Genetic contributions to the classification of renal cell cancer
Dijkhuizen, Trijntje

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CHAPTER 5

5. SUMMARY AND GENERAL DISCUSSION
The aim of any histopathological classification is to use morphological criteria to identify disease states which are biologically distinct, the recognition of which are of clinical value [31]. Different classification systems are currently in use for renal cell cancer (RCC) but their criteria are not always conclusive. This is not surprising since renal cancers display a heterogeneous morphology and their phenotype may change dramatically during progression [27]. The considerable diversity of RCC stresses the need for a more extended and refined classification than the commonly used WHO classification, which divides renal tumors into adenomas, carcinomas, and others, and does not allow extensive subtyping [2]. In 1986 Thoenes and Störkel proposed a new morphological classification for RCC, in which five different RCC subtypes are recognized, related to the basic cell types of the nephron from which they are derived [20]. Clear cell and chromophilic RCC arise from cells of the proximal part of the nephron, renal oncocytomas and chromophobe RCC find their origin in the intercalated cells of the collecting tubule, and Duct Bellini carcinomas are associated with the principle cells of the collecting tubule. Variants of the basic cell types, characterized by an accumulation of mitochondria (eosinophilic variants), are also mentioned. Three growth patterns: compact, tubulo-papillary and cystic are distinguished. Principally, in a given tumor, all growth patterns can occur simultaneously, but generally one of them predominates. There are relationships to the cell types, although not exclusive: clear cell and chromophobe RCC is predominantly related to compact growth, chromophilic RCC to tubulo papillary growth, renal oncocytoma to acinar growth, and Duct Bellini carcinoma to both compact and tubulopapillary growth.

Since it is generally accepted that genetic alterations, stable during cell division, are the fundamental cause of neoplastic transformation, the genetic profile of different subtypes of RCC may hold key information about oncogenesis, progression, and pathogenetic relationships of tumor types. Evidence is accumulating that RCC can be divided into genetically distinct entities. The concept of a genetic classification for renal neoplasms has been advocated by Kovacs and by us for several years [23,25-28]. A great advantage of a genetic approach is that, contrary to tumor phenotype, in general primary genetic changes are constant during tumor development and progression. Once present, these changes mark all descendent cells. Furthermore, distinct genetic changes may be associated with tumor progression. Assessment of the genetic constitution might prove to be a powerful diagnostic and prognostic tool in the clinical management of renal cancer. Establishment of the genetic constitution of different RCC subtypes might contribute to, and refine a morphological subtyping, but might also contradict existing morphological classifications and grading systems, possibly leading to a revised classification.

Combining the morphologic and genetic data of more than 200 cases of renal cancer, some of which have been discussed in the present thesis, and data extracted from the literature, we present an oncogenetic model for the development and progression of the heterogeneous group of RCC, depicted in Figure 1. Morphologic features are determined according to the classification proposed by Thoenes and Störkel [20,24]. In addition to morphological subtyping, we divided the different
RCC subtypes into two main groups, related to the embryologically different tissues of origin of the renal tubular system (see chapter 1). In Figure 1, the mature renal tubular system is represented as a white bar in which the proximal and distal tubule and the collecting tubule are recognized. The collecting tubule is separated from the proximal and distal tubule by a grey line, reflecting their different embryonal origin, i.e. the mesonephros and the metanephros, respectively. The next horizontal lane indicates the adenoma stage of the different subtypes, related to their presumed cell of origin. Each subtype is characterized by a distinct combination of genetic changes, reflecting its unique developmental pathway. In vertical direction, progression to a carcinoma stage, and subsequently progression to carcinomas of higher grade and stage, including sarcomatoid transformation is shown. The genetic changes associated with each of these processes are given. If determined, chromosome arms p (short) and q (long) are mentioned and the breakpoint designation, according to the recommendations of the International Standing Committee on Human Cytogenetic Nomenclature, is provided. The morphologic and genetic features of each of the five subtypes, in relation to histogenesis, pathogenesis, oncogenesis and classification, are discussed in detail in the sections below. Furthermore the correlations and differences between morphological and genetic subtyping will be emphasized.

