American Journal of Pathology, Vol. 149, No. 6, December 1996

Molecular Differential Diagnosis of Renal Cell Carcinomas by Microsatellite Analysis

Peter Bugert and Gyula Kovacs

From the Clinical Research Group, Laboratory of Molecular Oncology, Department of Urology, Ruprecht Karls University of Heidelberg, Heidelberg, Germany

Recent application of molecular cytogenetic techniques has resulted in a new type of genetic classification of renal cell tumors. The key aspect of the novel diagnostic concept is reflected by biologically distinct entities, each characterized by a specific combination of genetic changes. To work out a diagnostic/prognostic approach, we have applied polymorphic microsatellite markers for a quick analysis, based on polymerase chain reaction, of 82 tumor specimens. We compared the results to previously evaluated cytogenetic and histological data. All nonpapillary and chromophobe renal cell carcinomas, which make up approximately 90% of all malignant renal cell tumors, and a subset of renal oncocytomas were correctly diagnosed by detection of loss of heterozygosity at chromosomal sites 1, 2, and 3p. Allelic losses at chromosomal regions 8p, 9p, and 14q are associated with an advanced pathological stage of nonpapillary renal cell carcinomas. A loss of heterozygosity at chromosomes 6, 10, 13, 17, and 21, in addition to those at chromosomes 1 and 2, confirm the diagnosis of chromophobe renal cell tumors. Using this approach, the differential diagnosis of renal cell tumors could be carried out within 1 or 2 days. (Am J Pathol 1996, 149:2081–2088)

Renal cell carcinoma (RCC) is the most common malignant tumor arising from the kidney and affects approximately 7 in 100,000 adults. Approximately 40% of the patients have a metastatic disease at the time of diagnosis, and one-third of the remainder will progress during the first 2 years of the postoperative course. There is no adequate chemo- or immunotherapy for advanced disease. Therefore, it is important to establish a diagnosis, which is relevant for the biological behavior of the tumor, ie, for the clinical outcome. Generally, a RCC is diagnosed and its cytological features (clear, granular, chromophilic, chromophobe, oncocytic, mixed, or spindle cells) and growth patterns (solid, acinar, tubular, cystic, papillary, or mixed) are described.[1,2] However, renal cancers display a heterogeneous morphology and the phenotype may change dramatically during progression. These findings suggest that a transition between different cell types takes place and, therefore, the phenotype cannot be used for a correct diagnosis.[3,4] Even the same pathologist might obtain conflicting results in the same lesion, albeit at different times.

Recent advances in our understanding of how cancer develops have shown that tumor is a genetic disease resulting in the abnormal proliferation of a clone of cells. As soon as a gene or DNA alteration occurs in a progenitor cell, it marks all descendent cells for their entire life span. Carcinogenesis appears to be a multistep process in which a series of genetic alterations occur within one cell.[5] Primary genetic changes are associated with the development and growth of tumor, whereas additional changes are required for an autonomously growing clone of cells to metastasize. The combination of genetic alterations may be characteristic for one type of malignancy and, therefore, molecular markers may be used to identify distinct entities among tumors of an organ. Over the past decade, specific genetic alterations have been detected in RCC.[6–9] The evaluation of molecular cytogenetic as well as histopathological and clinical data resulted in a new type of classification of kidney cancers.[10] The key aspect of the novel diagnostic concept is reflected by genetically and biologically distinct entities.[3,4] The major advantage of the new classification is that

The Clinical Research Group is supported by the German Research Council.

Accepted for publication August 14, 1996.

Address reprint requests to Dr. Gyula Kovacs, Laboratory of Molecular Oncology, Department of Urology, Ruprecht Karls University, Im Neuenheimer Feld 365, D-69120 Heidelberg, Germany.

the genetic alterations associated with a given type of tumor are constant. Moreover, recent developments in DNA technologies offer simple methods to detect specific genetic changes and thus may allow a quick and correct diagnosis. A polymerase chain reaction (PCR)-based analysis of the DNA polymorphism by microsatellites might be the technique of choice until the genes are cloned.[11] To work out a diagnostic approach, we have applied polymorphic microsatellite markers mapped to tumor-specific deletions for the evaluation of renal cancers.

