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Distinctive renal cell tumor simulating atrophic kidney with 2 types of microcalcifications. Report of 3 cases☆,☆☆

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ABSTRACT

We report 3 cases of primary renal cell tumor simulating atrophic kidney with distinct gross, morphologic, immunohistochemical, and molecular genetic features. The tumors were retrieved out of more than 17 000 renal tumors from the Plzen Tumor Registry. Tissues for light microscopy had been fixed, embedded, and stained with hematoxylin and eosin using routine procedures. The tumors were further analyzed using immunohistochemistry, array comparative genomic hybridization, and human androgen receptor. Analyses of *VHL* gene and loss of heterozygosity (LOH) 3p were also performed. The patients were 2 women and 1 man, with ages ranging from 29 to 35 years (mean, 31.3 years). Grossly, the neoplasms were encapsulated and round with largest diameter of 3.5 cm (mean, 3.2 cm). Follow-up available for all patients ranged from 2 to 14 years (mean, 8 years). No aggressive behavior was noted. Histologically, akin to atrophic (postpyelonephritic) kidney parenchyma, the tumors were composed of follicles of varying sizes that were filled by eosinophilic secretion. Rare areas contained collapsed follicles. Each follicle was endowed with a small capillary. The stroma was loose, inconspicuous, and focally fibrotic. Two types of calcifications were noted: typical psammoma bodies and amorphous dark-blue stained calcified deposits. Immunohistochemically, tumors were strongly positive for cytokeratins (OSCAR), CD10, and vimentin, with weak immunopositivity for CAM5.2 and AE1-AE3. WT1 and cathepsin K were weakly to moderately focally to diffusely positive. Tumors were negative for cytokeratin 20, carbonic anhydrase IX, parvalbumin, HMB45, TTF1, TFE3, chromogranin A, thyroglobulin, PAX8, and ALK. Only 1 case was suitable for molecular genetic analyses. No mutations were found in the *VHL* gene; no methylation of *VHL* promoter was noted. No numerical aberrations were found by array comparative genomic hybridization analysis. LOH for chromosome 3p was not detected. Analysis of clonality (human androgen receptor) revealed the monoclonal nature of the tumor. We describe an unknown tumor of the kidney that (1) resembles renal atrophic kidney or nodular goiter of thyroidal gland; (2) contains a leiomyomatous capsule and 2 types of calcifications; (3) lacks mitoses, atypias, necroses, and hemorrhages and nearly lack Ki-67 positivity; and (4) so far showed benign biological behavior.

1. Introduction

We report 3 cases of a primary renal cell tumor microscopically simulating atrophic kidney with distinct gross, morphologic, and immunohistochemical features, which has not so far been reported, to the best of our knowledge. Comparative genomic hybridization (CGH) analysis and analysis of clonality using X-chromosomal inactivation pattern and human androgen receptor (HUMARA) locus were used to better understand the nature of this tumor and to distinguish it from the kidney with atrophic changes.

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2. Materials and methods

The tumors were retrieved out of more than 17 000 renal cases from the Plzen Tumor Registry. Two of 3 cases were sent to the authors (O.H., M.M.) for the second opinion, and case 3 was contributed by Dr Souza from Hospital Aliança, Salvador, Brazil. Tissues for light microscopy have been fixed in 4% formaldehyde and embedded in paraffin using routine procedures. Five-micrometer-thick sections were cut and stained with hematoxylin and eosin.

3. Immunohistochemistry

The immunohistochemical study was performed using a Ventana Benchmark XT automated stainer (Ventana Medical System, Inc, Tucson, Arizona). The following primary antibodies were used: epithelial membrane antigen (E29, monoclonal; 1:1000; DAKO, Carpinteria, California), cytokeratins (CKs; CAM5.2, monoclonal, 1:200; Becton-Dickinson, San Jose, California), Pan Ab-1 (AE1-AE3, monoclonal, 1:1000; BioGenex, San Ramon, California), CK7 (OV-TL12/30, monoclonal, 1:200; DAKO), CK18 (DC 10, monoclonal, 1:100; DAKO), CK19 (M 0888, monoclonal, 1:100; DAKO), CK20 (M7019, monoclonal, 1:100; DAKO), CK OSCAR (OSCAR, monoclonal, 1:2000; Covance, Princetown, New Jersey), CK NMF 116 (MNF 116, monoclonal, 1:100; DAKO), racemase/AMACR (P504S, monoclonal, 1:50; Zeta, Sierra Madre, California), vimentin (D9, monoclonal, 1:1000; NeoMarkers, Westinghouse, California), Ki-67 (MIB1, monoclonal, 1:1000; DAKO, Glostrup, Denmark), melanoma marker (HMB45, monoclonal, 1:200; DAKO), CD10 (56C6, monoclonal, 1:100; Abcam, Cambridge, UK), carbonic anhydrase IX (rhCA9, monoclonal, 1:100; RD Systems, Abingdon, GB, UK), TFE3 (polyclonal, 1:100; Abcam), TFE 3 (monoclonal, MRQ 37, RTU; Cell Marque, Rocklin, California), ALK (monoclonal, 5A4, 1:400; Novocastra, Newcastle, UK), cathepsin K (3F9, monoclonal, 1:100; Abcam), WT1 (GF-H2, monoclonal, 1:150; DAKO), synaptophysin (polyclonal, 1:400; Thermo Scientific, Cheshire, UK), chromogranin A (DAK A3, monoclonal, 1:300; DAKO), S 100 protein (polyclonal, 1:400; DAKO), TTF-1 (SPT24, monoclonal, 1:400; Novocastra), thyroglobulin (polyclonal, RTU; DAKO), parvalbumin (PARV-19, monoclonal, 1:500; Sigma, St Luis, Missouri), CD34 (monoclonal, QBEnd10, 1:100; DAKO), desmin (monoclonal, D33, 1:2000; DAKO), smooth muscle actin (monoclonal, 1A4, RTU; Ventana-Roche), PAX2 (polyclonal, 1:100; Invitrogen, Camarillo, California), and PAX8 (polyclonal, 1:100; Abcam). Appropriate positive controls were used.

