Molecular genetics of human renal cell tumours

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Abstract. This review discusses the impact of molecular genetics on the diagnosis and prognosis of renal cell tumours as well as the genetic changes in renal cell tumours associated with von Hippel-Lindau disease and end-stage kidney disease. The use of molecular techniques enables the division of renal cell tumours into genetically and biologically well defined entities. The new classification system distinguishes between papillary renal cell tumours, including papillary renal cell adenoma and papillary renal cell carcinoma, nonpapillary renal cell carcinoma, chromophobe renal cell carcinoma and renal oncocytoma. The advantage of the new classification system is that genetic markers, in contrast to the tumour phenotype, are constant during tumour progression and allow a precise diagnosis even from a small biopsy specimen.

Key words: end-stage kidney; general population; genetic classification; kidney cancer

Introduction

Renal cell tumours (RCTs) represent ~3% of all malignancies, with an incidence of disease of 7 per 100 000 individuals in the general population. An increased incidence of RCT has been observed in patients having long-term dialysis and acquired cystic disease of the kidneys [1], and nearly all individuals carrying the mutated von Hippel-Lindau (VHL) disease gene in the germline develop multiple bilateral renal cysts and renal cell carcinomas (RCC) if they live long enough. Familial occurrence of papillary and non-papillary RCC has been found in some cases with or without constitutional chromosomal translocations [2-4]. Sporadic renal cell carcinomas that develop in adults in the general population have a peak incidence in the sixth decade of life, but disease-associated RCTs develop at earlier ages.

There is no satisfactory screening method for early detection for RCTs. About 40% of patients have a metastatic disease at the time of diagnosis, and one-third of the remainder will develop distant metastases during the postoperative course. The most effective therapy for RCC localized to the kidney is surgery, whereas a metastatic tumour is practically incurable. The overall response to biological response modifiers is low, and the treatment is only palliative. The widespread use of ultrasound and CT scanning has increased the number of incidentally detected small asymptomatic RCCs. Most of them remain clinically quiescent for a long time, but some may metastasize during the first years of the postoperative course and become fatal. The biological potential of these small tumours, the development of second or third tumours after partial nephrectomy and the development of multiple tumours in patients without familial anamnesis are unresolved problems. Early detection increases the efficiency of surgical therapy but places the pathologist and oncologist in the challenging role of gleaning even more information from a small tumour specimen.

Recently, based on combined molecular cytogenetic, histological and clinical studies, we worked out a genetic diagnostic system for renal cell tumour [5]. The central idea of the new genetic classification is that the specific chromosomal or DNA changes associated with cancer-relevant genes, the alterations of which are associated with the transformation of the cell's behaviour from normal to neoplastic. The combination of such genetic changes reflects an altered function of a network of genes which are specifically involved in the development and progression of a given type of tumour. The advantage of the molecular genetic analysis is that it allows the diagnosis and progression of RCTs to be more accurately established.

Renal cell tumours in the general population

Papillary renal cell tumours

Papillary RCTs account for ~10% of renal cell neoplasms. Two subsets of papillary RCTs may be
separated on the basis of genetic changes [6]. The loss of the Y chromosome and combined trisomy of chromosomes 7 and 17 are the only aberrations in one group of papillary RCTs. As none of these tumours shows invasive or metastatic growth at the time of operation, these tumours should be diagnosed as papillary renal cell adenomas (RCA) irrespective of their size. The second group of papillary RCCs displays recurrent genetic changes in addition to those of RCAs, namely trisomy of chromosomes 3q, 8, 12, 16 and 20. About 60% of tumours belonging to this genetic subset had an infiltrative or metastatic growth at the time of nephrectomy. The association of additional karyotype alterations with malignant behaviour suggests that an accumulation of such genetic changes is a prerequisite for the aggressive behaviour of papillary RCCs. Some tumours may acquire complex genetic alterations very early during their growth (an adenoma–carcinoma sequence may be missed by genetic or histological examination), while others remain as an adenoma or undergo changes at a later stage of growth.

