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Commentary

Molecular Differential Diagnosis of Renal Carcinoma

From Microscopes to Microsatellites

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Introduction

Differential diagnosis of pathologically diverse renal neoplasms relies today on standard hematoxylin and eosin staining and a variety of cytochemical, immunohistochemical, and electron microscopic techniques. However, recent cytogenetic and molecular genetic analyses have provided new and important information on the origin, progression, and characteristics of these and other malignancies. To achieve proper diagnosis, it is therefore possible and, to some extent, quite desirable not only to describe the phenotypic features of neoplastic cells but also to use molecular genetic markers for their classification. These markers can help to identify alterations at the genetic level, which may precede morphological changes. In addition, interpretation of these genetic alterations is no longer a subjective matter but can easily be standardized.

Bugert and Kovács[1] describe their experience with the use of microsatellite markers as a diagnostic tool in the differential diagnosis of renal carcinomas. They were able to correctly diagnose all nonpapillary, papillary, and chromophobe carcinomas by assessment of loss of heterozygosity in 82 primary specimens.

Clear-Cell Carcinoma

Malignancies of the upper urinary tract account for approximately 27,000 of all cancers diagnosed each year in the United States. Clear-cell (nonpapillary) renal carcinoma (RCC) constitutes approximately 80% of all renal neoplasms and is the cause of death in 10,000 cases.[2] Histopathologically, the tumors display solid, trabecular, or cystic growth. However, tubulo- papillary or papillary patterns may also be found, and these cases might be mistaken for papillary renal cell cancer.[3] The neoplastic cells are clear in approximately 75% of cases, but the tumor can consist partially or completely of granular cells.[4] Although most cases are sporadic, a few notable exceptions have shown a hereditary pattern. In one family, an affected member exhibited a germ-line balanced translocation involving the chromosomal region 3p13-14.2.[5] Another important group of these tumors occur in patients affected by the von Hippel-Lindau (VHL) disease. Germ-line translocations and deletions on the short arm of chromosome 3 led to the discovery of the VHL gene, previously linked to chromosomal bands 3p25-26[6]; 96% of RCCs exhibit chromosomal losses in this area, and most sporadic tumors have been found to harbor point mutations of VHL or promoter methylation leading to inactivation of the gene.[7,8]

Other abnormalities in sporadic tumors have centered on chromosomal arm 5q, where cytogenetic characterization revealed a breakpoint at 5q22, near the site of the adenomatous polyposis coli (APC) gene. Furthermore, deletions of chromosomal arms 8p, 14q, and 6q and monosomy 9 have also been described in RCC.[3] Recently, homozygous deletions

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at 9p21-22, encompassing the tumor suppressor gene CDKN2/p16 have been found in 5% of RCCs.[9] Although no point mutations of p16 were discovered in this study, homozygous deletion and methylation now appear to be the most common mechanisms of p16 inactivation in many types of cancer.[10,11] Deletions or monosomies of chromosomes 1 and 2 are also invariably detected in renal chromophobe cancer, yet these changes are virtually absent in RCCs.[12]

With the progression of these cancers to higher stage and grade, more genetic changes occur.[13] Monosomy of chromosome 14 is present in only 8% of nonpapillary tumors smaller than 1 cm in diameter. However, the loss of this chromosome is more frequent in tumors greater than 3 cm; 56% exhibit monosomy 14 in these higher stages whereas 89% of grade 3 tumors also have loss of chromosome 14.[9] In males, loss of the Y chromosome has been observed in 26% of cases, a rate that is consistent with findings in other tumors in elderly patients.[3] These characteristic patterns can be used for the development of a progression model for RCC. Moreover, these studies now indicate that a correct diagnosis of RCC is feasible by microsatellite techniques. A tumor exhibiting LOH on chromosomal arm 3p (and additional losses on chromosomal arm 8p) without changes on chromosomes 1 and 2 is expected to be a clear RCC.

