

Chapter 2

Renal Cell Carcinoma: Pathologic and Molecular Assessment of Targets

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Evolution of the Classification of Renal Cell Carcinomas

Knowledge cannot be elaborated and transmitted in the form of isolated observations. For this reason, observations are grouped according to similar features, producing a classification. Classifications can differ depending on their objective and on changes in ways of thinking over time. In cancer, the evolution of knowledge reflects the pattern observed in the general evolution of human understanding, and accordingly cancer classifications are no more than a tool that require revision and refinement from time to time based on the gradual increase in knowledge. The microscopic characterization of renal cell carcinoma (RCC) started in the mid-nineteenth century (1) with the controversy aroused by Grawitz's hypothesis—in 1883, Grawitz stated that *alveolar* (clear cell) tumors, previously considered lipomas, originated in the neoplastic transformation of adrenal cortical residues into renal cortical. One year later, he confirmed his theory when he found ectopic adrenal cortex in the renal cortex. This theory was readily opposed by Sudek, who favored a renal tubular origin. The controversy between supporters and detractors of the Grawitz theory went on for decades. The term *hypernephroma* was introduced in 1909 and made reference to the adrenal origin. Support for the supposed adrenal origin started to grow weaker. Oberling et al.'s ultrastructural studies (2) finally brought the argument to a conclusion by demonstrating the tubular origin of RCC, in the proximal nephron.

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Initial Histological Classifications

For a long time, the mechanical model of disease (according to which man is a complex “machine” and disease is a fault in the machinery) and the limited therapeutic modalities (practically only surgery) resulted in a classification with few histological subtypes. The first international classifications unified all the historical histological types under the common denomination of renal adenocarcinoma; this could be a clear cell or a granular cell carcinoma, its architecture could be tubular, papillary, or cystic, and its appearance was rarely sarcomatoid [3]. Nevertheless, quite soon attempts began to be made to distinguish histological subtypes on the basis of their origins from different parts of the nephron, with efforts to correlate them with different clinical evolutions. Thus, Thoenes et al. described the *chromophobe renal cell carcinoma* [4] morphologically different from the clear cell carcinoma and regarded as probably originating in the intercalated cells of the distal nephron [5]. Subsequently many possible histological variants were described, and attempts made to identify their origin from different areas of the nephron by means of immunohistochemistry. Many of these histological subtypes failed to show a correlation with the clinical evolution, however, bringing into doubt the utility of such morphological classifications. In the wake of these failures, chromosomal studies and developing knowledge of familial RCC syndromes helped to change the scenario.

Chromosomal Findings in Familial Renal Cell Carcinomas: Impact on the Pathology and Therapy of Sporadic Cases

Approximately 2–3 % of RCCs occur within the context of a familial syndrome. These syndromes are characterized by early onset and/or multifocal/bilateral disease. Some are due to mutated or inactivated tumor suppressor genes and others to activated oncogenes. The recognition that each of these syndromes is associated with specific tumor phenotype, chromosomal changes, and gene alterations had a major impact on knowledge of RCC, and during recent years, various renal cancer syndromes have been characterized (Table 2.1).

Von Hippel–Lindau (VHL) Disease: This is the most frequent familial renal cancer syndrome, estimated to occur at rates of 1:36,000 to 1:45,500 population. It is associated with secondary *VHL* gene (3p25–26) changes. Missense mutations are the most common, but nonsense mutations, microdeletions/insertions, splice mutations, and large deletions also occur. The spectrum of clinical manifestations of VHL reflects the type of germline mutation [6].

Table 2.1 Hereditary renal cell carcinomas

Syndrome	Chr.	Gene	Protein	Tumor type	Extrarenal manifestations	
					Dermis	Other organs
von Hippel–Lindau	3p25	<i>VHL</i>	pVHL	Multiple, bilateral clear cell RCC, renal cysts	–	Hemangioblastoma of retina/cns, pheochromocytoma, pancreatic/renal cysts, neuroendocrine tumors, epididymal/parametrial cysts, tumors of the inner ear
Hereditary papillary RCC	7p31	<i>c-MET</i>	HGF-R	Multiple, bilateral papillary RCC (type 1)	–	–
HLRC	1q42	<i>FH</i>	FH	Papillary RCC (non-type 1)	Leiomyoma	Uterine leiomyoma/leiomyosarcoma
Hyperparathyroidism–jaw tumor (HP–JT)	1q25	<i>HRPT2</i>		Epithelial–stromal mixed tumors, papillary RCC	–	Tumors of the parathyroidea, fibro-osseous jaw tumors
Birt–Hogg–Dubé	17p11	<i>FLCN</i>	Folliculin	Multiple chromophobe RCC, oncocytic adenoma, papillary RCC	Facial fibrofolliculoma	Pulmonal cysts, spontaneous pneumothorax
Tuberous sclerosis	9q34 16p13	<i>TSC 1</i> <i>TSC 2</i>	Hamartin Tuberin	Multiple, bilateral angiomyolipomas, lymphangioleiomyomatosis, rare clear cell RCC	Angiofibroma, peau chagrin, subungual fibroma	Cardiac rhabdomyoma, adenomatous small intestine polyps, pulmonal/renal cysts, cortical tuber, subependymal giant cell astrocytomas
Constitutional translocation chr. 3	3p13–14	<i>FHIT</i>	FHIT	Multiple, bilateral clear cell RCC	–	–
Succinate dehydrogenase germline mutation	1p36	<i>SDHB</i>	SHDB	Renal tumors with unique morphology	–	Paraganglioma Papillary thyroid carcinoma

The typical renal manifestations of VHL disease are kidney cysts and clear cell renal cell carcinoma (ccRCC). Histological examination of macroscopically normal renal tissue may reveal several hundred independent tumors and cysts.

Hereditary Papillary Renal Carcinoma (HPRC): Trisomy or tetrasomy 7, trisomy 17, and loss of chromosome Y are the most common chromosomal changes, with a germline-activating mutation in the MET proto-oncogene (7q31–34) which can cause papillary renal cell carcinoma type 1 (type 1 pRCC), with cuboidal cells with scanty basophilic cytoplasm and low-grade nuclei [7].

Hereditary Leiomyomatosis and Renal Cell Cancer Syndrome (HLRCC): Some families have a linkage to 1q42.3–q43 [8]. At the genetic level, a germline loss-of-function mutation in the fumarate hydratase (FH) gene is present, and the typical kidney pathology is a papillary renal cell carcinoma type 2 (type 2 pRCC) with eosinophilic cells and high-grade nuclei. Recently, however, tubular and solid patterns and the presence of large nucleoli with perinucleolar halos have been described [9].

The Birt–Hogg–Dubé Syndrome (BHD): The BHD (FLCN) gene is located on 17q12–q11.2. It is associated with multiple cutaneous lesions (fibrofolliculomas, trichodiscomas, and acrochordons) and with an increased risk of renal cancers of various histological types, especially chromophobe renal cell carcinoma (chRCC), oncocytoma, and hybrid oncocytoma–chromophobe renal cell carcinoma, although ccRCC and pRCC can also be present [10].

Tuberous Sclerosis: The disease is associated with mutations in the *TSC1* (9q34) and *TSC2* (16p13) genes, leading to hyperactivation of the mTOR pathway. Although angiomyolipoma is the most characteristic kidney tumor in this syndrome, ccRCC and chRCC are also described [11].

Other familial syndromes are much more infrequent (Table 2.1).

Identification of the specific chromosomal and genetic alterations of the familial and hereditary syndromes as characteristics of the distinct histological subtypes of RCC has made it possible to confirm that a high percentage of the sporadic forms of these subtypes display the same genetic changes. The described morphological subtypes can be interpreted as an expression of specific genetic changes; accordingly, based on the morphology, distinct genetic pathways can be recognized. In view of the above considerations, additional entities were included in WHO's 2004 classification [12] (Table 2.2), which combined morphological and genetic characteristics and began to recognize some variations with evidence of different immunophenotypes or molecular changes with clinical implications. Thus, when developing target therapies against different genetic pathways, the histological subtype can help in selection of the drug.

