

Bilateral tubulocystic renal cell carcinomas in diabetic end-stage renal disease: first case report with cytogenetic and ultrastructural studies

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Abstract

Tubulocystic renal cell carcinoma (TC-RCC) is a rare renal tumor composed of well-differentiated tubules and cysts lined by neoplastic cells with eosinophilic cytoplasm and prominent nucleoli. The origin of the tumor cells is still controversial. TC-RCC typically arises unilaterally. Involvement of both kidneys by multifocal TC-RCC has not been reported. In this study we report the first case of bilateral and multifocal TC-RCC. Immunohistochemical, cytogenetic and ultrastructural studies suggest TC-RCC is closely related to papillary RCC.

Introduction

Tubulocystic renal cell carcinoma (TC-RCC) is a rare morphological variant of renal cell carcinoma (RCC) first described by Dr. George Farrow at a United States and Canadian Academy of Pathology annual meeting. MacLennan et al later reported 8 of 13 cases of renal tumors that displayed the morphologic features of TC-RCC.¹ Several reports since then described cases in detail.²⁻⁵ The tumors are most often incidentally detected and have a male predominance. The average age of the patients is 54-58 years old.²⁻⁵ The average size is 2-4.5 cm. The histogenesis of the tumor cells is still controversial. The tumor was initially thought to arise from collecting ducts.¹ However, the tumor cells are immunoreactive to markers of both proximal convoluted tubules (CD10, Racemase and Pax2) and distal convoluted tubules (CK19, parvalbumin, high molecular weight cytokeratin).

Ultrastructurally, the tumor cells show fea-

tures of both proximal convoluted tubules and intercalated cells.⁴ The tumor cells are shown cytogenetically to gain chromosomes 7, 17 and loss of chromosome Y.^{2,3} Gene expression profiles show the tumor cells of TC-RCC are similar to papillary RCC.^{2,3} The tumor has low malignant potential and low risk of distant metastasis.²⁻⁵ TC-RCC usually occurs unilaterally.⁴ Involvement of both kidneys by multifocal TC-RCC has not been reported in the English language literature. Here we present the immunohistochemical, cytogenetic and ultrastructural features of bilateral TC-RCC in a 62 year-old man with end stage renal disease on hemodialysis.

Case Report

A 62 year-old man with end stage renal disease on hemodialysis due to type II diabetes and hypertension presented with neck and chest pain. A computed tomography (CT) scan to exclude aortic dissection incidentally revealed bilateral complex renal masses less than 3.0 cm in greatest dimension. The patient reported two to three weeks of painless gross hematuria, but denied fever, flank pain, and lower urinary symptoms. Given the patient's comorbidities and the small size of the lesions, the patient was reluctant to undergo the recommended surgical intervention. Repeat CT scan of the abdomen 5 months later showed relative stability of the lesions with one cyst having grown from 1.1 to 1.3 cm. There was also a small nodular enhancing component considered suggestive of renal cell carcinoma. After an additional 6 months, a complete abdominal CT showed no growth of the cysts but heterogeneous attenuation suggested nodularity or thick septations consistent with malignancy.

After an additional 7 months (18 months after presentation), a repeat CT showed bilateral and multiple enhancing lesions: a 2.6×2.3 cm lesion and a 1.3×1.6 cm lesion in the left mid-kidney, a 1.8×1.7 lesion in the right upper pole, and a 1.5×1.6 cm lesion in the right mid-kidney (Figure 1A, B). Additional bilateral enhancing lesions did not show interval growth. Cystoscopy and urine cytology at that time were negative for malignancy. A CT urogram showed no excretion of contrast, limiting evaluation of the upper urinary tracts. At that time, with recurrent hematuria, and told of progression of the lesions, the patient elected to undergo bilateral laparoscopic radical nephrectomy.

