among others) are variable in previous studies.

The role of absence/presence of *VHL* gene mutation, *VHL* hypermethylation, or/and loss of heterozygosity (LOH) of chromosome 3p (LOH 3p) for diagnosis is widely discussed [5].

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We assembled a group of tumors with voluminous leiomyomatous stroma and clear cell morphology. The morphology, immunohistochemistry, and molecular biology of all tumors were examined as attempt to select RCCSMS from group of tumors with similar morphologic features.

2. Materials and methods

Of 17 700 renal tumors and tumor-like lesions in the institutional and consultation files of Sikl's Department of Pathology, Charles University, Plzen, Czech Republic, 15 cases of RCCs were retrieved. All were composed of epithelial cells with clear cytoplasm arranged in nests or tubules intermingled with abundant leiomyomatous stroma.

The tissue had been fixed in neutral formalin and embedded in paraffin; 4- to 5-μm-thick sections were cut and stained with hematoxylin and eosin.

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: CK 7 (OV-TL12/30, monoclonal, 1:200; DakoCytomation, Glostrup, Denmark), racemase/α-methylacyl-CoA racemase (AMACR) (P504S, monoclonal, 1:50; Zeta, Sierra Madre, CA), vimentin (D9, monoclonal, 1:1000; Neomarkers, Westinghouse, CA), carbonic anhydrase 9 (CANH 9) (rhCA9, monoclonal, 1:100; RD Systems, Minneapolis, MN), melanoma marker (HMB45, monoclonal, 1:200; Dako, Carpinteria, CA), and CD10 (56C6, 1:20; Novocastra, Burlingame, CA). Appropriate positive controls were used.

2.2. Molecular genetic study

2.2.1. Fluorescence in situ hybridization methods

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

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, Abbott Park, IL) (see Table 1) were mixed with water and Locus-Specific Identifier/Whole Chromosome Painting Hybridization buffer (Vysis) in a 1:2:7 ratio. An appropriate amount of probe mix was applied on specimen, covered with a glass coverslip, and sealed with rubber cement. The slide was incubated in the ThermoBrite instrument (StatSpin; Iris Sample Processing, Westwood, MA) with codenaturation 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 posthybridization wash solution (2× saline-sodium citrate/0.3% NP-40) at 72°C for 2 minutes. The slide was air dried in the dark, counterstained with 4′,6′-diamidino-2-phenylindole (Vysis), coverslipped, and immediately examined.

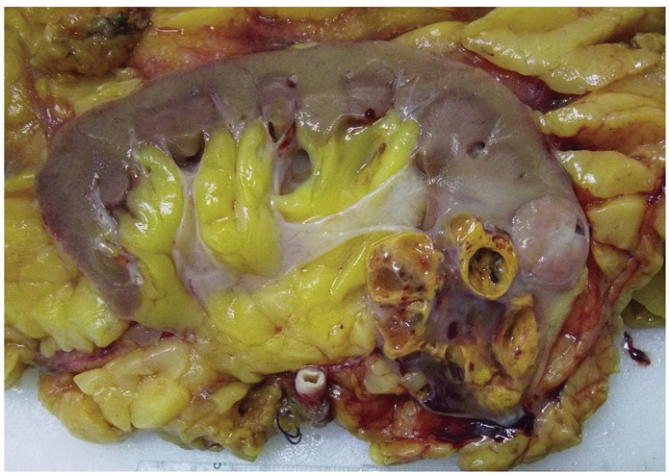

Fig. 1. Gross section of the kidney with 2 tumors. One was typical clear cell renal cell carcinoma (CCRCC) (yellow color), and the second was CCRCC (genetically confirmed) with abundant leiomyomatous stroma.

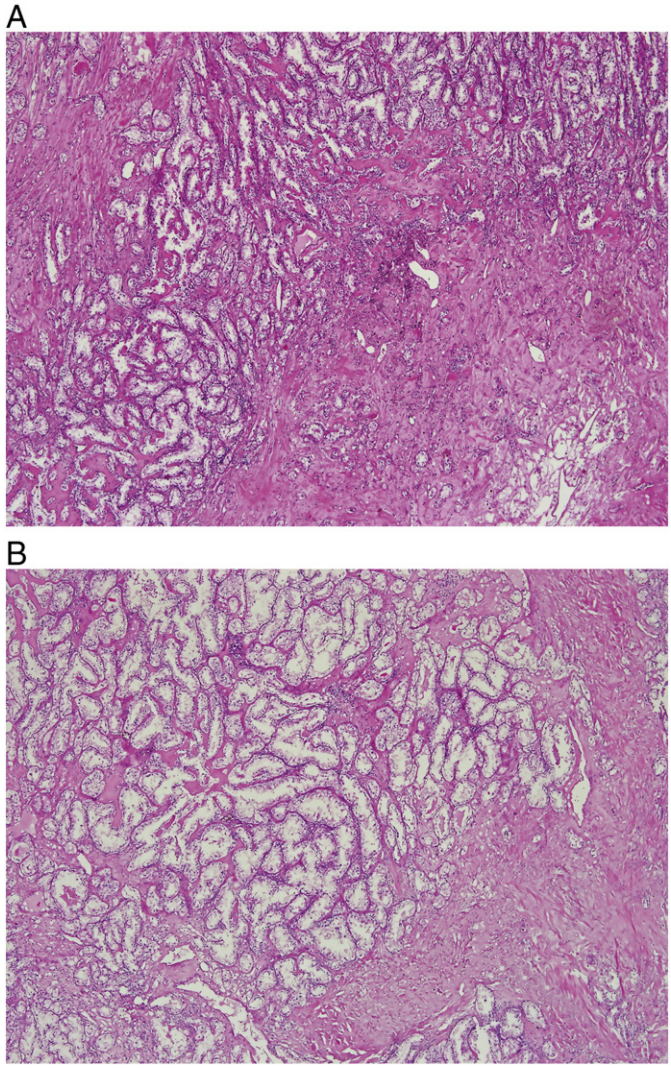

Fig. 2. Smooth muscle stroma was prominent in all cases. Epithelial component as well as stroma was identical in tumors, which were, after special examinations, considered as CCRCC (A), and in cases, which were diagnosed as "RCCLS" (B).

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 (4′,6′-diamidino-2-phenylindole/SpectrumGreen/SpectrumOrange) and single-band pass (SpectrumGreen/