Diagnostic Usefulness of Fluorescent Cytogenetics in Differentiating Chromophobe Renal Cell Carcinoma From Renal Oncocytoma

A Validation Study Combining Metaphase and Interphase Analyses

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Abstract

We investigated the usefulness of interphase fluorescence in situ hybridization (FISH) analysis to differentiate between 11 chromophobe renal carcinomas and 12 renal oncocytomas, showing different clinical outcomes, when compared with conventional metaphase cytogenetics by karyotyping.

Karyotypically, 3 chromophobe renal cell carcinomas showed losses of chromosomes, 3 were polyploid, 1 was normal, and 4 failed to grow. Of 12 oncocytomas, 5 showed a normal numeric karyotype and 6 additional structural rearrangements. FISH on chromophobe renal cell carcinomas showed a high percentage of cases (10/11 [91%]) with multiple numeric losses among chromosomes 1, 2, 6, 10, and 17; this interphase pattern was observed irrespective of the 3 different metaphase karyotypes. Of 12 oncocytomas, 11 (92%) revealed a normal numeric chromosomal status showing at least 2 chromosomes without aneusomy by interphase FISH.

The study demonstrates that indeed FISH performed on formalin-fixed, paraffin-embedded tissue can provide clinically useful information more reliably than karyotyping of most of these tumors.

Chromophobe renal cell carcinoma and renal oncocytoma are renal parenchymal neoplasms originating from the intercalated cells of the distal nephron with different behavior.[1-5] Although these tumors have several similarities in protein and gene expression profiling,[1,6] chromophobe renal cell carcinoma and renal oncocytoma show differing genetic abnormalities.[7] Chromophobe renal cell carcinoma is characterized by multiple chromosomal losses, whereas oncocytoma usually shows a normal numeric complement of chromosomes.[8] These findings were first noted using classic metaphase cytogenetic analyses,[9] and these studies have triggered the application of fluorescence in situ hybridization (FISH) analysis for the investigation of the chromosomal pattern of these tumors in formalin-fixed, paraffin-embedded tissues.[8,10]

After the description of the classic genetic abnormalities associated with chromophobe renal cell carcinoma and oncocytoma, other chromosomal rearrangements have been recognized in these tumors by cytogenetic metaphase analysis.[11,12] In these studies, oncocytoma was found on occasion to exhibit loss of the whole of chromosome 1 or of part of its short or long arm, loss of chromosome Y and/or chromosome 14, and, in a few tumors, structural rearrangements involving chromosome 11.[13-15] In addition to widespread chromosomal losses in chromophobe renal cell carcinoma, polyploidy and gains have been identified in some tumors.[11,16]

In previous studies we have shown, using FISH analysis, that chromophobe renal cell carcinoma usually exhibits loss of chromosomes 1, 2, 6, 10, and 17, and while loss of chromosome 1 may be seen in oncocytoma, multiple losses of chromosome 2, 6, 10, or 17 favor a diagnosis of chromophobe renal cell carcinoma.[8] Despite these findings,

it remains uncertain as to whether the breadth of genetic abnormalities now recognized in both of these tumors may act as a confounding influence on diagnostic FISH analysis, and, to date, no validation studies have been undertaken to investigate this. In this study we evaluated the relationship between patterns of chromosomal abnormalities observed in chromophobe renal cell carcinoma and oncocytoma by metaphase analyses and detection of chromosomal losses using interphase FISH analysis to determine whether FISH studies retain diagnostic usefulness in differentiating between these 2 tumor types, regardless of underlying constituent chromosomal abnormalities. The differential diagnosis is clinically important because renal oncocytoma is a benign renal tumor, different from chromophobe renal cell carcinoma that is usually a low-stage carcinoma.[2,17]

Materials and Methods

We studied 23 renal cell tumors originally diagnosed as chromophobe renal cell carcinomas (11 cases) **Image 1A**, **Image 1E**, and **Image 1I** and renal oncocytomas (12 cases) **Image 2A**, **Image 2E**, and **Image 2I**.

The histologic features of each of the tumors were reviewed to confirm the diagnosis. In addition, Hale colloidal iron staining was undertaken for each case, and 5-µm-thick sections from each of the tumors were immunostained with monoclonal antibodies to parvalbumin (clone PA-235, dilution 1:1,000; Sigma Chemical, St Louis, MO)[1] and S100A1 protein (monoclonal mouse antihuman S100A1, clone DAKO 100A1/1, dilution 1:50; Lab Vision, Fremont, CA).[18] All immunoreactions were developed using the EnVision peroxidase detection system (DAKO, Carpinteria, CA).

Karyotyping Analysis

For each case, karyotypes were prepared from specimens of tumor minced in collagenase overnight. After 5 days in culture, the cells were harvested in conformity with the standard procedure.[19] Chromosome preparations were G-banded, and tumor karyotypes were described according to the International System for Human Cytogenetic Nomenclature.

FISH Analysis and Tissue Microarray

Tissue microarrays were constructed using a tissue arraying instrument (Beecher Instruments, Hackensack, NJ). Tissue cylinders with a diameter of 0.6 mm were punched from each donor paraffin block in targeted areas corresponding to previously demarcated areas on the parallel H&E-stained slide. These tissue cores were then deposited into a recipient master paraffin block. The punches were placed 1 mm apart on the x-axis and 1.5 mm apart on the y-axis. Each microarray block contained a maximum of 79 punches.

