FISH Scoring on Paraffin Sections *Versus* Single-cell Suspension for Chromophobe Renal Carcinoma and Renal Oncocytoma

MATTEO BRUNELLI[1], DIEGO SEGALA[1], BRETT DELAHUNT[2], CLAUDIA PAROLINI[1], SAMANTHA BERSANI[1], LIANG CHENG[3], JOHN N EBLE[3], MARCO CHILOSI[1], STEFANO GOBBO[1] and GUIDO MARTIGNONI[1]

[1]*Department of Pathology and Diagnostic, University of Verona, Verona, Italy;*
[2]*Department of Pathology and Molecular Medicine, Wellington School of Medicine and Health Sciences, University of Otago—Wellington, Wellington South, New Zealand;*
[3]*Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN, U.S.A.*

Abstract. *Background: Sectioning of the nuclei on tissue sections may give an overestimate of monosomy, a feature diagnostic of chromophobe renal cell carcinoma versus renal oncocytoma. The aim of the study was to assess whether or not nuclear sectioning may distort the results obtained from interphase fluorescence in situ hybridization (FISH) comparing the data obtained from analysis of isolated nuclei derived from formalin-fixed, paraffin-embedded sections with histological sections from the adjacent sections from the same tumors. Patients and Methods: Five chromophobe renal cell carcinomas and five renal oncocytomas were recruited. Sections of 5 μm and 30 μm were cut for FISH to investigate chromosomes 1, 2, 6 10 and 17. Results: FISH of isolated nuclei from renal oncocytomas showed a mean increase of 3.0% for nuclei with two signals when compared to tissue sections. For chromosomes 2, 6, 10 and 17, isolated nuclei showed a mean increase of 4.9% of fluorescent signals over nuclei from tissue sections. FISH analysis of isolated nuclei from chromophobe renal cell carcinoma showed a similar counts. Conclusion: When a tumor section exhibits a borderline percentage of nuclei with single signals around the cut-off level on tissue sections, the test should be repeated on isolated nuclei to confirm chromosomal loss, diagnostic of chromophobe renal carcinoma.*

Among renal cell parenchymal tumors, chromophobe renal cell carcinoma and renal oncocytoma may exhibit common morphological features (1). Despite this, these tumors may be readily differentiated by genetic studies. In particular, chromophobe renal cell carcinoma has been shown to be characterized by multiple chromosomal losses (2,3), while renal oncocytoma usually displays a normal complement of chromosomes (1).

In earlier studies we confirmed the utility of interphase fluorescence *in situ* hybridization (FISH) analysis, using routine histological sections, in differentiating chromophobe renal cell carcinomas from oncocytoma, even in the presence of variable metaphase karyotypes (4). In this context, the presence of single fluorescent signals is of diagnostic importance; however, artifacts in sections of nuclei on tissue slides may give an overestimation of monosomy in some cases (5).

This study was undertaken to assess whether or not nuclear sectioning may distort the results obtained from interphase FISH. In this study, we have compared the results obtained for both renal oncocytomas and chromophobe renal cell carcinomas from analysis of isolated nuclei derived from formalin-fixed, paraffin-embedded sections with histological sections from the adjacent sections from the same tumors.

Patients and Methods

Patient selection and histopathology. Five cases of classic chromophobe renal cell carcinoma and five cases of renal oncocytomas, treated by radical nephrectomy, were identified from the files of the Department of Pathology, University of Verona, Italy. All formalin-fixed and paraffin-embedded blocks and corresponding hematoxylin and eosin-stained histological sections were retrieved and were reviewed by three pathologists (MB, SG and GM). Representative blocks from each case were selected for detailed study.