Genetic changes associated with normal kidney tissue:

Increasing evidence exists on the presence of clonal, mostly numerical, chromosome changes in apparently normal kidney tissue from patients with a normal constitutional karyotype [32,34,35,155,156]. Loss of the Y chromosome and trisomy of chromosomes 7 and 10 have frequently been described. These changes are not an in vitro artefact and are independent of the length of cell culture [32]. Trisomy 7 and 10 and loss of the Y chromosome are also observed in renal cell tumors, raising the question whether these cells in the kidney have some inherent propensity for neoplastic transformation. Some authors have proposed that trisomy 7 represents the first oncogenetic step in the development of renal tumors. However, several tumors genetically showing 3p deletions, do not have a trisomy 7. It has been shown that, in general, approx. 10% of kidney cells have trisomy 7 and neoplastic transformation occurs at similar frequencies in cells with and without trisomy 7 [157]. The presence of clonal and non clonal aberrations in apparently normal kidney tissue merely indicates a chromosome instability pattern or mosaicism, and this condition should not be considered as strictly neoplastic.

Renal cell cancer of the clear cell and chromophilic type:

Clear cell RCC also referred to as non papillary RCC is the most common form of RCC, comprising approximately 70-75% of cases. They show a male preponderance of approximately 2:1. Hereditary as well as sporadic cases of clear cell RCC are found. Hereditary RCC is characterized by the appearance of multiple and bilateral tumors and an early age of onset.
The Von Hippel-Lindau (VHL) cancer syndrome is the most common form of hereditary RCC [158]. Retinal, cerebellar and spinal haemangioblastomas, RCC, pheochromocytoma, and renal, pancreatic and epididymal cysts are specific findings associated with VHL disease. Hereditary RCC is also found in families with a constitutional balanced translocation involving chromosome 3 [159]. Sporadic cases usually present as solitary tumors. The distinction between clear cell adenomas and clear cell carcinomas is usually made on size, but the existence of clear cell adenomas is a debatable field. Some authors have proposed that clear cell tumors are malignant, regardless of their size [31]. The tumor mass of clear cell RCC is multicolored, with a predominantly yellow cut surface, in which white or gray foci are seen. Mostly the tumor cells show a solid growth pattern, but in a few cases a cystic appearance is seen. The cytoplasm is clear, due to an intensive intracytoplasmic accumulation of glycogen and lipids. The nuclei are condensed and hyperchromatic in well differentiated tumor cells, whereas in less differentiated tumor cells, polymorphic nuclei and prominent nucleoli appear. Other features of a higher grade in clear cell RCC are an increased cytoplasmic granularity or eosinophilia due to the augmentation of mitochondria. The latter can be found either in the vicinity of the nucleus or more or less diffusely distributed within the cytoplasm.

Loss of sequences of the short arm of chromosome 3 are a characteristic finding in hereditary as well as sporadic cases of clear cell RCC. In the dominantly inherited von Hippel-Lindau (VHL) cancer syndrome, the VHL gene, assigned to 3p25, is mutated in the germ line and in renal cell tumors of affected family members [158]. In families with a constitutional balanced translocation, the breakpoints of chromosome 3p in the t(3;6) and t(3;8) are 3p13 and 3p14.2,
respectively. In tumor tissue of translocation carriers the derivative chromosome containing the
distal 3p segment is consistently lost.
In sporadic cases the VHL-gene is mutated in 57% of cases, implying a significant but not exclusive
role for this gene in the development of sporadic clear cell RCC [158]. Other regions at 3p
frequently found to be lost are 3p12-14 and 3p21. Recent findings indicate that loss of at least two
of the regions mentioned above are necessary for kidney cells to develop into clear cell carcinomas,
and that loss of 3p21 is always involved [44]. In Figure 1 this double loss is pointed out as 3p=.
Tumors showing loss of either 3p12-14 or 3p25, depicted as 3p- in Figure 1, should be designated
clear cell adenomas [44]. Although loss of 3p sequences sometimes occurs as the sole genetic
change, and thus may be sufficient for the initiation of clear cell RCC development, it is usually
accompanied by other changes. The second most common abnormality observed in clear cell RCC
is gain of (part of) chromosome 5 [26]. Trisomy 5 or partial trisomy of the smallest overlapping
region (5q22-qter) occurs in 50% of cases. In several clear cell tumors trisomy of the 5q segment
arises concurrent with loss of 3p sequences by the formation of an unbalanced translocation
between chromosomes 3 and 5 [25]. Loss of the Y chromosome and trisomy 7 are also frequent
findings, but they appear in similar frequencies in normal kidney tissue. Therefore the role of these
chromosome changes in the development and/or progression of clear cell RCC is debatable. For
this reason, trisomy 7 and loss of the Y chromosome are placed between brackets in Figure 1.
Tumorprogression in clear cell RCC has been associated with a number of genetic changes. Loss
of chromosome 14 has been associated with higher grade and stage in several studies
[25,26,108,160,161]. A relation has been found between loss of 14q and increased genetic
instability, thus facilitating the formation of additional chromosome changes [104]. Other changes
involved in tumor progression in the clear cell subtype are loss of 6q, 8(p), 9, 10q, 11, 13, 17(p),
and 18q, and gain of chromosomes 12 and 20 [26,48,76,161-163]. Two linked structural changes
on 17p, being allelic loss and mutations of the p53 gene, assigned to 17p, generally occur late in
progression [85,164,165]. Mutations of the p53 gene have also been associated with sarcomatoid
transformation, an ultimate form of tumorprogression in renal cancer (see chapter 3.1.3)