Sections 5 µm thick were cut from the master block, stained with H&E, and reviewed to ensure the presence of chromophobe renal cell carcinoma and renal oncocytoma. Sections were then used for in situ hybridization.

We have previously demonstrated that tissue microarrays are a valid substitute for whole tissue sections in chromophobe renal cell carcinoma when FISH analysis is undertaken.[20] Concordance of results was almost perfect when the 3 neoplastic cores were analyzed. Thus, 3 tissue cores were prepared for tumor tissue and 2 for normal renal parenchyma for each case.[20] Overall, 33 cores of chromophobe renal cell carcinoma, 36 cores of renal oncocytoma, and 45 cores of normal renal tissue, sited adjacent to renal tumor, were prepared on 3 tissue microarray sections for subsequent study.

We performed interphase cytogenetic FISH analysis using a centromeric specific probe mapping to chromosomes 1 and 2 (SpectrumOrange, Abbott-Vysis, Des Plaines, IL) and 6, 10, and 17 (SpectrumGreen, Abbott-Vysis).

Sections, cut at 5-µm thickness, were prepared from the paraffin-embedded tissue microarrays. The paraffin was removed from the sections with two 10-minute washes in xylene. After hydrating in 100%, 85%, and 70% ethanol solutions (10 minutes), rinsing in distilled water (10 minutes), and twice in phosphate-buffer solution (pH 7, 10 minutes each), the slides were fixed in methanol-acetic acid 3:1 for 10 minutes and air dried. The sections were then treated in a 2× standard saline citrate (SSC) solution for 15 minutes at 37°C, dehydrated in consecutive 70%, 85%, and 100% ethanol solutions for 1 minute each, and then air dried. Next, the sections were bathed in 0.1 mmol/L citric acid (pH 6) solution at 85°C for 1 hour. Then they were again dehydrated in a series of ethanol solutions and dried. Tissues were digested by applying 0.75 mL of pepsin solution (Sigma; 4 mg/mL in 0.9% sodium chloride, pH 1.5) to each slide and incubating them in a humidified box for 30 minutes at 37°C. Next, the slides were rinsed with distilled water for a few seconds, dehydrated again in graded ethanol solutions, and dried. Centromeric probes for chromosomes 1, 2, 6, 10, and 17 were each diluted 1:100 in tDenHyb1 buffer (Insitus, Albuquerque, NM). Next, 10 µL of diluted probe was applied to each slide and the section coverslipped. Denaturation was achieved by incubating the slides at 80°C for 10 minutes in a humidified box; then hybridization was undertaken at 37°C for 3 hours. The coverslips were then removed, and the slides were immersed at room temperature in 0.5× SSC for 2 minutes and in 2× SSC for 2 minutes. The slides were air dried and counterstained with 10 µL of DAPI/Antifade (DAPI in Fluorguard, 0.5 µg/mL, Insitus). Slides were examined using an Axioplan (Zeiss, Jena, Germany) with appropriate filters for SpectrumOrange and SpectrumGreen and a UV filter for the DAPI nuclear counterstain. The signals were recorded with a CCD camera (AxioCam HRm, Zeiss).

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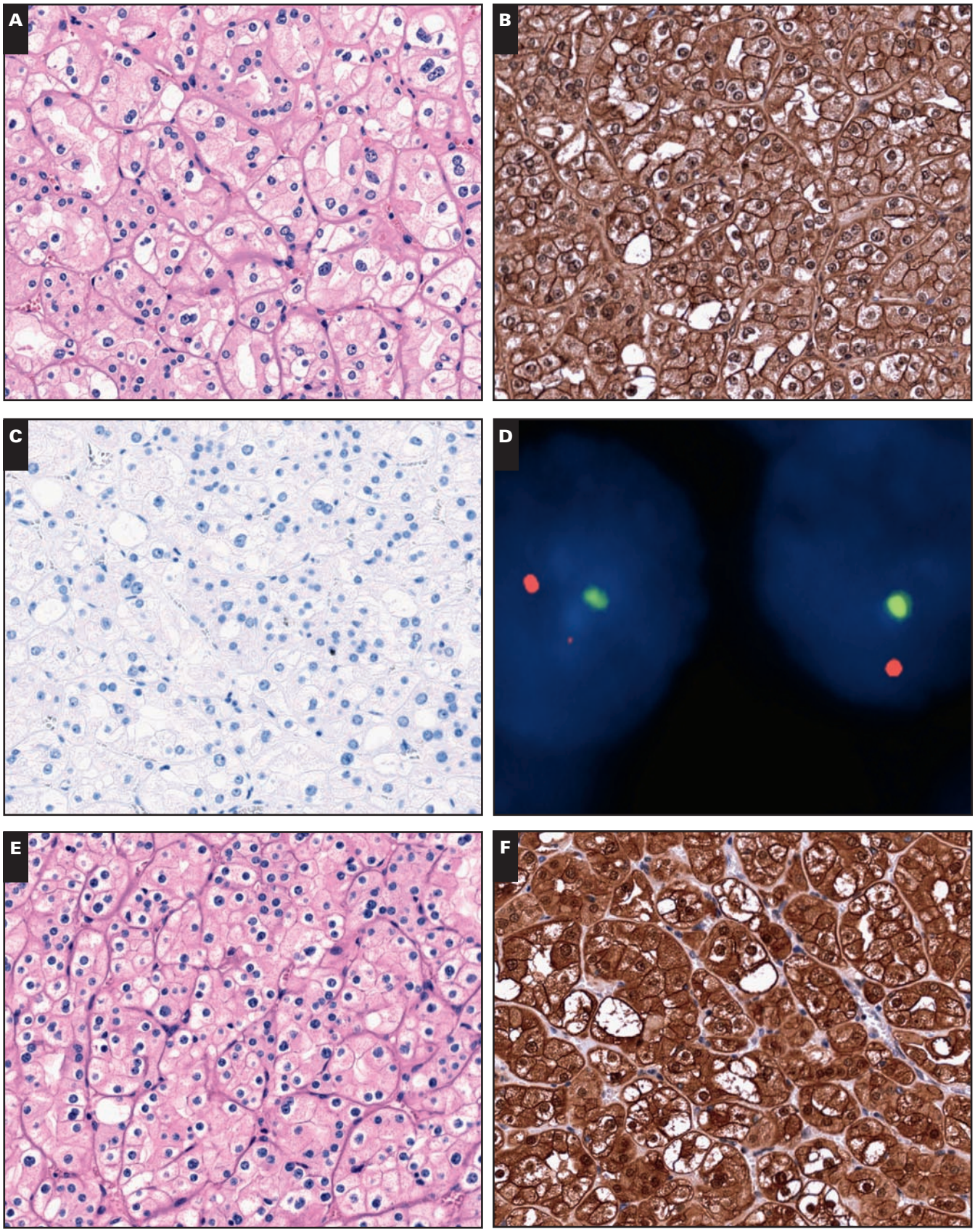