Table 2.2 Renal cell carcinoma classification

<i>WHO histological subtypes</i>
Clear cell renal cell carcinoma
Multilocular clear cell renal cell carcinoma
Papillary renal cell carcinoma
Chromophobe renal cell carcinoma
Carcinoma of the collecting ducts of Bellini
Renal medullary carcinoma
Xp11 translocation carcinomas
Carcinoma associated with neuroblastoma
Mucinous tubular and spindle-cell carcinoma
Renal cell carcinoma unclassified
<i>Other entities</i>
Tubulocystic carcinoma
Acquired cystic disease-associated carcinoma
Clear cell tubule-papillary carcinoma
Thyroid-like follicular carcinoma
Leiomyomatous renal cell carcinoma
Succinate dehydrogenase (SDHB) germline mutation-associated carcinoma
Anaplastic lymphoma kinase (ALK) translocation-associated carcinoma
Biphasic alveolosquamoid renal carcinoma cell carcinoma

Molecular Pathways in Renal Cell Carcinomas

Study of familial RCCs has identified the involvement of diverse molecular pathways, the main ones being those that mimic a hypoxic status [13], activating angiogenesis, and the mTOR pathway [14].

Pseudo-hypoxic Pathways in Renal Cell Carcinoma

VHL Pathway

The *VHL* gene (3p25.3) encodes the pVHL protein, which regulates HIF- α , a transcription factor involved in the response to oxygen changes. In the hypoxic situation, HIF is not degraded and activates several genes, including platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) that stimulate angiogenesis and inhibit tumor cell apoptosis [15]. In addition, it upregulates other growth factors (TGF α , EGFR, IGF) that stimulate autocrine cell growth or activate energy supply factors such as glucose transporter protein-1 (GLUT1) and erythropoietin.

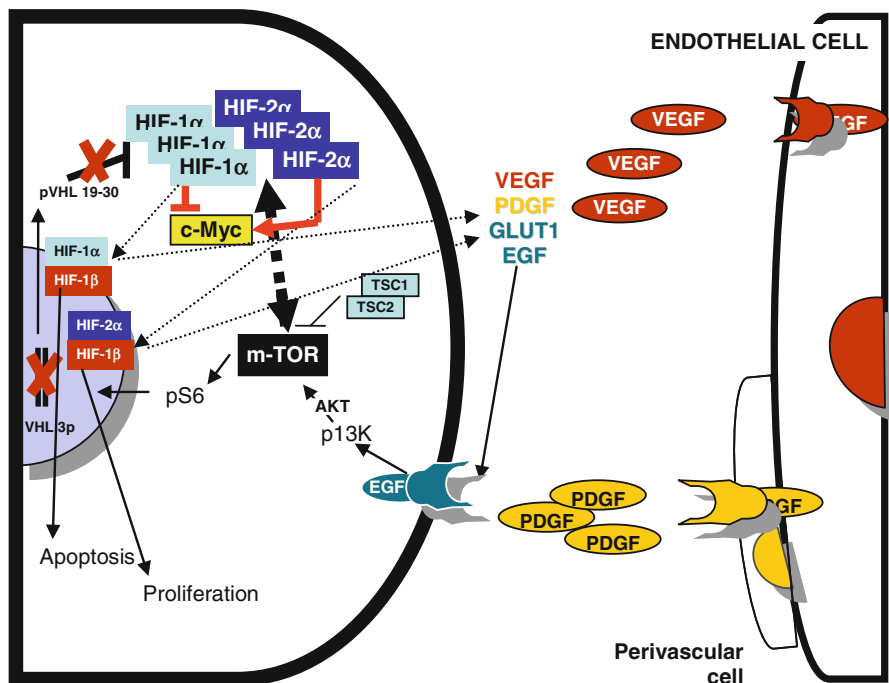


Fig. 2.1 Pseudo-hypoxic pathway. VHL loss with HIF-1 α and HIF-2 α accumulation and angiogenesis and proliferation increase

The mutation in 3p, present in 70–90 % of sporadic ccRCCs and less commonly in other subtypes, with inactivation of the *VHL* gene results in failure of the pVHL-E3 ubiquitin ligase complex that mediates HIF degradation. This leads to accumulation of HIF- α and binding to HIF-1 β , mimicking a hypoxia situation, and transcriptional activation of genes such as VEGF [16].

There are multiple forms of HIF- α , HIF-1 α , and HIF-2 α being those most commonly involved in RCC. Apoptosis is mediated by HIF-1 α , and proliferation is mediated preferentially by HIF-2 α , which displays elevated c-Myc activity, resulting in enhanced proliferation and resistance to replication stress [14, 17] (Fig. 2.1). Likewise, HIF-2 α can inhibit p53 through a growth factor receptor AKT-MDM2 pathway, contributing to the survival of RCCs during standard treatments such as ionizing radiation or chemotherapy [18].

Krebs Cycle and Pseudo-hypoxic Pathway

Fumarate Hydratase Pathway. The *fumarate hydratase* gene (FH) (1q42.3–q43) encodes the FH protein involved in the conversion of fumarate to malate in the Krebs cycle.

mTOR Pathway

Any pathway with HIF- α accumulation can upregulate the mammalian target of rapamycin or mTOR pathway.

mTOR is an intracellular serine/threonine protein kinase of 289 kDa belonging to the phosphatidylinositol kinase-related kinases coded in 1p36.2. It is involved in the monitoring of cellular nutrition, with effects on protein translation, angiogenesis, cell growth, and apoptosis [22]. mTOR exists in two multiprotein complexes: mTORC1 and mTORC2.

mTORC1 includes the regulatory associated protein of mTOR (RAPTOR). It can be activated by growth factors in the cellular membrane through Ras and PI3K and plays a role in the regulation of cell growth, proliferation, survival, and motility via the phosphorylation of S6K1 and 4E-BP1, which promote mRNA translations and ribosome biogenesis (Fig. 2.1) [23]. On the other hand, HIF-1 α represses mTORC1, thereby promoting the release of mTORC2 [18].

mTORC2 is a rapamycin-insensitive companion of mTOR (RICTOR). Knowledge of its functions and control is more limited. Recently the finding that it can directly phosphorylate Akt indicates that mTORC2 may modulate cell survival [24].

HIF-1 α seems to be regulated by mTORC1 and mTORC2, whereas HIF-2 α expression is mTORC2 dependent but mTORC1 independent [25].

TSC1/TSC2 Pathway

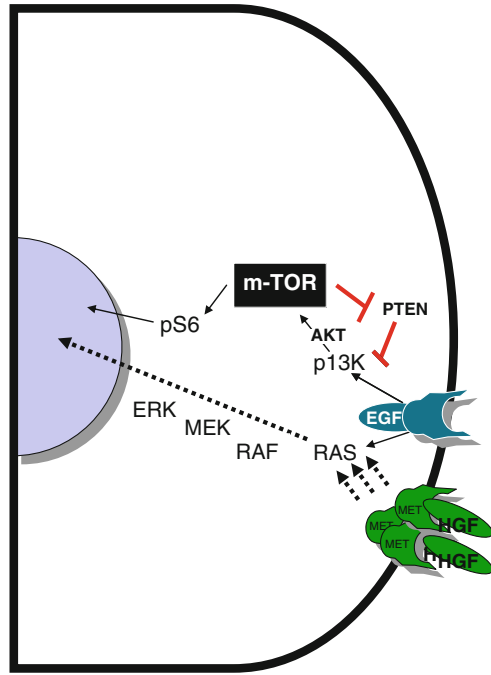
The complex TSC1 (9q34) and TSC2 (16p13.3) is a negative-regulating Rheb/mTOR/p70S6K cascade [26] (Fig. 2.1). The TSC2 loss results in HIF-1 α accumulation and pseudo-hypoxic pathway activation [27], which can explain the occasional association of angiomyolipoma with RCC in sporadic cases or in tuberous sclerosis [28].

c-MET Pathway in Renal Cell Carcinoma

The *MET* gene (7q31–34) is amplified in some RCCs. c-MET is a member of the receptor tyrosine kinase family; its ligand is the hepatocyte growth factor (HGF). Both are upregulated after renal injury and tissue repair via PI3-AKT and PI3-RAS-Erk. RCCs with a c-MET mutation presumably overactivate protein products of the *MET* gene, potentially driving uncontrolled growth [29] (Fig. 2.3).