Non-papillary renal cell carcinoma

The largest group of renal cell tumours, accounting for ~80% of the cases, belongs to this genetically well-defined entity. Most sporadic non-papillary RCCs are solitary tumours. The loss of the smallest overlapping region of chromosome 3p13-pter occurs in 98% of the tumours [7–9]. There is no doubt that the chromosome 3p region harbours tumour suppressor gene(s) responsible for the development of non-papillary RCC. The VHL gene has already been cloned from the chromosome 3p25.3 region [10]. As one allele of the VHL gene is deleted in nearly 100% of sporadic and VHL-associated RCCs, and the remaining allele is inactivated by mutation or hypermethylation in ~65% of the cases, the VHL gene has been suggested to be the RCC gene [11,12]. Reintroduction of the wild-type VHL into an RCC cell line carrying only the mutated VHL gene resulted in growth suppression of the tumour cells in nude mice [13]. However, some data suggest that another tumour suppressor gene, the RCC gene at chromosome 3p, is involved in the genetic changes of non-papillary RCCs. A terminal deletion at chromosome 3p21 or 3p23 would effectively eliminate the wild-type allele of the VHL gene. Recent microsatellite analysis of non-papillary RCCs has showed terminal deletion of an ~80–90 cM segment of chromosome 3p with a breakpoint cluster within 1 cM between loci D3S1603 and D3S1595 at chromosomal band 3p1.2 in 30% of cases [9]. These data suggest that another gene, the RCC gene, is located proximal to the VHL gene. It is likely that a homozygous expression of the mutated VHL gene is responsible for the development of renal cysts, and the inactivation of both alleles of the RCC gene located proximal to the VHL gene is required for the development of non-papillary RCCs in both sporadic and VHL-associated tumours. A partial trisomy of the chromosome 5q is the second most frequent alteration in non-papillary RCCs.

A non-homologous recombination between the chromosome 3p breakpoint region and chromosome 5q22 results in monosomy of 3p and partial trisomy of chromosome 5q22-qter in many cases [14]. In addition, the duplication of an ~1.5 Mb DNA segment distal to the MCC gene occurs in some cases without visible alteration of chromosomes 5. Thus, an overlapping region of at least 1.5 Mb occurs in ~70% of non-papillary RCCs [Kenck et al., submitted for publication].

Monosomy of the chromosome 6q23-qter (14%), 8p11-pter (22%) and 9 (14%) and chromosome 14q22-qter (50%) regions are additional genetic alterations [5]. There is no known oncogene or tumour suppressor gene (with the exception of the VHL gene) from these regions involved in the molecular mechanism of the development of non-papillary RCC. Comparing the incidence of the specific genetic alterations in cases with or without any metastatic growth at the time of operation suggested that alteration of genes at chromosomes 3p and 5q is associated with the tumour development, whereas alteration of genes at chromosomes 6q, 8p, 9 and 14q is responsible for the aggressive behaviour of non-papillary RCCs.

Chromophobe renal cell carcinoma

Chromophobe RCCs make up only 5% of renal cell tumours. By electron microscopy, chromophobe cells display characteristic cytoplasmic vesicles and a variable number of mitochondria. The number of vesicles and mitochondria seems to be correlated with the light microscopic pattern of ‘chromophobe’ and ‘eosinophilic’ cells. Chromosome analysis and recent comparative genomic hybridization studies have revealed a constant loss of chromosomes 1, 2, 6, 10, 13, 17 and 21 in 76–100% of cases and a frequent loss of the Y chromosome [15,16]. Recently, we confirmed these findings by microsatellite analysis on a large number of chromophobe RCCs [unpublished data]. These data suggest that at least 5–7 chromosomes should be lost before a clinically recognizable chromophobe RCC develops. In addition to the low chromosome number, chromophobe RCCs show a gross rearrangement of mitochondrial DNA [17]. Whether the unique morphology and neoplastic growth of chromophobe RCCs are the results of the extensive loss of specific chromosomal DNA sequences remains to be established.

Renal oncocytoma

Renal oncocytoma is a benign tumour of the kidney which makes up ~5% of renal tumours. It consists of large cuboidal cells with 'eosinophilic granular' cytoplasm. The oncocyes are densely packed with mitochondria. A genetic subset of renal oncocytomas shows a mixed population of cells with both normal and abnormal karyotype. Another subset reveals balanced translocation between chromosome 11q13 and other chromosomes. A third subset is marked by
Disease-associated renal cell tumours

VHL-associated non-papillary RCCs

The VHL disease is an autosomally inherited disorder that is generally manifested between the ages of 20 and 40 years and affects multiple organs. Its major lesions are haemangioblastoma of the central nervous system, renal, pancreatic and epididymal cysts, and pheochromocytomas [18]. Non-papillary RCC develops in ~30–50% of the cases. As suggested above, the mutational–deletional inactivation of the VHL gene might be responsible for the phacomatosis, i.e. for the developmental disturbances and tumour-like lesions, whereas an inactivation of the RCC gene at the more proximal chromosome 3p region is responsible for the development of malignant RCC. The genetic changes found in non-papillary RCCs arising in VHL patients are similar to those found in the general population. Deletion of the chromosome 3p13-pter segment (100% of the cases), trisomy of chromosome 5q, and deletion of chromosomes 6q, 8p, 9 and 14q are specific alterations in VHL-associated tumours as well [19].