Image 1 Case 1: **A**, Chromophobe renal cell carcinoma with losses of multiple chromosomes by metaphase karyotyping (H&E, ×20). **D**, Interphase fluorescence in situ hybridization (FISH) analysis revealed single fluorescent signals for chromosomes 2 (SpectrumOrange) and 17 (SpectrumGreen). Case 9: **E**, A rare case of chromophobe renal cell carcinoma showing gains of chromosomes by metaphase karyotyping (H&E, ×20).

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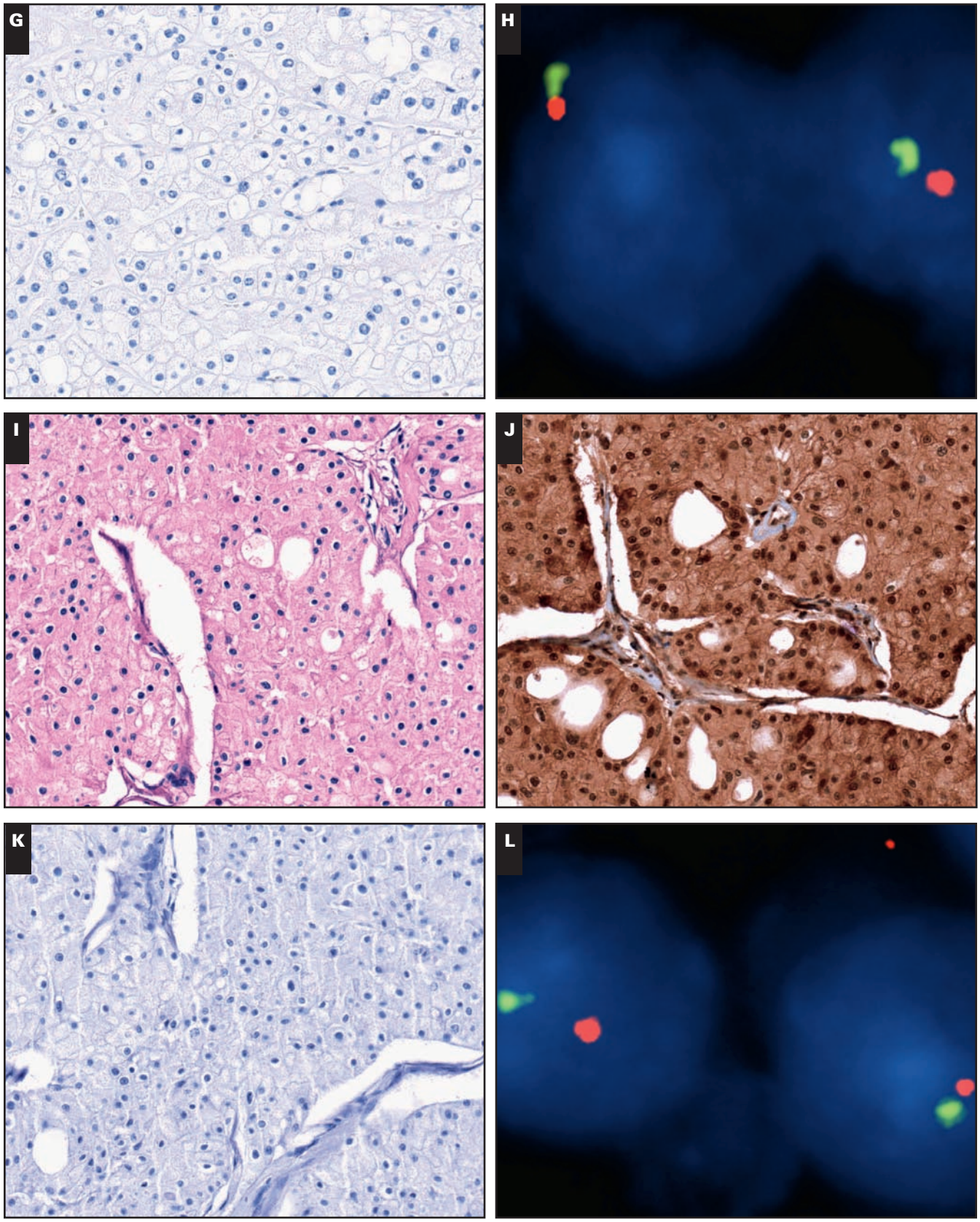