In order to confirm the histological diagnosis of the tumors further sections were cut from each block at 5 μm thickness for Hale's colloidal iron staining and for immunostaining with monoclonal antibodies to parvalbumin (clone PA-235, dilution, 1:1000; Sigma Chemical Co., St. Louis, MO, USA) (6) and S100A1 protein (Mouse Anti-Human S100A1, Monoclonal Antibody, Clone

Correspondence to: Professor Guido Martignoni, MD, Department of Pathology and Diagnostic, University of Verona, P.le Ludovico Scuro 10, 37134, Verona, Italy. Tel: +39 0458124323, Fax: +39 0458027136, e-mail: guidomart@yahoo.com, guido.martignoni@univr.it

Key Words: Renal cell carcinoma, chromophobe renal cell carcinoma, renal oncocytoma, fluorescence *in situ* hybridization, tissue sections, isolated nuclei.

1D5, diluition 1:800; Abnova Corporation, Taipei, Taiwan) (7). All the immunoreactions were developed using the Envision peroxidase detection system (DAKO, Carpinteria, CA, USA).

Following this, further serial sectioning was undertaken. Four serial sections of 5 μm thickness, five serial sections of 30 μm thickness, and a further four serial sections of 5 μm thickness were cut from the selected tissue block for each case. The first, sixth, and eighth 5 μm-thick sections were routinely stained with hematoxylin and eosin to ensure that each section consisted of at least 90% neoplastic tissue.

FISH on routine histological sections. Interphase cytogenetic FISH analysis was carried out using a centromeric-specific probe mapping to chromosomes 1, 2 (SpectrumOrange; Abbott-Vysis, Des Plaines, IL, USA), 6, 10 and 17 (SpectrumGreen; Abbott-Vysis). These probes were chosen as previous studies have shown a high frequency of loss of these chromosomes in chromophobe renal cell carcinoma, but an overall normal numerical complement of chromosomes in renal oncocytoma (4).

The 5 μm-thick sections were deparaffinized with two 10-minute washes in xylene. After hydrating in 100%, 85% and 70% ethanol solutions (10 min), rinsing once in distilled water (10 min), and twice in phosphate buffer solution (pH 7, 10 min each), the sections were fixed in methanolacetic 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 one minute each and then air dried. The sections were then bathed in 0.1 mM citric acid (pH 6) solution at 85˚C for 1 hour. They were again dehydrated in a series of ethanol solutions and dried. Tissues were digested by applying 0.75 ml of pepsin (Sigma Chemical Co.) solution (4 mg/ml in 0.9% NaCl, pH 1.5) to each slide and incubating them in a humidified box for 30 minutes at 37˚C. The slides were then rinsed with distilled water for few seconds, dehydrated again in graded ethanol solutions and dried. Centromeric probes were each diluted 1:100 in tDenHyb1 buffer (Insitus, Albuquerque, NM, USA). Ten microliters 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 4,6-diamidino-2-phenylindole (DAPI)/Antifade (DAPI in Fluorguard, 0.5 μg/ml; Insitus).

FISH on isolated nuclei extracted from thick paraffin-embedded tissue. Single-cell suspensions were prepared from sections cut from each tumour at 30 μm thickness. Sections were then de-waxed in Falcon tubes in 10 ml xylene for 2×20 minutes. After de-waxing, the tissue was rehydrated in 100% ethanol (15 minutes), 95% ethanol (15 minutes) and 70% ethanol (15 minutes). The cells were then washed in distilled water (2×15 minutes) and re-suspended in 0.5% pepsin solution (pH 1.5) at 37˚C for 10-20 minutes. After filtering to remove undigested tissue fragments, the nuclei were pelleted by centrifugation at 1200 rpm for 5 minutes, washed twice in phosphate-buffered saline (PBS) and resuspended in 500 μl of PBS. Ten microliters of the resulting suspension was cytocentrifuged on histological slides, resulting in isolated nuclei being deposited on the slides.

