USE OF FISH ANALYSIS FOR DIAGNOSIS OF RENAL CELL CARCINOMA SUBTYPES

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Background: Cytogenetic and molecular genetic techniques have been used in demonstrating the chromosomal abnormalities which characterize specific subtypes of renal cell carcinoma (RCC). The aim of this study was to determine the efficiency of fluorescent in situ hybridization (FISH) technique in characterizing various subtypes of RCC based on the presence of specific chromosome abnormalities found in each RCC subtype.

Materials and Methods: FISH was performed on touch imprint smears from eight renal cell carcinomas histologically confirmed by established criteria.

Results: In four tumors with histologic features of chromophobe renal cell carcinoma (ChRCC), interphase FISH was performed using centromeric probes for chromosomes 1, 2, 6, 10, 12, 17 and 21. All four ChRCC tumors showed one FISH signal corresponding to one copy number for each of these chromosomes. Two papillary RCCs included in this study showed trisomy 7 and 17, and loss of chromosome Y, using the corresponding chromosome centromeric probes. Similarly, we tested two clear cell RCCs for chromosome 3 short arm deletion with DNA probe 3p21.3. Both tumors showed loss of 3p21.3 signal.

Conclusion: We conclude that interphase FISH performed on touch imprint smears is a relatively simple, rapid and reliable method for detecting chromosome abnormalities which are specific for various subtypes of RCC.
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Key Words: Renal cell carcinoma, fluorescent in situ hybridization, interphase FISH, chromosome abnormalities.

Classification of renal cell carcinoma (RCC) into specific subtypes has traditionally been based on cytomorphologic features. However, recent advances in our understanding of the genetics of RCC have provided fresh impetus towards a combined morphologic and genetic classification of these neoplasms. Pioneering work by Kovacs[1] has served to emphasize the fact that genetic alterations transmitted during cell division are fundamentally involved not only in neoplastic transformation but also in determining the morphologic appearance of the RCC. Thus conventional RCC composed of clear and/or granular cells is characterized by chromosome 3 short arm deletion, while carcinomas with a predominant papillary pattern manifest trisomy of chromosome 7 and 17, along with loss of Y chromosome.

Chromophobe renal cell carcinoma (ChRCC) was first described by Thoenes et al. as a neoplasm characterized by a compact growth pattern of large tumor cells, having a translucent and reticular cytoplasm and distinct cell borders.[2-3] Electron microscopic studies have revealed that pale reticulated cytoplasm in cells of this tumor contains numerous unique microvesicles.[4] The tumor cells may also show variable staining for Hale's acid iron calloid.[2-3]

Although the diagnosis of ChRCC is relatively easy in experienced hands, difficulties may arise when the pathologist is either not familiar with this neoplasm or the neoplasm has atypical morphology. Ancillary studies such as electron microscopy, immunohistochemistry and DNA ploidy analysis may help arrival at a definitive diagnosis.[4-6] The last measurement typically reveals a hypodiploid DNA pattern, which correlates well with the recent cytogenetic and molecular genetic studies which indicate that ChRCC has a unique genetic profile characterized by monosomies of several chromosomes, especially chromosomes 1, 2, 6, 10, 13, 17 and 21.[7-10] In short, the diagnosis of ChRCC may be confirmed by demonstrating loss of specific chromosomes.

Kovacs et al.[7] employed microsatellite analysis for determining the genetic profile of renal carcinomas with excellent results. In this study, we use fluorescent in situ hybridization (FISH) analysis for detection of various genetic abnormalities which characterize the renal carcinoma subtypes. The FISH technique is increasingly

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FIGURE 1. FISH analysis on touch imprint smears from ChRCC (case #3). (A) The cells were hybridized with biotinylated chromosome 18 centromeric probe (large arrow) and digoxigenin-labelled chromosome 17 centromeric probe (small arrow). Cell nuclei in A and B are counterstained with DAPI. Chromosome 17 (ChRCC specific) is present in one copy and chromosome 18 is present in 2 copies. Similar dual color hybridizations are seen in (B) with digoxigenin-labelled chromosome 6 centromeric probe and biotin-labelled chromosome 12 centromeric probe, in (C) with biotinylated centromeric probes for chromosome 2 and in (D) with chromosome 1 centromeric probe; the nuclei in C and D are counterstained with propidium iodide. Cells with one FISH signal reflect monosomy for the respective chromosome corresponding to the centromeric probe.