Materials and Methods

Tumors and Histological Diagnosis

Sporadic RCCs were collected at the Department of Urology, University of Heidelberg, between May 1994 and September 1995. To increase the number of rare types of tumors, we have also analyzed chromophobe and papillary RCCs as well as renal oncocytomas collected earlier. Thus, 52 nonpapillary RCCs, 10 papillary RCCs, 10 chromophobe RCCs, and 10 renal oncocytomas were evaluated in this study. A macroscopically homogeneous part of the tumors was excised under sterile conditions. One part of this material was used for short-term culture, another part was frozen in liquid nitrogen and kept at −80°C, and the third part was processed for reference histology. The entire nephrectomy specimen was then subjected to a routine histological diagnosis. All histological specimens were reviewed by a pathologist (G. Kovacs) experienced in the new genetic classification system.[3] The pathological stage was then established for each tumor according to the UICC and AJCC classifications.[12,13] Results of the microsatellite analysis were then evaluated regarding the pathological stage. Briefly, T1 and T2 tumors with N0 and M0 were assigned as stage I and II, respectively, whereas tumors with T3a-c N0 M0 and any tumor with N1-3 and/or M1 were assigned to stage groups III and IV.

DNA Isolation

Analysis of tumor DNA without contamination by normal DNA is the most critical step to determine a loss of heterozygosity (LOH) by applying microsatellite markers. Therefore, for this study, DNA was extracted from short-term cultures of tumor cells. Briefly, tumor tissue was minced or cut into thin slices with a surgical scalpel and washed in RPMI 1640 medium. The small tumor pieces were then incubated in 0.1% collagenase dissolved in culture medium containing 10% fetal calf serum in a 25-cm^2 Falcon flask at 37°C until small cell clumps could be released by gentle shaking (usually 30 to 60 minutes). Tissue fragments were then washed and resuspended in medium and dispersed vigorously by a Pasteur pipette. The suspension was then allowed to sediment for 3 to 5 minutes. The supernatant containing single and damaged cells was discarded, and the interphase containing small tumor cell clusters was placed into 25-cm^2 Falcon flasks containing 5 ml of RPMI 1640 medium supplemented with 10% fetal calf serum in a CO_2 incubator at 37°C. Cells were monitored under an inverted microscope for growth and contamination with normal cells. DNA was extracted from cell cultures containing only tumor cells and also from corresponding normal kidney tissues after proteinase K digestion followed by phenol/chloroform extraction and ethanol precipitation. The DNA concentration of each sample was adjusted to 10 ng/μl, and 10 μl (100 ng) each were used as a template in the 20-μl PCR reactions.

It is also possible to use the same protocol of tumor cell isolation for routine diagnosis. As the interphase after sedimentation contains small cell clusters with minimal contamination of attached macrophages or endothelial cells, it could be used without culturing for DNA isolation and PCR assay. In our hands, tumor DNA isolated in this way contains far less than 10% of nucleic acid from normal cells. Incubation of the small tumor cell clusters for 5 to 10 minutes in the Petri dish or culture flask may further decrease the contamination. Macrophages attach to the surface within a couple of minutes and remain in the flask whereas tumor cells do not. Using this short approach, there is no need for handling tumor samples under sterile conditions, and the DNA is useful for the microsatellite assay.

Microsatellite Analysis

The DNA samples were coded and microsatellite analysis was carried out without knowledge of histological, cytogenetic, and clinical data. The polymorphic microsatellite markers used in this study were D1S1656 (1q), D1S162 (1p), D2S1391 (2q), D3S1560 (3p25-26), and D3S1300 (3p14.2) for the diagnostic primer set; D6S1040 (6q), D8S261 (8p), D9S171 (9p), D14S61 (14q24), and D14S73 (14q31) for the nonpapillary primer set; and D10S1239 (10q), D13S317 (13q), D17S783 (17p), D17S807 (17q), D21S1270 (21q), and D21S1436 (21q) for the chromophobe primer set. Microsatellite markers for loci D1S1656, D2S1391, and D3S1300 as well as for loci D1S162 and D3S1560 were used in multiplex PCR

Table 1. *Differential Genetics of Nonpapillary and Chromophobe Renal Cell Tumors and Renal Oncocytomas*

Type of tumor	Frequency of karyotype alterations (%)											
	−3p	+5q	−6q	−8p	−9	−14q	−1	−2	−10	−13	−17	−21
Nonpapillary RCC	98	70	14	22	14	41	–	–	–	–	–	–
Chromophobe RCC	25	–	88	25	18	–	100	95	88	95	76	88
Renal oncocytomas	–	–	–	–	–	40	40	–	–	–	–	–

reactions. Additional microsatellite markers were used in cases that were not informative for loci at chromosomes 1, 2, and 3p. Microsatellites localized to the smallest overlapping deletions were selected for this study. The locus D9S171 is flanking the putative tumor suppressor gene CDKN2/p16 at chromosome 9p21.[14]