FISH interpretation. Slides were examined using either an Axioplan (Zeiss, Germany) or an Olympus BX61 (Olympus, Hamburg, Germany) microscope, both with appropriate filters for SpectrumOrange and SpectrumGreen and a UV Filter for the DAPI nuclear counterstain. Both microscopes are fully motorized and all functions are completely automated, including focus, illumination, objective lens selection and filter wheels. A complete software command set allows for full computer control. The motorized drive permits movement in 0.01 μm increments, thus facilitating accurate movements in the z-axis. The signals were recorded using a CCD camera (both Axiocam HRm, Zeiss and Olympus Digital Camera, U-CMA D3).

To define the normal distribution of FISH signals in each of the ten tumors, normal renal parenchyma adjacent to tumor tissue was studied using all five probes. For each probe, 300 non-overlapping interphase nuclei from normal renal epithelium adjacent to either renal oncocytoma or chromophobe renal cell carcinoma tissue were examined. Two independent investigators (MB and SG) counted results in tissue sections and in isolated nuclei. If there was numerical discordance in results then a final result was obtained through simultaneous evaluation by both investigators. The percentage of epithelial nuclei containing 0, 1, 2, 3 or more signals was recorded for each probe.

In the tumours, chromosomal loss was defined as the percentage of nuclei with single signals numbering more than that of the normal tissue mean for that chromosome plus four times the normal tissue standard deviation for that chromosome. Thus, for comparison of FISH anomalies between chromophobe renal cell carcinoma and renal oncocytoma, the cut-off values used to determine chromosomal loss or gain, were respectively, >34% for nuclei with single signals and >9% for nuclei with 3 or more signals, and the tolerance limit around the cut-off was 9% for chromosomal loss and 4% for chromosomal gain.

No statistical analysis of data was carried out due to the small series used.

Results

All chromophobe renal cell carcinomas (Figure 1A and B) stained for Hale's colloidal iron and exhibited positive immunoexpression for parvalbumin (70-90% of neoplastic cells), but not for S100A1. All renal oncocytomas (Figure 1C) were negative for Hale's colloidal iron stain, but stained positively for S100A1 (70-90% of neoplastic cells) and for parvalbumin (80-90% of neoplastic cells).

The centromere copy number determined by FISH in isolated nuclei and in tissue sections of chromophobe renal cell carcinoma and renal oncocytoma, and mean values for each probe and tumor type are shown in Tables I and II.

FISH analysis of isolated nuclei from renal oncocytomas revealed a mean increase of 3% of nuclei showing two fluorescent signals when compared to results obtained from matched tissue sections for all five chromosomes examined. For chromosomes 2, 6, 10 and 17, there was a mean increase of 4.9% in fluorescent signals when compared to nuclei examined in tissue sections (Figure 1C).

FISH analysis of tissues from chromophobe renal cell carcinoma showed isolated nuclei and nuclei examined in