H, Interphase FISH analysis revealed single fluorescent signals for chromosomes 2 (SpectrumOrange) and 17 (SpectrumGreen). Case 5: **I**, Chromophobe renal cell carcinoma that did not grow in culture for metaphase analysis (H&E, ×20). **L**, Interphase FISH analysis revealed single fluorescent signals for chromosomes 2 (SpectrumOrange) and 10 (SpectrumGreen). All 3 chromophobe renal cell carcinomas showed immunoexpression of parvalbumin (**B**, ×20; **F**, ×20; and **J**, ×20) and negative immunoexpression for S100A1 (**C**, ×20; **G**, ×20; and **K**, ×20).

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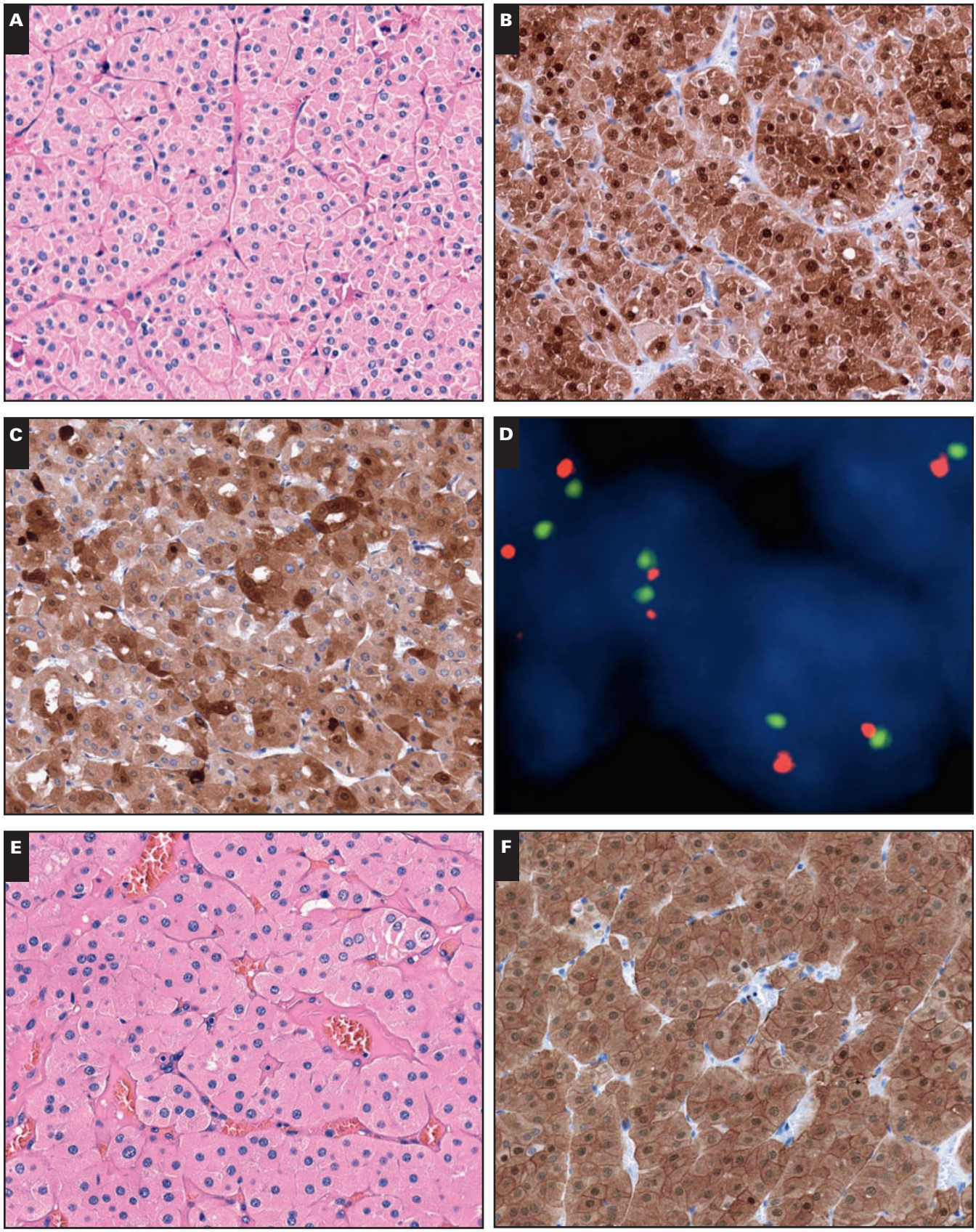

Image 2 Case 13: **A**, Renal oncocytoma with a normal complement of chromosomes by metaphase karyotyping (H&E, ×20). **D**, Interphase fluorescence in situ hybridization (FISH) analysis revealed double fluorescent signals for chromosomes 2 (SpectrumOrange) and 10 (SpectrumGreen). Case 17: **E**, Renal oncocytoma showing a mixture of metaphase rearrangements (H&E, ×20).