Chromophilic RCC, often referred to as papillary RCC, comprise 10-15% of RCC cases. They
show a strong male preponderance. The male to female ratio is approximately 8:1. Familial
cases of chromophilic or papillary RCC have been described [89,155], but the cause of the familial
appearance of this RCC subtype is not yet known. As in hereditary clear cell RCC, these tumors are
characterized by a frequent multiple and bilateral appearance and an early age of onset. Sporadic
cases of chromophilic RCC also may present as multiple/bilateral tumors (see chapter 3.12).

Chromophilic RCC can be divided into adenomas and carcinomas, in fact most adenomas
of the kidney are of the chromophilic/papillary type. It may be difficult to distinguish chromophilic
adenomas from carcinomas on histological grounds, and, as clear cell adenomas, they are usually
diagnosed on their size. Chromophilic adenomas tend to be beige- to white colored small tumor
masses (usually less than 3 cm). In chromophilic carcinomas an extensive greasy- brown colored
central necrosis resulting from consecutive hemorrhages is frequently seen. The tumor cells exhibit
centrally located small nuclei and the cytoplasm is covered with a few organelles only, especially
dioplasmatic reticulum. As a rule the tumor cells are arranged in a (tubulo) papillary architecture,
becoming solid in undifferentiated areas. In the latter, polymorphic nuclei with prominent nucleoli
and an eosinophilic or granular cytoplasm, due to an accumulation of mitochondria are seen. In rare instances chromophilic cell types contain fat and glycogen, resembling clear cells [20]. In these cases a papillary growth pattern may not be predominant.

Most chromophilic/papillary renal cell tumors are characterized by a unique combination of autosomal trisomies, in which trisomy 17 is the most consistent finding (see chapter 3.1.1). Adenomas specifically show a -Y,+7,+17 chromosomal pattern. In some cases also trisomy of chromosome 3(q) has been observed, but this might be an early reflection of malignant transformation [26]. In Figure 1 this is pointed out by (+3q). Subsequent gain of chromosomes 12, 16, and/or 20 marks the transition to a chromophilic/papillary carcinoma. The combined trisomy of chromosomes 7 and 17 as the sole autosomal change can be found in small as well as large tumors, whereas small tumors can have additional changes suggestive for malignant transformation. Therefore genetic analysis is mandatory in distinguishing chromophilic/papillary adenomas from carcinomas. Loss of the Y chromosome is observed in 85-93% of chromophilic/papillary tumors [75,166]. The high incidence of Y chromosome loss in this subtype, combined with the strong male preponderance, suggests that loss of specific sequences harboured on the Y chromosome probably is important in the development of this subtype. The incidence of -Y (85-93%) and trisomy 7 (87%) [75] is substantially higher than observed in apparently normal kidney tissue, and therefore are considered essential steps in the oncogenesis of chromophilic/papillary RCC. A multiple appearance has been associated with both familial and sporadic cases of chromophilic/papillary RCC. Cytogenetically, no differences are observed between hereditary and sporadic chromophilic/papillary tumors. Both show the characteristic combination of autosomal trisomies mentioned above. In a recent study, however, we demonstrated by chromosome 7 and 17 allelic imbalance studies, that differences do exist between the two. Sporadic cases of multiple chromophilic/papillary RCC show imbalance of the same chromosome 17 alleles in different tumors. Similar results have been published by Kovacs et al. [26]. It is highly unlikely that the same chromosome 17 is duplicated in so many tumors by chance. Furthermore, since most of the encountered tumors are adenomas, which have no metastatic potential, they cannot represent micrometastases. Therefore we propose that the first oncogenetic step in the development of sporadic chromophilic/papillary RCC occurs during kidney development as is discussed in chapter 3.1.2. In our model (Figure 1) the embryonal origin of these tumors is indicated by the big arrow, omitting the mature renal tubular stage. In the same survey, we found that in familial cases of chromophilic/papillary RCC, different alleles are randomly involved in chromosome 17 imbalance, suggesting that trisomy 17 is not the first oncogenetic step in the development of familial tumors (see chapter 3.1.2). At present we have no clue about the nature and localization of the inherited genetic defect responsible for the predisposition to develop chromophilic/papillary tumors in these families, and whether or not a similar genetic change also precedes gain of chromosome 17 in sporadic cases. Therefore, familial and sporadic chromophilic/papillary RCC are not mentioned as separate entities in Figure 1.