The phosphate and tensin homologue deleted on chromosome 10 (PTEN) is involved in negatively regulating the Rheb/mTOR/p70S6K cascade via PI3K inhi-

Fig. 2.3 c-MET pathway.
Mutation of c-MET
overactivate protein products
of the *MET* gene, potentially
driving uncontrolled growth



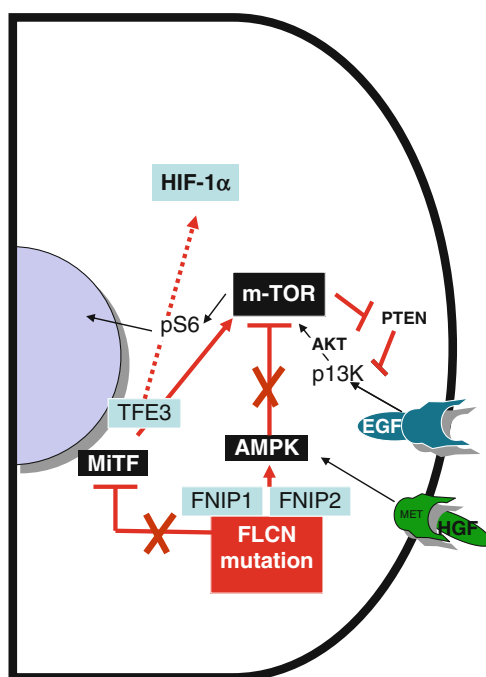
bition [22]. Individuals with a germline mutation of the *PTEN* gene (Cowden syndrome) have a risk of tumors in the breast, thyroid, endometrium, and kidney [30].

FLCN Pathway in Renal Cell Carcinoma

FLCN forms a complex with folliculin-interacting proteins (FNIP1 and FNIP2). These components bind to AMP-activated protein kinase (AMPK). AMPK acts to sense cellular energy and assists in the regulation of the mTOR activity level. In tumors that are noted to have FLCN alterations in both alleles, mTOR activation (mTORC1 and mTORC2) and also increased TFE3 transcriptional activity has been observed [31] (Fig. 2.4).

TFE3 is a member of the MiT family of transcription factors (TFE3, TFEB, MITF, and TFEC), which are overexpressed in RCC for translocations in chromosomes 1 and X, t(X:1)(p11.2;p34), and chromosomes 6 and 11, t(6;11)(p21;q13). These translocations create active fusion proteins with MiT transcription factor activity but without their normal regulation [32], conditioning mTOR pathway activation and increase in HIF-1 α [33].

Fig. 2.4 FLCN pathway. Folliculin gene mutations deregulate mTOR activity and increased TFE3 transcriptional activity



Renal Cell Carcinoma Pathology According to Molecular Pathway

Pseudo-hypoxic Pathway

Association with VHL Gene Changes and TSC1/TSC2 Loss

Like the majority of patients with TSC1/TSC2 loss, the familial and sporadic cases with *VHL* gene changes can develop ccRCC [34]. This neoplasm consists of clear cytoplasm (empty) cells (Fig. 2.5). Cells of high nuclear grade can acquire an eosinophilic aspect due to the higher mitochondrial content. The most frequent arrangement is a solid pattern, though tubular and occasionally cystic patterns can also be present. Papillary areas are very rarely observed. Sarcomatoid transformation is observed in 5 % of cases [35]. A prominent vascular stroma is typical. Expression of CAIX and CD10 occurs in the majority of cases [36].

In multilocular ccRCC, 3p deletion is present in 74 % of cases and *VHL* gene mutation in 25 % [37]; for this reason it can be considered a variant of classical ccRCC of low aggressivity.

In addition to the *VHL* gene, other parts of chromosome 3 can be lost, such as 3p12, 3p14, and 3p21, which contain the *PBRM1* gene, with truncating mutations in 41 % of cases of ccRCC [38]. Other chromosomes affected are 5q, 9p, and 14q.

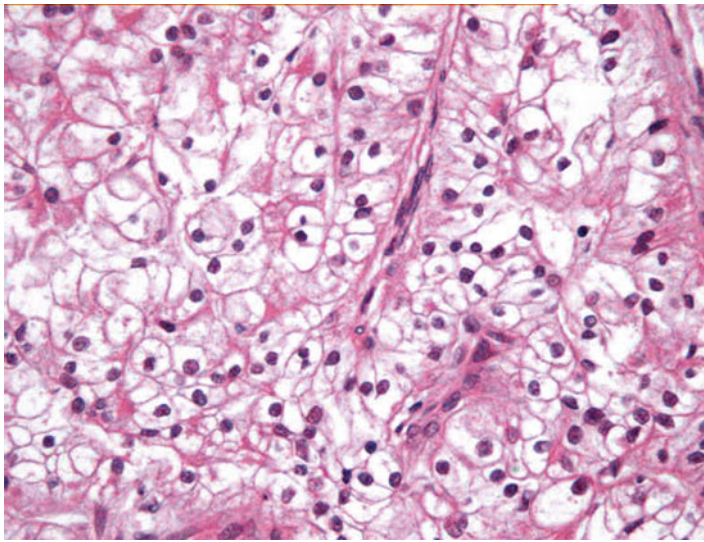


Fig. 2.5 Clear cell RCC. The cells have an empty cytoplasm for lipids and glycogen dissolution during the technical handling of the tumor

Around 10 % of cases of sporadic ccRCC do not have the *VHL* gene mutation, and some of them have somatic *NF2* gene (22q12.2) mutations [39].

Association with Krebs Cycle Mutations

These carcinomas fulfill the Warburg model of cancer because they depend on anaerobic glycolysis instead of oxidative phosphorylation [40].

HLRCC patients (with *FH* gene mutation) and 42 % of those with sporadic papillary RCC have a similar histological subtype to type 2 pRCC, with eosinophilic cells of high nuclear grade and pseudostratified nucleus in papillary cores [41] (Fig. 2.6).

Lack of expression of cytokeratin 7 and positive alpha-methylacyl-CoA race-mase (AMACR) are typical. The sporadic type 2 pRCC has a higher frequency of allelic imbalance on 9p21 and a lower frequency of trisomy 17q, which is typical for the other pRCCs [42]. Other changes are in 1p, 3p, and 5q. At present there are doubts over whether HLRCC and sporadic type 2 pRCC are in fact the same entity or not and whether they follow the same pathway as the familial forms. The International Society of Urogenital Pathology (ISUP) has considered HLRCC to be a separate entity from the other histological subtypes, and the majority of authors regard sporadic type 2 pRCC as a heterogeneous variant [43].

Another mutation in the Krebs cycle is at the level of *SDHB* genes. This mutation is associated with the risk of development of succinate dehydrogenase germline mutation-associated carcinoma (SDHB RCC), a variant also characterized by eosinophilic cells with vacuoles and entrapped normal tubules in the periphery [21] (Fig. 2.7).

