Molecular Genetic Evidence for the Independent Origin of Multifocal Papillary Tumors in Patients with Papillary Renal Cell Carcinomas

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Abstract

Purpose: In patients with papillary renal cell carcinoma, it is not uncommon to find two or more anatomically distinct and histologically similar tumors at radical nephrectomy. Whether these multiple papillary lesions result from intrarenal metastasis or arise independently is unknown. Previous studies have shown that multifocal clear cell renal cell carcinomas express identical allelic loss and shift patterns in the different tumors within the same kidney, consistent with a clonal origin. However, similar clonality assays for multifocal papillary renal cell neoplasia have not been done. Molecular analysis of microsatellite and chromosome alterations and X-chromosome inactivation status in separate tumors in the same patient can be used to study the genetic relationships among the coexisting multiple tumors.

Experimental Design: We examined specimens from 21 patients who underwent radical nephrectomy for renal cell carcinoma. All patients had multiple separate papillary lesions (ranging from 2 to 5). Eighteen patients had multiple papillary renal cell carcinomas. Seven had one or more papillary renal cell carcinomas with coexisting papillary adenomas. Genomic DNA samples were prepared from formalin-fixed, paraffin-embedded tissue sections using laser-capture microdissection. Loss of heterozygosity assays were done for six microsatellite polymorphic markers for putative tumor suppressor genes on chromosomes 3p14 (D3S1285), 7q31 (D7S522), 9p21 (D9S171), 16q23 (D16S507), 17q21 (D17S1795), and 17p13 (TP53). X-chromosome inactivation analyses were done on the papillary kidney tumors from three female patients. Fluorescence *in situ* hybridization analysis was done on the tumors of selected patients showing allelic loss at loci on chromosome 7 and/or chromosome 17.

Results: Twenty of 21 (95%) cases showed allelic loss in one or more of the papillary lesions in at least one of the six polymorphic markers analyzed. A concordant allelic loss pattern between each coexisting kidney tumor was seen in only 1 of 21 (5%) cases. A concordant pattern of nonrandom X-chromosome inactivation in the coexisting multiple papillary lesions was seen in two of three female patients. A discordant pattern of X-chromosome inactivation was seen in the tumors of the other female patient. Fluorescence *in situ* hybridization showed that the majority of tumors analyzed had gains of chromosomes 7 and 17. Two patients had one tumor with chromosomal gain and another separate tumor that did not.

Conclusion: Our data suggest that, unlike multifocal clear cell renal cell carcinomas, the multiple tumors in patients with papillary renal cell carcinoma arise independently. Thus, intrarenal metastasis does not seem to play an important role in the spread of papillary renal cell carcinoma, a finding that has surgical, therapeutic, and prognostic implications.

Approximately 10% to 15% of renal cell carcinomas are of the papillary type. Papillary renal cell carcinoma is more frequently multifocal than other types of renal cell carcinoma (1–5). Papillary adenomas are common benign lesions of the kidney, which are characterized by a papillary or tubular architecture, a low nuclear grade, and a size smaller than 5 mm. Previous

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studies have shown multifocal clear cell renal cell carcinomas to be of a common clonal origin with multifocality presumably arising secondary to intrarenal metastasis (6, 7). However, similar clonality studies have not been done on papillary neoplasms of the kidney despite the fact that these tumors are the most frequently multifocal renal cancers. Treatment with nephron-sparing surgery or nonsurgical intervention such as radiofrequency ablation is increasingly being employed for renal tumors, especially small ones (8–11). In this clinical setting, clearly defining the genetic relationships among the multifocal papillary lesions and assessing the malignant potential of each lesion could have important diagnostic, surgical, and prognostic implications. In addition, understanding the nature of tumor multifocality can serve to further our understanding of the genetic basis of tumor progression in papillary renal neoplasms. In this study, molecular analysis of microsatellite and chromosomal alterations and X-chromosome inactivation status in separate papillary renal neoplasms from the same patient was used to assess the molecular genetic relationships among the coexisting multiple tumors.

Materials and Methods

Patients. Eighteen men and three women with multifocal papillary tumors of the kidney (papillary renal cell carcinomas and papillary adenomas) underwent nephrectomy from 1991 to 2003. None of the patients was known to have the hereditary papillary renal carcinoma syndrome. Three patients had end-stage renal disease. The patients had a mean age of 60 years (range, 26-83 years). All patients had two or more papillary neoplasms including at least one papillary renal cell carcinoma. All tumors were confined to the kidney. Pathologic staging was done according to the 2002 tumor-node-metastasis classification system (12). Eleven patients had stage pT_{1a} lesions, eight patients had pT_{1b} lesions, and three patients had pT_2 lesions. The mean diameter of the largest tumor from each patient was 4.9 cm (median, 5.0 cm; range, 1.0-10.0 cm).

Tissue samples and microdissection. Archival surgical materials from 21 patients with papillary renal cell carcinoma (3 female patients and 18 male patients) having two or more separate papillary tumors accessioned from 1991 to 2003 were retrieved from the surgical pathology files of the Department of Pathology and Laboratory Medicine of the Indiana University School of Medicine (Indianapolis, IN), the Department of Pathology of Case Western Reserve University (Cleveland, OH), the Department of Pathology and Molecular Medicine of Wellington School of Medicine and Health Science (Wellington, New Zealand), the Department of Pathology of Corboda University (Corboda, Spain), and the Department of Pathology of the University of Verona (Verona, Italy). This study included a total of 52 separate papillary tumors including 42 papillary renal cell carcinomas and 10 papillary adenomas.

Histologic sections were prepared from formalin-fixed, paraffin-embedded tissue and were stained with H&E for microscopic evaluation. From these slides, the multiple papillary neoplasms were reviewed by a single pathologist (L.C.). Laser-assisted microdissection of the separate tumors was done (Fig. 1) on unstained sections using a PixCell II laser-capture microdissection system (Arcturus Engineering, Mountain View, CA) as previously described (13–16). Approximately 400 to 1,000 cells of each tumor were microdissected from the 5-μm histologic sections. Normal tissue from each case was microdissected as a control.

