

**CYTOGENETICS IN THE DIAGNOSIS AND UNDERSTANDING OF RENAL  
NEOPLASIAS**

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There are 150,000 new cases of renal cell carcinoma (RCC) diagnosed every year worldwide, representing 1.9% of all malignant tumors. It has been estimated that there will be 35,710 new cases and 12,480 deaths due to RCC in the United States this year, which is one of the highest rates in the world, along with that of Europe. Although the incidence of RCC is modest compared to that of other tumors, renal cancer is a disease that it is frequently detected in advanced stage and it is difficult to treat. Oberling in 1960 demonstrated that these neoplasms originate from renal tubular epithelium, a finding that was the first step in developing a valid classification system of renal tumors.

Recently, cytogenetic and molecular biology techniques have been used to study these tumors and have demonstrated a clear relationship between RCC and the presence of chromosomal alterations. These findings have led to a better understanding of the pathogenesis of RCC and to the development of new classification systems, including that of Heidelberg (1997) which integrated morphologic, clinical and cytogenetic elements. The last classification system from the World Health Organization (WHO) in 2004 includes recently described entities and incorporates morphologic, clinical, and genetic correlations.

These molecular biology techniques include in situ hybridization (ISH), particularly fluorescent in situ hybridization (FISH), which has been used in the study of various tumors for the detection of numeric and structural chromosomal alterations. Enumerating chromosome copies with centromeric probes has been performed using chromogenic detection methods since the 1990s, the chromogenic detection of unique sequences such as the HER-2 oncogene in breast cancer has shown some advantages over FISH, including lower cost and ease of application in conventional pathology laboratories, because it does not require use of a fluorescent microscope and histologic evaluation of the tissue under study can be performed concomitantly.

We evaluated the use of CISH, chromosome in situ hybridization in the diagnosis of renal tumors.

### ***Tissue Samples***

The histology slides and paraffin blocks from 91 cases of various types of renal tumors were obtain for the study. Each case was reviewed and classified according to the 2004 WHO classification. The cases under study included 37 cases of PRCC, 28 of which were type 1 (9 sporadic cases, 11 hereditary, and 8 of unknown history) and 9 of which were type 2. The 54 remaining cases represented a substantial number of different diagnoses within the spectrum of renal tumors, including 18 clear cell RCC, 10 oncocytomas, 8 chromophobe renal cell carcinoma, 4 medullary carcinomas, 6 collecting duct (of Bellini) carcinomas (CDC), 4 hybrid tumors, 2 metanephric adenomas, and 2 carcinomas which could not be classified.

## **CISH**

CISH was done on 4 µm thick archival formalin-fixed paraffin-embedded (FFPE) tissue sections following the technique of Tanner et al.

In order to obtain satisfactory visualization of the chromosome alterations, a count of 100 to 500 nuclei was performed in randomly selected fields from each of the slides based on the size of the sections and the degree of nuclear overlapping, in accordance with Hopman's recommendations. The final result was an average expressed as a percentage of nuclei with 2, 3, or 4 signals (spots), classifying them as disomic, trisomic, and tetrasomic, respectively.

We scored the intensity of the stain with 1, 2, or 3 crosses: +/+++, when the signals could only be seen with the X40 and X60 objectives; ++/+++, when they could be seen with the X20 objective; and +++/+++, when they were visible with the X1