utilized to detect numerical and structural changes in a variety of malignancies.[11-15] Recently, interphase FISH analysis was used to demonstrate trisomy 7 and 17 in association with loss of sex chromosomes in papillary RCC.[16-21] FISH has the advantage of being a relatively simple and rapid technique and is ideally suited for detection of specific numerical chromosomal abnormalities usually seen in these neoplasms.

Materials and Methods

Eight nephrectomy specimens with histologically confirmed RCCs in which material for FISH studies was available were identified. The diagnosis of various subtypes of RCC was made according to established histologic criteria.[2-4] Nuclear grading of all carcinomas was performed according to the criteria determined by Fuhrman et al.[22]

Touch imprint smears were made from fresh RCC tumors, air dried and fixed twice for 5 minutes each in 3:1 methanol:acetic acid. For FISH analysis, touch imprints were pretreated in 2 x SSC solution at 37°C for 30 minutes, followed by dehydration in 70%, 80%, and 95% ethanol for 2 minutes each. The nuclei were then denatured in 70% formamide/2 x SSC solution, pH 7.0 at 70°C for 2 minutes. An appropriate amount of chromosome centromeric or single gene probe, 1.5 µL for single color or 50:50 mixture of two different probes for dual color detection, was denatured in Hybrisol VI for 5 minutes at 72°C. The denatured probe was then placed on the slide for overnight hybridization at 37°C in a humidified chamber. After a rapid wash in 2 x SSC, pH 7.0 solution at 72°C for 5 minutes, the detection of hybridization proceeded by applying either rhodamine-labelled antidigoxigenin or FITC-avidin and counterstaining with DAPI or propidium

FIGURE 2. Dual color FISH analysis on touch imprint smears from papillary RCC (case #1). (A) Cells were hybridized with digoxigenin-labelled chromosome 7 and biotin-labelled chromosome 17 centromeric probes. (B) Cells were hybridized with biotin-labelled chromosome X and digoxigenin-labelled chromosome Y probes. Cells with three FISH signals reflect trisomy and with one FISH signal represent monosomy. Trisomy 7 and 17 are present in A and loss of chromosome Y is seen in B.

iodide, respectively. For dual color detection, rhodamine-labelled antidigoxigenin was applied before FITC-avidin. The slides were scanned by a Ziess Axiophot fluorescence microscope and images were captured on Powergene Probe System (Perceptive Scientific System, Texas, USA). Chromosome centromeric probes used in this study were chromosomes 1, 2, 6, 10, 17, X, Y, 3p21.3 and Quint-Essential 13/21 dual color cocktail probe (chromosome 13 appears as green and 21 as red). These probes were purchased from Oncor, Inc. (Gaithersburg, MD). FISH was performed according to the manufacturer's instructions (Oncor Instruction Manual Catalog No. S1340-KIT; S1332-DR and S1333-BF; Edition 4.1, Feb 1996, Gaithersburg, MD). A minimum of 100-200 interphase cells were scored by two technologists for the presence of FISH signal. The normal cut-off for each probe used in the study was determined by hybridization to normal cells according to the method of Schad and Dewald.[23]

Results

Eight RCC tumors were included in this study. Four tumors were of chromophobe type and had cells with "classical" morphology in two, while the remaining two were predominantly composed of eosinophilic cells. The nuclear grade was two in all cases. Two tumors were of papillary type and two had morphologic features of conventional RCC.