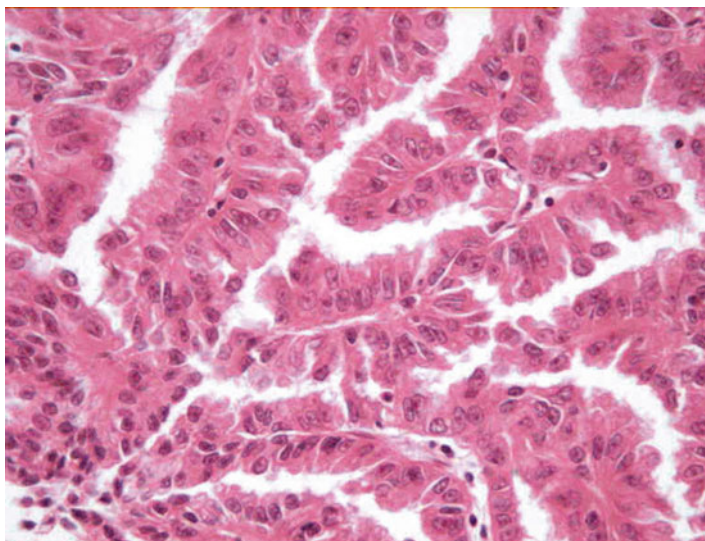
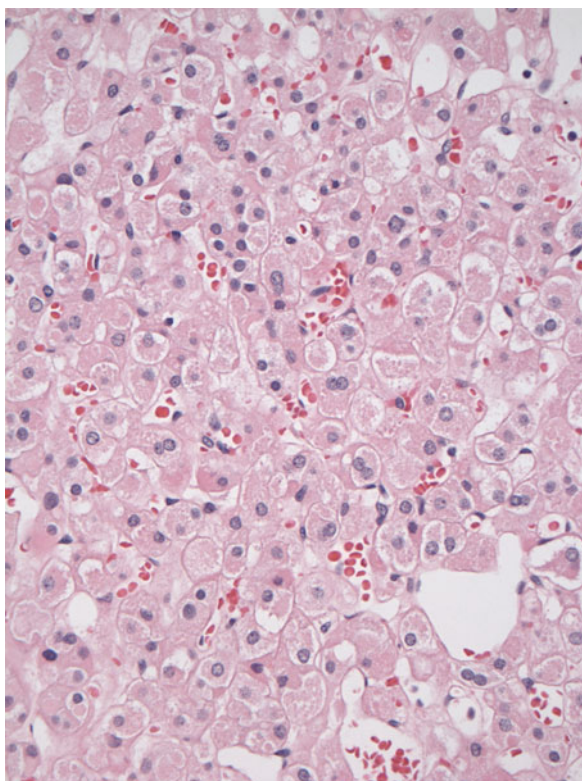


Fig. 2.6 Papillary type 2 RCC. Eosinophilic cells with nucleolus with a pseudostratified papillary arrangements

Fig. 2.7 Succinate dehydrogenase (SDHB) germline mutation-associated RCC. The cells have an eosinophilic cytoplasm with occasional vacuolization and bland nucleus (courtesy Dr. K. Trpkov—Calgary)



c-MET Pathway

HPRC and the sporadic cases are characterized by a type 1 pRCC defined by a monolayer of basophilic-cuboidal cells with scant cytoplasm, regular nuclei, and small nucleoli around capillary cores in 50–70 % of the entire tumor (Fig. 2.8). Expression of AMACR is also present [44]. Approximately 75 % of the sporadic forms have trisomy 7q31, which contains genes for *c-MET* and ligand *HGF*, but an activating *MET* mutation is seen in only 13 % of these sporadic cases [45]. In addition, gains in chromosome 17q (full trisomy, isochromosome 17q, or duplication of 17q21-qter) are typical.

Mucinous tubular and spindle-cell RCC is composed of small basophilic-cuboidal cells with round and elongated tubules and spindle cells with mucinous stroma (Fig. 2.9). It has some similarities with type 1 pRCC, with gains in 12q, 16q, 17, and 20q and losses in 1, 4, 6, 8, 9, 13, 14, 15, and 22, but no gains in 7 or 17 [46].

The RCC with PTEN mutation in Cowden syndrome is, in the majority of cases, similar to type 1 pRCC [47].

Tubulocystic RCC is composed of packed tubules and cysts lined by cuboidal or hobnail cells with eosinophilic cytoplasm and large nuclei showing prominent nucleoli (Fig. 2.10). The expression of AMACR and the gains in chromosomes 7 and 17 [48] are considered by some authors to suggest that it is closely related to type 1 pRCC [49].

FLCN Pathway

Mutation or loss of the wild-type allele of the *FLCN* gene has been identified in 70 % of BHD families, with associated risk of development of various RCC subtypes, especially chRCC, oncocytomas, and hybrid oncocytoma–chromophobe renal cell carcinoma. However, this mutation is present in only 10.9 % of the sporadic cases [50].

The cells of chRCC are larger than those of ccRCC. They display polyhedral outlines with good delimitation of the cellular membrane (giving them a vegetal cell appearance) and abundant pale reticular cytoplasm. Numerous, sometimes invaginated vesicles of 150–300 nm in diameter are present, resembling those of type B intercalated cells in the cortical collecting duct. The cytoplasm can be clear or eosinophilic according to the quantity of mitochondria [51] (Fig. 2.11). The architecture is solid, in sheets, and with a trabecular distribution. Losses in chromosomes Y, 1, 2, 6, 10, 3, 17, and 21 are typical of this RCC. The massive chromosomal losses lead to a hypodiploid DNA index. In spite of losses in chromosomes 10 and 17, there are no alterations in PTEN [52], and mutation of the *TP53* tumor suppressor gene is present in only 27 % of cases.

Overexpression of c-kit mRNA is found in not only chRCC but also oncocytomas, and differential expression of c-kit in renal tumors makes it an excellent immunohistochemical marker for diagnosis [53].

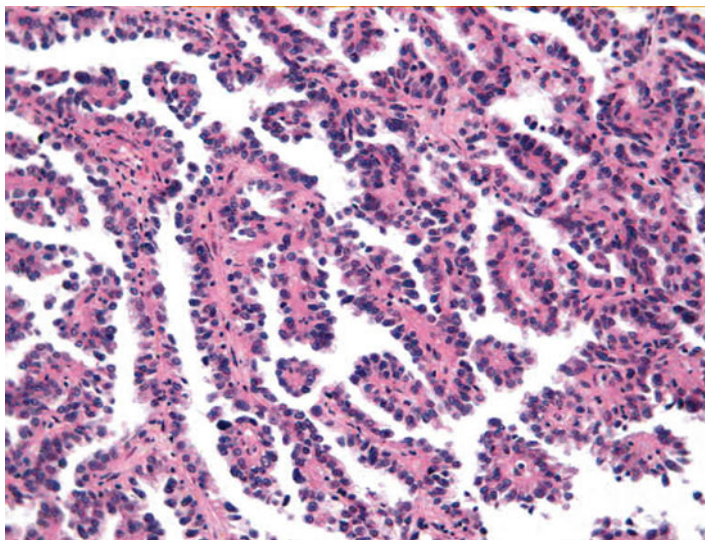


Fig. 2.8 Papillary type 1 RCC. Cuboidal cells with small nucleus and no evident nucleoli with scant cytoplasm (basophilic cells) arranged in a papillary way

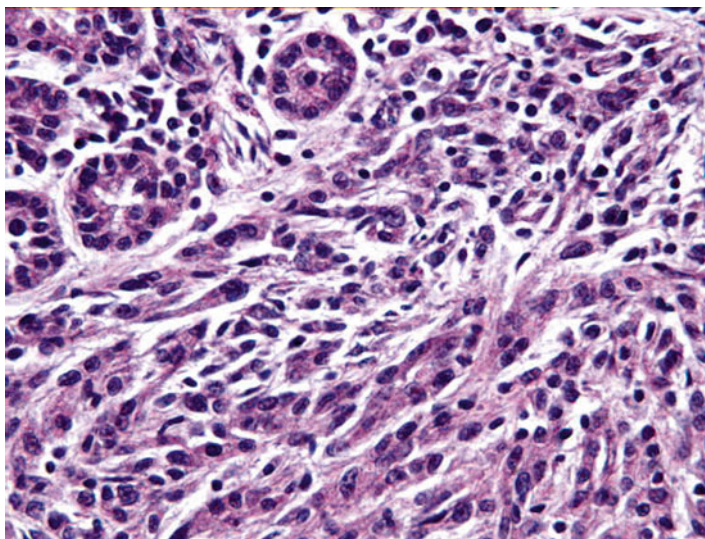


Fig. 2.9 Mucinous tubular and spindle-cell RCC. A neoplasm with bland nucleus cuboidal aspect (basophilic cells) arranged in a tubular way and with areas of spindle appearance for compression and mucinous stroma