Papillary Carcinoma

Papillary renal carcinoma constitutes approximately 10 to 14% of all renal cell neoplasms.[3,14] They represent the predominant neoplasm among lesions under 3 cm in diameter.[15] These tumors consist histologically of papillary or tubulopapillary formations, but these features are not absolutely characteristic for papillary carcinomas.[3] They cannot be distinguished from clear RCC by ultrastructural or immunohistochemical means.[16] Cytogenetically, however, a unique constellation of chromosomal abnormalities, profoundly different from the pattern of clear RCCs, can be found. Regardless of size, papillary carcinomas show frequent loss of chromosome Y (93% of cases) and trisomy 7 and 17 (75 and 80% of cases, respectively).[3] In addition, clinically more aggressive tumors exhibit trisomy of chromosomes 16, 12, and 20 and partial loss of chromosome 14. Therefore, it has been concluded that the first set of changes occurs early in the genesis of papillary cancers and that the second set of chromosomal abnormalities indicates progression of the tumor and the acquisition of a high-grade phenotype.[16]

Differential diagnosis between clear and papillary RCC has great significance because of the different survival in these two groups of tumors. Many studies now suggest that papillary carcinomas have a far worse prognosis compared with clear RCCs.[17] It is therefore mandatory to attempt a proper assessment of the diagnosis. With the knowledge of the different chromosomal alterations in these types of tumors, and modern microsatellite analysis based on polymerase chain reaction, a molecular differential diagnosis now appears feasible. The findings of loss of chromosome Y and trisomy of chromosomes 7 and 17 by cytogenetic analysis or fluorescence *in situ* hybridization are consistent with the molecular genetic diagnosis of a papillary RCC. Additional losses on chromosomal arm 14q indicate a higher grade associated with a poor prognosis in the first group. The absence of microsatellite losses (except for chromosome Y) at loci screened for clear and chromophobe carcinoma indicates a high probability of papillary renal carcinoma.

Chromophobe Tumors

A third group of kidney tumors is chromophobe renal carcinoma, accounting for approximately 4 to 5% of all renal neoplasms.[3,16] Whereas clear and papillary RCC are thought to arise from the proximal tubule, this tumor type has been related to intercalated cells of the collecting duct.[18] Microscopic evaluation reveals a pale, reticular cytoplasm in two-thirds of the cases. In the remaining one-third of cases, eosinophilic and finely granular cytoplasm predominates. Findings by electron microscopy are characteristic and define this tumor type by the detection of microvesicles.[19] Early cytogenetic investigations revealed a hypodiploid karyotype.[20] In another study, 11 chromophobe carcinomas, investigated by restriction fragment length polymorphism, showed loss of material of chromosomal arms 3p, 5q, 17p, and 17q.[21] Finally, comparative genomic hybridization revealed loss of chromosomes 1 and 2 in 100 and 95% of cases, respectively.[22] The combination of these losses is unique to this renal epithelial tumor. By applying the same chromosomal markers for this tumor type as for clear and papillary RCC, it may be possible to establish the correct diagnosis for chromophobe carcinoma.

The use of microsatellite markers is especially helpful to distinguish between clear, papillary, and chromophobe carcinomas as pointed out in the article by Bugert and Kovács,[1] and 90 to 95% of renal carcinomas can be diagnosed relatively easily and accurately.

Oncocytoma

Renal oncocytoma, an essentially benign neoplasm, which is also thought to arise from the distal nephron, is diagnosed in 3 to 7% of all solid renocortical tumors. Perhaps the most intriguing ultrastructural feature of these neoplasms is the fact that their cytoplasm is packed with mitochondria. This is not only true for renal oncocytomas but also for salivary, parathyroid, adrenal, and other tumors of this kind.[23] Contrary to the statement by Bugert and Kovács,[1] there is at least some evidence for loss of chromosomal material in oncocytomas. In one study, fluorescence *in situ* hybridization revealed that ten of twenty of the tumors of male patients lost chromosome Y.[24] Five of those tumors displayed additional loss of chromosome 1, and two other tumors exhibited gain of chromosome 12. The tumors of four female patients with chromosomal abnormalities (40% of tumors studied) had loss of chromosome 1, whereas one tumor displayed gain of chromosome 1 and another gain of chromosome 12. As in most cancers, loss or gain of these chromosomes might be involved in the pathogenesis of these tumors. Another recent investigation from our laboratory demonstrated frequent loss of heterozygosity (LOH) on chromosomal arms 1p (57%), 8p, 14, and 19q in thirteen renal oncocytomas.[25] These chromosomal changes can also be regarded as relatively specific, with alterations of this pattern indicating the diagnosis of renal oncocytoma. However, complete microsatellite analysis of all chromosomal arms has not yet been completed in all types of renal cancers.