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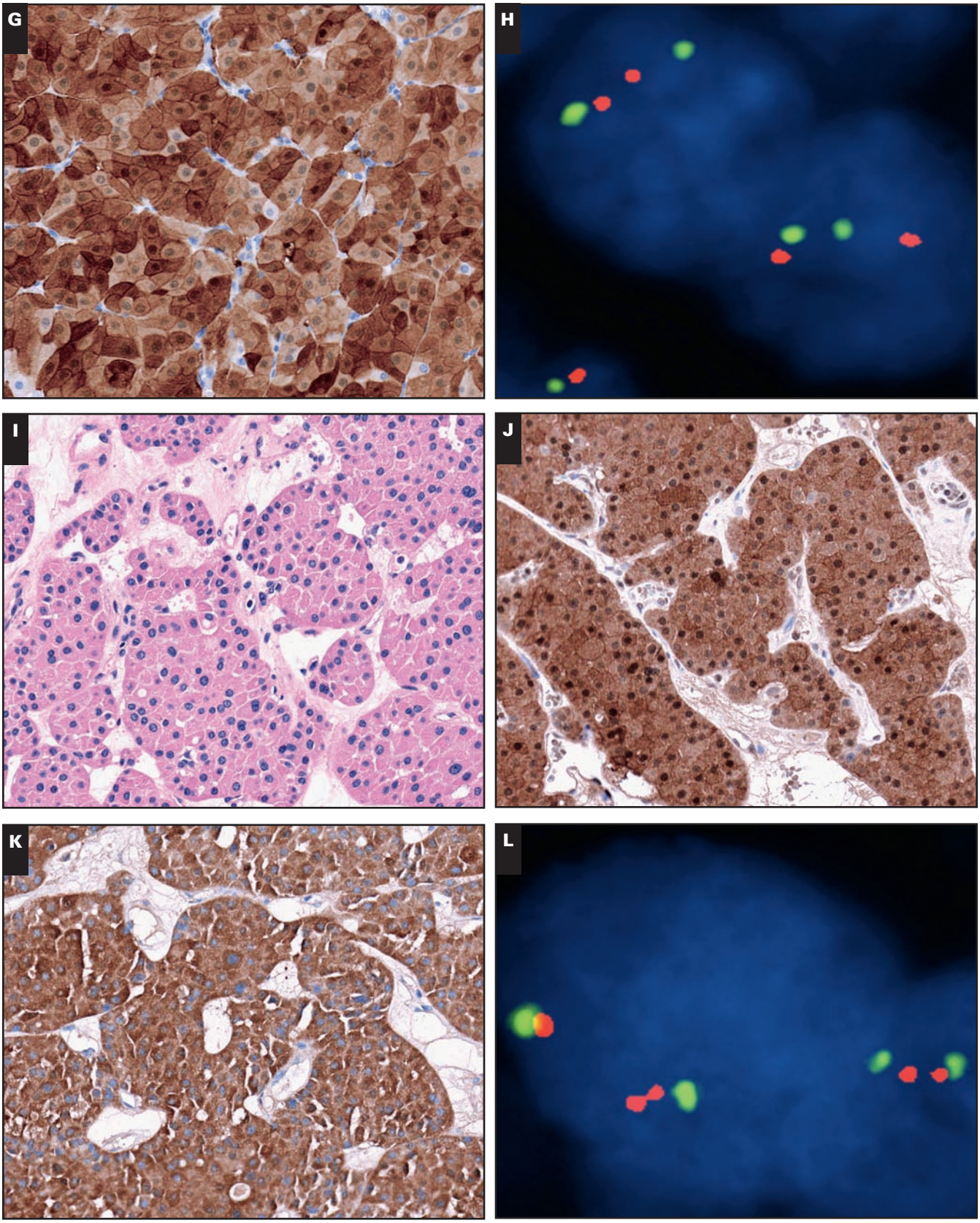

H, Interphase FISH analysis revealed double fluorescent signals for chromosomes 2 (SpectrumOrange) and 10 (SpectrumGreen). Case 21: **I**, Renal oncocytoma showing a normal complement of chromosomes with additional rearrangements involving chromosome 11. **L**, Interphase FISH analysis revealed double fluorescent signals for chromosomes 2 (SpectrumOrange) and 10 (SpectrumGreen). Renal oncocytomas revealed positive immunoexpression for parvalbumin (**B**, ×20; **F**, ×20; and **J**, ×20) and S100A1 (**C**, ×20; **G**, ×20; and **K**, ×20).