To perform 10 standard PCR reactions, 20 pmol of forward strand primer were 5′-labeled with 10 μCi of [γ-^{32}P]ATP in a 10-μl kinase reaction with 10 U of T4 kinase (MBI-Fermentas, Heidelberg, Germany) for 1 hour at 37°C, followed by 10 minutes at 80°C to inactivate the enzyme. A PCR mixture for 10 standard reactions contained 20 μl of 10X PCR buffer (100 mmol/L Tris/HCl, pH 8.3, 500 mmol/L KCl, 15 mmol/L $MgCl_2$, 1% bovine serum albumin), 20 μl of 10X dNTP (2 mmol/L each of dATP, dCTP, dGTP, and dTTP), 20 pmol of reverse primer, 10 μl of radiolabeled forward primer, 10 μl of 10% Tween 20, 1 μl of *Taq* polymerase (5 U/μl; Perkin Elmer, Norwalk, CT) adjusted to a final volume of 100 μl with sterile water. A 10-μl aliquot of this PCR mixture was added to 10 μl of template DNA, which results in a final concentration of 10 mmol/L Tris/HCl, 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, and 100 nmol/L each of forward and reverse primer. Cycling was performed in a PTC100 thermal cycler (MJ Research, Watertown, MA) with an initial denaturing step for 2 minutes at 95°C followed by 28 cycles of 94°C for 40 seconds and 55°C for 30 seconds. After a final extension step at 72°C for 10 minutes, 10 μl of sequencing stop solution was added to the reaction. The PCR products were separated on 5% denaturing polyacrylamide gels for 1.5 to 2 hours at a constant 80 W. The dried gels were exposed to x-ray films for 1 to 16 hours at room temperature without using screens.

Experimental Design

As with leukemias, the new diagnosis is related to specific genetic alterations, which mark subsets of renal cell tumors.[3,4] Each type has a unique natural history with a strong impact on the clinical course.[10] We must constantly be aware that we are not dealing with a single disease called RCC or its cytomorphological subtypes but with genetically well characterized entities with distinct molecular pathology. Although the molecular basis of genetic changes, with exception of the von Hippel-Lindau disease (VHL) gene, is not yet established, the combination of DNA alterations determine unequivocally distinct types of renal cell tumors.[10] The crucial point in the differential diagnosis of renal cell tumors is the detection of the LOH at chromosome 3p (Table 1). This genetic alteration occurs in a large proportion (98%) of nonpapillary RCCs of sporadic as well as hereditary origin, similar to the frequency with which the Philadelphia chromosome, ie, the *bcr* rearrangement is observed in chronic myelogeneous leukemia.[6,7,15] A LOH at chromosome 3p has not been found in papillary renal cell tumors and in renal oncocytomas, but it occurs in approximately 25% of chromophobe RCCs.[8,9,16] However, a monosomy of chromosome 1 and 2, which occurs in 100 and 95% of chromophobe RCCs, respectively, has not been found in papillary and nonpapillary RCCs.[9,10] Monosomy of chromosome 1 has also been found in a subset of renal oncocytomas.[17,18] Thus, it is possible to separate nonpapillary and chromophobe RCCs and some renal oncocytomas and exclude papillary renal cell tumors by detection of LOH at chromosomal sites 1, 2, and 3p (Figure 1). As chromophobe RCCs display additional losses of chromosomes 6, 10, 13, 17, and 21, a detection of LOH at these chromosomal regions will unequivocally confirm the diagnosis of chromophobe RCC.[9] Comparative genetic and clinical data suggested that loss of chromosome 3p and also the gain of chromosome 5q sequences are associated with the development of nonpapillary RCCs, whereas LOH at chromosome 6q, 8p, 9, and 14q occur at a higher frequency in advanced stages of the disease.[3,4] Thus, microsatellite analysis of these regions will not only confirm the diagnosis of a nonpapillary RCC but may also give information about the biological behavior, ie, about the prognosis. Approximately one-third of renal oncocytomas