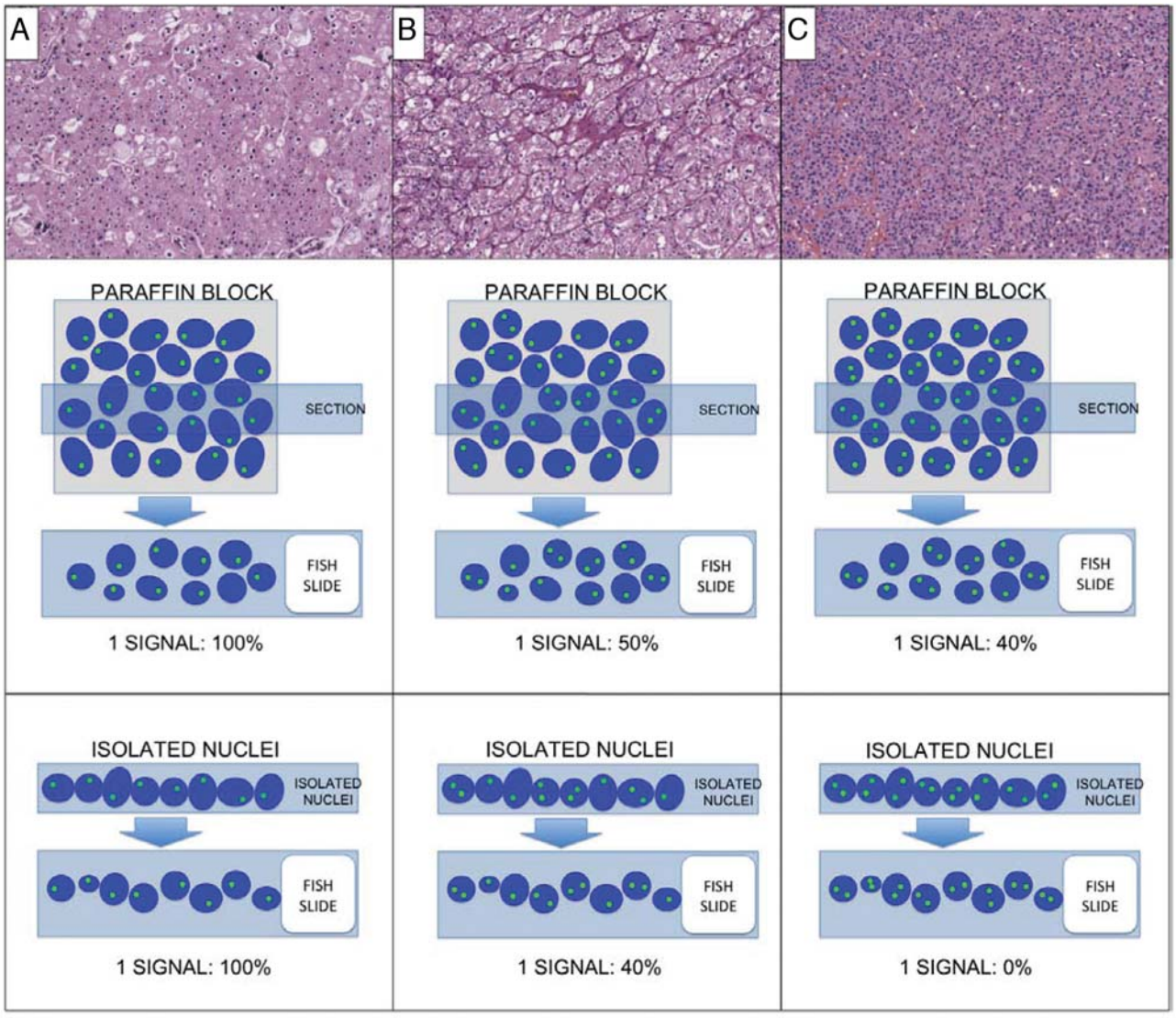

Figure 1. *A: FISH analysis of tissues from chromophobe renal cell carcinoma showing isolated nuclei and nuclei examined in tissue sections having similar percentages of nuclei harboring single signals. B: FISH analysis of tissue from chromophobe renal cell carcinoma showing isolated nuclei and nuclei examined in tissue section having percentages for chromosomes approaching the low level cut-off for interpretation of the finding as chromosomal loss. C: FISH analysis of tissues from renal oncocytoma showing isolated nuclei without significant truncation of nuclei and matched tissue section with partial artefactual nuclei with chromosomal losses.*

tissue sections to have similar percentages of nuclei harboring single signals (mean −1% to +1%) for all chromosomes tested. Analysis of chromosomes 2, 6, 10 and 17 in isolated nuclei revealed almost corresponding mean percentages (mean <1%) when compared to the results obtained from tissue sections (Figure 1A).

Four out of five chromophobe renal cell carcinomas displayed losses of two to five chromosomes when the percentages from both isolated nuclei and those from tissue sections were evaluated (Figure 1B). The remaining case (case no. 5), in spite of exibiting the morphological and immunophenotypical features of classic chromophobe renal cell carcinoma, had a normal numerical chromosomal pattern.