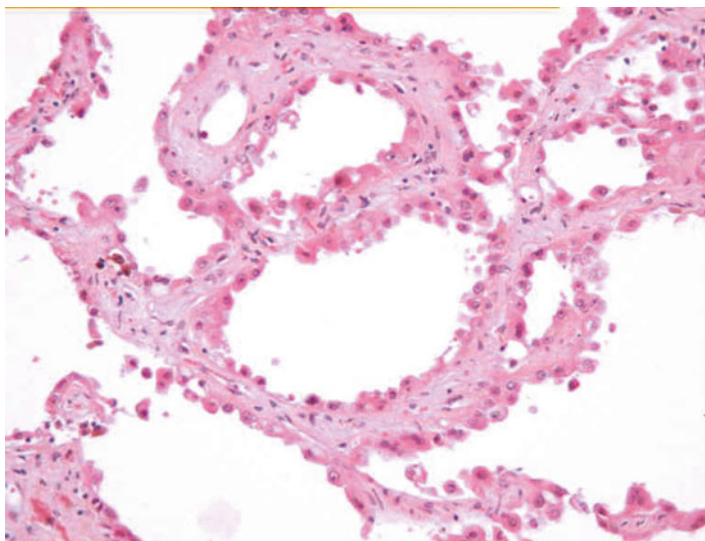


Fig. 2.10 Tubulocystic RCC. Neoplasm with cystic arrangement lined by cuboidal or hobnail cells with scant eosinophilic cytoplasm and occasionally large nuclei with evident nucleoli

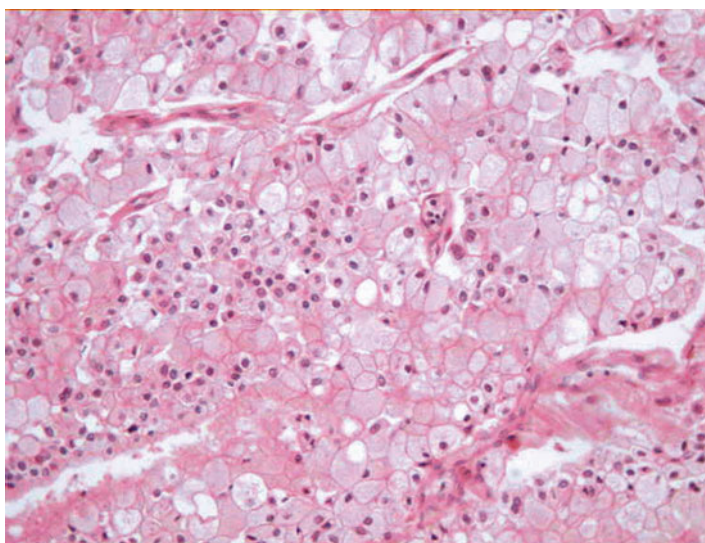


Fig. 2.11 Chromophobe RCC. Large cells with evident cellular outline with granular (clear-like) cytoplasm

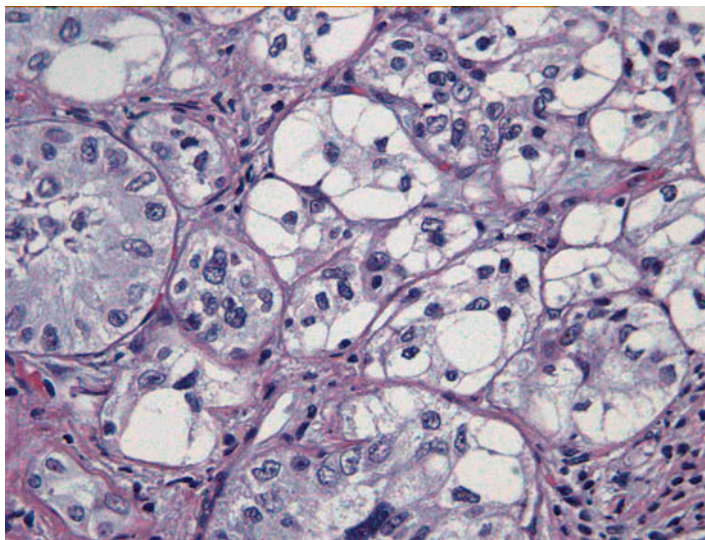


Fig. 2.12 MiT germline mutation RCC. Large cells with clear and eosinophilic cytoplasm, large nucleus, and solid or tubular arrangement

In folliculin-deficient RCC, increased TFE3 transcriptional activity has been found, and this represents a connection with the *MiT germline mutation RCC* [31]. RCCs with TFE3 accumulation display an increase in pS6, activation of the mTOR pathway, and HIF-1 α expression [33], but transactivation of the *MET* promoter by ASPL-TFE3 fusion protein has also been reported [54]. TFEB-associated RCCs express HMB45 and melanocytic markers. The morphology of the MiT germline mutation RCC is characterized by large and bizarre clear and eosinophilic cells, some papillary areas, calcifications, and a biphasic pattern in some cases [43] (Fig. 2.12).

Undefined Pathway

Collecting duct (Bellini) RCC (cdRCC) and medullary RCC (mRCC) are infrequent neoplasms characterized by very atypical cells and an overlapping appearance. Eosinophilic cells are present in a solid, papillary, or cribriform arrangement with desmoplasia in cdRCC (Fig. 2.13) while marked inflammatory cells are observed in the stroma in mRCC [43] (Fig. 2.14).

The molecular genetic abnormalities in these tumors are heterogeneous, and there have been few studies on the topic. Recently immunohistochemical loss of INI1 was found in 15 % of cdRCC [55], and many alterations suggestive of mRCC have been observed in the *INI1* gene (hSNF5/BAF47), a remodeling gene of cell differentiation [43].

Some of these tumors have high expression of c-MET and HIF-1 α , and for this reason, some authors also relate them to the pseudo-hypoxic pathway [54].

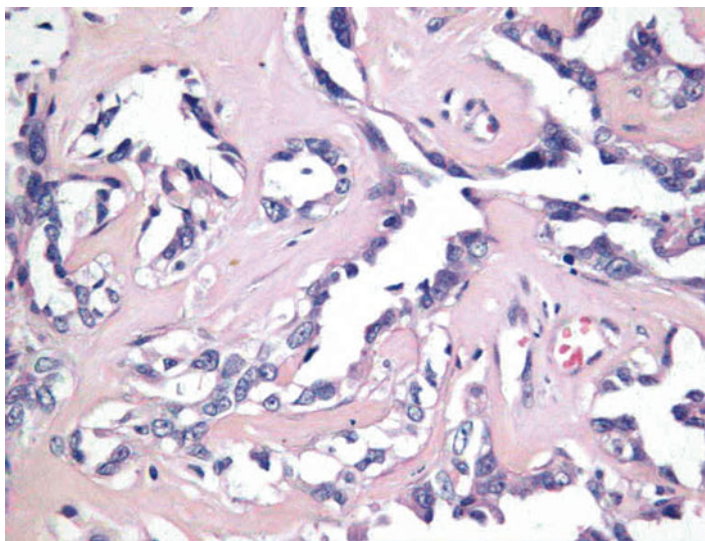


Fig. 2.13 Collecting duct RCC. High-grade carcinoma with tubular pattern in a desmoplastic stroma

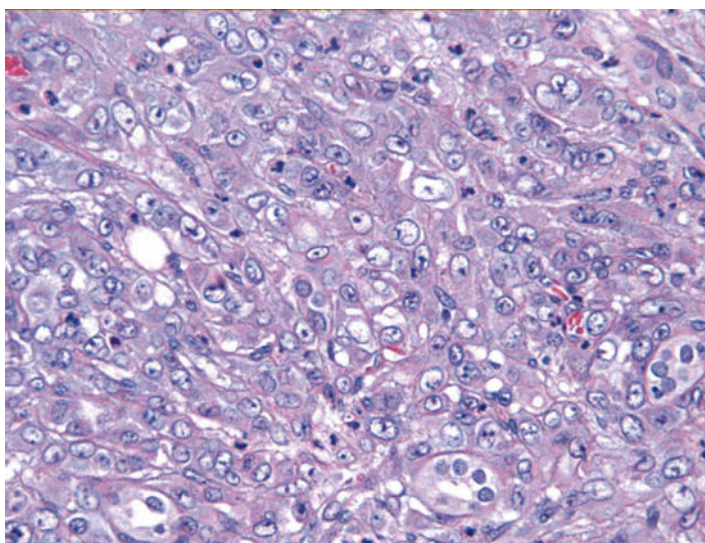


Fig. 2.14 Medullary RCC. Undifferentiated high-grade carcinoma in a solid pattern with some inflammatory cells. Notice sickle-cell erythrocytes

Other Pathological Renal Cell Carcinomas Entities

Other morphological subtypes of RCC with different chromosomal and molecular features have been reported, but the series of these other types are few in number and small; accordingly, conclusive data have not yet been obtained. The entities most frequently cited in the literature are discussed below:

Acquired Renal Cystic Disease-Associated Carcinoma: Patients with end-stage renal disease can have different RCC subtypes [56], but in those with acquired cystic disease of the kidney, the typical composition is large eosinophilic cells with a rounded nucleus and large nucleoli arranged in variety of architectural patterns; in addition, calcium oxalate crystals are observed within the tumors [57] (Fig. 2.15). These carcinomas express AMACR. At the molecular genetic level, gains in chromosomes 1, 2, 6, and 10 and monosomies 3, 9, and 16 are reported, suggesting a distinction from the other RCCs [58].