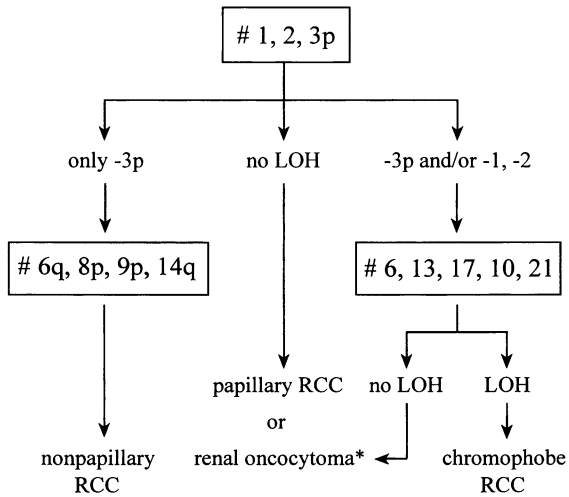

Figure 1. *Schematic illustration of molecular differential diagnosis of renal cell tumors by microsatellite analysis. Screening the tumor samples with polymorphic markers from the chromosomal regions 1, 2, and 3p differentiates nonpapillary RCCs (only −3p) from chromophobe RCCs (−1, −2, and sometimes −3p); the rest belong to papillary renal cell tumors or renal oncocytomas. Subsequently, nonpapillary RCCs can be characterized for progression-associated genetic changes at chromosomes 6q, 8p, 9p, and 14q and chromophobe RCCs for additional LOH at chromosomes 6, 10, 13, 17, and 21. *In a subgroup of renal oncocytomas, a combination of loss of the Y chromosome and monosomy of chromosome 1 occurs, which can be delineated by LOH at chromosome 1 and lack of LOH at all other autosomal regions analyzed.*

show a loss of the Y chromosome and monosomy of chromosome 1 and 14.[10,17,18] Based on the LOH at other specific chromosomal regions such as chromosome 2, 3p, 6, 8, 10, etc, these tumors can be separated from nonpapillary and chromophobe RCCs. Papillary renal cell tumors, which make up approximately 10% of epithelial kidney tumors, are characterized by highly specific combination of trisomies of chromosomes 3q, 7, 8, 12, 16, 17, and 20.[8,10] However, an allele duplication cannot be unequivocally diagnosed with microsatellite markers.

Results

Molecular Differential Diagnosis of Renal Cell Tumors

We used a diagnostic set of polymorphic microsatellites mapped to chromosomes 1, 2, and 3p in a multiplex PCR reaction for the differential diagnosis of eighty-two renal cell tumors. Applying this screening set of markers, we found LOH at chromosome 3p in fifty-four cases. Fifty-two tumors showed only 3p deletion, whereas two tumors showed also an additional LOH at chromosome 1p and 2. Eleven of eighty-two tumors, including those two with chromosome 3p loss, showed LOH at chromosome 1p and/or chromosome 2. Thereafter, we applied the nonpapillary set of primers mapped to chromosomes 6q, 8p, 9, and 14q to all tumors showing LOH at chromosome 3p in the first round. Tumors with chromosome 3p alterations displayed additional changes at chromosomes 6q, 8p, 9, and 14q, with the exception of the two cases showing LOH at chromosomes 1p and 2 during the first round of amplification. These two cases, together with nine additional tumors showing LOH at chromosome 1p and/or 2 were then subjected to the analysis with the chromophobe set. Ten tumors had LOH at chromosomes 6, 10, 13, 17, and 21 in addition to those found in the first round. One of the eleven tumors showing LOH at chromosome 1 retained heterozygosity at all other regions tested with both the nonpapillary and chromophobe primer sets. This tumor was therefore diagnosed as an oncocytoma. All fifty-two tumors with chromosome 3p deletion and additional changes at chromosomal regions of the nonpapillary set or lack of alteration at chromosomal sites of the chromophobe set were diagnosed as a nonpapillary RCC. All ten tumors showing chromosome 1p and/or 2 alterations in the first round and also LOH with the chromophobe primer set were diagnosed as a chromophobe RCC. Some examples are shown in Figure 2. Those tumors without any changes after analysis with all three sets of markers were designated as papillary renal cell tumor or renal oncocytoma. The molecular diagnosis, which was obtained by a molecular geneticist (P. Bugert) from coded DNA samples, matched the previously established cytogenetic and histological diagnosis of tumors.