For four out of five renal oncocytomas, the number of the five chromosomes tested for all cases was normal when fluorescent signals on isolated nuclei were evaluated (Figure 1C). In one case (case no. 9), however, the percentages for chromosomes 1 and 17 approached the low level cut-off for interpretation of the findings as chromosomal loss.

Table I. *Interphase FISH scoring percentage on nuclei from tissue histological sections and isolated nuclei.*

Chromophobe renal cell carcinoma

	Chromosome 1						Chromosome 2						Chromosome 6						Chromosome 10						Chromosome 17					
	Tissue sections			Isolated nuclei			Tissue sections			Isolated nuclei			Tissue sections			Isolated nuclei			Tissue sections			Isolated nuclei			Tissue sections			Isolated nuclei		
Signals	1	2	≥3	1	2	≥3	1	2	≥3	1	2	≥3	1	2	≥3	1	2	≥3	1	2	≥3	1	2	≥3	1	2	≥3	1	2	≥3
Case no.																														
1	82	16	2	84	13	3	68	30	2	81	17	2	85	12	3	82	16	2	74	24	2	79	18	3	78	17	5	83	15	2
2	50	47	3	43	55	2	46	52	2	40	58	2	47	51	2	39	58	3	50	48	2	43	54	3	45	52	3	43	55	2
3	81	16	3	82	15	3	76	15	9	78	20	2	83	14	3	85	13	2	77	20	3	81	16	3	85	13	2	82	15	3
4	82	15	3	85	12	3	80	15	5	83	14	3	81	14	5	84	13	3	79	16	4	84	12	4	84	14	2	85	13	2
5	26	71	3	20	78	2	26	72	2	18	80	2	28	69	3	23	75	3	24	72	4	21	77	2	22	75	3	20	77	3
Mean	64.2	33.0	2.8	62.8	34.6	2.6	59.2	36.8	4.0	60.0	37.8	2.2	64.8	32.0	3.2	62.6	35.0	2.6	60.8	36.0	3.0	61.6	35.4	3.0	62.8	34.2	3.0	62.6	35.0	2.4

Renal oncocytoma

	Chromosome 1						Chromosome 2						Chromosome 6						Chromosome 10						Chromosome 17					
	Tissue sections			Isolated nuclei			Tissue sections			Isolated nuclei			Tissue sections			Isolated nuclei			Tissue sections			Isolated nuclei			Tissue sections			Isolated nuclei		
Signals	1	2	≥3	1	2	≥3	1	2	≥3	1	2	≥3	1	2	≥3	1	2	≥3	1	2	≥3	1	2	≥3	1	2	≥3	1	2	≥3
Case no.																														
6	24	74	2	33	65	2	34	64	2	27	70	3	32	65	3	29	68	3	35	63	2	29	68	2	38	60	2	22	75	3
7	28	69	3	30	68	2	31	66	3	32	65	3	26	72	2	31	67	2	32	65	3	28	70	2	36	61	3	25	73	2
8	24	73	3	20	77	3	37	61	2	34	64	2	30	68	2	25	73	2	33	64	3	25	73	2	35	62	3	23	75	2
9	27	71	2	37	61	2	50	48	2	54	45	1	31	67	2	24	73	3	49	49	2	52	47	1	44	53	3	35	63	2
10	25	72	3	33	64	3	35	63	2	31	67	2	37	62	1	27	71	2	33	64	3	27	70	3	36	62	2	25	73	2
Mean	25.6	71.8	2.6	30.6	67.0	2.4	37.4	60.4	2.2	35.6	62.2	2.2	31.2	66.8	2.0	27.2	70.4	2.4	36.4	61.0	2.6	32.2	65.6	2.0	37.8	59.6	2.6	26.0	71.8	2.2

For chromosomal gains of three or more fluorescent signals, no significant differences between FISH results from isolated nuclei and tissue sections were observed. Chromophobe renal cell carcinoma had a small proportion of nuclei showing polysomy (2 to 9% of neoplastic cells) when compared to renal oncocytoma.