Detection of loss of heterozygosity. The dissected cells were deparaffinized with xylene and ethyl alcohol. PCR was used to amplify genomic DNA at six specific loci on five different chromosomes: 3p14 (D3S1285), 7q31 (D7S522), 9p21 (D9S171), 16q23 (D16S507), 17q21 (D17S1795), and 17p13 (TP53). Previous studies have shown that loss of heterozygosity (LOH) at these loci occurs frequently in renal cell carcinomas (6, 17–27). The tumor suppressor gene fragile histidine triad (*FHIT*) locus is present at 3p14 (D3S1285). Chromosomal region 7q31 (D7S522) contains the aphidicolin-inducible fragile site *FRA7G*. D9S171 includes regions of the putative tumor suppressor gene *p16*. D16S507 corresponds to the *CDH13* (H-cadherin) gene. The 17q21 (D17S1795) locus includes a putative tumor suppressor gene. The TP53 locus corresponds to the gene encoding the p53 protein. Mutations of the *p53* gene are the most common genetic abnormalities in cancer (28). PCR amplification and gel electrophoresis were done as previously described (14, 15, 29–32). The criterion for allelic loss was complete or nearly complete absence of one allele in tumor DNA (14, 15, 29–32). PCRs for each polymorphic microsatellite marker were repeated at least twice from the same DNA preparations and the same results were obtained.

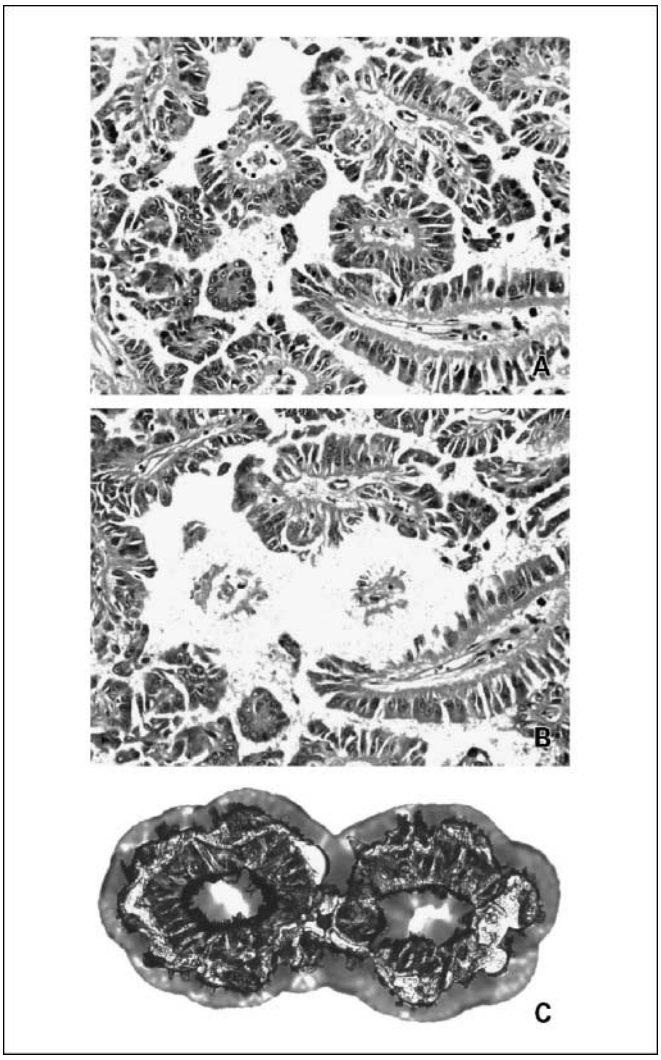

Fig. 1. Laser microdissection of papillary renal cell carcinoma from patients with multifocal papillary tumors. *A,* tumor before microdissection; *B,* tumor after microdissection; *C,* laser-captured papillary renal cell carcinoma cells.

Analysis of allelic loss pattern. When the genetic material in a patient was found to be homozygous for the polymorphic markers (i.e., showing only one allele in the normal control tissue), the case was considered noninformative. Patients with genetic material that was informative (i.e., showing two alleles in the normal control tissue) were divided into two categories. Their DNA may show no allelic deletions in

the tumor, retaining two different alleles of similar intensity on autoradiographs, or show absence of one allele. DNA sampled from the cells of separate papillary neoplasms demonstrating identical allelic loss patterns is compatible with a common clonal origin whereas different patterns of allelic deletions are compatible with independent clonal origins of these tumors (29, 30, 32, 33).

Detection of X-chromosome inactivation. X-chromosome inactivation analysis was done on papillary tumors from three female patients as previously described (30, 34, 35). DNA samples were prepared from each tumor from the same patient. The dissected cells were placed in 15 μL of buffer [10 mmol/L Tris-HCl, 1 mmol/L EDTA, 1% Tween 20, and 0.2 mg/mL of proteinase K (pH 8.3)] and incubated overnight at 37°C. The PCR products were separated by electrophoresis at 1,600 V for 4 to 7 hours. The bands were visualized after autoradiography with Kodak X-OMAT film (Eastman Kodak Company, Rochester, NY) for 8 to 16 hours.

Analysis of X-chromosome inactivation. The cases were considered to be informative if two *AR* allelic bands were detected after PCR amplification in normal control samples that had not been treated with *Hha*I. In tumor samples, nonrandom X-chromosome inactivation was defined as a complete or a nearly complete absence of an *AR* allele after *Hha*I digestion, which indicated a predominance of one allele. Tumors were considered to be of the same clonal origin if the same *AR* allelic inactivation pattern was detected in each separate tumor. Tumors were considered to be of independent origin if alternate predominance of *AR* alleles after *Hha*I digestion (different allelic inactivation patterns) was detected in each tumor (34–36).

Fluorescence* in situ *hybridization. Fluorescence *in situ* hybridization (FISH) analysis was done as previously described (37–39). Five-micron-thick sections were cut from paraffin-embedded blocks. α-Satellite centromeric DNA probes for chromosomes 7 and 17 were from Vysis (Downers Grove, IL). The CEP7/17 probes labeled with spectrum green were diluted with tDenHyb1 (Insitus, Alburquerque, NM) in a ratio of 1:100. Five microliters of diluted probes were added to the slide in the reduced light condition. The slides were counterstained with 10 μL of 4′,6-diamidino-2-phenylindole/Antifade (4′,6-diamidino-2-phenylindole in Fluorguard, 0.5 μg/mL, Insitus) for 2 minutes and covered with a 50 × 22 mm coverslip and were sealed. The slides were examined using a MetaSystem Axioplan 2 System (Metasystem Group, Inc., Belmont, MA).