Why do these tumors exhibit a high frequency of chromosomal changes and still act basically benign? One possible explanation is that the first step of tumorigenesis leading to oncocytoma is the loss of a putative tumor suppressor gene or activation of a proto-oncogene, which directly or indirectly causes chromosomal instability. Perhaps, despite a slight growth advantage over normal cells, oncocytomas might subsequently lose too many other important genes during cell division, and therefore, the fastest growing cells might undergo apoptosis before metastatic potential could emerge. In support of this notion, mismatch repair deficiency and genomic sequence instability occur in the tumors of patients with hereditary nonpolyposis colon cancer, yet these patients appear to have a better outcome than those with sporadic tumors. Neoplastic growth is always a very complex event, and we usually look at a single, not necessarily key, genetic event. Although the reason for numeric and structural chromosomal alterations in these tumors is unknown, they may still provide invaluable help in the differential diagnosis of oncocytomas.

Collecting Duct Carcinoma

Collecting duct carcinoma (CDC) is one of the rarest renal epithelial neoplasms, making up only 1% of all renal tumors. It is also thought to arise from the distal nephron. CDC is a clinically aggressive tumor, usually occurring in a younger population than is typical for clear or papillary RCC. It has a great tendency to metastasize into lymph nodes, bone, and liver, often leading to death rapidly despite surgical intervention.[26]

Recently, a few efforts have been made to better characterize these tumors. Frequent loss of chromosomal arms 8p and 13q could be shown in 6 cases by using microsatellite techniques based on the polymerase chain reaction.[27] The same approach in another study revealed numerous chromosomal alterations in 18 CDCs. The most frequently affected chromosomal arm was 1q, displaying LOH in 57% of informative cases. Also, chromosomal arms 6p, 8p, 9p, and 21q exhibited LOH up to 45%. High-density mapping of chromosomal arm 1q demonstrated a region of minimal deletion at 1q32.1-32.2, implicating a putative tumor suppressor gene that might play a key role in the development or the progression of CDCs.[28] The frequent LOH on chromosomal arms 1p, 8p, and 19q, in oncocytoma and the loss of chromosomal material at arms 1q and 6p in CDCs are both unique findings and seem to be characteristic for these tumors. Diagnosis based upon microsatellite analysis with informative markers on these chromosomes should enable investigators to assess the histological type of a renal tumor more precisely.

Summary

In the last decade, specific chromosomal alterations have been associated with different tumor types. These aberrations were originally detected by karyotyping and then by more sophisticated cytogenetic analysis. A few karyotypic alterations can be directly linked to distinct malignancies, such as the Philadelphia chromosome in acute lymphoblastic leukemia, loss of distal chromosome 3p14 in small-cell lung cancer, the loss of distal chromosome 11p13 in Wilms' tumor, and loss or rearrangement of the short arm of chromosome 3 in clear and chromophobe RCC. The relative specificity of the latter findings enabled investigators to diagnose an occult renal clear-cell carcinoma from a supraclavicular lymph

Table 1. *Selected Frequency of Loss of Chromosomal Material in Renal Carcinoma*

Chromosomal arm	Clear-cell carcinoma[3,9,14]	Papillary carcinoma[3,22]	Chromophobe carcinoma[21,22]	Oncocytoma[25]	Collecting duct carcinoma[25]
1p			100%	57%	
1q			100%		57%
2			95%		
3p	96%		56%		
5q			40%		
6p					45%
8p	22%			44%	41%
9p	33%				33%
14q	41%	36%*		46%	
17p			55%		
17q					
19q				43%	
21q				44%	40%
Y (in males only)	26%	93%			