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FISH Interpretation

Fluorescent in situ signals were evaluated according to previous reports.[8] Briefly, signals from 100 to 200 neoplastic nuclei were counted for each tumor. Chromosomal loss was defined as the percentage of nuclei with single signals greater than the normal tissue mean for that chromosome within 4 times the normal tissue standard deviation for that chromosome. Thus, for centromeric chromosomes 1, 2, 6, 10, and 17, percentages of single signals greater than 33%, 35%, 29%, 31%, and 34%, respectively, were considered to indicate chromosomal loss. Chromosomal gain was defined as the percentage of nuclei with 3 or more signals greater than the normal tissue mean for that chromosome within 4 times the normal tissue standard deviation for that chromosome. Thus, for centromeric chromosomes 1, 2, 6, 10, and 17, percentages of 3 or more signals greater than 11%, 13%, 12%, 14%, and 11%, respectively, were considered to indicate chromosomal gains.

Results

Histochemical and Immunohistochemical Findings

All chromophobe renal cell carcinomas stained for Hale colloidal iron and exhibited positive immunoexpression for parvalbumin **Image 1B**, **Image 1F**, and **Image 1J** but not for S100A1 **Image 1C**, **Image 1G**, and **Image 1K**. All renal oncocytomas were negative for Hale colloidal iron stain. All stained positively for S100A1 **Image 2C**, **Image 2G**, and **Image 2K**, while 11 (92%) of 12 showed positive expression for parvalbumin **Image 2B**, **Image 2F**, and **Image 2J**.

Interphase and Metaphase Findings

The results of interphase and metaphase analyses are summarized in **Table 1** and **Table 2**. Karyotypically, 3 chromophobe renal cell carcinomas showed multiple chromosome losses (Image 1A), 3 showed polyploidy (Image 1E), 1 tumor exhibited a normal karyotype, and in 4 cases, there was failure of cell culture growth (Image 1I). Of the 3 polyploid tumors, 2 displayed additional combined metaphase with losses of chromosomes.

Of the 12 renal oncocytomas studied, 5 showed a normal karyotype (46,XY or 46,XX) (Image 2A). Six tumors had a normal numeric karyotype with additional minor structural chromosomal rearrangements; among these, 2 renal oncocytomas showed rearrangements on chromosome 11 (Image 2I) and 1 a metaphase with multiple losses (Image 2E). One case showed 47,XX,+7.

Table 1
Interphase Fluorescence In Situ Hybridization: Percentage of 1, 2, and 3 or More Fluorescent Signals in Chromophobe RCCs and Renal Oncocytomas

	Chromosome								
	1			2			6		
Case No.	1 Signal (%)	2 Signals (%)	≥3 Signals (%)	1 Signal (%)	2 Signals (%)	≥3 Signals (%)	1 Signal (%)	2 Signals (%)	≥3 Signals (%)
RCC									
1	76	19	5	69	30	1	57	37	6
2	41	54	5	65	25	10	68	25	7
3	58	38	4	42	47	11	27	66	7
4	58	38	4	27	67	6	76	23	1
5	64	32	4	84	12	4	79	18	3
6	12	80	8	18	76	6	23	68	9
7	24	65	11	4	83	13	78	17	5
8	33	57	10	27	67	6	30	64	6
9	27	69	4	41	54	5	80	12	8
10	33	55	12	23	42	35	32	57	11
11	54	42	4	76	22	2	78	12	10
Renal oncocytoma									
12	20	67	13	13	76	11	24	68	8
13	25	66	9	33	59	8	20	78	2
14	33	64	3	32	47	21	12	78	10
15	25	66	9	29	63	8	16	77	7
16	25	66	9	35	58	7	20	78	2
17	27	64	9	33	59	8	20	78	2
18	26	66	8	54	38	8	18	81	1
19	32	66	2	32	66	2	28	70	2
20	32	65	3	35	57	8	20	78	2
21	80	12	8	12	78	10	26	72	2
22	80	19	1	12	82	6	14	77	9
23	86	10	4	10	88	2	22	77	1

RCC, renal cell carcinoma.

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Interphase FISH on chromophobe renal cell carcinomas showed losses of 2 or more chromosomes in 10 cases and gains of multiple chromosomes in 1 (Table 1). All 3 cases of chromophobe renal cell carcinomas showing multiple monosomy by metaphase karyotyping displayed multiple losses of chromosomes 1, 2, 6, 10, and 17 by interphase FISH **Image 1D**. Moreover, the 4 chromophobe renal cell carcinomas that failed to grow in culture displayed multiple chromosomal losses by interphase FISH **Image 1L**. Of 3 chromophobe renal cell carcinomas with a polyploidy pattern by karyotyping analysis, 2 revealed at least 2 losses of chromosomes **Image 1H**.

FISH analysis on renal oncocytomas revealed normal content of all chromosomes in 6 cases **Image 2D**. In 5 cases, there was loss of 1 chromosome (chromosome 1 in 3 cases, chromosome 2 in 1 case, and chromosome 10 in 1 case), and in the single remaining case, losses of 2 chromosomes (chromosomes 2 and 10) were observed. The 2 renal oncocytomas showing rearrangements on chromosome 11 on metaphase analysis displayed an overall normal content of chromosomes by interphase FISH **Image 2H** and **Image 2L**.