In situ *hybridization analysis.* The criteria for evaluating the FISH signals were previously described (37–39). Fifty to 200 nuclei were scored for α-satellite signals observed with the fluorescence microscope at ×400 magnification. As much as possible, signals from solitary nuclei were counted, but groups of two or three adjacent but not overlapping nuclei were occasionally included in the counts. Nuclei were counted when the entire nuclear circumference had a round-to-oval contour and showed no evidence of fragmentation. Two signals of the same size in close proximity, not connected by a link, were counted as two signals. A diffuse signal was regarded as a signal if it was contiguous and within an acceptable boundary. Two small signals connected by a visible link were counted as one signal. Overlapping nuclei and nuclei with uncertain signals were not counted. There was no significant variation in hybridization efficiency when different areas of the slides were examined. The number of signals visualized in nuclei was tabulated from areas of the slides in which nuclear overlap was minimal. Cells bearing one, two, and three signals were counted separately. Normal tissue on the same slides was also counted as a control.

Results

Twenty of the 21 (95%) patients with multifocal papillary renal neoplasms showed allelic loss in one or more of their papillary tumors (Table 1). The number of loci lost in a single tumor ranged from one to five. Nearly all of the tumors (n = 52) from the 21 patients showed different patterns of allelic loss.

The frequencies of allelic losses in the informative papillary renal cell carcinomas were 31% (13 of 42) with D3S1285, 36% (15 of 42) with D7S522, 50% (20 of 40) with D9S171, 26% (11 of 42) with D16S507, 41% (15 of 37) with D17S1795, and 14% (6 of 42) with TP53. The frequencies of allelic losses in the informative papillary adenomas were 0% (0 of 10) with D3S1285, 10% (1 of 10) with D7S522, 50% (5 of 10) with D9S171, 30% (3 of 10) with D16S507, 29% (2 of 7) with D17S1795, and 20% (2 of 10) with TP53.

The allelic loss patterns at the six loci varied among the multifocal papillary carcinomas that were analyzed, consistent with independent origin. The LOH patterns from coexisting carcinomas and adenomas were very different, which is also consistent with independent origin. A concordant allelic loss pattern between each of coexisting kidney tumors was seen in only 1 of 21 (5%) patients (case 13). One patient showed a concordant pattern of allelic loss between two papillary adenomas; however, three coexisting papillary renal cell carcinomas in this patient each displayed an LOH pattern different from the adenomas and from each other.

A discordant pattern of nonrandom X-chromosome inactivation was seen in the tumors of one female patient, consistent with the independent origin of the multiple tumors (case 20; Fig. 2). A concordant pattern of nonrandom X-chromosome inactivation in the coexisting multiple papillary lesions was seen in two of three female patients (cases 16 and 21).

FISH analysis was done on 13 tumors from 6 patients showing LOH at loci on chromosome 7 and/or chromosome 17 (Fig. 3). Trisomy 7 was observed in all 6 cases in 12 of 13 tumors. Trisomy 17 was observed in all 6 cases in 12 of 13 tumors. Disomy was seen in a single tumor in two patients. In case 6, one tumor showed disomy of chromosome 7 and the other showed trisomy of chromosome 7. In case 11, one tumor showed disomy 17 and the other showed trisomy 17. Thus, the FISH data support an independent origin for the multifocal tumors in these two cases.

Discussion

Tumor multifocality occurs in 7% to 25% of renal cell carcinoma cases (40–42) with papillary renal cell carcinomas being the histologic type most commonly having coexisting multiple separate tumors (1–5). Previous clonality studies by LOH analysis of multifocal clear cell renal cell carcinomas by Miyake et al. (6) and Junker et al. (7) have shown that these tumors seem to arise from a common clonal origin with multifocality thought to arise secondary to intrarenal metastasis. Similar studies on multifocal papillary renal neoplasms, however, have not been previously reported despite the fact that papillary renal cancers are more likely than clear cell renal cell carcinomas to be multifocal. In this study, we studied 21 patients with multifocal papillary tumors of the kidney, including both papillary renal cell carcinomas (n = 42) and papillary adenomas (n = 10), using LOH, X-chromosome inactivation, and FISH analyses to assess tumor clonality. We found that, unlike multifocal clear cell renal cell carcinomas, coexisting separate papillary tumors of the kidney seem to arise independently, suggesting a mechanism other than intrarenal metastasis for the

Table 1. LOH, X-chromosome inactivation, and FISH analyses of multifocal papillary renal neoplasms