In addition to loss of DNA sequences on these chromosomes, clear-cell carcinoma shows partial trisomy on 5q and papillary carcinoma exhibits trisomy of chromosomes 7 and 17. Data on papillary and chromophobe carcinomas are only available from studies with relatively few tumors. Molecular analysis was not performed on every chromosomal arm for every tumor type.
*In advanced tumors.

node metastasis by analysis of G-banded metaphase chromosomes obtained from this mass.[29] A similar report based also on cytogenetic findings was published earlier.[30]

Karyotypic changes, however, detect only gross alterations visible to an observer. With more refined diagnostic tools, such as microsatellite analysis, other, even smaller, well defined lesions can be analyzed. A summary of the known frequencies of chromosomal losses is given in Table 1. The combination of certain LOH patterns has shown great promise in the differential diagnosis of renal tumors. The transfer of molecular genetics from the laboratory to surgical pathology and other clinical departments is a meaningful event and a challenging task. Molecular pathology is certain to become important in the diagnosis of tumors with unclear histology. Diagnosis based widely upon staining techniques and determination of a patient's prognosis by staging and grading alone will be increasingly accompanied by molecular genetic methods. Pathology may be on the verge of the greatest change since the introduction of the microscope.

References

1. Bugert P, Kovács G: Molecular differential diagnosis of renal cell carcinomas by microsatellite analysis. Am J Pathol 1996, 149:2081–2088
2. Jennings SB, Linehan WM: Renal, perirenal, and ureteral neoplasms. Adult and Pediatric Urology. Edited by JY Gillenwater, JT Grayhack, SS Howards, JW Duckett. St. Louis, MO, Mosby, 1996, pp 643–694
3. Kovács G: Molecular differential pathology of renal cell tumours. Histopathology 1993, 22:1–8
4. O'Toole KM, Brown M, Hoffmann P: Pathology of benign and malignant kidney tumors. Urol Clin North Am 1993, 20:193–205
5. Cohen AJ, Li FP, Berg S Marchetto DJ, Tsai S, Jacobs SC, Brown RS: Hereditary renal cell carcinoma associated with a chromosomal translocation. N Engl J Med 1979, 301:592–595
6. Latif F, Tory K, Gnarra J, Yao M, Duh F-M, Orcutt ML, Stackhouse T, Kuzmin I, Modi W, Geil L, Schmidt L, Zhou F, Li H, Wei MH, Chen F, Glenn G, Choyke P, Walther MM, Weng Y, Duan D-SR, Dean M, Glavac D, Richards FM, Crossey PA, Ferguson-Smith MA, Le Paslier D, Chumakov I, Cohen D, Chinault AC, Maher ER, Linehan MW, Zbar B, Lerman MI: Identification of the von Hippel-Lindau disease tumor suppressor gene. Science 1993, 260:1317–1320
7. Gnarra J, Tory K, Weng Y, Smidt L, Wei MH, Li H, Latif F, Liu S, Chen F, Duh F-M, Lobensky I, Duan DR, Florence C, Poza Hi R, Walther MW, Bander NH, Grossman HB, Brauch H, Pomer S, Brooks JD, Isaacs WB, Lehrman MI, Zbar B, Linehan WM: Mutations of VHL in sporadic renal cell carcinoma. Nature Genet 1994, 7:85–90
8. Herman JG, Latif F, Weng Y, Lerman MI, Zbar B, Liu S, Samid D, Duan D-SR, Gnarra JR, Linehan WM, Baylin SB: Silencing of the VHL tumor-suppressor gene by methylation in renal cell carcinoma. Proc Natl Acad Sci USA 1994, 91:9700–9704
9. 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
10. Cairns P, Polascik TJ, Eby Y, Tokino K, Califano J, Merlo A, Mao L, Herath J, Jenkins R, Westra W, Rutter JL, Buckler A, Gabrielson E, Tockman M, Cho KR, Hedrick L, Bova GS, Isaacs W, Koch W, Schwab D, Sidransky D: Frequency of homozygous deletion at p16/CDKN in primary human tumors. Nature Genet 1995, 11:210–212