Little is known about the genetic basis of tumor progression in chromophilic/papillary carcinomas. In a recent paper (see chapter 2.1.1), we observed that tumorprogression is related to gain of chromosome 20 and loss of the extra copy of chromosome 17 or loss of 17p. No mutations of the \( p53 \) gene have been observed in this subtype, suggesting that the \( p53 \) gene most likely does not play an important role in the progression of chromophilic/papillary RCC. Other genetic changes...
which have been associated with tumor progression in chromophilic/papillary RCC, aside from those related to the transition from adenomas into carcinomas, are loss of 6q, 9, 11, 14q, and 21 and gain of chromosome 8 [26,76,107,108].

A small subset of chromophilic RCC is characterized by translocations of the X chromosome at Xp11.2. Thusfar, a specific t(X;1)(p11.2;q21) has been observed in eight cases, in some of which as the sole cytogenetic aberration. In others it is accompanied by other changes, including trisomy 17. The genes involved in the t(X;1)(p11.2;q21) have recently been cloned [121,122] and it has been shown that the translocation results in a fusion of the transcription factor TFE3 on the X chromosome, with a novel gene, designated PRCC, on chromosome 1. Variants of this translocation have also been described. A literature review of these cases is summarized in chapter 3.2.2. Chromophilic tumors showing Xp11.2 translocations have a tendency to occur in children and young adults. Morphologically these tumors are characterized by the deposition of fat and glycogen in their cytoplasm to an extent that the cells resemble large clear cells. A papillary growth pattern, usually found in chromophilic/papillary tumors, may not predominate in these neoplasms, leading to a misdiagnosis of clear cell RCC, when the commonly accepted criteria of the WHO are applied. A diagnosis based on cell type or genetic constitution would correctly point to a chromophilic variant. In Figure 1 these tumors are depicted as a distinct entity, but, since some of them also show trisomy 17 in addition to an Xp11.2 translocation, they may arise through an "ordinary" chromophilic/papillary adenoma stage, as is marked by an arrow.

Comments on the above mentioned findings:

Although clear cell RCC and chromophilic/papillary RCC both are derived from cells of the same part of the renal tubule, and have a similar antigenic phenotype, the differences in genetic changes associated with the development of these neoplasms suggest that their oncogenesis is different as well as their histogenesis. A major contributing factor to this difference might be that clear cell RCC arises from mature renal tubular cells, whereas chromophilic/papillary tumors most likely have an embryonal origin. The fact that mixed tumors, comprising both clear cell and chromophilic/papillary areas, exist does not contradict this assumption. Genetic analysis of these tumors reveal the typical chromosome changes of the respective tumor types ([50], and unpublished observations). A number of additional changes, mostly related to tumor progression, are similar for both subtypes [26,28,46,53,104-108]. Common changes observed in both subtypes are loss of 6q, 9, 11, 14q, 17p, and gain of chromosomes 12 and 20. Whether shared, progression related, genetic changes, as observed in these subtypes, point to a partly common oncogenetic pathway because of their common origin, i.e. the metanephros, has to be elucidated.