Case no.	Tumors	Allelic loss						X-chromosome inactivation	FISH	
		Microsatellite markers							Chromosome 7	Chromosome 17
		D3S1285	D7S522	D9S171	D16S507	D17S1795	TP53			
1	PRCC 1	▼	▲	▲	⇕	⇕	⇕			
	PRCC 2	⇕	⇕	▲	⇕	⇕	⇕			
2	PRCC 1	▼	▲	▼	⇕	⇕	⇕		T	T
	PRCC 2	⇕	▲	▼	⇕	⇕	⇕		T	T
	PRCC 3	⇕	▼	⇕	⇕	⇕	⇕		T	T
3	PRCC 1	⇕	⇕	▼	▲	▲	⇕			
	PRCC 2	⇕	▼	▼	▲	⇕	⇕			
4	PRCC 1	⇕	⇕	NI	⇕	▲	⇕		T	T
	PRCC 2	⇕	⇕	NI	▼	▲	⇕		T	T
5	PRCC 1	⇕	⇕	⇕	▲	⇕	⇕			
	PRCC 2	⇕	⇕	▼	▲	⇕	⇕			
	PRCC 3	⇕	▼	▼	⇕	⇕	⇕			
6	PRCC 1	⇕	⇕	⇕	⇕	▼	⇕		T	T
	PRCC 2	⇕	⇕	▲	⇕	▼	⇕		D	T
7	PRCC 1	▲	⇕	⇕	⇕	⇕	⇕			
	PRCC 2	▼	⇕	⇕	▲	▼	⇕			
8	PRCC 1	⇕	⇕	⇕	⇕	▼	⇕			
	PRCC 2	⇕	▼	⇕	⇕	▼	⇕			
9	PRCC 1	▲	⇕	▼	⇕	⇕	⇕			
	PRCC 2	▲	⇕	▼	⇕	▼	⇕			
10	PRCC 1	⇕	⇕	▲	⇕	⇕	⇕			
	PRCC 2	⇕	⇕	▲	▲	▼	⇕			
11	PRCC 1	▲	▲	⇕	▼	⇕	⇕		T	D
	PRCC 2	▲	▲	▼	⇕	▼	▲		T	T
12	PRCC 1	▲	⇕	⇕	⇕	⇕	⇕		T	T
	PRCC 2	▲	▲	▲	⇕	⇕	⇕		T	T
13	PRCC 1	⇕	⇕	⇕	⇕	NI	▲			
	PRCC 2	⇕	⇕	⇕	⇕	NI	▲			
14	PRCC 1	⇕	⇕	▲	⇕	▼	⇕		T	T
	PRCC 2	⇕	⇕	▲	⇕	⇕	⇕		T	T
15	PRCC 1	⇕	⇕	⇕	⇕	⇕	⇕			
	PA 1	⇕	⇕	⇕	⇕	⇕	⇕			
16	PRCC 1	⇕	⇕	⇕	⇕	⇕	⇕	▲		
	PRCC 2	▲	▼	▼	⇕	▼	⇕	▲		
	PRCC 3	▲	▼	⇕	⇕	⇕	⇕	▲		
	PA 1	⇕	⇕	▼	⇕	▼	⇕	▲		
	PA 2	⇕	⇕	▼	⇕	▼	⇕	▲		
17	PRCC 1	⇕	⇕	⇕	⇕	⇕	⇕			
	PRCC 2	⇕	⇕	⇕	⇕	▼	▲			
	PA 1	⇕	⇕	⇕	⇕	⇕	⇕			
18	PRCC 1	▲	⇕	▲	▼	NI	▲			
	PA 1	⇕	⇕	▲	▲	NI	▲			
19	PRCC 1	⇕	⇕	⇕	⇕	▼	⇕			
	PA 1	⇕	⇕	⇕	⇕	⇕	⇕			
20	PRCC 1	⇕	▼	▲	▲	NI	⇕	▼		
	PRCC 2	⇕	▼	⇕	▲	NI	⇕	▲		
	PA 1	⇕	⇕	⇕	▲	NI	▲	▼		
	PA 2	⇕	▼	▼	▲	NI	⇕	▼		
21	PRCC 1	⇕	▲	⇕	⇕	⇕	⇕	▲		
	PRCC 2	⇕	⇕	⇕	⇕	⇕	⇕	▲		
	PA 1	⇕	⇕	⇕	⇕	⇕	⇕	▲		
	PA 2	⇕	⇕	▼	⇕	⇕	⇕	▲		

Abbreviations: PRCC, Papillary renal cell carcinoma; PA, Papillary adenoma; NI, Noninformative; FISH, Flourescence in situ hybridization; T, Trisomy; D, Disomy; ⇕, Both allele present; ▲, loss of lower allele; ▼, loss of upper allele.

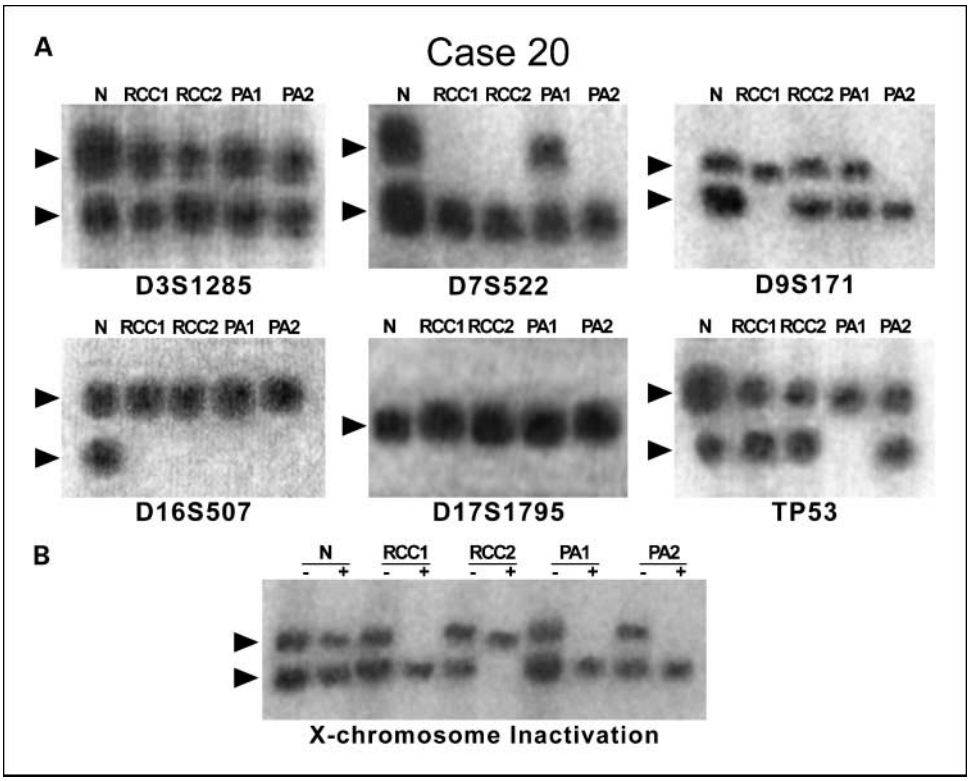

Fig. 2. Representative results (*Case 20*) of LOH analysis (*A*) and X-chromosome inactivation analysis (*B*). *A,* DNA was prepared from normal tissue and from multiple separate papillary tumors, amplified by PCR using polymorphic markers D3S1285, D7S522, D9S171, D16S507, D17S1795, and TP53, and separated by gel electrophoresis. *B,* cells from separate papillary tumors show different patterns of X-chromosome inactivation, consistent with independent origin. Arrows, allelic bands; N, normal tissue (control); RCC, renal cell carcinoma; PA, papillary adenoma; +, after *Hha* I endonuclease digestion; −, without *Hha* I endonuclease digestion (control).

presence of multifocal disease in some cases of multicentric papillary renal cell carcinoma.