Discussion

In an earlier study, we evaluated chromosomal losses in chromophobe renal cell carcinomas utilizing whole sections taken from formalin-fixed paraffin-embedded tissue (1). We found that losses of chromosomes 1, 2, 6, 10 and 17 were frequent in both classic and eosinophilic chromophobe renal cell carcinoma. While loss of chromosome 1 occasionally occurred in sporadic oncocytomas (8-10), these tumors consistently failed to show losses of chromosomes 2, 6, 10 and 17. We also observed that utilizing tissue microarrays of chromophobe renal cell carcinomas, an increase in the number of cores analyzed gave an increased concordance of results with those obtained from whole tissue sections (11). We have also validated the utility of interphase FISH analysis on routine histological sections in differentiating chromophobe renal cells from oncocytoma, showing differing metaphase karyotypes (4). In these previous studies, we had observed that 15% to 20% of tumors had a count of nuclear fluorescent signals that was close to the cut-off level, raising the possibility that sectioning of nuclei on tissue slides may give an overestimation of monosomy in these tumors.

In the present study, we assessed the results of FISH analysis on isolated nuclei obtained from chromophobe renal cell carcinomas and renal oncocytomas and compared the results with those observed on matched histological tissue sections from paraffin blocks of the same tumors. From our results, it is apparent that for chromophobe renal cell carcinomas, when single signals for each chromosome are frequently observed, *i.e.* are present in 50-90% of nuclei, the analysis performed on both tissue sections and isolated nuclei has comparable results and both are of diagnostic utility (11). From this, it would appear that although an appropriate cut-off point based on the number of normal nuclei is always required, when a tumor shows a high level of monosomy, assessment of routine histological sections is not biased by nuclear sectioning. In a previous study, Iqbal *et al.* obtained similar results by FISH using centromeric probes

Table II. *Differences between the percentage of single, double and three or more fluorescent signals in nuclei in favor of analysis of tissue sections versus isolated nuclei.*

Chromophobe renal cell carcinoma.

	Chromosome 1			Chromosome 2			Chromosome 6			Chromosome 10			Chromosome 17		
Signals	1	2	≥3	1	2	≥3	1	2	≥3	1	2	≥3	1	2	≥3
Case no.															
1	2	−3	1	13	−13	0	−3	3	−1	5	−6	1	5	−2	−3
2	−7	8	−1	−6	6	0	−8	7	1	−7	6	1	−2	3	−1
3	1	−1	0	2	5	−7	2	−1	−1	4	−4	0	−3	2	1
4	3	−3	0	3	−1	−2	3	−1	−2	5	−4	0	1	−1	0
5	−6	7	−1	−8	8	−7	−5	6	0	−5	5	−2	−2	2	0
Mean	−1.4	1.6	−0.2	0.8	1.0	−3.2	−2.2	2.8	−0.6	0.4	−0.6	0.0	−0.2	0.8	−0.6

Renal oncocytoma

	Chromosome 1			Chromosome 2			Chromosome 6			Chromosome 10			Chromosome 17		
Signals	1	2	≥3	1	2	≥3	1	2	≥3	1	2	≥3	1	2	≥3
Case no.															
6	9	−9	0	−7	6	1	−3	3	0	−6	5	0	−8	15	1
7	2	−1	−1	1	−1	0	5	−5	0	−4	5	−1	−9	12	−1
8	−4	5	0	−3	3	0	−5	5	0	−8	7	−1	−12	13	−1
9	10	−10	0	4	−3	−1	6	−5	1	3	−2	−1	−9	10	−1
10	8	−8	0	−4	4	0	−10	9	1	6	6	0	−9	11	0
Mean	5.0	−4.6	−0.2	−1.8	1.8	0.0	−1.4	1.4	0.4	−1.8	4.2	−0.6	−9.4	12.2	−0.4

for chromosomes 1, 2, 6, and 10 on touch imprint smears from six histologically proven chromophobe renal cell carcinomas (12). All six tumors had one FISH signal corresponding to one copy number for each of these chromosomes. In their study, the percentage of cells with one FISH signal ranged from 48 to 88% for chromosome 1, 36 to 89% for chromosome 2, 26 to 98% for chromosome 6, and 64 to 99% for chromosome 10. They concluded that interphase FISH performed on touch imprint smears is a simple, rapid, and reliable method for detecting chromosome abnormalities specific for chromophobe renal cell carcinoma (12).

