Understanding familial and non-familial renal cell cancer

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Molecular genetic analysis of familial and non-familial cases of conventional renal cell carcinoma (RCC) revealed a critical role(s) for multiple genes on human chromosome 3. For some of these genes, e.g. VHL, such a role has been firmly established, whereas for others, definite confirmation is still pending. Additionally, a novel role for constitutional chromosome 3 translocations as risk factors for conventional RCC development is rapidly emerging. Also, several candidate loci have been mapped to other chromosomes in both familial and non-familial RCCs of distinct histologic subtypes. The MET gene on chromosome 7, for example, was found to be involved in both forms of papillary RCC. A PRCC–TFE3 fusion gene is typically encountered in t(X;1)-positive non-familial papillary RCCs and results in abrogation of the cell cycle mitotic spindle checkpoint in a dominant-negative fashion, thus leading to RCC. Together, these data turn human RCC into a model system in which different aspects of both familial and non-familial syndromes may act as novel paradigms for cancer development.

RENAL CELL CARCINOMAS

Renal cell carcinomas (RCCs) represent 85% of all renal neoplasms, and the overall incidence increases significantly between the ages of 50 and 70 (1,2). RCCs comprise a heterogeneous group of tumors. Based on their location within the nephron and the cell type from which the tumors originate, a detailed classification system was introduced in which epithelial cells of the proximal part of the renal tubule give rise to clear cell carcinomas (also called non-papillary RCCs) and chromophobe RCCs (also called papillary RCCs) and the collecting tubule of the nephron gives rise to chromophobe RCCs, renal oncocytomas and Duct Bellini carcinomas (3). More recently, another classification system for RCCs was proposed and has been denoted the ‘Heidelberg classification’ (4). In this classification system, renal cell tumors are subdivided into benign and malignant parenchymal neoplasms and, where possible, each subcategory is linked to the most commonly documented (cyto)genetic abnormalities. Thus, benign tumors have been subclassified into metanephric adenoma and adenofibroma, papillary renal cell adenoma, and renal oncocytoma. The malignant tumors are subclassified into common or conventional RCCs, papillary RCCs, chromophobe RCCs, collecting duct RCCs, and unclassified RCCs (Fig. 1). Although RCC mostly occurs in a sporadic form, several inherited RCC syndromes and familial RCC cases have been reported (5).

NON-FAMILIAL RENAL CELL CARCINOMAS

Conventional RCC: the chromosome 3 connection

Conventional RCCs comprise the major subgroup of RCCs. Loss of sequences of the short arm of chromosome 3 is a characteristic finding in this type of tumor and was observed as the sole karyotypic change in about 10% of the cases. Therefore, it has been suggested that such losses may be related to the early stages of tumor development (http://cgap.nci.nih.gov/chromosomes/mitelman). The frequently noted loss of 3p sequences suggested the presence of one of more tumor suppressor gene(s) on this chromosomal arm relevant for RCC development (6). Through loss of heterozygosity (LOH) studies in sporadic RCCs, three common regions of allelic loss could be defined: 3p12–14, 3p21–22, and 3p25–26 (7). Additionally, it was found that allelic losses in
adenomas occurred in either 3p25 or 3p12–14, whereas those in carcinomas occurred in 3p21 together with losses in 3p25 or 3p12–14, or both. These results indicated that loss of 3p21 may be related to malignancy. Chromosome transfer studies revealed that 3p12–14 sequences could suppress tumorigenic properties of RCC-derived cell lines, thus implying the presence in this region of a gene(s) involved in tumor development. Similar results were obtained for the 3p21 region. Recent data, in which LOH studies were performed on renal tumors with allele titration assay (ATA) and comparative genome hybridization (CGH) techniques, indicated that this chromosomal region can be divided into two separate subregions, 3p21.3–3p22 and 3p21.2, of which the first is most frequently deleted (8). This same region was also implicated in several other histologically distinct cancers and was found to coincide with homozygous deletions in lung and breast tumors (9,10). Several candidate genes in this region are currently under investigation. The third tumor suppressor region on 3p is 3p25–26. The most relevant RCC-related gene within this region is the Von Hippel Lindau (VHL) gene.