Genetic Alterations Associated with the Pathological Stage of Nonpapillary RCCs

As nonpapillary RCCs used in this study were operated recently, an evaluation of the impact of secondary genetic changes on the 5-year survival of these patients is not yet available. However, it is possible to compare the genetic changes to the stage of progression at the time of nephrectomy. In this study, we found LOH at the chromosome 6q region in 8 of 44 informative cases (18%), at chromosome 8p in 18 of 50 cases (36%), at chromosome 9 in 10 of 43 tumors (23%), and at chromosome 14q in 16 of 52 tumors (31%). We have evaluated the genetic changes in nonpapillary RCCs of stage I and II *versus* stage III and IV at the time of surgery (Table 2). LOH at chromosome 6q did not show any correlation with the stage of tumors. However, an increased frequency of LOH at chromosomal regions 8p, 9p, and 14q was found in the group of tumors with stage III and IV in comparison with those

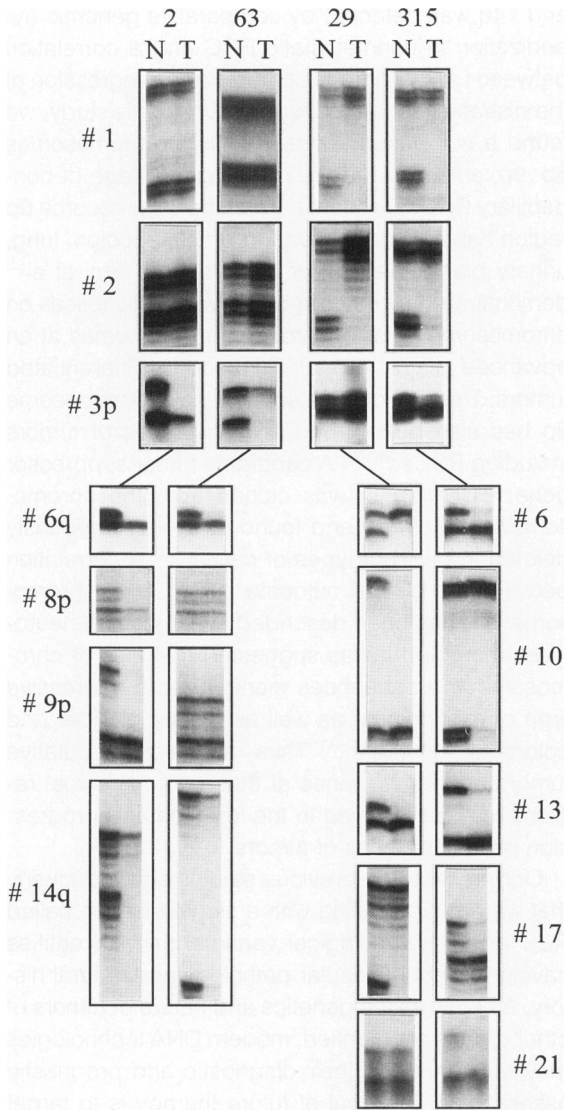

Figure 2. *Result of a molecular differential diagnosis by microsatellite analysis. Two examples for nonpapillary (cases 2 and 63) and two for chromophobe (cases 29 and 315) RCCs are shown. The screening of the normal (N) and tumor (T) samples was performed with markers from the chromosomal regions 1, 2, and 3p. Both nonpapillary tumors showed LOH only at 3p and, when further analyzed with microsatellite markers, at chromosomal regions 6q, 8p, 9p, and 14q. The chromophobe RCCs showed LOH at chromosome 1 and 2 and additionally at 3p in case 315. Additional allelic losses have been found in the chromophobe RCCs at chromosomal regions 6, 10, 13, 17, and 21.*

with stage I and II. Only 2 of 21 nonpapillary RCCs with stage I and II showed LOH at chromosome 14q, whereas 14 of 31 tumors with stage III and IV had LOH at chromosome 14q.

Discussion

The major aim of this study was to establish a quick, reproducible approach for the differential diagnosis of RCCs. For genetic diagnosis and prognosis to become a practical approach, multiple tumor suppressor gene loci or genes involved in the genetics of distinct types of tumors must be identified. Previously, we have shown that highly specific cytogenetic, DNA, and mitochondrial DNA alterations separate entities among kidney cancer.[10] This type of genetic stratification was a prerequisite for the diagnostic and prognostic approach applied in this study. For example, the LOH at chromosome 1 occurs in approximately 5 to 7% of RCCs in general. Therefore, LOH at chromosome 1p has not been proven to be a specific genetic alteration in previous allelotyping studies.[11] However, LOH at chromosome 1 becomes a highly specific genetic alteration when data are evaluated according to the new classification; it occurs in 100% of chromophobe RCCs, which make up 5% of all renal tumors, and also in a subset of renal oncocytomas.[9,10,17,18]

Table 2. *Genetic Changes and the Pathological Stage of Nonpapillary Renal Cell Carcinomas*

Tumor stage groups	Allelic loss at chromosome			
	6q	8p	9p	14q
I and II	3/17*	4/19	1/20	2/21
III and IV	5/27	14/31	9/23	14/31

*LOH/informative cases.