In 7 of 11 renal oncocytomas with a predominantly normal karyotype and in 2 renal oncocytomas showing a mixture of metaphase rearrangements, a normal complement of chromosomes was seen on FISH analysis. In the cases with loss of chromosome 1, 1 tumor had a normal karyotype, whereas the other 2 showed numerous translocations. In the remaining 3 cases, loss of 1 chromosome (chromosome 2) was seen in a tumor exhibiting multiple chromosomal monosomies on karyotyping, and in the other 2 tumors, which had normal karyotypes, loss of chromosomes involving chromosome 10 or chromosomes 2 and 10 was observed.

Chromosome					
10			17		
1 Signal (%)	2 Signals (%)	≥3 Signals (%)	1 Signal (%)	2 Signals (%)	≥3 Signals (%)
78	10	12	62	32	6
77	20	3	61	34	5
26	66	8	71	22	7
82	17	1	69	27	4
50	48	2	42	50	8
70	22	8	76	21	3
49	49	2	10	86	4
66	22	12	68	22	10
66	31	3	55	41	4
30	58	12	22	40	38
50	36	14	66	31	3
10	78	12	28	66	6
30	67	3	32	59	9
32	48	20	32	58	10
25	71	4	33	59	8
31	67	2	34	60	6
29	69	2	28	69	3
50	47	3	18	81	1
59	36	5	30	63	7
31	63	6	34	57	9
20	77	3	24	68	8
22	78	0	33	57	10
22	66	12	24	66	10

Discussion

In our study we concluded the following: (1) Interphase FISH analysis demonstrates multiple chromosomal losses irrespective of the spectrum of alterations found by karyotyping in chromophobe renal cell carcinomas. (2) Renal oncocytomas often show normal DNA content by interphase and metaphase analyses. (3) The loss of 2 or more of chromosomes 1, 2, 6, 10, and 17 favors the diagnosis of chromophobe renal cell carcinomas over that of renal oncocytoma. (4) The cytogenetic complement of chromophobe renal cell carcinomas that fail to grow in culture may be partially defined by interphase FISH. (5) These findings validate the diagnostic usefulness of fluorescent cytogenetics in differentiating chromophobe renal cell carcinoma from renal oncocytoma on routinely available formalin-fixed, paraffin-embedded tissue.

Classic cytogenetic analysis, based on banding techniques, has been proved to be an invaluable tool for the detection of chromosomal abnormalities in tumor samples.[9,21-26] In many cases, however, the lack of availability of fresh material, the low mitotic index of a number of tumor types, and/or limitations in the numbers of neoplastic cells present within tumor samples impose restrictions on the use of this technology for routine diagnostic purposes.[27] FISH is a cytogenetic technique that can be used to detect and localize the presence or absence of specific DNA sequences on chromosomes in routine formalin-fixed, paraffin-embedded tissue.[27] While the detection of genetic abnormalities is limited to the range of probes used, the application of this technique to archival tissues emphasizes its value in the routine assessment of tumors for diagnostic purposes.[27-31]

In previous studies, we have demonstrated that FISH analysis of archival tissue permits distinction between oncocytoma and chromophobe renal cell carcinoma, which is of particular value in tumors showing ambiguous morphologic features.[8] It is now recognized that although oncocytoma and chromophobe renal cell carcinomas have what has been considered to be typical genetic rearrangements on karyotypic analysis, occasionally, atypical genetic abnormalities may be encountered that cannot be predicted by histologic examination. Although our studies have shown FISH analysis to be of

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Table 2
Chromophobe RCC and Renal Oncocytoma: Combining Results From Interphase FISH and Metaphase Karyotyping Analyses

	FISH/Chromosome					
	1	**2**	**6**	**10**	**17**	**Karyotyping**
RCC						
1	Loss	Loss	Loss	Loss	Loss	37,X,–X,–1,–2,–6,–7,–12,–15,–21,–22[2]
2	Loss	Loss	Loss	Loss	Loss	38,X,–Y,–3,–8,–9,–10,–11,–13,–22[2]
3	Loss	No loss	No loss	No loss	Loss	38-40,X,–X,–1,–2,–4,–5,–6,–7,–10,–17,–22[2]
4	Loss	No loss	Loss	Loss	Loss	No growth
5	Loss	Loss	Loss	Loss	Loss	No growth
6	No loss	No loss	No loss	Loss	Loss	No growth
7	No loss	No loss	Loss	Loss	No loss	No growth
8	No loss	No loss	No loss	Loss	Loss	73<4n>,XX,YY,–1–1,–2,–3,–6,–9,–10,–11,–12,–14,–15,–16,–17, –18,–19,–20,–21,–22,–22[1]
9	No loss	Loss	Loss	Loss	Loss	78-90<4n>
10	No loss	Gains	No loss	No loss	Gains	78-90<4n>,XX,–Y,–Y[5]/46,XY[10]
11	Loss	Loss	Loss	Loss	Loss	46,XX[2]
Renal oncocytoma						
12	No loss	No loss	No loss	No loss	No loss	46,XX,t(6;15)(p21;q21),t(6;9)(p12;p24)[2]
13	No loss	No loss	No loss	No loss	No loss	46,XY[2]
14	No loss	No loss	No loss	No loss	No loss	46,XX,del(9)(q21q22)(20)[2]
15	No loss	No loss	No loss	No loss	No loss	47,XX,+7[20]
16	No loss	No loss	No loss	No loss	No loss	46,XY[20]
17	No loss	Loss	No loss	No loss	No loss	38,X,–Y,–1,–3,–4,–5,–11,–11,–14,–16,+add(17)(q25),–21[1]/46,XY[5]
18	No loss	Loss	No loss	Loss	No loss	46,XY[20]
19	No loss	No loss	No loss	Loss	No loss	46,XX,der(12)t(12;?)(q12;?)[20]
20	No loss	No loss	No loss	No loss	No loss	46,XY[2]
21	Loss	No loss	No loss	No loss	No loss	46,XY,t(3;?19)(p11;?p13),der(11)t(3;11)(p14;p15)[2]
22	Loss	No loss	No loss	No loss	No loss	X,–Y,+inv(7)(p22;q11),+12,+17,+20[2]/46,XY,del(12)(q14q21)[2]
23	Loss	No loss	No loss	No loss	No loss	46,XY[2]