Histopathology findings

The left kidney and perinephric fat measured 10.5×7.5×4.5 cm and weighed 125.5 grams. The right kidney and perinephric fat

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measured 11×8×4.5 cm and weighed 190 grams. On cut section, there were multifocal unencapsulated sharply demarcated gray spongy cystic lesions ranging from 0.3 to 2.5 cm in diameter in renal cortex and renal medulla (Figure 1C, D). The cystic lesions contained clear serous fluid. The renal capsules and perinephric fat were grossly uninvolved. Simple cortical cysts were also seen separately. Numerous black multifaceted calculi ranging from 0.1 to 0.2 cm in greatest dimension were present within both renal pelvises (Figure 1C, D). On microscopic examination, the cystic lesions were composed of tightly packed tubules and cysts separated by bland fibrous stroma (Figure 2A-D). The cysts were lined by a single layer of cuboidal to columnar cells, often with a *hobnail* appearance. The cells had large round to oval nuclei (Fuhrman grade 2 to 3), prominent nucleoli, and abundant eosinophilic cytoplasm (Figure 2C, D). No desmoplastic reaction or cellular ovarian-type stroma was present. No areas of solid or papillary growth was seen in either kidney. Papillary

renal cell carcinoma was not present. Pathologic stage was pT1 for both kidneys.

Immunostains for vimentin, PAX-2, AE1/AE3, p63, p504S (AMACR), 34 beta E12, cytokeratin 8/18, EMA, cytokeratin 7, cytokeratin 20, and CD10 were performed using a Dako Autostainer with prior heat induced epitope retrieval in a water bath with buffered Citrate (pH 6.0) and EDTA (pH 8.0) (Table 1). The tubulocystic carcinoma cells showed diffuse strong immunoreactivity (Figure 3) for AMACR, AE1/AE3, CK8/18, CD10 and PAX2, and focally strong immunoreactivity for CK7 (Figure 3D), EMA, Vimentin and 34BE12. No immunoreactivity was seen for P63 and CK20. The tubule-containing and cystic areas of the tumor demonstrated equal immunoreactivity for pan-cytokeratin, CK8/18 and AMACR. Immunoreactivity for 34BE12, CK7, CD10 and EMA was greater in the tubule-containing areas, relative to the cystic areas.

The uninvolved renal parenchyma bilaterally showed extensive global glomerular sclerosis, interstitial fibrosis, tubular atrophy with thickening of tubular basement membrane, and focal interstitial chronic inflammation. The non-sclerotic glomeruli showed diffuse nodular mesangial expansion, with numerous Kimmelstiel-Wilson lesions highlighted by Periodic Acid Schiff (PAS) stain. *Onion-skin* thickening of the arterioles was noted. Both kidneys showed multiple simple cysts. These changes are consistent with the patient's clinical history of end-stage renal disease in the setting of hypertension and diabetes.

Fluorescence *in situ* hybridization for chromosome 7 and 17

Fluorescence in situ hybridization (FISH) was performed on a representative section from the left kidney tubulocystic carcinoma using methods described earlier.² Briefly, 4.0 micrometer sections from a representative, formalin-fixed, paraffin-embedded block were cut and utilized for FISH studies. To confirm the cytogenetic abnormalities reported previously in a case of TC-RCC,² a UroVysion bladder cancer kit containing probes for chromosome 7 (CEP7, spectrum green), and chromosome 17 (CEP17, spectrum aqua) (Abbott Molecular/Vysis Cat. no.36-161070) was used.

The FISH data were analyzed using methods described before.⁶ The corresponding hematoxylin and eosin slides were reviewed before the FISH study and areas of tumor and normal renal tubules were marked. One hundred nuclei from normal and tumor areas were examined for signals from probes under the fluorescence microscope with 1000 magnification. Normal renal tubules were used as a control. Definitions of chromosomal gain and loss of chromosomes 7, 17 were based on the Gaussian model and related to the non-neoplastic controls. Any tumor with a signal score

beyond the cut-off value was considered to have a gain or loss of the specific chromosome. The cut-off values for each probe were set at mean +3 SD of the control values. Gains of chromosomes 7 and 17 were present in 11% and 35% of the tubulocystic RCC cells. Adjacent normal renal tubule cells showed gains of chromosomes 7 and 17 in 1% and 0% of cells, respectively.