Until now, only one tumor suppressor gene, the recently cloned VHL gene, has been shown to be involved in a specific manner in the genetics of renal cell tumors. However, the VHL gene is mutated in only approximately 50% of the nonpapillary RCCs, whereas LOH at chromosome 3p occurs in 98% of cases.[15,19] A mutation of the p53 tumor suppressor gene has been found in only 25% of the chromophobe RCCs (unpublished data), whereas the loss of chromosome 17p occurs in 76% of all cases.[9] Therefore, a cytogenetic or DNA analysis is more efficient in the differential diagnosis of renal tumors than the mutation analysis of the VHL and p53 genes or other known tumor-related genes.

Current morphological classifications use the granular or eosinophilic cellular phenotype to characterize renal cell tumors.[1,2] Granular or eosinophilic cells may occur in genetically distinct types of tumors. The cytomorphological features of eosinophilic nonpapillary RCCs and eosinophilic chromophobe RCCs as well as of renal oncocytoma may be very similar, and a histological diagnosis in cases with overlapping phenotype remains often uncertain. However, a differential diagnosis is highly significant, given the implications for the expected clinical course of the disease. Patients with a nonpapillary RCC have a poor prognosis; less than 50% of the

patients are alive 5 years after the diagnosis.[20] Recent clinical follow-up showed that nearly 100% of the patients with a chromophobe RCC have a 5-year survival.[21] Renal oncocytoma is a benign tumor, which never metastasizes. Therefore, it is of clinical importance to establish the correct diagnosis in each individual case. This study shows that all nonpapillary and chromophobe RCCs, which make up approximately 90% of malignant renal cell tumors as well as a subset of renal oncocytomas could be correctly assigned to genetic subtypes when using special sets of microsatellite markers. We used short-term cultures of tumors for this study to be sure of having tumor DNA without contamination of normal DNA. However, it is possible to separate tumor cells by an enzymatic/mechanical approach within 1 or 2 hours. After gentle sedimentation of the cell clusters, cell suspension containing more than 90% of tumor cells may be separated, which is useful for the microsatellite assay described here. It is also possible to isolate DNA from paraffin-embedded material within 5 hours. By applying three sets of markers simultaneously, a microsatellite analysis could be carried out within 1 or 2 days. A higher diagnostic output and easier material handling might be achieved by using the recently developed automated fragment-length analysis systems. The sensitive fluorescence detection system will allow the determination of allelic duplications occurring in papillary renal cell tumors.

When diagnosis has been established, the question of clinical outcome of the disease becomes important. The most effective therapy for tumors confined to the kidney is surgery, whereas a metastatic tumor is practically incurable. Therefore, we have to estimate the patients' chance to survive, especially in cases with a cancer confined to the kidney. It is possible to estimate the prognosis by clinical and pathological staging at the time of nephrectomy.[1,12,13,20] However, more often than one would desire, a significant degree of doubt remains as to the nature and biological potential of a given lesion. By introducing modern imaging diagnostic techniques, an increasing number of tumors confined to the kidney are detected incidentally, and the pathological staging is not informative in such cases. Therefore, it is imperative to find molecular markers predicting the clinical outcome of patients having a RCC localized to the kidney.

Comprehensive cytogenetic analysis and an allelotyping study showed that loss of chromosome 8p, 9, and 14q regions is associated with the development nonpapillary RCCs.[10,11] A prevalent loss of DNA sequences at chromosome 3p, 6q, 8p, 9, 13q, and 14q was detected by comparative genomic hybridization in nonmetastatic RCC and a correlation between LOH at chromosome 9p and progression of the disease has been suggested.[22] In this study, we found a correlation between LOH at chromosomes 8p, 9p, and 14q and the pathological stage of nonpapillary RCC. Frequent LOH at the chromosome 8p region has also been found in prostate, colon, lung, urinary bladder, and liver cancers.[23–25] Emi et al[25] demonstrated a higher frequency of allelic losses on chromosome 8p in hepatocellular carcinomas at an advanced stage and also in poorly differentiated tumors. The loss of DNA sequences at chromosome 9p has also been found in many types of tumors including RCCs.[26–29] A candidate tumor suppressor gene, CDKN2/p16, was cloned from the chromosomal region 9p21 and found to be homozygously deleted in different types of cancer.[30] A correlation between the clinical outcome and LOH at chromosome 14q has been described in a subset of neuroblastomas, and it was suggested that loss of chromosome 14q sequences marks a more aggressive type of meningioma as well as urinary bladder and colorectal tumors.[31–34] Thus, alteration of putative tumor suppressor genes at these chromosomal regions may be involved in the initiation and progression of distinct types of tumors.