Clear Cell Tubulopapillary Renal Cell Carcinoma: This tumor was initially described in end-stage kidneys but has recently also been detected in nonterminal kidney disease. In 50 % of cases, a pronounced cystic component is observed; solid, tubular, and microcystic areas are also present. The tumor cells show a clear cytoplasm and low-grade nuclear atypia, with the nucleus situated toward the surface of the papillary tufts [59] (Fig. 2.16). They show neither deletion of 3p nor trisomies of chromosomes 7 and 17 [59].

Thyroid-Like Follicular Renal Cell Carcinoma: Very few cases of this entity have been reported. It has a follicular architecture resembling that of follicular carcinoma of the thyroid and is composed of cells showing low-grade pleomorphism with

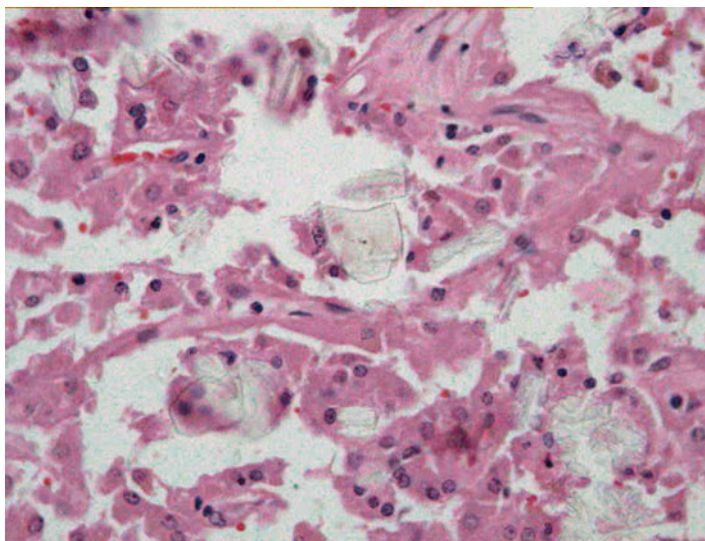


Fig. 2.15 Acquired cystic disease-associated RCC. Eosinophilic cells with calcium oxalate crystals

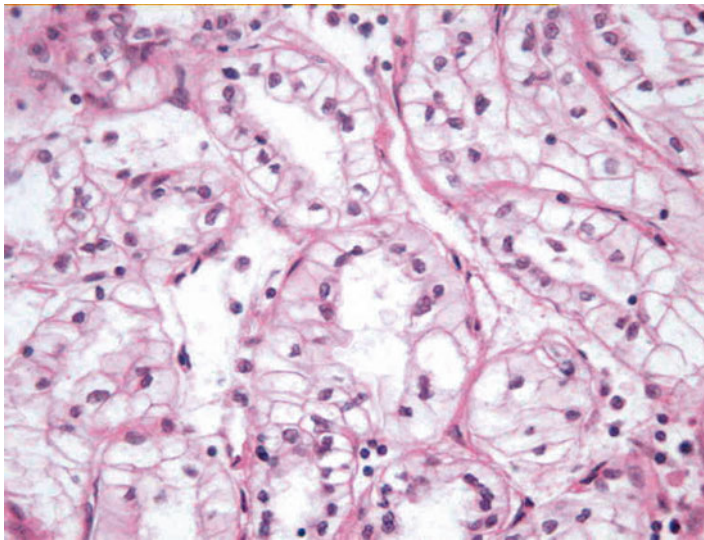


Fig. 2.16 Clear cell tubulo(papillary) RCC. Cuboidal clear cells with tubular arrangement (in some areas can be papillary) with apical nucleus localization

amphophilic to eosinophilic cytoplasm. Gene expression profiling has revealed widespread underexpression or overexpression involving chromosomes 1, 2, 3, 5, 6, 10, 11, 16, and 17 [60].

Leiomyomatous Renal Cell Carcinoma: This entity is composed of tubular aggregates of neoplastic clear cells intermixed in a prominent leiomyomatous proliferation. There is controversy over the chromosome 3 status [61].

Anaplastic Lymphoma Kinase (ALK) Translocation-Associated Renal Cell Carcinoma: This entity displays structural karyotypic abnormalities involving the ALK locus on chromosomal band 2p23 [62]; two cases of VCL (*vinculin*)-ALK fusion have been detected, and two each of TPM3-ALK and EML4-ALK fusions.

Biphasic Alveolosquamoid Renal Cell Carcinoma: There is a dual cell population, and the larger tumor cells with squamous features are arranged in well-demarcated islands, with the smaller cells surrounding them. Partial or complete losses of chromosomes 2, 5, 6, 9, 12, 15, 16, 17, 18, and 22 and partial gains of chromosomes 1, 5, 11, 12, and 13 have been reported [63].

Unclassified Renal Cell Carcinoma

This diagnostic category is for renal cell carcinomas that are impossible to classify as any of the other histological subtypes. It includes pure sarcomatoid RCCs without any evidence of the cellular origin, oncocytic RCCs without sufficient features for a precise diagnosis, any mixture of histological subtypes except oncocytoma–chromophobe varieties, and all RCCs with an unidentifiable morphology.

This diagnostic category can include different biologic entities, and for this reason in each individual case, the prognosis correlates only with the stage and grade [64].

Conclusions

After the identification of the chromosomal and molecular bases of the RCC familial syndromes and the discovery that these correspond with concrete morphological variants, it appeared that these same molecular alterations were present in the sporadic forms, with similar alterations being found in 90 % of sporadic ccRCCs and between 5 and 15 % of the other variants. In recent years, investigations have centered on the development of new therapies based on the consequences of HIF accumulation, the c-MET and FLCN mutations, and the pseudo-hypoxic status that they induce. On this basis it has been possible to reclassify RCCs according to the molecular pathway (Table 2.3) in order to help in therapeutic decision making.

However, not all the sporadic RCCs follow these pathways, and research continues. Recently some deletions in histone-modifying genes immediately next to the *VHL* gene have been detected in ccRCC. This observation has shifted biological interest away from hypoxia-induced epigenetic regulation and specifically toward the methylation of histone 3 and chromatin structure [65], opening potential avenues for new therapeutic approaches [66].