Nearly every tumor examined displayed a different allelic loss pattern at the six loci examined in this study, suggesting that each separate tumor arose independently and that coexisting tumors are not clonally related. Only one patient (case 13) had two papillary renal cell carcinomas with an identical pattern of LOH. By contrast, LOH analysis of multifocal clear cell renal cell carcinomas has shown these tumors to be of a common clonal origin with multifocality presumably occurring secondary to intrarenal metastasis (6, 7). The molecular and cytogenetic properties of these two distinct histologic types of renal cancer have been extensively studied and each is characterized by different characteristic genetic alterations (43–50). Whereas clear cell renal cell carcinomas frequently show loss of DNA regions on chromosomal arms 3p, 14q, 9p, 8p, 6q, and 5q, papillary renal cell carcinomas often exhibit chromosomal polysomies, most frequently gains of chromosomes 7 and 17. Thus, with these contrasting genetic abnormalities, it is not surprising that the mechanisms responsible for tumor multifocality in these two types of renal cell carcinoma may be different as well.

Junker et al. (51) analyzed five cases of multifocal papillary renal tumors by comparative genomic hybridization and found at least one identical alteration in four of the five cases; however, this technique can only detect DNA copy number aberrations that span 2 to 10 Mb or more and, thus, is not ideal for clonality analysis. These results are also not surprising given the well-characterized and specific chromosomal copy number increases commonly seen in most cases of papillary renal cell carcinoma. Because papillary renal cell carcinoma is characterized by gains of whole chromosomes, microsatellite analysis may not detect LOH at every locus; however, clonally related tumors should still be expected to display the same allelic loss patterns. We found only 1 of 21 patients with multifocal papillary tumors of the kidney to have identical allelic loss patterns in each tumor. Alternatively, it is possible that one tumor resulted from a clonal metastasis from a specific, unsampled subpopulation of tumor cells within another tumor or that different allelic loss patterns among multifocal papillary tumors may represent clonal divergence after intrarenal spread rather than true oligoclonality. This may have occurred in cases 16 and 21 as the allelic loss patterns were variable among the multiple tumors; however, each showed the same pattern of nonrandom X-chromosome inactivation.

The most consistently informative marker of the clonal composition of neoplastic disorders in females is the nonrandom pattern of X-chromosome inactivation (34). Unfortunately, only 3 of the 21 patients in this study were female, limiting the subset of multifocal tumors that could be analyzed by X-chromosome inactivation analysis. One case (case 20) shows a discordant pattern of nonrandom X-chromosome inactivation among the four tumors in this patient, clearly demonstrating an independent origin for these multicentric tumors. Zhuang et al. (52) studied multifocal papillary renal cell carcinomas in patients with hereditary papillary renal cell carcinoma syndrome and showed a nonrandom duplication of the chromosome bearing a mutated *MET* gene in these patients. They analyzed 10 separate tumors (five from each kidney) in one patient using X-chromosome inactivation analysis and found evidence of independent clonal origins in at least two tumors from each kidney (52). Although our patient population did not include patients with hereditary papillary renal carcinoma, our results are consistent with these findings, suggesting that