Electron microscopy

A formalin-fixed sample of the specimen was used for the EM study. The specimen was transferred from neutral buffered formalin to 3% Sorenson's phosphate buffered glutaraldehyde and washed in 0.1M Sorenson's phosphate buffer (pH 7.3). The tissue was then post-fixed in 2% osmium tetroxide, dehydrated in a graded series of ethanol. Samples were

Table 1. Antibodies and antigen retrieval methods used for immunostaining.

Antibody	Clone	Supplier	Titer	Buffer
Vimentin	V9	DAKO	1:1000	EDTA
Pax-2	Rabbit	Invitrogen	1:250	EDTA
AE1/AE3	AE1/AE3	DAKO	1:400	EDTA
p63	BC4A4	Biocare	1:1	EDTA
p504s	Rabbit-p	Biocare	1:1	EDTA
CK8/18	Ucd/pr10.11	Invitrogen	1:100	Citrate
EMA	E29	DAKO	1:400	Citrate
CK7	out112/30	DAKO	1:1000	Citrate
CK20	ks20.8	DAKO	1:400	Citrate
CD10	56C6	Cell Marque	1:25	EDTA
34βe12	34 e12	DAKO	1:200	Citrate

Table 2. Immunohistochemical profiles of tubulocystic (TC-RCC) and papillary renal cell carcinomas (PRCC): (-) indicates negative; (+/-) negative or focal positive; (+) positive.

	AMACR	AE1/AE3	CK7	CK8/18	CD10	PAX2	CAIX	EMAVimentin	34βE12	P63	CK20
TC-RCC	+	+/-	+/-	+	+	+/-	+/-	+/-	+/-	-	-
PRCC	+	+/-	+	+	+	+	+	+	+/-	-	-

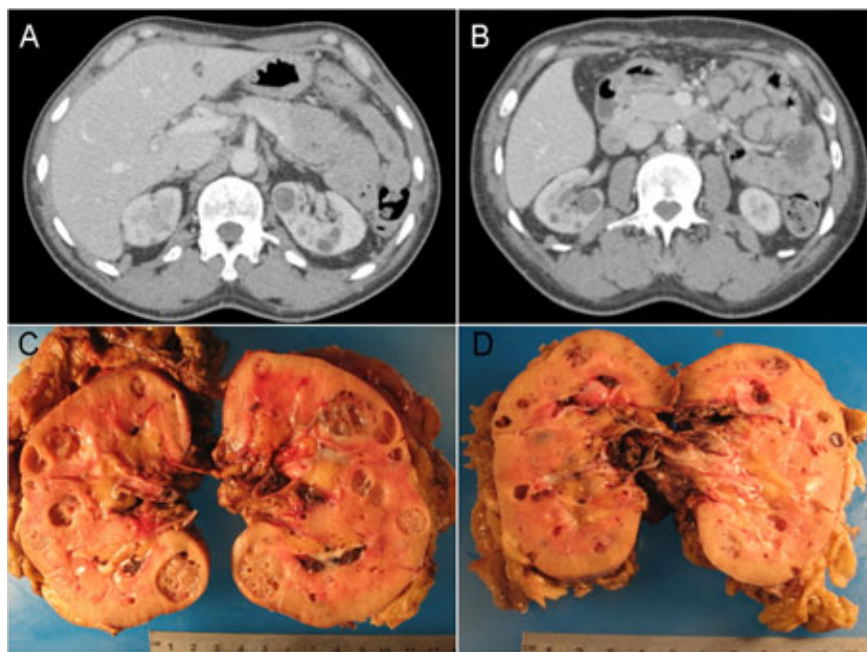


Figure 1. Radiologic imaging and gross photograph of tubulocystic renal cell carcinoma. Cystic lesions without contrast enhancement within the left (A) and right kidney (B). Sharply-demarcated cystic lesions of varying sizes with involvement of both cortex and medulla, are present in both the left (C) and right (D) kidneys. Black calculi were present in the renal pelvis bilaterally.

then transferred to propylene oxide, incubated in mixtures of propylene oxide and eponate 812 (Electron Microscopy Sciences), and embedded in eponate 812 at 60 degrees centigrade. 1.0 micron-thick sections were cut for orientation and 70 nm sections were made for ultrastructural examination. These sections were placed on copper grids and stained with uranyl acetate and lead citrate and examined in a JEM 1010 electron microscope.