**VHL: a classical tumor suppressor gene**

The VHL gene is an example of a classical tumor suppressor gene and was identified after linkage analysis and comparison of constitutional deletions in patients with the VHL syndrome (see below; 11). These patients may develop different types of cancer, among which are conventional RCCs. In agreement with Knudson’s two-hit model (12), inactivation of the VHL gene was observed in 100% of the tumors analyzed in VHL families and in a significant proportion of sporadic conventional RCCs (13). Moreover, transfection studies with the wild-type VHL gene revealed suppression of growth in both VHL-deficient RCC cell lines and in Vhl-null RCC cells, thus supporting the tumor suppressor function of this gene (14,15). Inactivation of the gene may occur through mutation or deletion, but silencing by promoter hypermethylation has also been observed in about 20% of the tumors (16). To evaluate whether VHL gene inactivation is essential for tumor formation, the patterns and extent of allelic losses on 3p were compared in a set of conventional RCCs both with and without VHL mutations. This study revealed that, although the frequency of 3p12–22 LOH was similar in both tumor types, LOH at 3p25–26 was significantly less than LOH at 3p12–22 in tumors without VHL mutation, whereas LOH at 3p25–26 and 3p12–22 was similar in tumors with VHL mutation (17–19). These results support the presence of both VHL-dependent and VHL-independent tumorigenic pathways and indicate that inactivation of a tumor suppressor gene at 3p12–22 may play an important role in both these pathways.
The VHL protein forms a complex with elongin B, elongin C, CUL2, NEDD8 and RBX1 (20–23). This complex closely resembles the so-called Skp1/Cdc53 or Cullin/F-box (SCF) complex known to be involved in ubiquitin-mediated proteolysis in yeast (24). Recent studies revealed that the VHL protein can also bind to one of the subunits of the hypoxia-inducible factor (HIF) and target this factor for destruction in the presence of oxygen (25). HIF, in turn, controls the expression of several proteins, including two surface transmembrane carbonic anhydrases (CAs) (CA9 and CA12) and proteins involved in angiogenesis [i.e. vascular endothelial growth factor (VEGF), and erythropoietin (EPO)] via oxygen-dependent ubiquitination (26,27). The two CA proteins were first identified in VHL-defective tumors. Since they are overexpressed not only in these tumors, but also in other tumor types, a general role for these proteins in tumorigenesis was suggested. It has been hypothesized that the CA enzymes may be involved in maintaining an extracellular low pH in tumors, which appears to be essential for tumor growth and spread (28). Increased concentrations of VEGF and EPO in VHL-defective cancer cells, irrespective of ambient oxygen concentrations, have implicated the indirect involvement of VHL (via HIF) in tumor angiogenesis (29). VHL-associated neoplasms are typically hypervascular and, on occasion, associated with excessive production of red blood cells. Also, other functions of the VHL protein have been reported, including a role in cell cycle exit control, fibronectin and extracellular matrix binding, and post-transcriptional regulation of target genes through mRNA stability effects (30–33).