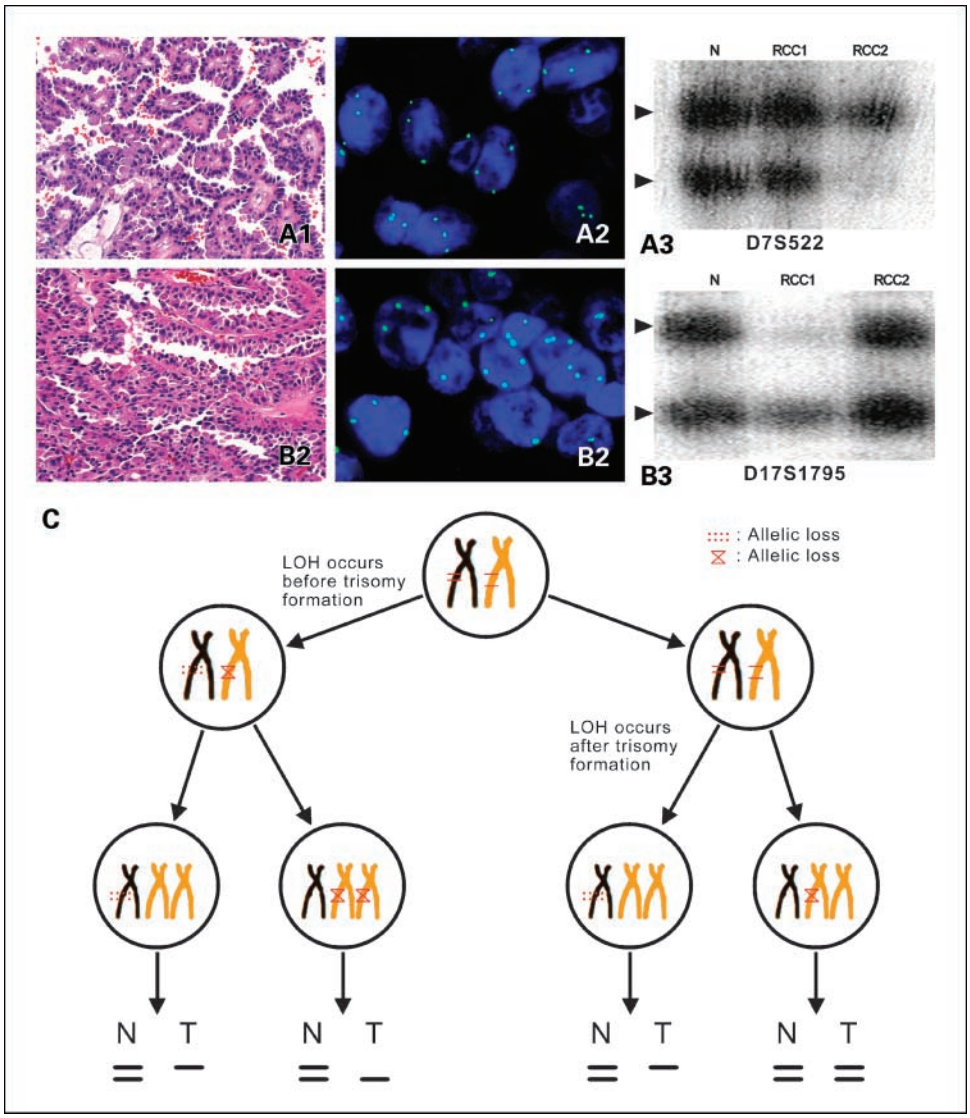

Fig. 3. *A* and *B,* histologic, FISH, and LOH features of papillary renal cell carcinoma. *A1* to *A3*, case 12; *B1* to *B3*, case 14. *A1* and *B1*, papillary renal cell carcinoma composed of branching papillae and covered with a single layer of cells with eosinophilic cytoplasm. *A2* and *B2,* FISH of the corresponding tumor with a centromeric probe of chromosomes 7 and 17 showed groups of tumor cells with trisomic chromosome 7 (*A2*) or 17 (*B2*; *green signals*). *A3* and *B3*, microsatellite analysis on chromosomes 7 (D7S522) and 17 (D17S1795) showed different LOH patterns between different tumor foci. *C,* proposed mechanism resulting in trisomic chromosomes 7 and 17. Acentromeric misdivision resulting in a nondisjunction of the pair of chromosomes during cell mitosis resulted in trisomic daughter cells. =, microsatellite loci of different origins and different sizes. LOH could occur before the trisomy formation (*C, left*) in which case allelic loss of either the upper or lower allele would be shown (*C, bottom left*). LOH could occur after trisomy formation (*C, right*), in which case only allelic loss on the nonduplicated chromosome could be detected. Allelic loss on only one copy of the duplicated chromosome would not be detectable by LOH analysis (*C, bottom right*).

multifocal sporadic papillary renal cell carcinomas may arise by a similar mechanism. Future studies involving a greater number of female patients could provide further insights on the genetic relationships among multifocal papillary renal tumors.

Our findings are concordant with the findings of Henn et al. (53) who reported a case of papillary renal cell carcinoma with six multifocal and bilateral lesions. Karyotype analysis of each tumor revealed cytogenetic heteroclonality favoring the assumption that each tumor arose independently. Multifocality in papillary renal tumors is often seen bilaterally without evidence of metastasis to other organs (4, 54), a finding that suggests independent origin. Multifocality in papillary renal cell carcinoma has been shown not to be associated with stage, grade, or histologic subtypes (i.e., type 1 versus type 2; refs. 55–57), a finding that is again consistent with independent origin. Whereas papillary adenomas are low-grade tumors (<5 mm in diameter) and are benign, the malignant potential of small papillary renal cell carcinomas (0.5-1.5 cm) if left unresected remains uncertain. In a series of 344 patients treated with nephron-sparing surgery, Krejci et al. (58) showed that patients with clear cell renal cell carcinoma had significantly worse cancer-specific survival than patients with papillary renal cell carcinoma. It is possible that these differences might be explained, in part, by decreased local recurrence due to a decreased incidence of intrarenal metastasis in papillary renal cell carcinomas as compared with their clear cell counterparts.

Radical nephrectomy has long been considered the gold standard therapy for renal cell carcinoma. With advances in radiologic imaging, an increasing proportion of renal cancers are discovered incidentally with a high proportion of these tumors being small (40). As a result, nephron-sparing surgical techniques have become widely employed for renal cancer therapy, even in patients with a normally functioning contralateral kidney, and there is increasing interest in the urologic and radiologic communities in another form of conservative therapy for renal neoplasms—computed tomography–guided radiofrequency ablation of tumors *in situ* (8, 9, 11, 40). The main objection to conservative surgery for renal cell carcinoma is concern over local recurrence due to the possible presence of small tumors with malignant potential in the portion of kidney left behind. Additional tumors undetectable by conventional radiologic examination have been found in

13% to 25% of kidneys resected by radical nephrectomy (40, 42, 59). These tumors become important in the setting of nephron-sparing surgery or computed tomography–guided radiofrequency ablation because if intrarenal metastasis is the mechanism by which renal carcinomas become multicentric, then these radiologically undetectable lesions likely have malignant potential as they would be derived from the same clone of neoplastic cells as the main tumor. Additional lesions that arise independently from the main tumor may be of less importance when considering conservative surgery for renal cell carcinoma. However, the malignant potential of the multiple tumor foci needs to be assessed. Based on our data, we suggest that some multifocal papillary tumors of the kidney arise independently and that these separate lesions are not clonally related and, thus, are not a result of intrarenal metastasis.

Papillary renal cell carcinomas are characterized cytogenetically by trisomy of chromosomes 7 and 17 in the majority of cases. Despite the chromosomal trisomies, previous studies have shown allelic losses on these chromosomes at the D7S522 and D17S1795 loci (17, 20). Six of our patients with tumors showing LOH at these loci ($n = 13$) were analyzed with FISH to detect trisomies of chromosomes 7 and 17 (Fig. 3). Interestingly, trisomy of chromosome 7 was observed in 12 of 13 tumors. One patient (case 6) had one tumor with disomy of chromosome 7 and another tumor with trisomy of chromosome 7, further demonstrating the genetic differences between these coexisting separate tumors. Trisomy of chromosome 17 was also observed in 12 of 13 tumors. The one patient with a tumor showing disomy of chromosome 17 (case 11) had a coexisting tumor with trisomy of chromosome 17, again highlighting the genetic differences between multicentric papillary tumors. Similar results were found by Zhuang et al. (52) who examined multifocal papillary renal cell carcinomas in patients with hereditary papillary renal carcinoma by FISH and found that separate tumors from the same patient harbor either disomy or trisomy of chromosome 17. We propose two mechanisms by which LOH can be observed at loci on trisomic chromosomes (Fig. 3C). Allelic loss can occur on either chromosome copy before trisomy formation, in which case LOH at either allele could be shown regardless of which chromosome is duplicated. Alternatively, allelic loss can occur after trisomy formation, in which case only LOH on the nonduplicated chromosome would be detectable on LOH analysis. It is unknown which of the two proposed mechanisms is most common in these cases; however, it is likely that in papillary renal tumors, LOH is involved in the development and/or progression of disease even when allelic loss occurs on a chromosome that is present in more than two copies.

In conclusion, our data suggest that, unlike multifocal clear cell renal cell carcinomas, multifocal papillary tumors of the kidney can and often do arise independently. Thus, intrarenal metastasis does not seem to play as important a role in the spread of papillary renal cell carcinoma. Whereas the precise mechanism responsible for the independent and multicentric origin of these papillary tumors remains uncertain, the decreased incidence of intrarenal spread with this histologic type of renal cell carcinoma may potentially be diagnostically, therapeutically, and prognostically important.