Ultrastructural examination revealed the presence of two types of cells. The type I cells (Figure 4A-C) were composed of cuboidal cells bound on their luminal surfaces by tight junctions. The nuclear contour of these cells ranges from irregular (Figure 4A) to oval (Figure 4B). Nucleoli were prominent (Figure 4B). There were well-formed brush borders with long microvilli with prominent rootlets (Figure 4C). The cytoplasm of these cells had numerous mitochondria, many of which contained dense plaques (Figure 4C). Lipid material and membrane bound granules were also present. These cells were bound by basement membrane material. Interstitial material consists of fibroblasts, collagen, and blood vessels. These ultrastructural features are characteristic of proximal convoluted tubules. Type I cells were seen in the tubular component of the TC-RCC. The type II cells (Figure 4D) were present in a single layer, with desmosomal junctions and luminal surfaces bound by tight junctions. The cytoplasm of these cells contained rough endoplasmic reticulum, mitochondria, and lipid droplets. The surfaces of these type II cells were devoid of microvilli (Figure 4D). The base of the glands was bound by basement membrane material. These ultrastructural features are characteristic of distal nephrons. Type II cells were seen in the cystic component of the TC-RCC. No cells had ultrastructural characteristics of both type I and type II cells.

Case follow up

The patient died of non-tumor related causes 16 months post-bilateral nephrectomy. No tumor recurrence or metastasis was noted by the time of his death.

Discussion

Tubulocystic carcinoma of the kidney (TC-RCC) is a peculiar rare kidney tumor with male predominance, and characteristic gross and microscopic features.²⁻⁵ It usually occurs as a single focus, or multifocally within one kidney. To our knowledge, bilateral and multifocal tubulocystic renal cell carcinoma has not been reported. Here we report the first case of bilateral TC-RCC associated with end-stage renal disease. Only two unilateral TC-RCC cases have been identified in the setting of

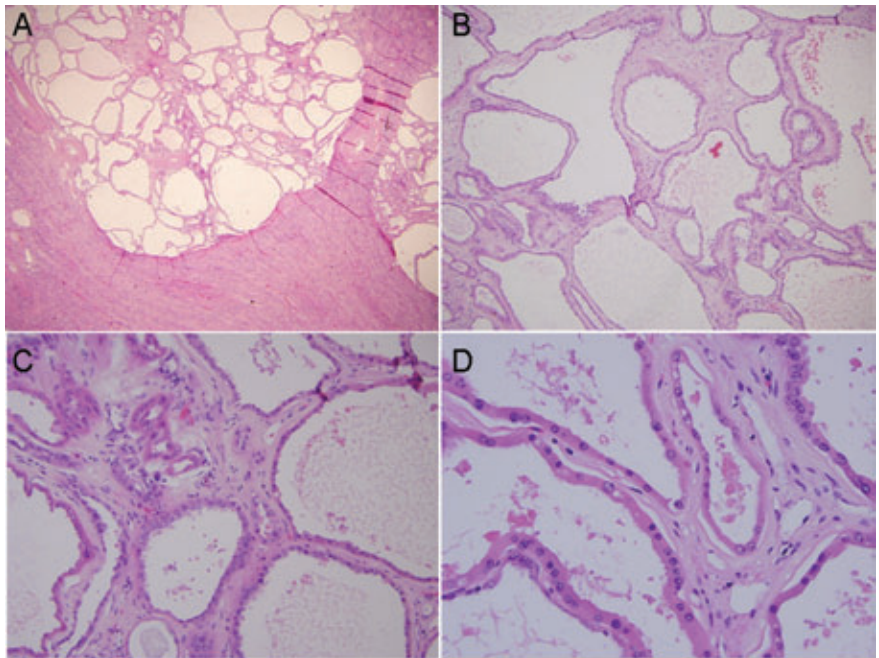


Figure 2. Representative histologic features of tubulocystic renal cell carcinoma. Tubulocystic RCC is composed of tubules and cysts of varying size. (A) Two adjacent foci of tubulocystic RCC in the renal cortex and medulla of the left kidney (20×). (B) The cysts contain clear serous fluid (40×). (C) and (D) flat to cuboidal cells with abundant eosinophilic cytoplasm line the tubules and cysts (100×). (D) The tumor cells have occasional prominent nucleoli (100×).