Other candidate genes on 3p

The 3p12–14 region is one of the other chromosomal regions that may harbor (a) tumor suppressor gene(s) relevant for RCC. Using a functional genetic assay, tumor suppressive activity could be demonstrated for this chromosomal region. Subsequent physical mapping of suppressing and non-suppressing DNA fragments divided this region into two parts: 3p12 and 3p14.2, designated NRC-1 and NRC-2 (non-papillary renal carcinoma-1 and -2) (34,35), respectively. Within NRC-2, the tumor suppressive region was further narrowed down to a genomic segment which contains both exons 9 and 10 of the FHIT (fragile histidine triad; see below) gene (35). Tumor suppressive activity of NRC-1 was also observed in VHL-defective RCC cell lines, and it was hypothesized that NRC-1 may act either downstream from VHL in the same pathway or in a pathway unrelated to VHL (36). Also, a function in apoptosis was suggested, but the exact role that NRC-1 plays in kidney cancer awaits the identification of candidate gene(s) in this region. Finally, NRC-1 coincides with a homozygous deletion observed in a lung cancer cell line (34). Thus, it was proposed that this region may contain either multiple genes, each involved in different tumor types, or a single broadly acting tumor suppressor gene.

RASSF1A was first identified as a candidate tumor suppressor gene for lung and breast cancer located in the 3p21.3 region (37,38). Recently, silencing by hypermethylation of RASSF1A was also demonstrated in 90% of primary conventional RCCs, and therefore a role in RCC development was suggested (39). This suggestion was supported by growth suppression of several RCC-derived cell lines after exogenous RASSF1A expression (38,39). Moreover, mutants of this gene exhibited significantly reduced growth suppressive activity (39). Other researchers reported silencing of RASSF1A by hypermethylation in 20% of RCCs showing 3p21 allelic loss. It was, therefore, suggested that this gene may act in a haplo-insufficient fashion or that inactivation of an other tumor suppressor gene(s) precede(s) RASSF1A insufficiency (40). RASSF1A shows significant homology (55% identity) to the mouse RAS effector protein Nore1 and to the rat orthologous protein Maxp1. Nore1 interacts with Ras in a GTP-dependent manner (37). The presence of conserved domains in the RASSF1A protein, known to play important roles in several signal transduction pathways, supports a role for RASSF1A in RAS signaling (37,38). As such, inactivation of the RASSF1A gene may have the same cellular effect as oncogenic RAS mutations (38).

The gene for the transforming growth factor-β (TGF-β) type II receptor (TβR-II) has been mapped to chromosome 3p22. This receptor forms a complex with the TβR-I receptor to which TGF-β binds. In general, TGF-β is a potent inhibitor of epithelial cell growth and plays a central role in cell cycle control. By binding to the TGF-β receptor complex its biological activity is exerted. The majority of epithelial tumors, however, are insensitive to the inhibitory effects of TGF-β, and it was demonstrated that this insensitivity is often correlated with malignant progression of the tumor (41). This TGF-β insensitivity in cancer cells is most commonly due to alterations in the TβR-II gene. Rescue of wild type TβR-II activity by gene transfection restored TGF-β responsiveness and reduced the growth rate of cancer cell lines. The same observation was made in different subtypes of human RCC and in a mouse RCC cell line (42,43). Transfection of TβR-II in a TGF-β-insensitive mouse RCC cell line restored TGF-β sensitivity and reduced tumorigenicity, strongly suggesting a role for TβR-II and the TGF-β regulated pathway in RCC development (41). Further support for such a role was obtained through altered expression of proteins downstream in the TGF-β signaling pathway, such as SMAD2 and SMAD4 (44). Also remarkable are the elevated TGF-β plasma levels observed in peripheral blood samples of RCC patients (45,46). In this context, it is of interest to note that an elevated level of another transforming growth factor, TGF-α, has been observed in VHL-deficient cancer cells (47). TGF-α shows no homology to TGF-β and acts, in contrast to TGF-β, as a growth-stimulating factor. Despite these differences, they act synergistically in inducing transformation and, as such, may both be involved in RCC-related pathways.

The DRR1 (down regulated in renal cell carcinoma) gene was identified in the chromosomal 3p21.1 region. A reduced expression of this gene in different tumor types, among which are conventional RCCs, and growth suppression after transfection of DRR1-negative cell lines with wild-type DRR1, supported a role for this gene in RCC development (48). Another candidate gene, located on 3p26.2, is OGG1 (8-oxoguanine DNA glycosylase), involved in DNA repair. One study demonstrated LOH for this gene in about 85% of the conventional RCCs and the presence of an inactivating mutation in one RCC (49).