Our present and previous studies showed clearly that we are not dealing with a single disease called RCC or its morphological variants but with entities having distinct molecular pathology and natural history. As soon as the genetics and nature of tumors of other organs are clarified, modern DNA technologies may be applied to their diagnostic and prognostic assessment. The goal of future therapy is to target tumor suppressor genes. Introducing new molecular approaches in cancer treatment will require a new type of diagnosis. Therefore, it is important to incorporate our actual knowledge of the genetics of tumors into the process of diagnostic evaluation. The histopathological analysis remains important, but the new techniques of molecular biology are providing new tools of extraordinary power to sharpen the diagnosis and give it a molecular biological interpretation. Once the genes are identified, they may be used for diagnostic screening, for estimating the clinical outcome, and ultimately for a possible individual gene therapy.

Acknowledgments

We thank Dr. G. Staehler for providing tumor tissues and Dr. H. F. Otto making histopathological data available.

References

1. Thoenes W, Storkel S, Rumpelt HF: Histopathology and classification of renal cell tumors (adenomas, oncocytomas, and carcinomas). Pathol Res Pract 1989, 181: 125–143
2. Murphy WM, Beckwith JB, Farrow GM: Tumors of the Kidney, Bladder, and Related Urinary Structures. Washington, DC, AFIP, 1994, pp 92–192
3. Kovacs G: Molecular differential pathology of renal cell tumors. Histopathology 1993, 22:1–8
4. Wilhelm M, Krause U, Kovacs G: Diagnosis and prognosis of renal cell tumors: a molecular approach. World J Urol 1995, 13:143–148
5. Fearon ER, Vogelstein B: A genetic model for colorectal tumorigenesis. Cell 1990, 61:759–767
6. Zbar B, Brauch H, Talmadge C, Linehan M: Loss of alleles of loci on the short arm of chromosome 3 in renal cell carcinoma. Nature 1987, 327:721–724
7. Kovacs G, Frisch S: Clonal chromosome abnormalities in tumor cells from patients with sporadic renal cell carcinomas. Cancer Res 1989, 49:651–659
8. Kovacs G, Füzesi L, Emanuel A, Kung H: Cytogenetics of papillary renal cell carcinomas. Genes, Chromosomes & Cancer 1991, 3:249–255
9. Speicher M, Schoell B, du Manoir S, Ried T, Kovacs A, Störkel S, Cremer T, Kovacs G: Loss of chromosomes 1, 2, 6, 10, 13, 17, and 21 in chromophobe renal cell carcinomas revealed by comparative genomic hybridization. Am J Pathol 1994, 145:356–364
10. Kovacs G: Molecular cytogenetics of renal cell tumors. Adv Cancer Res 1993, 62:89–124
11. Thrash-Bingham CD, Greenberg RE, Howard S, Bruzel A, Bremer M, Goll A, Salazar H, Freed JJ, Tartof KD: Comprehensive allelotyping of human renal cell carcinomas using microsatellite DNA probes. Proc Natl Acad Sci USA 1995, 92:2854–2858
12. Hermanek P, Sobin LH: TNM Classification of Malignant Tumors, ed 4. Berlin, Springer, 1992, pp 148–150
13. Beahrs OH, Henson DE, Hutter RV, Kennedy BJ: Manual for Staging of Cancer, ed 4. Philadelphia, JB Lippincott, 1992, pp 201–204
14. Weaver-Feldhaus J, Gruis NA, Neuhausen S, Le Paslier D, Stockert E, Skolnick MH, Kamb A: Localization of a putative tumor suppressor gene by using homozygous deletions in melanomas. Proc Natl Acad Sci USA 1994, 91:7563–7567
15. 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, 55:5383–5385
16. Kovacs G, Wilkens L, Papp T, deRiese W: Differentiation between papillary and nonpapillary renal cell carcinomas by DNA analysis. J Natl Cancer Inst 1989, 81:527–530