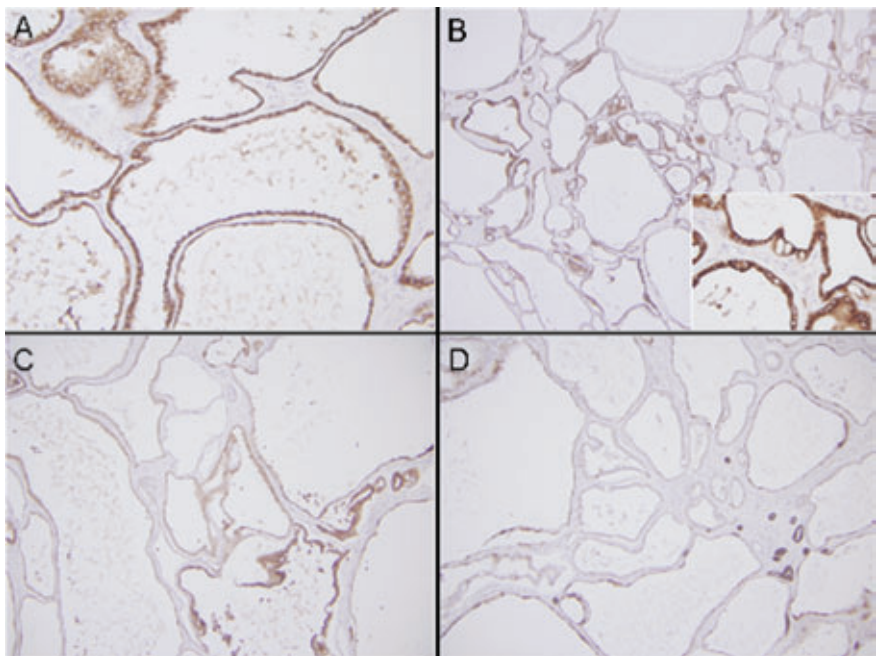


Figure 3. Characteristic immunohistochemical features of tubulocystic renal cell carcinoma. (A) Diffuse strong immunoreactivity for AMACR (100×), (B) CK8/18 (40×; Insert, original magnification 200×), (C) CD10 (40×), and (D) focally strong positivity for CK7 (40×).

end-stage renal disease in the five published case series.^{2-5,7}

Multiple subtypes of RCC occur in end-stage renal disease, most frequently papillary RCC, but also clear cell RCC, clear cell papillary RCC, TC-RCC, chromophobe RCC, and oncocytoma.⁸ In up to 70% of cases more than one tumor was identified in a single kidney.⁸ More than four different types of renal cell entities were recently reported in a single kidney, including renal oncocytosis with chromophobe RCC, papillary RCC, clear cell papillary and cystic RCC, and TC-RCC, demonstrating that TC-RCC is a part of RCC spectrum seen in end stage kidney disease together with PRCC, clear cell RCC, etc.⁷ The occurrence of multiple types of RCC in a single kidney suggests that these subtypes are related.⁷

Electron microscopy of this case demonstrated that the neoplastic cells have two cell types: cuboidal type I cells, with tight junctions and well formed brush borders, and flat type II cells, with desmosomal junctions but without microvilli. The long microvilli of the type I cells are associated with rootlets (Figure 4C), a feature seen in the proximal convoluted tubules described by Jennette *et al.*⁹ These type I cells are seen in the tubular component of the tumors in this case and were described by

Amin.⁴ The type II cells are seen in the cystic component of the tumor, and resemble cells of the thin descending limb of Henle.⁹ We did not observe any cells with ultrastructural characteristics of both type I and type II cells.