Based on the frequent loss of chromosome 3 sequences in conventional RCCs, the chromosome 3 candidate genes dis-
Cussed above are suggested to be specific for this subtype of RCC. However, silencing of the *RASSF1A* gene by promoter hypermethylation was also observed in papillary RCCs, and genetic alterations in the *TβR-II* gene were also detected in other RCC subtypes (40,50). In addition, *VHL* and *FHIT* locus loss was found in all RCC subtypes, but differed in pattern and prognostic significance (51). Thus, an overlap in tumorigenic pathways between the different RCC subtypes should be emphasized.

**Papillary RCC: an impaired mitotic checkpoint**

In papillary RCCs, a combination of gain of chromosomes 7 and 17 and loss of the Y chromosome have been found. In addition, t(X;1)(p11;q21) and variants thereof have repeatedly been encountered in a subgroup of these tumors (52). Positional cloning of the translocation breakpoint revealed an in-frame fusion of the *TFE3* gene on the X chromosome to a novel gene, *PRCC*, on chromosome 1 (53–55). *TFE3* is a ubiquitously expressed helix-loop-helix transcription factor belonging to the family of E-box binding factors, characterized by the presence of a dimerization domain, a DNA-binding domain, and two transactivation domains (56). Also, *PRCC* is a ubiquitously expressed nuclear protein characterized by a relatively high proline content, but without homology to any other known protein (54,57). The in-frame fusion results in two fusion genes, *TFE3PRCC* and *PRCCTFE3*, which are both expressed in t(X;1)-positive tumor cells (54). The fusion protein PRCCTFE3 retains all the domains of TFE3 necessary for transcriptional activation. It was found that addition of the N-terminal 156 amino acids of PRCC to the C-terminal part of TFE3 significantly elevates the transcriptional activity of this fusion protein as compared to wild-type TFE3 (57). Furthermore, transfection of conditionally immortalized mouse renal proximal epithelial cells, from which these chromophilic tumors are thought to arise, revealed that exogenous PRCCTFE3 expression can bypass temperature-induced growth arrest and differentiation (58).

Via yeast two-hybrid screening, it was found that PRCC interacts with MAD2B, a human homolog of the yeast MAD-family protein Mad2, a component of the mitotic spindle checkpoint (59,60). Mitotic checkpoint proteins have been shown to be essential for delaying the onset of anaphase and thus preventing mis-segregation of chromosomes during metaphase (61–65). The MAD2B protein has recently been found to bind indirectly to the anaphase-promoting complex (APC) (66) and to act as an inhibitor of Cdh1-mediated activation of APC (63,67). To substantiate the role of human MAD2B in the control of cell division, we microinjected recombinant MAD2B protein into one blastomere of the two-cell stage of *Xenopus laevis* embryos. Indeed, this overexpression resulted in mitotic arrest, whereas the non-injected part of the embryo continued to divide normally (Fig. 2). As a control, MAD2B protein was injected together with an antibody directed against MAD2B. These latter embryos showed normal mitotic activities, thus confirming the anticipated role of human MAD2B in the mitotic spindle checkpoint.