In the present study, we observed that FISH analysis on isolated nuclei from renal oncocytoma specimens usually reveals a mean increase of 3% of nuclei with two fluorescent signals compared to those from matched tissue sections. For chromosomes 2, 6, 10 and 17, analysis of isolated nuclei revealed a mean increase of 4.9% of fluorescent signals when compared to results obtained from tissue sections.

Chromophobe renal cell carcinomas with normal complements of chromosomes, such as our case no. 5, do exist but are in the minority (1) and the presence of very few neoplastic nuclei in a whole tumor with chromosomal gains does not usually reach significance without impacting on the differential diagnosis between chromophobe renal cell carcinoma and renal oncocytoma (4).

The technique of examining sections and that of examining isolated nuclei may also differ in how many nuclei of endothelial cells, fibroblasts and other non-neoplastic cells are counted, and this issue may in part influence the results. This problem is partially overcome by using conservative criteria for abnormality when scoring nuclei by using the DAPI filter (5); we evaluated only nuclei with homogeneous sizes and representing the biggest in maximum diameter among the overall population, thus avoiding potential bias such as small spindle-shaped nuclei or small lymphocytic-like nuclei.

Similar studies of other tissues from the urologic field, comparing data from sections with data from naked nuclei have been performed on neoplastic and non-neoplastic prostate lesions by Qian *et al*. (13). The authors found that in prostatic carcinoma, isolated nuclei had more chromosomal tetrasomy than tissue sections and intratumor heterogeneity of chromosomal anomalies was identified in some cases by FISH analysis of tissue sections but not in isolated nuclei. Tibiletti elegantly demonstrated that the cut-off values depend both on the types of probes used and on the types of target nuclei when evaluating the specificity of interphase FISH for detection of chromosome aberrations in tumour pathology using different tissue (5). For the aforementioned reasons, we cannot compare our data with that others.

From this study, it is apparent that there is a trend in the percentage of chromosomal loss for each of the chromosomes examined with chromosome 17>chromosome 6>chromosome 10>chromosome 2>chromosome 1. These findings lead us to

propose that in order to differentiate between these two tumor types, an initial set of centromeric probes should include those for chromosomes 6, 10 and 17. This could be particularly useful in those cases where there is only a small amount of tissue available for pathological examination.

From this study, we conclude that FISH analysis of isolated nuclei from chromophobe renal cell carcinoma and renal oncocytoma is a useful tool for the differentiation of these two neoplasms. We have also shown that for the chromosomes studied, FISH analysis on isolated nuclei from renal oncocytoma specimens exhibited a mean increase of 3% of nuclei with two fluorescent signals when compared to those from matched tissue sections. In particular for chromosomes 2, 6, 10 and 17, isolated nuclei had a mean increase of 4.9% of fluorescent signals over nuclei evaluated on tissue sections. In view of this, we would recommend that when a tumor exhibits a borderline percentage of nuclei with single signals around the diagnostic cut-off level on tissue sections by FISH, the test should be repeated using isolated nuclei in order to confirm or refute the presence of chromosomal loss.

Acknowledgements

Financial Support was received from MiUR, Diagnostica molecolare in Oncologia and Banco di Sardegna. This study was presented in part at SIAPEC-IAP Meeting, Bari, Italy, September 2008.

References

1 Brunelli M, Eble JN, Zhang S, Martignoni G, Delahunt B and 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 *18*: 161-169, 2005.