Table 2.3 Proposed renal cell carcinoma classification according to molecular pathway

Pseudo-hypoxic pathway
<i>VHL</i> pathway— <i>clear (empty) cells</i>
Clear cell renal cell carcinoma
Multilocular clear cell renal cell carcinoma
Krebs cycle mutations— <i>granular (eosinophilic) cells</i>
Papillary type 2 renal cell carcinoma
SDHB germline mutation-associated carcinoma
<i>C-MET</i> pathway— <i>basophilic-cuboidal cells</i>
Papillary type 1 renal cell carcinoma
Mucinous tubular and spindle renal cell carcinoma
Tubulocystic carcinoma
<i>FLCN</i> pathway— <i>large cells</i>
Chromophobe renal cell carcinoma
Hybrid oncocytoma—chromophobe renal cell carcinoma
MiT family renal cell carcinomas
Undefined pathway
Collecting duct renal cell carcinoma
Medullary renal cell carcinoma
Tubulopapillary clear cell renal cell carcinoma
Unclassified renal cell carcinomas
Pure sarcomatoid renal cell carcinoma
Mixed cellular types no chromophobe and oncocytoma
Oncocytic tumors without characteristics of typical subtype

References

1. Delahunt B, Thornton A. Renal cell carcinoma. A historical perspective. *J Urol Pathol*. 1996;4:31–49.
2. Oberling C, Rivière M, Haguénan F. Ultrastructure of the clear cells in renal carcinomas and its importance for the determination of their renal origin. *Nature*. 1960;186:402–3.
3. Bennington JL, Beckwith JB. Tumors of the kidney, renal pelvis, and ureter, Atlas of tumor pathology, Second series, vol. 12. Washington: AFIP; 1975. p. 130.
4. Thoenes W, Störkel S, Rumpelt HJ. Human chromophobe cell renal carcinoma. *Wichows Arch B Cell Pathol Incl Mol Pathol*. 1985;155:277–87.
5. Störkel S, Pannen B, Thoenes W, Staert PV, Wagner S, Drenckhalm D. Intercalated cells as a probable source for the development of renal oncocyoma. *Wichows Arch B Cell Pathol Incl Mol Pathol*. 1988;56:185–9.
6. Maher ER, Kaelin WG. von Hippel-Lindau disease. *Medicine*. 1997;76:381–91.
7. Kovacs G, Fucsi L, Emanuel A, Kung HF. Cytogenetics of papillary renal cell tumors. *Genes Chromosomes Cancer*. 1991;3:249–55.
8. Kiuru M, Launonen V, Hietala M, Aittomäki K, Vierimaa O, Salovaara R, Arola J, Pukkala E, Sistonen P, Herva R, Aaltonen LA. Familial cutaneous leiomyomatosis is a two-hit condition associated with renal cell cancer of characteristic histopathology. *Am J Pathol*. 2001;159:825–9.
9. Merino MJ, Torres-Cabala C, Pinto P, Linehan WM. The morphologic spectrum of kidney tumors in hereditary leiomyomatosis and renal cell carcinoma (HLRCC) syndrome. *Am J Surg Pathol*. 2007;31:1578–85.
10. Pavlovich CP, Walther MM, Eyler RA, Hewitt SM, Zbar B, Linehan WM, Merino MJ. Renal tumors in the Birt-Hogg-Dubé syndrome. *Am J Surg Pathol*. 2002;26:1542–52.
11. Sampson JR, Patel A, Mee AD. Multifocal renal cell carcinoma in sibs from a chromosome 9 linked (TSC1) tuberous sclerosis family. *J Med Genet*. 1995;32:848–50.
12. Eble JN, Sauter G, Epstein JI, Sesterhenn IA. World Health Organization classification of tumours. Pathology and genetics tumours of the urinary system and male genital organs. Lyon: IARC Press; 2004.
13. Bratslavsky G, Sudarshan S, Neckers L, Linehan WM. Pseudohypoxic pathways in renal cell carcinoma. *Clin Cancer Res*. 2007;13:4667–71.
14. Smaldone MC, Maranchie JK. Clinical implications of hypoxia inducible factor in renal cell carcinoma. *Urol Oncol*. 2009;27:238–45.
15. Hacker KE, Rathmell WK. Emerging molecular classification in renal cell carcinoma: implications for drug development. *Target Oncol*. 2010;5:75–84.
16. Kaelin Jr WG. New cancer targets emerging from studies of the Von Hippel-Lindau tumor suppressor protein. *Ann NY Acad Sci*. 2010;1210:1–7.
17. Gordan JD, Lal P, Dondeti VR, Letrero R, Parekh KN, Oquendo CE, Greenberg RA, Flaherty KT, Rathmell WK, Keith B, Simon MC, Nathanson KL. HIF- α effects on c-Myc distinguish two subtypes of sporadic VHL-deficient clear cell renal carcinoma. *Cancer Cell*. 2008;14:435–46.
18. Keith B, Johnson RS, Simon MC. HIF1 α and HIF2 α : sibling rivalry in hypoxic tumour growth and progression. *Nat Rev Cancer*. 2011;15(12):9–22.
19. Sudarshan S, Linehan WM, Neckers L. HIF and fumarate hydratase in renal cancer. *Br J Cancer*. 2007;96:403–7.
20. Gottlieb E, Tomlinson IP. Mitochondrial tumour suppressors: a genetic and biochemical update. *Nat Rev Cancer*. 2005;5:857–66.
21. Gill AJ, Pachter NS, Chou A, Young B, Clarkson A, Tucker KM, Winship IM, Earls P, Benn DE, Robinson BG, Fleming S, Clifton-Bligh RJ. Renal tumors associated with germline SDHB mutation show distinctive morphology. *Am J Surg Pathol*. 2011;35:1578–85.
22. Balsat M, Cornillon J. [m-TOR inhibitors: biology and use in the treatment of haematological diseases]. *Bull Cancer*. 2011;98:935–43.

23. Kim DH, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM. mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell*. 2002;110:163–75.
24. Zhou H, Luo Y, Huang S. Updates of mTOR inhibitors. *Anticancer Agents Med Chem*. 2010;10:571–81.
25. Toschi A, Lee E, Gadir N, Ohh M, Foster DA. Differential dependence of hypoxia-inducible factors 1 alpha and 2 alpha on mTORC1 and mTORC2. *J Biol Chem*. 2008;283:34495–9.
26. Martignoni G, Pea M, Reghellin D, Gobbo S, Zamboni G, Chilosi M, Bonetti F. Molecular pathology of lymphangioleiomyomatosis and other perivascular epithelioid cell tumors. *Arch Pathol Lab Med*. 2010;134:33–40.
27. Brugarolas JB, Vazquez F, Reddy A, Sellers WR, Kaelin Jr WG. TSC2 regulates VEGF through mTOR-dependent and -independent pathways. *Cancer Cell*. 2003;4:147–58.
28. Jimenez RE, Eble JN, Reuter VE, Epstein JI, Folpe AL, de Peralta-Venturina M, Tamboli P, Ansell ID, Grignon DJ, Young RH, Amin MB. Concurrent angiomyolipoma and renal cell neoplasia: a study of 36 cases. *Mod Pathol*. 2001;14:157–63.
29. Giubellino A, Linehan WM, Bottaro DP. Targeting the Met signaling pathway in renal cancer. *Expert Rev Anticancer Ther*. 2009;9:785–93.
30. Shuch B, Ricketts CJ, Vocke CD, Komiya T, Middleton LA, Kauffman EC, Merino MJ, Metwalli AR, Dennis P, Linehan WM. Germline PTEN mutation Cowden syndrome: an underappreciated form of hereditary kidney cancer. *J Urol*. 2013;190:1990–8.
31. Linehan WM, Ricketts CJ. The metabolic basis of kidney cancer. *Semin Cancer Biol*. 2013;23:46–55.
32. Malouf GG, Camparo P, Molinié V, Dedet G, Oudard S, Schleiermacher G, Theodore C, Dutcher J, Billefont B, Bompas E, Guillot A, Boccon-Gibod L, Couturier J, Escudier B. Transcription factor E3 and transcription factor EB renal cell carcinomas: clinical features, biological behavior and prognostic factors. *J Urol*. 2011;185:24–9.
33. Argani P, Hicks J, De Marzo AM, Albadine R, Illei PB, Ladanyi M, Reuter VE, Netto GJ. Xp11 translocation renal cell carcinoma (RCC): extended immunohistochemical profile emphasizing novel RCC markers. *Am J Surg Pathol*. 2010;34:1295–303.
34. Kucejova B, Peña-Llopis S, Yamasaki T, Sivanand S, Tran TA, Alexander S, Wolff NC, Lotan Y, Xie XJ, Kabbani W, Kapur P, Brugarolas J. Interplay between pVHL and mTORC1 pathways in clear-cell renal cell carcinoma. *Mol Cancer Res*. 2011;9:1255–65.
35. Algaba F, Akaza H, López-Beltrán A, Martignoni G, Moch H, Montironi R, Reuter V. Current pathology keys of renal cell carcinoma. *Eur Urol*. 2011;60:634–43.
36. Al-Ahmadie HA, Alden D, Fine SW, Gopalan A, Touijer KA, Russo P, Reuter VE, Tickoo SK. Role of immunohistochemistry in the evaluation of needle core biopsies in adult renal cortical tumors: an ex vivo study. *Am J Surg Pathol*. 2011;35:949–61.
37. Halat S, Eble JN, Grignon DJ, Lopez-Beltran A, Montironi R, Tan PH, Wang M, Zhang S, MacLennan GT, Cheng L. Multilocular cystic renal cell carcinoma is a subtype of clear cell renal cell carcinoma. *Mod Pathol*. 2010;23:931–6.
38. Varela I, Tarpey P, Raine K, et al. Exome sequencing identifies frequent mutation of the SWI/SNF complex gene PBRM1 in renal carcinoma. *Nature*. 2011;469:539–42.
39. Dalgliesh GL, Furge K, Greenman C, et al. Systematic sequencing of renal carcinoma reveals inactivation of histone modifying genes. *Nature*. 2010;463:360–3.
40. Linehan WM. Genetic basis of kidney cancer: role of genomics for the development of disease-based therapeutics. *Genome Res*. 2012;22:2089–100.
41. Delahunt B, Eble JN. Papillary renal cell carcinoma: a clinicopathologic and immunohistochemical study of 105 tumors. *Mod Pathol*. 1997;10:537–44.
42. Sanders ME, Mick R, Tomaszewski JE, Barr FG. Unique patterns of allelic imbalance distinguish type 1 from type 2 sporadic papillary renal cell carcinoma. *Am J Pathol*. 2002;161:997–1005.
43. Srigley JR, Delahunt B, Eble JN, Egevad L, Epstein JI, Grignon D, Hes O, Moch H, Montironi R, Tickoo SK, Zhou M, Argani P, ISUP Renal Tumor Panel. The International Society of Urological Pathology (ISUP) Vancouver classification of renal neoplasia. *Am J Surg Pathol*. 2013;37:1469–89.