In contrast to PRCC, the MAD2B protein is predominantly located in the cytoplasm (59). After co-transfection, however,
both proteins were found to be located in the nucleus, suggesting transport of MAD2B to the nucleus through its interaction with PRCC. When examining the subcellular localization of the PRCCTFE3 and TFE3PRCC fusion proteins in conjunction with MAD2B, the distribution patterns of TFE3PRCC and MAD2B remained unchanged. Surprisingly, the transport of MAD2B to the nucleus was impaired when expressed together with PRCCTFE3, suggesting lack of interaction in spite of retention of the MAD2B interaction domain within the PRCC moiety of the fusion protein (59). Based on these observations, it was tested whether t(X;1)-positive tumor cells might have a defective mitotic checkpoint. Therefore, t(X;1)-positive cell lines were treated with the mitotic arrest inducer nocodazole. Similarly, PRCCTFE3 and TFE3PRCC transfected kidney HEK293 cells were treated with nocodazole. In all cases, mitotic indexes and ploidy levels were assayed after treatment. In the t(X;1)-positive tumor cell lines and in the PRCCTFE3 transfectedants, clear shifts were observed as compared to controls, indicative of a mitotic checkpoint defect. In contrast, TFE3PRCC and empty vector transfectedants did not show such shifts, indicating that only PRCCTFE3 expression abrogates mitotic checkpoint control in these cells (59). Taken together, these data suggest that expression of PRCCTFE3 leads to impairment of mitotic checkpoint control through interference with MAD2B binding and thus, in a dominant-negative fashion, to papillary RCC development.

Cytogenetic studies revealed variant translocations in which TFE3 is fused to other genes such as RCC17 on chromosome 17, PSF on chromosome 1, and NonO (p54\textsuperscript{arb}) on the X chromosome (68,69). As PSF and NonO (p54\textsuperscript{arb}) both encode pre-mRNA splicing factors, a similar role was suggested for PRCC (70).

**FAMILIAL RENAL CELL CARCINOMAS**

**The VHL cancer syndrome**

The VHL cancer syndrome is a dominantly inherited disease characterized by the development of vascular tumors (hemangioblastomas) in the retina and central nervous system, conventional RCCs, and pheochromocytomas (71). The risk of RCC in VHL disease is >70% by the age of 60 years. However, not all germline VHL mutations are associated with such a risk of RCC development. There is a marked genotype–phenotype correlation between VHL gene mutations and manifestations of the different phenotypes. Based on these differences, VHL families were divided into two major subgroups: VHL types 1 and 2. The first represents the most common group, and is characterized by a predisposition to develop retinal angiomas, central nervous hemangioblastomas, and conventional RCCs. The second group was subdivided into 2A and 2B. VHL type 2A is the next most common form and is characterized by a predisposition to develop pheochromocytomas in addition to the manifestations of VHL type 1. The rarest form is VHL type 2B, which is characterized by a predisposition to develop pheochromocytomas with little or no tendency to develop RCCs (72). Genetic alterations in the VHL gene in VHL type 1 families mostly constitute deletion, nonsense or frameshift mutations, whereas 90% of VHL mutations found in the VHL type 2 families are missense mutations. A few missense mutations have been described that specifically lead to VHL type 2A. Further investigation is warranted to explain these phenotype–genotype relationships.

**Constitutional translocations: another chromosome 3 connection**

Until recently, two conventional RCC families with constitutional chromosome 3 translocations had been described in the literature. In the first family, a t(3;8)(p14;q24) translocation was found in several family members, including 10 RCC patients (73). Six of these patients developed bilateral RCC and two of them developed thyroid carcinoma. In one family member, a benign renal cyst was diagnosed at the age of 32. In the second family, several siblings carried a constitutional t(3;6)(p13;q25) translocation, the only member of the family over 50 years of age carrying the translocation developed multiple bilateral RCCs (74).

Positional cloning revealed that the FHIT gene spans the chromosome 3 breakpoint in the RCC-related constitutional 3;8 translocation (75). This gene co-localizes with the most common inducible fragile site within the human genome, FRA3B (76). Several reports have supported a tumor suppressor function for this gene by demonstrating hemi- or homozygous loss or reduced expression of FHIT protein in a variety of neoplasms, including kidney, lung, cervix, esophagus, colon, stomach and pancreas tumors. Therefore, a general role in tumor development was suggested (77–81). Transfection studies confirmed that FHIT expression may indeed reduce the tumorigenicity of FHIT-defective cancer cells. These assays also demonstrated that mutant FHIT could suppress tumorigenicity, indicating that other function(s) of this protein may be related to tumor development (82). The exact role of FHIT in tumor development, however, still remains to be established (83).