2 Amin MB, Paner GP, Alvarado-Cabrero I, Young AN, Stricker HJ, Lyles RH and Moch H: Chromophobe renal cell carcinoma: histomorphologic characteristics and evaluation of conventional pathologic prognostic parameters in 145 cases. Am J Surg Pathol *32*: 1822-1834, 2008.

3 Martignoni G, Pea M, Brunelli M, Chilosi M, Zamo A, Bertaso M, Cossu-Rocca P, Eble JN, Mikuz G, Puppa G, Badoual C, Ficarra V, Novella G and Bonetti F: CD10 is expressed in a subset of chromophobe renal cell carcinomas. Mod Pathol *17*: 1455-1463, 2004.

4 Brunelli M, Delahunt D, Gobbo S, Tardanico R, Eccher A, Bersani S, Cossu-Rocca P, Parolini C, Balzarini P, Menestrina F, Cheng L, Eble JN and Martignoni G: Diagnostic utility of fluorescent cytogenetic in differentiating chromophobe renal cell carcinoma from renal oncocytoma: a validation study combining metaphase and interphase analyses. Am J Clin Pathol *133*: 116-126, 2010.

5 Tibiletti MG: Specificity of interphase fluorescence *in situ* hybridization for detection of chromosome aberrations in tumor pathology. Cancer Genet Cytogenet *155*: 143-148, 2004.

6 Martignoni G, Pea M, Chilosi M, Brunelli M, Scarpa A, Colato C, Tardanico R, Zamboni G and Bonetti F: Parvalbumin is constantly expressed in chromophobe renal carcinoma. Mod Pathol *14*: 760-767, 2001.

7 Rocca PC, Brunelli M, Gobbo S, Eccher A, Bragantini E, Mina MM, Ficarra V, Zattoni F, Zamo A, Pea M, Scarpa A, Chilosi M, Menestrina F, Bonetti F, Eble JN and Martignoni G: Diagnostic utility of S100A1 expression in renal cell neoplasms: an immunohistochemical and quantitative RT-PCR study. Mod Pathol *20*: 722-728, 2007.

8 Paner GP, Lindgren V, Jacobson K, Harrison K, Cao Y, Campbell SC, Flanigan RC and Picken MM: High incidence of chromosome 1 abnormalities in a series of 27 renal oncocytomas: cytogenetic and fluorescence *in situ* hybridization studies. Arch Pathol Lab Med *131*: 81-85, 2007.

9 Nagy A, Buzogany I and Kovacs G: Microsatellite allelotyping differentiates chromophobe renal cell carcinomas from renal oncocytomas and identifies new genetic changes. Histopathology *44*: 542-546, 2004.

10 Meyer PN, Cao Y, Jacobson K, Krausz T, Flanigan RC and Picken MM: Chromosome 1 analysis in chromophobe renal cell carcinomas with tissue microarray (TMA)-facilitated fluorescence *in situ* hybridization (FISH) demonstrates loss of 1p/1 which is also present in renal oncocytomas. Diagn Mol Pathol *17*: 141-144, 2008.

11 Brunelli M, Delahunt B, Ficarra V, Gobbo S, Eccher A, Cossu-Rocca P, Zattoni F, Cheng L, Eble JN and Martignoni G: Utility of tissue microarrays for assessment of chromosomal abnormalities in chromophobe renal cell carcinoma. Anal Quant Cytol Histol *31*: 401-409, 2009.

12 Iqbal MA, Akhtar M, Ulmer C, Al-Dayel F and Paterson MC: FISH analysis in chromophobe renal cell carcinoma. Diagn Cytopathol *22*: 3-6, 2000.

13 Qian J, Bostwick DG, Takahashi S, Borell TJ, Brown JA, Lieber MM and Jenkins RB: Comparison of fluorescence *in situ* hybridization analysis of isolated nuclei and routine histological sections from paraffin-embedded prostatic adenocarcinoma specimens. Am J Pathol *149*: 1193-1199, 1996.

Received May 10, 2011
Revised July 15, 2011
Accepted July 18, 2011