Renal cell tumours in acquired cystic disease of the kidney (ACDK)

Renal cell tumours are 50 times more frequent in individuals with ACDK than in the general population. ACDK develops more frequently in males than in females, in a ratio of 3:1, and RCCs also develop preferentially in male patients, in a ratio of 7:1. Multiple tumours arise in ~50% of cases and bilateral occurrence of RCCs was observed (by macroscopic examination of the kidneys) in ~10% of the patients with end-stage kidney disease (ESKD). Although most of the cancers that have been reported are small, it is unknown how many are malignant. In reviews of cases of RCCs associated with ACDK, 20–27% were recorded as having metastasized at the time the reports were published. Taking into consideration the fact that the number of patients with ESKD is increasing worldwide and also that the number of cases with RCCs is underestimated, the development of RCCs in end-stage kidneys is a nephrological and oncological problem of ever-increasing magnitude. Although the morphological pattern of these tumours is similar to those in the general population, a differential diagnosis between non-papillary RCCs, papillary RCCs, chromophobe RCCs and renal oncocytomas proves difficult in many cases, even for an experienced pathologist. Histological studies have suggested that the incidence of genetic subtypes of RCCs arising in ESKD differs from those observed in the general population: non-papillary and papillary RCCs each account for 50% of tumours in ESKD, whereas they account for 80 and 10% respectively of tumours in patients without renal disease.

Many factors have been implicated in the pathogenesis of ACDK and tumour development but not one has been confirmed. Little information is available on the genetic make-up of renal cell tumours in end-stage kidneys. Trisomy of chromosome 16 was detected in four of seven papillary RCCs analysed cytogenetically. The loss of the Y chromosome occurred in four of six tumours obtained from male patients. The incidence of these alterations is similar to that observed in papillary RCCs in non-dialysis patients. However, a combined trisomy of chromosomes 7 and 17, which are highly characteristic first steps of genetic changes for papillary RCCs in the general population, has been found in only one of the seven papillary renal cell tumours from end-stage kidneys [I. Ishikawa, personal communication]. We have recently analysed six RCCs obtained from four patients with ESKD by karyotyping. Two of the tumours showed a deletion of chromosome 3p whereas the remaining four tumours had a normal karyotype. These data suggest that the initial step for ESKD-associated renal cell tumours might be different from those developing in individuals without ESKD [for review see 20].

Conclusions

It is now possible to separate genetically homogeneous groups among renal cell tumours. The present genetic stratification of RCCs has many advantages over morphologically based classifications. The most important finding is the discrimination between papillary and non-papillary RCCs. Other classifications describe papillary RCCs as a morphological variant of kidney cancer. Papillary RCCs, however, represent a unique entity and have not only molecular genetics but also a natural history distinct from those of non-papillary RCCs. Papillary RCCs have a precursor lesion–adenoma–carcinoma sequence and are almost all multiple tumours. On the other hand, non-papillary RCCs develop from differentiated, potential stem cells of the tubular system and are, except for rare hereditary cases, solitary tumours. The difference in their natural history and biology has an impact on current surgical therapy and clinical follow up.

Molecular cytogenetic studies have also established the diagnosis of true renal cell adenomas. Tumours with combined trisomy of chromosomes 7 and 17 as the only autosomal change are always benign tumours and should be diagnosed as papillary renal cell adenoma. Adenomas may reach a large size without changing their biological behaviour. All non-papillary RCCs are malignant tumours irrespective of their size.

The most important advantage of the new classification is that the genetic analysis yields a more accurate and reproducible diagnosis. We did not find any renal cell tumours showing a mixture of genetic alterations. Recent developments in diagnostic DNA technology
offer some new tools to detect specific genetic changes in tumours. Fragment length analysis in paired normal and tumour tissues by using microsatellites will be the technique of choice until the genes from the specific chromosomal regions are cloned. We use this technique for differential diagnosis and prognosis assessment of renal cell tumours routinely in our laboratory. However, as mentioned above, the genetic changes and the biology of renal cell tumours in ESKD are not yet known, and the PCR-based diagnostic system is not useful for their diagnosis. We need a consorted molecular analysis on a large number of renal cell tumours and tumour-like lesions arising in patients with chronic renal failure in order to establish the genetics and biology of these special types of tumours.

References