Additional positional cloning of the 3;8 translocation revealed TRC8 as the breakpoint-spanning gene on chromosome 8 (84). TRC8 shows similarity to the *Drosophila patched* (PTC) gene, which is a segment polarity gene. PTC plays an important role in embryonic development through inhibition of the Sonic Hedgehog pathway, which, in turn, targets the expression of proteins involved in the Wingless-Wnt and TGF-β pathways. Inactivation of the human homolog of this patched gene (PATCH) has led to growth stimulation, indicating that this gene may also function as a tumor suppressor (85). Very recently, it was found that the *Drosophila* Trc8 protein physically interacts with the *Drosophila* Vhl protein, thereby linking TRC8 and VHL in a common cellular pathway (86).

Molecular analysis of the 3;6 translocation revealed that the chromosome 3 breakpoint is different from that of the 3;8 translocation (87). As yet, no genes have been identified at this breakpoint.

**More chromosome 3 translocation-positive RCC families**

More recently, two novel Dutch conventional RCC families with chromosome 3 translocations were detected. In the first family, five members over three generations carrying a
constitutional t(2;3)(q35;21) translocation developed RCCs (88,89). Three patients developed multiple tumors, two of them in both kidneys. One patient was diagnosed through a screening program (90). Multiple small cystic and solid tumors were found in both kidneys. One additional translocation carrier in this family developed bladder cancer. In the second family, a t(3;6)(q12;q15) translocation was transmitted and four patients with conventional RCC were diagnosed (91). An additional third RCC family was identified but has not yet been described in detail (92). Within this family, a t(3;4)(p13;p16) translocation was found in three generations. One translocation carrier developed RCC. Finally, seven RCC patients over three generations were encountered in a Polish family with a constitutional t(2;3)(q35;q21) translocation (93), and four RCC patients over four generations in a Japanese family with a constitutional t(1;3)(q32;q13.3) translocation (94). Two members of the Japanese family died of gastric cancer and exocrine pancreatic cancer, respectively. It is not known whether these latter two patients also carried the translocation.

Positional cloning of the familial t(2;3)(q35;q21) translocation revealed that this translocation disrupts a novel gene DIRC2 (disrupted in renal cancer 2) (95). This gene encodes a predicted multimembrane protein which represents a new member of the major facilitator superfamily (MFS) of transporters. Most likely, this protein acts as an anion–cation transporter. RNA studies revealed that the DIRC2 gene is expressed in several tissues and, importantly, also in the proximal tubules of the nephron. In general, anion–cation transporters are known to be involved in the secretion of xenobiotics (96). Disruption of one DIRC2 allele may result in reduced or defective transit of these molecules across the renal proximal tubular epithelium and, consequently, in accumulation within the cells. As some of these molecules may be toxic, increased exposure to these molecules may impose stress conditions upon these renal cells. Mutation analysis of the remaining allele in different translocation carriers within the 2;3 family revealed, besides a non-RCC linked polymorphism, no genetic changes. Also, in a panel of sporadic RCCs and blood samples from different non-VHL and non-MET (see below) hereditary RCC cases, no mutations could be detected.

In the Polish RCC family, the DIRC1 gene was found to be disrupted by the breakpoint on chromosome 2. This gene does not share any homology with other known genes. RNA studies revealed that this gene is ubiquitously expressed at a low level, also in the kidney. The chromosome 3 breakpoint in this family was mapped to 3q21. As yet, no genes have been identified at this breakpoint (97).