Renal oncocytomas, chromophobe RCC and Duct Bellini carcinomas:

Renal oncocytoma or renal cell adenoma of the oncocytic type, comprising approximately 4% of RCC, is an essentially benign neoplasm, although cases with local or distant metastasis have been described [5]. The male to female ratio is 2.5:1. Renal oncocytomas are solitary, in rare instances multiple, well circumscribed, slightly lobulated solid tumors with a tan-brown cut surface and larger tumors exhibit a stellate central scar. Focal hemorrhage and invasion of adjacent
structures may be present, but these tumors do not exhibit necrosis [24]. Microscopically, the tumor consists of cells with abundant granular eosinophilic cytoplasm. The centrally located nuclei are generally round and vesicular, but focal areas may have marked nuclear atypia, probably as a result of polyploidization. The cells are usually arranged into solid nests (acinar growth pattern) and sheets of trabeculae that are separated by loose edematous fibrous stroma. Ultrastructurally the cytoplasm is packed with numerous round mitochondria, rich in cristae. The mitochondria of oncocytomas are larger than the mitochondria of other renal cell neoplasms.

Renal oncocytomas find their origin in the intercalated cells of the collecting tubule, which is substantiated by the shared expression of carbonic anhydrase C (CAC) and band-3 protein. However, tumor associated loss of antigen might occur [152]. Therefore not all oncocytic cells are positive for the above mentioned antigens.

Little is known about the genetic changes responsible for the development of renal oncocytomas, but different authors have observed alterations of the mitochondrial DNA. Telomere shortening and telomeric associations have been observed in renal oncocytomas [73]. Cytogenetic analysis of these neoplasms has revealed a variety of chromosomal patterns, suggesting the existence of distinct subsets [26,67,69-71,132-134,136,138,146]. Several cases display mixed populations of cells with normal and abnormal karyotypes, which fail to show any cytogenetic similarity [26]. Others seem to reveal a consistent pattern of genetic changes. They can be divided into 1) tumors showing the combined loss of chromosomes 1 and X/Y and 2) tumors revealing translocations involving chromosome 11, with breakpoint 11q13. Recently Sinke et al [147] mapped this oncocytoma specific 11q13 breakpoint between D11S443/D11S146 and the BCL1 locus, using two t(5;11)-positive renal oncocytomas. The 11q13 breakpoint of a third case, revealing a t(9;11)(p23;q13) was found to be located in the same region (unpublished data). Tumor-specific translocations have been described in solid tumors, some of which result in the production of chimeric transcription factors. A notable example is the finding of a specific (X;1) translocation in a subset of chromophilic RCC (described above). A similar mechanism might play a role in the development of the oncocytomas characterized by translocations involving breakpoint 11q13. The fusion of 11q13 with either 5q35 or 9p23 may result in a new fusion gene, the expression of which is responsible for uncontrolled growth. Direct activation of a putative oncogene by juxtaposition to active promotor/enhancer sequences is another possible mechanism.

Loss of chromosomes 1 and X or Y probably results in loss of tumor suppressor gene(s) responsible for the development of this subset of oncocytomas [167]. Recent findings indicate that especially loss of 1p is important in these neoplasms, suggesting the presence of a tumor suppressor gene at this chromosomal region [149]. No morphological differences have been observed between the genetically different subsets of oncocytomas. Nevertheless, the observed distinct genetic changes might have a biological meaning, one of which could be the reported malignant potential of some of the oncocytomas published thusfar. As is discussed in chapter 4.3, a subset of renal oncocytomas might represent the benign counterpart of chromophobe carcinomas. A proposed adenoma carcinoma sequence for these neoplasms will be presented in detail in one of the sections below.

**Chromophobe RCC** was first described in humans by Thoenes et al. [124]. This subtype accounts for 2-5% of cases and no male preponderance is found [130]. As holds for renal
oncocytomas these tumors find their origin in the intercalated cells of the collecting tubules. Depending on their size, chromophobe tumors consist of one or more solid tumor nodules with a slightly lobulated surface. The cut surface appears homogeneously orange. An uniformly pale cut surface interspersed with a few hemorrhages is a very characteristic gross feature of the well differentiated tumor type, whereas a slightly brown-colored cut surface is usually associated with less differentiated tumors. Microscopically, the basic chromophobe cell type is characterized by large polygonal cells with a transparent, not clear, but slightly reticulated cytoplasm. The nuclei are central or slightly eccentric and usually moderate or substantial in size with marked variation in a single tumor. Clearly recognizable nucleoli are present. Ultrastructurally the cytoplasm of chromophobe cells is crowded with glycogen deposits and numerous, sometimes invaginated, vesicles that resemble those of a subset of intercalated cells of the renal collecting tubule from which chromophobe RCC develops. The origin of the microvesicles is not yet known but the outer membrane of mitochondria have been proposed to be a probable source [168]. A few glycogen particles are found free in the cytoplasm, between the microvesicles and a few mitochondria with a reasonably large number of crista-like or vesicular inner membranes occur loosely scattered at the cell periphery. There are two cytomorphological variants of chromophobe RCC: the typical variant, as described above, and the eosinophilic variant. The latter differs from the typical variant in its higher content of mitochondria, which are also somewhat larger on average. These mitochondria take up an appreciable proportion of the cytoplasm and contain abundant crista-like or vesicular internal membranes. The cytoplasmic microvesicles are present in great numbers, either between the mitochondria or in small mitochondria-free areas. Both variants show a strong positive reaction with Hale's acid iron colloid stain, which is probably the most important diagnostic feature for chromophobe RCC. In addition chromophobe tumors are positive for carbonic anhydrase C, but they do not express band-3 protein.