44. Tickoo SK, Reuter VE. Differential diagnosis of renal tumors with papillary architecture. *Adv Anat Pathol*. 2011;18:120–32.
45. Schmidt L, Junker K, Nakaigawa N, et al. Novel mutations of the MET proto-oncogene in papillary renal carcinomas. *Oncogene*. 1999;18:2343–50.
46. Cossu-Rocca P, Eble JN, Delahunt B, Zhang S, Martignoni G, Brunelli M, Cheng L. Renal mucinous tubular and spindle carcinoma lacks the gains of chromosomes 7 and 17 and losses of chromosome Y that are prevalent in papillary renal cell carcinoma. *Mod Pathol*. 2006;19:488–93.
47. Mester JL, Zhou M, Prescott N, Eng C. Papillary renal cell carcinoma is associated with PTEN hamartoma tumor syndrome. *Urology*. 2012;79:1187.
48. Yang XJ, Zhou M, Hes O, Shen S, Li R, Lopez J, Shah RB, Yang Y, Chuang ST, Lin F, Tretiakova MM, Kort EJ, Teh BT. Tubulocystic carcinoma of the kidney: clinicopathologic and molecular characterization. *Am J Surg Pathol*. 2008;32:177–87.
49. Zhou M, Yang XJ, Lopez JI, Shah RB, Hes O, Shen SS, Li R, Yang Y, Lin F, Elson P, Sercia L, Magi-Galluzzi C, Tubbs R. Renal tubulocystic carcinoma is closely related to papillary renal cell carcinoma: implications for pathologic classification. *Am J Surg Pathol*. 2009;33:1840–9.
50. Gad S, Lefèvre SH, Khoo SK, Giraud S, Vieillefond A, Vasiliu V, Ferlicot S, Molinié V, Denoux Y, Thiounn N, Chrétien Y, Méjean A, Zerbib M, Benoît G, Hervé JM, Allègre G, Bressac-de Paillerets B, Teh BT, Richard S. Mutations in BHD and TP53 genes, but not in HNF1beta gene, in a large series of sporadic chromophobe renal cell carcinoma. *Br J Cancer*. 2007;29(96):336–40.
51. Thoenes W, Störkel S, Rumpelt H-J, Moll R, Baum HP, Werner S. Chromophobe cell renal carcinoma and its variants—a report on 32 cases. *J Pathol*. 1988;155:277–87.
52. Sükösd F, Digon B, Fischer J, Pietsch T, Kovacs G. Allelic loss at 10q23.3 but lack of mutation of PTEN/MMAC1 in chromophobe renal cell carcinoma. *Cancer Genet Cytogenet*. 2001;128:161–3.
53. Huo L, Sugimura J, Tretiakova MS, Patton KT, Gupta R, Popov B, Laskin WB, Yeldandi A, Teh BT, Yang XJ. C-kit expression in renal oncocytomas and chromophobe renal cell carcinomas. *Hum Pathol*. 2005;36:262–8.
54. Williamson SR, Eble JN, Cheng L. Molecular pathology of kidney tumors. In: Cheng L, Eble JN, editors. *Molecular surgical pathology*. New York: Springer; 2013. p. 171–212.
55. Elwood H, Chaux A, Schultz L, et al. Immunohistochemical analysis of SMARCB1/INI-1 expression in collecting duct carcinoma. *Urology*. 2011;78:474.
56. Tickoo SK, dePeralta-Venturina MN, Harik LR, Worcester HD, Salama ME, Young AN, Moch H, Amin MB. Spectrum of epithelial neoplasms in end-stage renal disease: an experience from 66 tumor-bearing kidneys with emphasis on histologic patterns distinct from those in sporadic adult renal neoplasia. *Am J Surg Pathol*. 2006;30:141–53.
57. Rioux-Leclercq NC, Epstein JI. Renal cell carcinoma with intratumoral calcium oxalate crystal deposition in patients with acquired cystic disease of the kidney. *Arch Pathol Lab Med*. 2003;127:E89–92.
58. Cossu-Rocca P, Eble JN, Zhang S, Martignoni G, Brunelli M, Cheng L. Acquired cystic disease-associated renal tumors: an immunohistochemical and fluorescence in situ hybridization study. *Mod Pathol*. 2006;19:780–7.
59. Gobbo S, Eble JN, Grignon DJ, Martignoni G, MacLennan GT, Shah RB, Zhang S, Brunelli M, Cheng L. Clear cell papillary renal cell carcinoma: a distinct histopathologic and molecular genetic entity. *Am J Surg Pathol*. 2008;32:1239–45.
60. Jung SJ, Chung JI, Park SH, Ayala AG, Ro JY. Thyroid follicular carcinoma-like tumor of kidney: a case report with morphologic, immunohistochemical, and genetic analysis. *Am J Surg Pathol*. 2006;30:411–5.
61. Srigley JR, Delahunt B. Uncommon and recently described renal carcinomas. *Mod Pathol*. 2009;22 Suppl 2:S2–23.
62. Debelenko LV, Raimondi SC, Daw N, Shivakumar BR, Huang D, Nelson M, Bridge JA. Renal cell carcinoma with novel VCL-ALK fusion: new representative of ALK-associated tumor spectrum. *Mod Pathol*. 2011;24:430–42.

63. Petersson F, Bulimbasic S, Hes O, Slavik P, Martínek P, Michal M, Gomolčáková B, Hora M, Damjanov I. Biphasic alveolosquamoid renal carcinoma: a histomorphological, immunohistochemical, molecular genetic, and ultrastructural study of a distinctive morphologic variant of renal cell carcinoma. *Ann Diagn Pathol.* 2012;16:459–69.
64. Lopez-Beltran A, Kirkali Z, Montironi R, Blanca A, Algaba F, Scarpelli M, Yorukoglu K, Hartmann A, Cheng L. Unclassified renal cell carcinoma: a report of 56 cases. *BJU Int.* 2012;110:786–93.
65. Hakimi AA, Chen YB, Wren J, Gonen M, Abdel-Wahab O, Heguy A, Liu H, Takeda S, Tickoo SK, Reuter VE, Voss MH, Motzer RJ, Coleman JA, Cheng EH, Russo P, Hsieh JJ. Clinical and pathologic impact of select chromatin-modulating tumor suppressors in clear cell renal cell carcinoma. *Eur Urol.* 2013;63:848–54.
66. Catto JW, Shariat SF. The changing face of renal cell carcinoma: the impact of systematic genetic sequencing on our understanding of this tumor's biology. *Eur Urol.* 2013;63:855–7.

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