11. Merlo A, Herman JG, Mao L, Lee DJ, Gabrielson E, Burger P, Baylin SB, Sidransky D: 5′ CpG island methylation is associated with transcriptional silencing of the tumor suppressor p16/CDKN2/MTS1 in human cancers. Nature Med 1995, 1:686–692
12. Kovács G: Molecular cytogenetics of renal cell tumors. Adv Cancer Res 1993, 62:89–124
13. Kovács G, Frisch S: Clonal chromosome abnormalities in tumor cells from patients with sporadic renal cell carcinomas. Cancer Res 1989, 49:651–659
14. Thoenes W, Störkel S, Rumpelt HJ: Histopathology and classification of renal cell tumors (adenomas, oncocytomas and carcinomas): the basic cytological and histopathological elements and their use for diagnostics. Pathol Res Pract 1986, 181:125–143
15. Cristol DS, McDonald JR, Emmett JL: Renal adenomas in hypernephromatous kidneys: a study of their incidence, nature, and relationship. J Urol 1946, 55:18–27
16. Weiss LM, Gelb AB, Medeiros J: Adult renal epithelial neoplasms. Am J Clin Pathol 1995, 103:624–635
17. Fuhrmann SA, Lasky LC, Limas C: Prognostic significance of morphologic parameters in renal cell carcinoma. Am J Surg Pathol 1982, 6:655–663
18. Störkel S, Steart PV, Drenckhahn D, Thoenes W: The human chromophobe cell renal carcinoma: its probable relation to intercalated cells of the collecting duct. Virchows Arch B Cell Pathol 1989, 56:237–245
19. Thoenes W, Störkel S, Rumpelt H-J: Chromophobe cell renal carcinoma and its variants: a report on 32 cases. J Pathol 1988, 155:277–287
20. Kovács A, Kovács G: Low chromosome number in chromophobe renal cell carcinomas. Genes Chromosomes & Cancer 1992, 4:167–168
21. Kovács A, Störkel S, Thoenes W, Kovács G: Mitochondrial and chromosomal DNA alterations in human chromophobe renal cell carcinomas. J Pathol 1992, 167: 273–277
22. Speicher M, Schoell B, du Manoir S, Ried T, Kovács A, Störkel S, Cremer T, Kovács 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
23. Lieber MM: Renal oncocytoma. Urol Clin North Am 1993, 20:355–359
24. Brown JA, Takahashi S, Alcaraz A, Borell TJ, Anderl K, Qian J, Persons DL, Bostwick DG, Lieber M, Jenkins RB: Fluorescence *in situ* hybridization analysis of renal oncocytoma reveals frequent loss of chromosomes Y and 1. J Urol 1996, 156:31–35
25. 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
26. Dimopoulos MA, Logothetis CJ, Markowitz A, Sella A, Amato R, Ro J: Collecting duct carcinoma of the kidney. Br J Urol 1993, 71:388–391
27. Schoenberg M, Cairns P, Brooks JD, Marshall FF, Epstein JI, Isaacs WB, Sidransky D: Frequent loss of chromosome arms 8p and 13q in collecting duct carcinoma (CDC) of the kidney. Genes Chromosomes & Cancer 1995, 12:76–80
28. Steiner G, Cairns P, Polascik TJ, Marshall FF, Epstein JI, Sidransky D, Schoenberg MP: High-density mapping on chromosomal arm 1q in renal collecting duct carcinoma: region of minimal deletion at 1q32.1-32.2. Cancer Res 1996 (in press)
29. Dal Cin P, Sciot R, De Wever I, Van Damme B, Van den Berghe H: Diagnosis of primary renal cell carcinoma in a left supraclavicular lymph node by chromosome analysis. J Urol 1996, 156:171–172
30. Peier AM, Meloni AM, Sandberg AA, Leong SP, Carroll PR: Cytogenetic findings in a metastatic renal cell carcinoma. Cancer Genet Cytogenet 1995, 80:168–169