Genetic data on chromophobe RCC are limited, but almost all cases described thusfar are characterized by extensive nonrandom chromosome losses [60,61,71,126,127]. Loss of chromosomes 1, 2, 6, 10, 13, 17, 21, and X/Y is consistently found in these cases. Telomeric associations and telomere shortening have also been observed [73]. Furthermore, molecular analysis of this subtype has revealed gross alterations of the mitochondrial DNA (mtDNA) [54]. Genetic changes related to tumor progression and metastatic behavior within chromophobe carcinomas are not yet known. Thusfar only one cytogenetically analyzed case of a chromophobe metastasis has been described (see chapter 4.1). In this case, next to the extensive chromosome losses specifically assigned to the chromophobe subtype, also structural changes were observed. Whether or not these changes are related to metastatic growth has to await further studies in RCC metastases. Therefore they are not included in Figure 1.

Duct Bellini carcinomas, or collecting duct carcinomas, are rare tumors representing approx 1% of all RCC. They find their origin in the principal cells of the collecting duct system. At presentation, these tumors are usually smaller than other RCC subtypes, but they appear to be aggressive neoplasms, often with metastatic disease at diagnosis, and rapid progression despite surgical intervention. Duct Bellini carcinomas are usually localized to the renal medulla with distortion of the pelvicalyceal system, often with irregular extensions into the adjacent renal cortex.
They generally are white colored and firm at cut surface and lack necrosis and hemorrhage. The growth pattern is mainly tubular combined with a microcystic, pseudopapillary, and solid pattern. The basic cell type exhibits medium-sized tumor cells with a basophilic, sometimes light cytoplasm due to pronounced formation of endoplasmic reticulum and glycogen deposits. Anaplastic nuclei are usually found.

Genetic data on Duct Bellini carcinomas is limited, but these tumors frequently show an aneuploid DNA content. Cytogenetic data published on a few cases thusfar have revealed conflicting results [63]. Molecular analysis has shown that 8p and 13q are frequently lost in these neoplasms [64]. In a recent study, loss of chromosome 1, region 1q32.1-q32.2, appeared to be a consistent finding in Duct Bellini carcinomas [169]. Therefore tumor suppressor genes located at these three chromosome regions might be involved in the development of Duct Bellini carcinoma.