Results of the FISH analysis are summarized in Tables 1, 2 and 3. All four cases of ChRCC showed one signal corresponding to one copy number for chromosomes 1, 2, 6, 10, 13, 17 and 21. The percent cells with one FISH signal ranged from 48%-88% (chromosome 1); 36%-89% (chromosome 2); 26%-98% (chromosome 6); 64%-99% (chromosome 10); 60-75% (chromosome 13); 63%-73% (chromosome 17) and 60%-75% (chromosome 21).

Representative FISH images from case #3 are shown in

FIGURE 3. FISH analysis on touch imprint smears from clear cell RCC (case #1). The cells were hybridized with digoxigenin-labelled 3p21.3 single gene probe, the nuclei are counterstained with DAPI. Cells with one FISH signal represent 3p deletion.

TABLE 1. *Summary of chromosome copy number in chromophobe RCC as detected by FISH using chromosome centrometic probes for 1, 2, 6, 10, 13, 17 and 21.*

Single FISH Signal							
Cent. Probes*	1	2	6	10	13	17	21
Case #	(%)	(%)	(%)	(%)	(%)	(%)	(%)
1	16	15	26	64	56	40	56
2	87	89	98	99	–	82	73
3	56	68	87	80	60	63	60
4	88	87	78	84	75	73	75

*Normal cut-off for these probes in our laboratory is around 3%-5% for one signal and 0.2%-0.6% for three signals.

TABLE 2. *Trisomy of chromosomes 7 and 17 and loss of Y chromosome in papillary RCC using FISH.*

Cent. Probes	7			17			X/Y (dual color)	
FISH Signal (%)	2	3	4	2	3	4	XY	XO
Case #1	28	13	58	47	50	3	2	98
Case #2	16	77	–	38	58	–	18	75

*Normal cut-off for these probes in our laboratory is around 3%-5% for one signal and 0.2% -0.6% for three signals.

TABLE 3. *Chromosome 3p deletion in clear cell (RCC) detected by FISH.*

Probe	3p21.3	
FISH signal (%)	1	2
Case #1	60.8	42
Case #2	34	65

Figure 1. The centromeric probes for chromosomes 12 and 18 were used as internal controls, since these chromosomes are not usually involved in ChRCC. Chromosomes 12 and 18 revealed two signals in virtually 100% of the cells. Chromosomes 6 and 17, on the other hand, only showed one signal indicating monosomy. In two cases, FISH results were also confirmed by cytogenetic analysis. In case #2, the karyotype analysis revealed: 35-38, X, -X, -1, -2, -6, -10, -13, -17, -21 [cp13] and in case #3 the karyotype was 35-38, X, -X, -1, -2, -6, -10, -13, -16, -17, -21 [cp12].

FISH results on two papillary RCCs are shown in Table 2. Both these cases showed trisomy 7 and 17 with loss of Y chromosome. Representative FISH images from case #1 are shown in Figure 2. In this case, the percentage of cells with trisomy 7 was 13%, trisomy 17 was 50% and loss of Y was 98%, and in case 2, trisomy 7 was seen in 77% of cells and trisomy 17 in 58%, respectively. Loss of Y chromosome was observed in 75% of cells.

FISH results on the touch imprints from two RCC tumors classified as clear cell type histologically are presented in Table 3. Both these tumors showed deletion in the chromosome 3 short arm as detected by the 3p21.3 DNA probe, which specifically hybridizes to a locus on chromosome 3 short arm (Figure 3). In case #1 (Table 3), 60.8% of cells showed only one signal for the probe 3p21.3 indicating a 3p deletion; similarly in case #2, 34% of cells showed a deletion of the 3p21.3 region.