References

1. Kovacs G, Kovacs A. Parenchymal abnormalities associated with papillary renal cell tumors: a morphologic study. J Urol Pathol 1993;1:301–12.
2. Baltaci S, Orhan D, Soyupek S, Beduk Y, Tulunay O, Gogus O. Influence of tumor stage, size, grade, vascular involvement, histological cell type and histological pattern on multifocality of renal cell carcinoma. J Urol 2000;164:36–9.
3. Schlichter A, Wunderlich H, Junker K, Kosmehl H, Zermann DH, Schubert J. Where are the limits of elective nephron-sparing surgery in renal cell carcinoma? Eur Urol 2000;37:517–20.
4. Wunderlich H, Schlichter A, Zermann D, Reichelt O, Kosmehl H, Schubert J. Multifocality in renal cell carcinoma: A bilateral event? Urol Int 1999;63:160–3.
5. Dimarco DS, Lohse CM, Zincke H, Cheville JC, Blute ML. Long-term survival of patients with unilateral sporadic multifocal renal cell carcinoma according to histologic subtype compared with patients with solitary tumors after radical nephrectomy. Urology 2004;64:462–7.
6. Miyake H, Nakamura H, Hara I, et al. Multifocal renal cell carcinoma: evidence for a common clonal origin. Clin Cancer Res 1998;4:2491–4.
7. Junker K, Thrum K, Schlichter A, Muller G, Hindermann W, Schubert J. Clonal origin of multifocal renal cell carcinoma as determined by microsatellite analysis. J Urol 2002;168:2632–6.
8. Filipas D, Fichtner J, Spix C, et al. Nephron-sparing surgery of renal cell carcinoma with a normal opposite kidney: long-term outcome in 180 patients. Urology 2000;56:387–92.
9. Lau WK, Blute ML, Weaver AL, Torres VE, Zincke H. Matched comparison of radical nephrectomy vs nephron-sparing surgery in patients with unilateral renal cell carcinoma and a normal contralateral kidney. Mayo Clin Proc 2000;75:1236–42.
10. Barbalias GA, Liatsikos EN, Tsintavis A, Nikiforidis G. Adenocarcinoma of the kidney: nephron-sparing surgical approach vs. radical nephrectomy. J Surg Oncol 1999;72:156–61.
11. Zagoria RJ, Hawkins AD, Clark PE, et al. Percutaneous CT-guided radiofrequency ablation of renal neoplasms: factors influencing success. Am J Roentgenol 2004;183:201–7.
12. Greene FL, Page DL, Fleming ID, et al. American Joint Committee on Cancer Staging Manual. 6th ed. New York: Springer; 2002.
13. Kernek KM, Ulbright TM, Zhang S, et al. Identical allelic losses in mature teratoma and other histologic components of malignant mixed germ cell tumors of the testis. Am J Pathol 2003;163:2477–84.
14. Brandli DW, Ulbright TM, Foster RS, et al. Stroma adjacent to metastatic mature teratoma after chemotherapy for testicular germ cell tumors is derived from the same progenitor cells as the teratoma. Cancer Res 2003;63:6063–8.
15. McCarthy RP, Zhang S, Bostwick DG, et al. Molecular genetic evidence for different clonal origins of epithelial and stromal components of phyllodes tumor of the prostate. Am J Pathol 2004;165:1395–400.
16. Cheng L, MacLennan GT, Zhang S, Wang M, Pan CX, Koch MO. Laser capture microdissection analysis reveals frequent allelic losses in papillary urothelial neoplasm of low malignant potential of the urinary bladder. Cancer 2004;101:183–8.
17. Balint I, Fischer J, Ljungberg B, Kovacs G. Mapping the papillary renal cell carcinoma gene between loci D17S787 and D17S1799 on chromosome 17q21.32. Lab Invest 1999;79:1713–8.
18. Willers CP, Siebert R, Bardenheuer W, et al. Genetic instability of 3p12-p21-specific microsatellite sequences in renal cell carcinoma. Br J Urol 1996;77:524–9.
19. Wilhelm M, Bugert P, Kenck C, Staehler G, Kovacs G. Terminal deletion of chromosome 3p sequences in nonpapillary renal cell carcinomas: a breakpoint cluster between loci D3S1285 and D3S1603. Cancer Res 1995;55:5383–5.
20. Shridhar V, Sun QC, Miller OJ, Kalemkerian GP, Petros J, Smith DI. Loss of heterozygosity on the long arm of human chromosome 7 in sporadic renal cell carcinomas. Oncogene 1997;15:2727–33.
21. Schraml P, Struckmann K, Bednar R, et al. CDKNA2A mutation analysis, protein expression, and deletion mapping of chromosome 9p in conventional clear-cell renal carcinomas: evidence for a second tumor suppressor gene proximal to CDKN2A. Am J Pathol 2001;158:593–601.
22. Schraml P, Muller D, Bednar R, et al. Allelic loss at the D9S171 locus on chromosome 9p13 is associated with progression of papillary renal cell carcinoma. J Pathol 2000;190:457–61.
23. Reiter RE, Anglard P, Liu S, Gnarra JR, Linehan WM. Chromosome 17p deletions and p53 mutations in renal cell carcinoma. Cancer Res 1993;53:3092–7.
24. Pesti T, Sukosd F, Jones EC, Kovacs G. Mapping a tumor suppressor gene to chromosome 2p13 in metanephric adenoma by microsatellite allelotyping. Hum Pathol 2001;32:101–4.
25. Grady B, Goharderakhshan R, Chang J, et al. Frequently deleted loci on chromosome 9 may harbor several tumor suppressor genes in human renal cell carcinoma. J Urol 2001;166:1088–92.
26. Girgin C, Tarhan H, Hekimgil M, Sezer A, Gurel G. P53 mutations and other prognostic factors of renal cell carcinoma. Urol Int 2001;66:78–83.
27. Contractor H, Zariwala M, Bugert P, Zeisler J, Kovacs

G. Mutation of the p53 tumour suppressor gene occurs preferentially in the chromophobe type of renal cell tumour. J Pathol 1997;181:136–9.

28. Levine AJ, Momand J, Finlay CA. The p53 tumour suppressor gene. Nature 1991;351:453–6.

29. Cheng L, Bostwick DG, Li G, et al. Allelic imbalance in the clonal evolution of prostate carcinoma. Cancer 1999;85:2017–22.