**A proposed adenoma-carcinoma sequence for chromophobe RCC**

Comparing the morphologic and genetic features of chromophobe carcinomas and renal oncocytomas, a marked degree of similarity is observed, which is not surprising since they have the same progenitor. The intercalated cells of the collecting tubule give rise to both tumortypes, reflected by the shared expression of carbonic anhydrase C. The lack of band 3 protein expression in chromophobe RCC might be due to tumor related loss of antigen. Oncocytomas and the eosinophilic variant of chromophobe RCC show an accumulation of enlarged and morphologically altered mitochondria. Alterations of the mitochondrial DNA (mtDNA) have been observed in chromophobe carcinomas as well as in renal oncocytomas. The characteristic microvesicles, said to be exclusive for chromophobe RCC, have been observed in small amounts in some oncocytomas (Störkel, personal communication), but their origin is presently unknown. Telomeric associations and telomere shortening have been observed in both subtypes. Comparison of the genetic profile of oncocytomas with chromophobe carcinomas reveals that loss of chromosomes 1 and X/Y is shared by both. In addition to loss of chromosomes 1 and X/Y, chromophobe carcinomas show extensive nonrandom whole chromosome losses, involving chromosomes 2, 6, 10, 13, 17, and 21. The above mentioned findings suggest that chromophobe carcinomas and renal oncocytomas are closely related entities. As is depicted in Figure 2, renal oncocytomas characterized by the combined loss of chromosomes 1 and X/Y might be chromophobe adenomas, explaining why occasionally oncocytomas show a malignant behavior. MtDNA changes and loss of chromosomes 1 and X/Y may be the first oncogenetic steps in their development. Progression from chromophobe adenomas via eosinophilic chromophobe carcinomas to typical chromophobe carcinomas most likely occurs through subsequent chromosome losses, involving chromosomes 2, 6, 10, 13, 17, and 21, and additional mtDNA changes. The latter might result in a sequential breakdown of mitochondria. In addition, characteristic microvesicles accumulate in the cytoplasm of the cells. Evidence substantiating this proposed oncogenetic pathway are 1) the finding of an oncocytoma containing chromophobe cell nests [151], pointing to a possible transition between both tumor types, 2) the cytogenetic analysis of a case of oncocytoma revealing multiple chromosome losses, among which chromosomes 1, 2, 6, 17, 21, and Y [154], and 3) LOH studies, showing loss of chromosome regions 1p, 2pq, 13q, 17pq, Xpq, in one case of oncocytoma, and loss of 1pq, and
21q in another [149], abnormalities specifically assigned to chromophobe carcinomas (see also chapter 4.3).

**CONCLUDING REMARKS AND FUTURE PERSPECTIVES**

Cytogenetic and molecular genetic studies of RCC have shown that these tumors can be divided into genetically distinct entities. A specific combination of genetic changes marks each subtype, whereas other changes have been related to progression to a more malignant phenotype. There is indeed a close relationship between morphological subtyping according to Thoenes and Störkel, and genetic subtyping of RCC. However, the genetic profile of renal cancers enholds much more information than a morphological subtyping can provide. Renal cell adenomas are difficult, maybe even impossible, to recognize on histological grounds, and the size of a given tumor, often used as a diagnostic criterium, might not be representative for its clinical behavior. The recognition of a distinct adenoma stage in clear cell RCC, characterized by a single deletion in either 3p25 or 3p12-14 (depicted as 3p- in Figure 1) is therefore a tremendous step forward in achieving a proper diagnosis [44]. The same holds for chromophilic adenomas, revealing a -Y, +7, +17 chromosomal pattern without additional changes. The proposed embryonal origin for sporadic chromophilic tumors (chapter 3.1.2), and the observed differences between familial and sporadic cases of this subtype are major contributions to the proposed onco-developmental pathway of these tumors. However, since we do not know, at present, which genetic events, other than -Y, +7, +17, are...
involved in the development of familial cases of chromophilic RCC, and whether they also contribute to the development of sporadic cases, familial chromophilic RCC is not mentioned as a separate entity in Figure 1. The proposed adenoma-carcinoma sequence for chromophobe RCC (depicted in detail in Figure 2), reassigning a subset of oncocytomas to the chromophobe subtype, might have diagnostic and prognostic consequences, and also contributes to our understanding of its oncogenesis (see chapter 4.3).

Tumor progression is the result of an accumulation of genetic changes. Genetic changes related to this process, however, do not occur randomly and their appearance may depend on the primary aberration and on the specific tumor type involved. The knowledge of specific progression related genetic changes may provide valuable prognostic information. In RCC several genetic events have been associated with the progression of clear cell and chromophilic tumors, some of which seem to be unique for one of the subtypes, whereas others are shared by both. Common progression related genetic changes are loss of 6q, 9, 11, 14q, 17p, and gain of chromosomes 12 and 20. In addition, clear cell RCC specifically shows gain of 5q, loss of 8p, 10q, 13, and 18q, and mutations of the \( p53 \) gene, whereas the chromophilic/papillary subtype shows gain of 3q, 8, and 16, and loss of 21 (see also chapters 2, 3.1.1 and 3.1.3). No pertinent data are available on progression related genetic changes in other subtypes of RCC thusfar.