17. Polascik TJ, Cairns P, Epstein JI, Füzesi L, Ro JY, Marshall FF, Sidransky D, Schoenberg M: Distal nephron renal tumors: microsatellite allelotype. Cancer Res 1996, 56:1892–1895
18. Presti JC, Moch H, Reuter VE, Huynh D, Waldman FM: Chromosome 1 and 14 loss in renal oncocytomas. J Urol 1996, 154:414A
19. Gnarra JR, Tory K, Weng Y, Schmidt L, Wie MH, Li H, Latif F, Liu S, Chen F, Duh FM, Lubensky I, Duan DR, Florence C, Pozzatti R, Walther MM, Bander NH, Grossman HB, Brauch H, Pomer S, Brooks JD, Isaacs WB, Lerman MI, Zbar B, Linehan WM: Mutation of the VHL tumour suppressor gene in renal carcinoma. Nature Genet 1994, 7:85–90
20. Weiss LM, Gelb AB, Medeiros LJ: Adult renal epithelial neoplasms. Am J Clin Pathol 1995, 103:624–635
21. Crotty TB, Farrow GM, Lieber MM: Chromophobe cell renal carcinoma: clinicopathological features of 50 cases. J Urol 1995, 154:964–967
22. Moch H, Presti JC, Sauter G, Buchholz N, Jordan P, Mihatsch MJ, Waldman FM: Genetic aberrations detected by comparative genomic hybridisation are associated with clinical outcome in renal cell carcinoma. Cancer Res 1996 56:27–30
23. Trapman J, Sleddens HFBM, van der Weiden MM, Dinjens WNM, Konig JJ, Schroder FH, Faber PW, Bosman FT: Loss of heterozygosity of chromosome 8 microsatellite loci implicates a candidate tumor suppressor gene between loci D8S87 and D8S133 in human prostate cancer. Cancer Res 1994, 54:6061–6064
24. Knowles MA, Elder PA, Williamson M, Cairns JP, Shaw ME, Law MG: Allelotype of human bladder cancer. Cancer Res 1994, 54:531–538
25. Emi M, Fujiwara Y, Nakajima T, Tsuchiya E, Tsuda H, Hirohashi S, Maeda Y, Tsuruta K, Miyaki M, Nakamura Y: Frequent loss of heterozygosity for loci on chromosome 8p in hepatocellular carcinoma, colorectal cancer, and lung cancer. Cancer Res 1992, 52:5368–5372
26. Fountain JW, Karayiorgou M, Ernstoff MS, Kirkwood JM, Vlock DR, Titus-Ernstoff L, Bouchard B, Vijayasaradhi S, Dracopoli NC: Homozygous deletions within human chromosome band 9p21 in melanoma. Proc Natl Acad Sci USA 1992, 89:10557–10561
27. Olopade OI, Buchhagen DL, Malik K, Sherman J, Nobori T, Bader S, Nau MM, Gazdar AF, Minna JD, Diaz MO: Homozygous loss of the interferon genes defines the critical region on 9p that is deleted in lung cancers. Cancer Res 1993, 53:2410–2415
28. Cairns P, Tokino K, Eby Y, Sidransky D: Homozygous deletions of 9p21 in primary human bladder tumors detected by comparative multiplex polymerase chain reaction. Cancer Res 1994, 54:1422–1424
29. Cairns P, Tokino K, Eby Y, Sidransky D: Localization of tumor suppressor loci on chromosome 9 in primary human renal cell carcinomas. Cancer Res 1995, 55: 224–227
30. Kamb A, Gruis NA, Weaver-Feldhaus J, Liu Q, Harshman K, Tavtigian S, Stockert E, Day RS, Johnson BE, Skolnick MH: A cell cycle regulator potentially involved

in genesis of many tumor types. Science 1994, 264: 436–440

31. Fong C, White PS, Peterson K, Sapienza C, Cavenee WK, Kern SE, Vogelstein B, Cantor AB, Look AT, Brodeur GM: Loss of heterozygosity for chromosomes 1 or 14 defines subsets of advanced neuroblastomas. Cancer Res 1992, 52:1780–1785
32. Grundy D, Rutter J, Gusella J, Rustgi A, Houseal T, Menon A: Identification of a putative tumor suppressor locus in 14q24–32 that is involved in formation and progression of solid tumors. Cytogenet Cell Genet 1994, 66:14–15
33. Chang WY, Cairns P, Schoenberg MP, Polascik TJ, Sidransky D: Novel suppressor loci on chromosome 14q in primary bladder cancer. Cancer Res 1995, 55: 3246–3249
34. Young J, Leggett B, Ward M, Thomas L, Buttenshaw R, Searle J, Chenevix-Trench G: Frequent loss of heterozygosity on chromosome 14 occurs in advanced colorectal carcinomas. Oncogene 1993, 8:671–675