Tumor cells in this case expressed markers of proximal convoluted tubule differentiation (CD10, P504S/racemase, Pax 2), as well as markers of distal nephron differentiation (CK7, CK19, 34BE12).^{4,7,10} Interestingly, we found the intensity of immunostaining between the tubule-like and cystic areas to be similar for pan-cytokeratin, CK8/18 and AMACR. Staining for 34BE12, CK7, CD10 and EMA was stronger in the tubule-like areas, relative to the cystic areas. The ultrastructural characteristics and immunoprofiles of these tumors suggest that TC-RCC represents a renal tumor with dual proximal tubule and distal nephron differentiation.

Our cytogenetic study of this case showed gains of chromosomes 7 and 17, consistent with the previous report.² Interestingly, 20 cases of papillary RCC had the same gain of chromosomes 7 and 17 in Zhou's analysis.² 10 of 12 TC-RCC had gain of chromosome 7, and 8 of 12 TC-RCC gain of chromosome 17.² Papillary RCC also expresses CK7, CK8/18, CK19, CD10 and Pax2.^{2,4,10}

Conclusions

In conclusion, TC-RCC is an unusual renal cell carcinoma with characteristic gross and microscopic features. The immunophenotype and cytogenetics of papillary RCC and tubulocystic RCC are very similar (Table 2), suggesting a developmental link between these two entities.² Ultrastructural findings and immunoprofile suggest that TC-RCC may represent a renal tumor with dual differentiation towards both proximal tubules and distal nephrons. This case is the first report of bilateral multifocal TC-RCC associated with end-stage renal disease.

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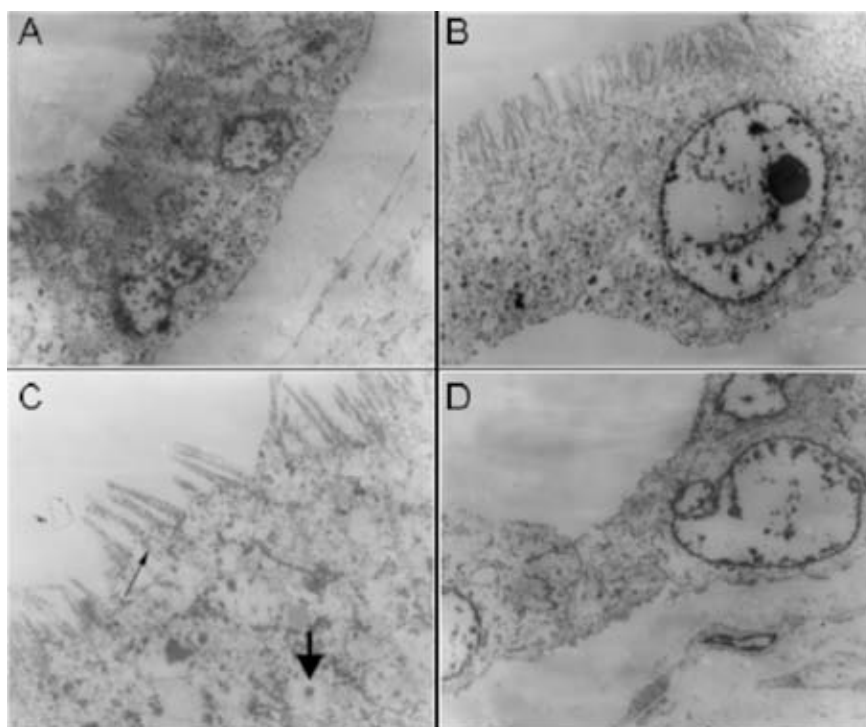


Figure 4. Ultrastructural features of tubulocystic carcinoma. Type I cells (A) and (B) at 8250 \times and at 27,500 \times (C). Type I cells have irregular nuclear contours (A), well-formed microvilli (A-B), rootlets (C, small arrow), and prominent nucleoli (B). Type I cells contain numerous mitochondria, many of which have dense plaques (C, larger arrow). Type II cells (D) lack microvilli and contain numerous mitochondria in dense plaques (11,000 \times).