Chromosome 3 translocations as risk factors

Two of the above-mentioned RCC families were identified in a series of 10 Dutch families with constitutional chromosome 3 translocations that were filed for disorders other than RCC (92). The number of affected patients within this cohort is not yet determined. In the other published families, support the notion that germline chromosome 3 translocations may act as predisposing factors in the development of RCC. Although the positions of the breakpoints vary between the seven RCC families (Table 1), they all map in the proximal p- and q-arm regions of chromosome 3. This breakpoint position-related phenomenon may explain the variation in penetrances and other phenotypic differences (i.e. the development of non-RCC malignancies; see below) between the seven RCC families. In addition, genes at or near these breakpoints may play a critical role(s) in RCC development (see above). Together, these notions may have consequences for the identification of persons at risk, the development of genetic counseling strategies, and (clinical) patient management. Periodic ultrasound screening and genetic counseling are already being actively pursued in some of the ‘at-risk’ families that have been identified so far, allowing early RCC detection and surgical intervention (90).

A three-step model for familial conventional RCC

LOH analyses were performed in several of the translocation-positive RCC families (Table 1). The results obtained revealed frequent loss of the derivative chromosome 3. Subsequent VHL gene analyses revealed 10 novel mutations in 20 tumor samples analyzed. Based on these combined molecular (cyto)genetic findings, a three-step model for RCC development was proposed (89) (Fig. 3). In this model, the first hit is the occurrence of a germline chromosome 3 translocation (98). Non-disjunctional loss of the derivative chromosome that carries the 3p segment represents the second step. The third step involves a somatic mutation in the remaining 3p allele of an RCC-related tumor suppressor gene(s), i.e. the VHL gene. Other, as yet unidentified, RCC-related tumor suppressor gene(s) on 3p may also be affected (99). The three-step model may also explain the observed reduced penetrance as compared to that in the VHL disease; that is, an extra step is required. Besides kidney cancer, other types of cancer were also diagnosed in some of the RCC families. Genetic alterations affecting 3p have been reported for the sporadic forms of each of these different tumor types, as well as in tumors from other organs (100–103). Thus, the first two steps for the hereditary RCCs may also affect other tissues and organs.

Familial papillary RCC

Germline mutations in the MET gene on chromosome 7 were identified (104) in a hereditary form of papillary RCC. MET belongs to the family of tyrosine kinases, the members of which

<table>
<thead>
<tr>
<th>Translocation</th>
<th>Number of RCC cases</th>
<th>3p lossa</th>
<th>VHL mutationsa</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(3;8)(p14;q24)</td>
<td>10</td>
<td>5/5</td>
<td>2/2</td>
<td>73</td>
</tr>
<tr>
<td>t(3;6)(p13;q25.1)</td>
<td>1</td>
<td>5/5</td>
<td>ND</td>
<td>74</td>
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<tr>
<td>t(2;3)(q35;q21)</td>
<td>5</td>
<td>7/8</td>
<td>5/7</td>
<td>88–90</td>
</tr>
<tr>
<td>t(3;6)(q12;q15)</td>
<td>4</td>
<td>2/5</td>
<td>1/7</td>
<td>91</td>
</tr>
<tr>
<td>t(3;4)(p13;p16)</td>
<td>1</td>
<td>ND</td>
<td>ND</td>
<td>92</td>
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<td>t(2;3)(q33;q21)</td>
<td>7</td>
<td>2/2</td>
<td>ND</td>
<td>93</td>
</tr>
<tr>
<td>t(1;3)(q32;q13.3)</td>
<td>4</td>
<td>4/4</td>
<td>2/4</td>
<td>94</td>
</tr>
</tbody>
</table>

aThe numbers of tumors in which loss of 3p or VHL mutations were observed. The numbers after the slashes represent the numbers of tumors that were informative for these analyses.