30. Cheng L, Gu J, Ulbright TM, et al. Precise microdissection of human bladder carcinomas reveals divergent tumor subclones in the same tumor. Cancer 2002;94:104–10.

31. Cheng L, Shan A, Cheville JC, Qian J, Bostwick DG. Atypical adenomatous hyperplasia of the prostate: a premalignant lesion? Cancer Res 1998;58:389–91.

32. Cheng L, Song SY, Pretlow TG, et al. Evidence of independent origin of multiple tumors from patients with prostate cancer. J Natl Cancer Inst 1998;90:233–7.

33. Paterson RF, Ulbright TM, MacLennan GT, et al. Molecular genetic alterations in the laser-capture microdissected stroma adjacent to bladder carcinoma. Cancer 2003;98:1830–6.

34. Gu J, Roth LM, Younger C, et al. Molecular evidence for the independent origin of extra-ovarian papillary serous tumors of low malignant potential. J Natl Cancer Inst 2001;93:1147–52.

35. Cheng L, Jones TD, McCarthy RP, et al. Molecular genetic evidence for a common clonal origin of urinary bladder small cell carcinoma and co-existing urothelial carcinoma. Am J Pathol 2005;166:1533–9.

36. Cheng L, Gu J, Eble JN, et al. Molecular genetic evidence for different clonal origin of components of human renal angiomyolipomas. Am J Surg Pathol 2001; 25:1231–6.

37. Brunelli M, Eble JN, Zhang S, Martignoni G, Cheng L. Gains of chromosomes 7, 17, 12, 16, and 20 and loss of Y occur early in the evolution of papillary renal cell neoplasia: a fluorescent *in situ* hybridization study. Mod Pathol 2003;16:1053–9.

38. Brunelli M, Eble JN, Zhang S, Martignoni G, Cheng L. Metanephric adenoma lacks the gains of chromosomes 7 and 17 and loss of Y that are typical of papillary renal cell carcinoma and papillary adenoma. Mod Pathol 2003;16:1060–3.

39. Brunelli M, Eble JN, Zhang S, Martignoni G, Delahunt B, Cheng L. Eosinophilic and classic chromophobe renal cell carcinomas have similar frequent losses of multiple chromosomes from among chromosomes 1, 2, 6, 10, and 17, and this pattern of genetic abnormality is not present in renal oncocytoma. Mod Pathol 2005;18:161–9.

40. Nissenkorn I, Bernheim J. Multicentricity in renal cell carcinoma. J Urol 1995;153:620–2.

41. Hafez KS, Fergany AF, Novick AC. Nephron sparing surgery for localized renal cell carcinoma: impact of tumor size on patient survival, tumor recurrence and TNM staging. J Urol 1999;162:1930–3.

42. Cheng WS, Farrow GM, Zincke H. The incidence of multicentricity in renal cell carcinoma. J Urol 1991;146: 1221–3.

43. Kovacs G, Akhtar M, Beckwith BJ, et al. The Heidelberg classification of renal cell tumours. J Pathol 1997; 183:131–3.

44. van den Berg E, van der Hout AH, Oosterhuis JW, et al. Cytogenetic analysis of epithelial renal-cell tumors: relationship with a new histopathological classification. Int J Cancer 1993;55:223–7.

45. Thrash-Bingham CA, Greenberg RE, Howard S, et al. Comprehensive allelotyping of human renal cell carcinomas using microsatellite DNA probes. Proc Natl Acad Sci U S A 1995;92:2854–8.

46. Steiner G, Sidransky D. Molecular differential diagnosis of renal carcinoma: from microscopes to microsatellites. Am J Pathol 1996;149:1791–5.

47. Kovacs G. Molecular differential pathology of renal cell tumours. Histopathology 1993;22:1–8.

48. Presti JC, Jr., Rao PH, Chen Q, et al. Histopathological, cytogenetic, and molecular characterization of renal cortical tumors. Cancer Res 1991;51: 1544–52.

49. Wilhelm M, Veltman JA, Olshen AB, et al. Array-based comparative genomic hybridization for the differential diagnosis of renal cell cancer. Cancer Res 2002;62:957–60.

50. Bugert P, Kovacs G. Molecular differential diagnosis of renal cell carcinomas by microsatellite analysis. Am J Pathol 1996;149:2081–8.

51. Junker K, Hindermann W, Schubert J, Schlichter A. Differentiation of multifocal renal cell carcinoma by comparative genomic hybridization. Anticancer Res 1999;19:1487–92.

52. Zhuang Z, Park WS, Pack S, et al. Trisomy 7-harbouring non-random duplication of the mutant MET allele in hereditary papillary renal carcinomas. Nat Genet 1998; 20:66–9.

53. Henn W, Zwergel T, Wullich B, Thonnes M, Zang KD, Seitz G. Bilateral multicentric papillary renal tumors with heteroclonal origin based on tissue-specific karyotype instability. Cancer 1993;72:1315–8.

54. Dal Cin P, van Poppel H, van Damme B, Baert L, van den Berghe H. Cytogenetic investigation of synchronous bilateral renal tumors. Cancer Genet Cytogenet 1996;89:57–60.

55. Delahunt B, Eble JN. Papillary renal cell carcinoma: a clinicopathologic and immunohistochemical study of 105 tumors. Mod Pathol 1997;10:537–44.

56. Mejean A, Hopirtean V, Bazin JP, et al. Prognostic factors for the survival of patients with papillary renal cell carcinoma: meaning of histological typing and multifocality. J Urol 2003;170:764–7.

57. Kletscher BA, Qian J, Bostwick DG, Andrews PE, Zincke H. Prospective analysis of multifocality in renal cell carcinoma: influence of histological pattern, grade, number, size, volume and deoxyribonucleic acid ploidy. J Urol 1995;153:904–6.

58. Krejci KG, Blute ML, Cheville JC, Sebo TJ, Lohse CM, Zincke H. Nephron-sparing surgery for renal cell carcinoma: clinicopathologic features predictive of patient outcome. Urology 2003;62:641–6.

59. Mukamel E, Konichezky M, Engelstein D, Servadio C. Incidental small renal tumors accompanying clinically overt renal cell carcinoma. J Urol 1988;140:22–4.

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