FISH, fluorescence in situ hybridization; RCC, renal cell carcinoma.

clinical value, uncertainty remains as to whether variations in tumor karyotype can produce confounding results that bring into question the usefulness of FISH analysis in distinguishing between these 2 tumor types.

The oncocytomas in our series showed a variety of genetic arrangements, which parallels the reported genetic spectrum for these tumors. Three of our cases of oncocytoma showed monosomy of chromosome 1, which has also been observed in previously reported studies.[8,14,15,32-34] In addition, 1 tumor was found to have rearrangement of chromosome 11, which is a feature seen less frequently in these tumors.[35,36] Among renal oncocytomas, our FISH studies showed a normal complement of chromosomes among chromosomes 2, 6, 10, and 17 in 9 of 12 cases, and this finding confirms our earlier observations that losses of 2 or more chromosomes 1, 2, 6, 10, and 17 favor a diagnosis of chromophobe renal cell carcinoma over oncocytoma.

In this study, we also detected a variety of genetic abnormalities in our chromophobe renal cell carcinomas by interphase analysis, which again reflects the spectrum of genetic abnormalities previously reported for this tumor type.[4,8,37,38] Similarly, and in accord with our previous findings from other tumors, we found that 91% of chromophobe renal cell carcinomas showed multiple losses among whole chromosomes 1, 2, 6, 10, and 17 by interphase FISH analysis. Specifically, all 3 cases of chromophobe renal cell carcinomas showing multiple monosomy by metaphase karyotyping displayed multiple losses of chromosomes 1, 2, 6, 10, and 17 by interphase FISH. Moreover, the 4 chromophobe renal cell carcinomas that failed to grow in culture displayed a diagnostic complement of multiple chromosomal losses by interphase FISH, thus reinforcing the value of FISH analysis in cases in which standard karyotyping is not possible owing to unavailability of tissues or failure of analytic techniques.

Among the group of the 3 chromophobe renal cell carcinomas showing karyotypic polyploidy, we found multiple chromosomal losses by FISH in 2 cases. These apparently conflicting findings between FISH and karyotyping might be explained by either of 2 hypotheses. Tumors are predominantly composed of monosomic cells with a minor component of hyperdiploid cells that may show a selective advantage during cell culture for metaphase analysis. As a consequence, there would be a prevalence of cells with a single centromeric signal on tissue section by interphase FISH analysis, whereas metaphase analysis would show a hyperdiploid clone. A further explanation could be that with the techniques used in this study, we could not fully exclude the influence of tumor heterogeneity, with samples being derived from 2 separate clones coexisting in the same tumor.

In the remaining case of chromophobe renal cell carcinoma showing karyotypic polyploidy in this study, interphase FISH analysis showed chromosomal gains. It is recognized

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that chromosomal gains may characterize some chromophobe renal cell carcinomas, although this is apparently a rare event.[8,11,12,16,39,40] It would seem that, while characteristic interphase chromosomal loss is diagnostic of chromophobe renal carcinoma, aberrant patterns exist, and limited chromosomal gains do not exclude this diagnosis. Moreover, commonly a tumor may maintain the characteristic chromosomal pattern of multiple chromosomal monosomies in addition to whole polyploidy DNA status[41-43]; this phenomenon justifies the presence of chromosomal gains by interphase analysis, although their numbers are in minority with respect to other chromosomes not characteristically involved in the pathogenesis of the specific tumors.[44]

We conclude that chromophobe renal carcinomas usually display multiple chromosomal losses by FISH analysis despite a variable spectrum found by karyotyping, whereas renal oncocytomas usually show normal DNA content by interphase and metaphase analyses. In cases in which atypical results are obtained from karyotype analysis, the observed FISH profile is in accord with the known spectrum of unusual abnormalities reported from these tumors. Despite the presence of karyotypic changes in both of these forms of renal neoplasia, FISH analysis is a useful tool that facilitates differentiation between these 2 tumor types. This remains particularly important in cases of renal tumor biopsies when limited tissue is available for analysis and the results are required to inform a conservative nephron-sparing surgical approach.[45]

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