ND, not determined.
play important roles in transmitting signals from the cellular surface to the nucleus (105). Most MET mutations turned out to be missense mutations located within the tyrosine kinase domain. Germline missense mutations were also detected in patients without a clear family history of papillary RCC, and it was therefore suggested that the MET gene may be a low-penetrance cancer-causing gene with RCC onset at relatively late ages (106). MET acts as a transmembrane receptor for the hepatocyte growth factor (HGF), and binding of this growth factor induces a growth-stimulating signaling cascade via RAS. It was suggested that mutations in the cytoplasmic kinase domain of the MET gene might lead to constitutive activation without ligand binding and, thus, to continued activation of the signaling cascade leading to out-of-control cellular proliferation. Transfection studies in combination with biological assays indeed showed that tumorigenicity is related to enzymatic activation of MET (107). Another member of the MET family, designated RON, was identified in one of the candidate tumor suppressor regions on 3p. RON encodes a tyrosine kinase receptor for the macrophage-stimulating protein (MSP), which is structurally related to HGF. As the MET and HGF genes are both located on 7q, and the RON and MSP genes both on 3p, a subtype-specific role was suggested for the MET–HGF signaling pathway in papillary RCC development and the RON–MSP signaling pathway in conventional RCC development. Recently, it was reported that point mutations in the RON gene analogous to those found in the MET gene and overexpression of the RON gene induce malignant transformation, tumor formation, and metastasis (108).

**Familial oncocytoma**

Only a few familial cases of renal oncocytomas have been described (109). Affected members in these families often develop multiple and/or bilateral oncocytomas. No metastases were observed. Little is known about their genetic background. In one patient with bilateral multifocal renal oncocytomas and cysts, a constitutional reciprocal translocation, t(8;9)(q24.1;q34.3), was observed in conjunction with a rare constitutional VHL missense mutation (110). Remarkably, the chromosome 8 breakpoint occurs in the same region as in the above-described RCC-related t(3;8)(p14;q24) translocation. The chromosome 9 breakpoint occurs in the region containing the TSC1 locus. Fluorescence in situ hybridization (FISH) analysis revealed that the breakpoint maps telomeric to the TSC1 locus and that, most likely, this gene is not involved. The involvement of TRC8 on the chromosome 8 breakpoint was not tested and can, as yet, not be excluded.

**The Birt–Hogg–Dubeé syndrome**

The Birt–Hogg–Dubeé (BHD) syndrome represents an inherited autosomal genodermatosis characterized by benign tumors in the hair follicles and has been associated with renal and other types of neoplasia. Linkage analysis in several of these families indicated that BHD is genetically heterogeneous and that one locus for BHD lies within chromosomal band 17p11.2 (111,112). Several candidate genes within this region are currently being tested for their involvement in this familial disease.

**PERSPECTIVES**

The role of several (fusion) genes in the development of different subtypes of familial and non-familial RCCs has been well established. Further investigations should be aimed at clarifying the role of additional candidate (fusion) genes that have been, or will be, identified in the near future and to establish relevant genotype–phenotype correlations. Novel cell and animal model systems (including RNA interference and conditional gene knock-outs and knock-ins) may be employed for further functional studies (113), whereas recently developed high-throughput genomics technologies (including microarrays for genomic and expression profiling and proteomics) may be applied for gene and signal transduction pathway identification and, ultimately, diagnostic/prognostic purposes.

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**Figure 3.** Schematic illustration of the alternative three-step tumorigenesis model in hereditary RCCs with constitutional chromosome 3 translocations.
(114). The recent finding that constitutional chromosome 3 translocations may act as risk factors for RCC development awaits the identification of novel RCC families and comprehensive cohort studies for more refined risk assessment and presymptomatic genetic counseling purposes.

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REFERENCES


Bodmer, D., Weterman, M.A.J., Eleveld, M.J., Janssen, I.,

Zajaczek, S., Gronwald, J., Kata, G., Borowka, A., Lubinski, J. and Huebner, K. (2001) The DIRC1 gene at chromosome 2q33 spans a familial RCC-associated t(2;3)(q33;q21)

debre, D., Heinrich, M., van der Meijden, A.P.J., Koolen, M.I., Hulsbergen-van der Kaa, C.,