Discussion

Renal carcinoma subtypes may be characterized by specific karyotypic and genetic abnormalities. The recently proposed Heidelberg classification of renal tumors attempts to incorporate morphology and genetic data for each subtype of RCC.[24] Pathologic diagnosis of ChRCC is usually based on recognition of characteristic morphologic features, such as abundant translucent reticulated cytoplasm, prominent cell borders, perinuclear cytoplasmic clear zone and the presence of balloon cells. However, the cellular morphology of ChRCC is not monomorphous and may show considerable variation within a given tumor and from one tumor to the other. Indeed, in one study, three cell patterns were recognized, any one of which may predominate. Furthermore, some of the ChRCCs are composed of eosinophilic cells which mimic closely the cells seen in granular cell carcinoma and renal oncocytoma. In ChRCC, cells with abundant translucent cytoplasm may also mimic clear cell carcinoma. A definitive distinction between these tumors is crucial since the clinical behavior and prognosis is substantially different. Ancillary studies, such as immunohistochemistry and electron microscopy, may be helpful. Chromophobe RCC may also be recognized by positive staining of the tumor cells for Hale's

acid iron colloid stain, although staining may be quite variable.[2-4]

Several molecular genetic studies on ChRCC have been published. Initially, a variety of genetic abnormalities such as an interstitial deletion of the short arm of chromosome 11, trisomies of chromosomes 7, 12, 16, 18, 19 and 20, and alteration of chromosome 11q were reported.[25-26] Schwerdtle et al.[27] described a high frequency (70%) of loss of heterozygosity (LOH) at chromosome 14q. This finding, however, has not been confirmed by other studies. By using CGH analysis, Speicher et al.[10] found specific loss of chromosomes 1, 2, 6, 10, 13, 17 and 21 in ChRCC. These findings have been confirmed by karyotyping studies.[8] In a detailed microsatellite study of 42 ChRCCs, Bugert and colleagues demonstrated loss of chromosomes 1, 2, 6, 10, 13 and 17 in up to 95% of the tumors.[7] In all but one tumor, monosomies of multiple chromosomes were noted. The authors concluded that loss of specific chromosomes may be used to confirm the diagnosis of ChRCC. These findings correlate well with DNA studies on ChRCC which reveal a consistent finding of hypodiploid clones. The presence of these clones has also been suggested as an important diagnostic criterion for ChRCC. [5-6]

Clear cell RCCs of the kidney, which represent approximately 80% of the renal cell carcinomas, are characterized by either complete loss of chromosome 3 or structural rearrangements of the short arm of chromosome 3, affecting the 3p13-3pter region.[28-31] A high frequency of LOH on 3p is also a characteristic feature of clear cell RCCs.[26,29-33] As the tumor undergoes further growth and progression, it may acquire additional karyotypic abnormalities, such as partial trisomy 5q, trisomy 7, monosomy 6q, 8p, 9 and allelic loss at chromosome 14q.[1] Partial or full trisomy 5 has been reported to be present in 50% of cases.[29-31] In this study, we have demonstrated abnormalities resulting in deletion of the 3p13-3pter region, which were detected by FISH on freshly prepared touch imprints from both clear cell RCCs confirming the histological diagnosis.

Papillary carcinomas of the kidney are shown to be characterized by trisomies of 7, 12, 16, 17 and 20;[33-36] however, combined trisomy of chromosomes 7 and 17 and loss of Y is suggested to be an initial event.[1] These alterations are present in both papillary adenoma and carcinoma. In the latter, however, additional karyotype alterations occur as trisomy 12, 16 and 20, which may determine the growth potential and clinical behavior.[33] In a number of studies, FISH has been demonstrated to be an efficient method for detecting trisomy 7, 17 and loss of Y chromosome in paraffin-embedded tissues from papillary RCC.[16,18-21,36] In this study, we have shown that the FISH technique can efficiently detect trisomies of chromosomes 7, 17 and loss of Y on touch imprint smears of papillary RCC.

We observed some variation in the percentage of cells with one FISH signal in the case of ChRCC and three FISH signals in papillary RCC. This variation is to be expected due to karyotypic heterogeneity within these tumors, but different cells within a tumor share common chromosome abnormalities with different frequencies. This correlates well with our previous cytogenetic findings, where we have shown heterogeneity in the clonal cell population.[8]

FISH is a powerful technique for detecting numerical abnormalities in interphase cells from touch imprints. Our findings in this study have shown that FISH may also be an efficient technique for detecting monosomies of specific chromosomes characteristic of various subtypes of RCC, and this may be used as an adjunct to histopathologic diagnosis.

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