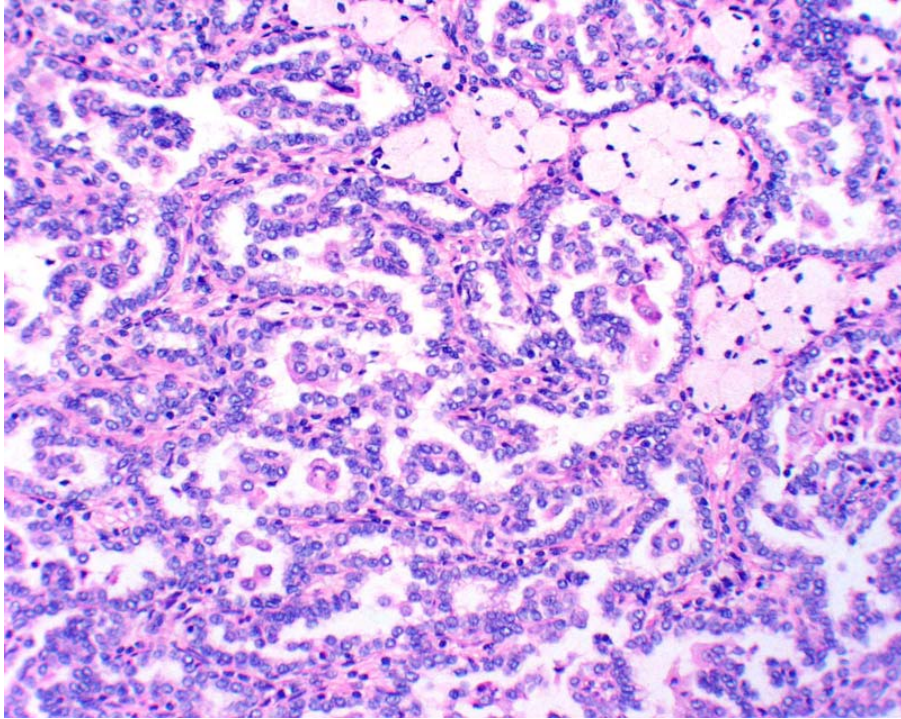
## **Results**

Our study demonstrated that gains in chromosomes 7 and 17 are more frequent in PRCC than in nonpapillary RCC. Specifically, polysomy for chromosomes 7 and 17 was observed in 100% and 54.5% of cases of familial type 1 PRCC, respectively, higher figures than those obtained in cases of type 2 PRCC or nonpapillary RCC. These findings confirmed the differences in cytogenetic profile between type 1 and type 2 PRCC.

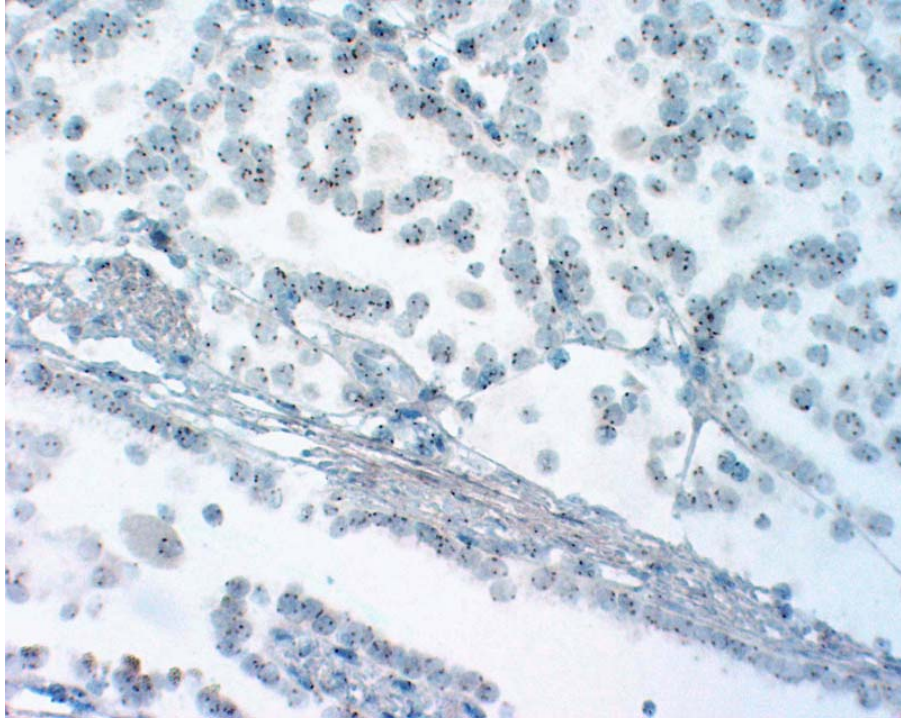
This was the first study in which chromogenic in situ hybridization was used to evaluate numeric alterations of specific chromosomes in RCC, and our results correspond to those reported in prior studies in which different techniques were used. In sporadic type 1 PRCC, we found a lack of numeric alterations of chromosome 17, data that has not been previously reported and that may prove helpful in the differentiation of these tumors. We found no alterations in the 2 metanephric adenomas studied, a finding that may prove useful in the differential diagnosis between these tumors and PRCC.

We found chromosome 7 trisomy in a case of ChRCC, which is one of the alterations that has been described in this type of tumor, in addition to hypodiploidy of various chromosomes, which is its most characteristic feature.

We conclude that CISH can be a very useful tool in the identification of numeric abnormalities in specific chromosomes and can improve the diagnosis of renal tumors. There is a clear correlation between chromosome 7 aneuploidy identified by CISH and familial type 1 PRCC. The absence of chromosome 17 aneuploidy can help identify cases of sporadic type 1 PRCC. The identification of chromosome 7 aneuploidy in clear cell RCC with sarcomatous features as well as in one unclassified tumor suggests a high grade and the possibility of new genetic alterations as the tumor progresses.



**Papillary Renal Cell carcinoma type I**



**PRCC Chromogenic In Situ Hybridization showing trisomy for chromosome 7**

## RESULTS

RESULTS									
Histology	Cases	Chromosome 7 Aneuploidy	%	Chromosome 7 Diploidy	%	Chromosome 17 Aneuploidy	%	Chromosome 17 Diploidy	%
<b>TYPE 1 PRCC</b>	28	15	53.6	13	46.4	7	25	21	75
Herededitary	11	11	100	0	0	6	54.5	5	45.5
Sporadic	9	3	33.3	6	66.7	0	0	9	100
Undetermined	8	1	12.5	7	87.5	1	12.5	7	87.5
<b>TYPE 2 PRCC</b>	9	3	33.3	6	66.7	2	22.2	7	77.8
<b>OTHERS</b>	54	6	11.1	48	88.9	3	5.6	51	94.4

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