4. Molecular genetic study

4.1. DNA extraction

DNA from formalin-fixed paraffin-embedded (FFPE) tumor and nontumor tissues was extracted using a QIAsymphony DNA Mini Kit (Qiagen, Hilden, Germany) on automated extraction system (QIAsymphony SP; Qiagen), according to the manufacturer's supplementary protocol for FFPE samples (purification of genomic DNA from FFPE tissue using the QIAamp DNA FFPE Tissue Kit and Deparaffinization Solution). Concentration and purity of isolated DNA was measured using NanoDrop ND-1000 (NanoDrop Technologies Inc, Wilmington, Delaware). DNA integrity was examined by amplification of control genes in a multiplex polymerase chain reaction (PCR).[1]

4.2. Array CGH

4.2.1. A microarray processing

A CytoChip Focus Constitutional (BlueGnome Ltd, Cambridge, UK) was used for analysis. CytoChip Focus Constitutional uses BAC technology and covers 143 regions of known significance with 1-Mb spacing across a genome. Probes are spotted in triplicates. First, 400 ng of DNA was labeled using the Fluorescent Labeling System (BlueGnome Ltd). The procedure included Cy3 labeling of a test sample and Cy5 labeling of a reference sample. The labeled reference and the test sample were mixed, dried, and hybridized overnight at 47°C using Arrayit hybridization cassette (Arrayit Corporation, Sunnyvale, California). Posthybridization washing was done using SSC buffers with increasing stringency. Dried microarray was scanned with InnoScan 900 (Innopsys, Carbonne, France) at a resolution of 5 μm.

4.2.2. Image and data analysis

Scanned image was analyzed and quantified by BlueFuse Multi software (BlueGnome Ltd). BlueFuse Multi uses Bayesian algorithms to generate intensity values for each Cy5- and Cy3-labeled spot on the array according to an appropriate.gal file. The results were annotated using BlueFuse Multi, as well. Cutoff values for log 2 ratio were set to −0,193 for loss and to 0.170 for gain.[2]

4.3. VHL gene mutation analysis and promoter methylation

Mutation analysis of coding sequence and exon-intron junctions of the *VHL* gene was performed using PCR and direct sequencing. Detection of promoter methylation was performed via methylation-specific PCR, as described by Herman et al.[3] Bisulfite conversion, PCR primers, and setup are shown in a previous study.[4]

4.4. 3p loss of heterozygosity analysis

For LOH analysis of neoplastic tissue DNA, 10 short tandem repeat markers (D3S666, D3S1270, D3S1300, D3S1581, D3S1597, D3S1600, D3S1603, D3S1768, D3S2338, and D3S3630) located on the short arm of chromosome 3 (3p) were chosen from the database (GenBank UniSTS). The method is described in details in a previous study.[4]

4.5. Analysis of clonality using HUMARA locus

Clonality analysis was performed according to the previously described method based on the digestion of differentially methylated X-chromosomal DNA with methylation-sensitive restriction enzyme followed by PCR amplification of a CAG repeat located in HUMARA. DNA from tumor and nontumor tissues was digested by restriction enzyme *Hha*I and amplified as described previously.[5]

DNA from tumor and nontumor tissues was digested by restriction enzyme *Hha*I and amplified as described previously.[6]

Polymerase chain reaction products were examined by a fragment analysis on Abi3130*xl*. Peak heights of the 2 alleles were measured for each specimen. A corrected ratio (CR) was assessed by dividing the ratio (allele 1/allele2) of the digested sample by the ratio (allele1/allele 2) of the undigested sample. A final clonality ratio was determined by dividing the CR of the tumor DNA by the CR of the nontumor DNA. Sample was considered monoclonal if the final ratio was higher than 1.5 or lower than 0.66.

5. Results

5.1. Case 1

A 30-year-old woman has been followed up for 11 years for hyperurikemia and nephrolithiasis. Radical right nephrectomy was performed. The tumor was located in the renal cortex between the upper pole and part of the kidney. No infiltration into the adjacent perirenal structures or into the renal sinus was noted. There was no relationship of the lesion to the renal pelvis. The tumor was excised fragmented in 8 pieces, and estimated overall size of the tumor was 3 cm in largest diameter.

Grossly, it was difficult to appreciate whether tumor was encapsulated because of the surgical fragmentation of the excised tissue. At follow-up, 14 years after nephrectomy, the patient is