Sarcomatoid transformation is an ultimate form of tumor progression in RCC and may occur in any of the subtypes. The diagnosis of sarcomatoid RCC on morphological grounds may be difficult and incorrect, since the histologic appearance of the carcinoid areas, on which these tumors usually are judged, might not represent the sarcomatoid part of the tumor (see chapter 3.1.3). Assessment of the genetic profile of these tumors may prove to be a valuable diagnostic tool as to tumor subtype, but also in distinguishing sarcomatoid RCC from true renal sarcomas. Taken together, the genetic constitution of the different subtypes of RCC, as shown in Figure 1, enhold valuable diagnostic, and prognostic information, and genetic analysis of RCC is therefore important for the individual patient affected with this disease. Furthermore, assessment of the distinct genetic profiles observed in RCC has improved our understanding of its oncogenesis and of pathogenetic relationships between tumor subtypes. Presently, the diagnosis of RCC is mainly based on histopathological examination. Considering the close relation between genetic subtyping of RCC and the morphological classification of Thoenes and Störkel, we feel that this classification should be adapted in routine pathologic examination of these neoplasms. However, for the diagnosis of a renal adenoma, either clear, chromophilic/papillary, or oncocytic, genetic analysis is mandatory. In the near future the morphological diagnosis will be increasingly accompanied by cytogenetic and molecular genetic methods, improving the diagnosis, and having prognostic significance. Once the genes, involved in the development and progression of the different subtypes of RCC, have been identified, genetic analysis might be transferred to routine pathology and used in the clinical management of individual patients. Furthermore, identification of the as yet unknown genes, and their encoded proteins, may form the basis for new and improved therapies in the future.
Summary of Figure 1:

A PROPOSED ONCOGENETIC MODEL FOR RENAL CELL CANCER

The compiled morphologic and genetic data of RCC extracted from our survey and from the literature is depicted in a proposed oncogenetic model for RCC (Figure 1). The white bar represents the normal mature renal tubular system with on the left the proximal and distal tubule, and on the right the collecting tubule. Both are separated from each other by a grey line, indicating their different embryonal origin, i.e. the metanephros and the mesonephros respectively. In the horizontal lane below that, the adenoma stages of the different subtypes of RCC are given. Progression to a carcinoma stage and subsequent progression to a higher grade are indicated in vertical direction. Clear cell and chromophilic/papillary tumors arise from the proximal/distal tubule, whereas renal oncocytomas, chromophobe carcinomas, and Duct Bellini carcinomas find their origin in the collecting tubule. The genetic changes, known thusfar to be involved in the different tumor subtypes and progression stages, are given. Clear cell adenomas are characterized by a single 3p deletion, either in 3p25 or 3p12-14, depicted as 3p- in Figure 1. Progression to clear cell carcinomas is associated with a subsequent deletion of 3p21 (3p= in Figure 1). Trisomy 5q is
another frequent finding in clear cell carcinomas, and occurs independent of tumor grade. Trisomy 7 and loss of the Y chromosome are placed between brackets, since these genetic changes have been found in similar frequencies in normal kidney tissue, and their contribution to the oncogenesis of clear cell carcinomas is therefore debatable. Genetic changes reflecting a higher grade in clear cell carcinomas are: loss of 6q, 8, 9, 10q, 11, 13, 14, 17(p), and 18q, gain of chromosomes 12 and 20 and mutations of the \( p53 \) gene. Chromophilic/papillary adenomas are characterized by trisomy 7 and 17 and loss of the Y chromosome. In some adenomas also trisomy of 3q is observed, but this might be an early sign of malignant transformation, indicated as (+3q) in Figure 1. The big arrow omitting the mature renal tubular stage, reflects the proposed embryonal origin of these neoplasms (chapter 3.1.2). Chromophilic/papillary carcinomas reveal additional trisomies of chromosomes 3q, 12, 16, and 20, and progression to a higher grade is associated with loss of 6q, 9, 11, 14q, 17(p), and 21, and gain of chromosome 8. A small subset of chromophilic tumors is characterized by translocations involving chromosome X, with breakpoint Xp11.2. In some of these tumors also trisomy of 7 and 17 is observed. "True" renal oncocytopas are characterized by translocations involving chromosome 11, breakpoint 11q13, and changes of the mitochondrial DNA (mtDNA). Chromophobe adenomas reveal the combined loss of chromosomes 1 and X/Y, and mtDNA changes. Chromophobe carcinomas show additional loss of chromosomes 2, 6, 10, 13, 17, and 21. Duct Bellini carcinomas are characterized by loss of 1q, 8p, and 13. Genetic changes associated with a higher grade in chromophobe carcinomas and Duct Bellini carcinomas have not been assessed.
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