Current insights in renal cell cancer pathology

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Abstract

In recent years molecular biologists and pathologists have described new entities of renal cell cancer (RCC) with a totally different morphology and biology among the histotypes of renal carcinoma, but always referring to the same renal cancer disease. The evidence of a distinct biological behavior and long-term prognosis among these makes the correct pathological diagnosis of renal cancer critically important for the clinician. Advances in understanding of the pathogenesis, behavior, and importance of prognostic factors for RCC have paved the way for a revision of its classification and staging. We reviewed the role of histological classification, microscopic tumor necrosis, microscopic venous invasion, lymph node involvement and, particularly, pathological stage. In our series of patients who underwent renal surgery for neoplasm, a retrospective study established the predictive role of tumor size on recurrence rate, compared with other known prognostic factors, and we conclude that histological grade, pathological stage and tumor size remain relevant prognosticators in early stage RCC patients. In order to optimize the management of patients with RCC it is necessary to develop an interdisciplinary approach (surgeon, radiologist, pathologist, oncologist) and find new prognostic parameters at molecular and cellular levels. Many efforts are ongoing to integrate molecular data (from tissue microarrays) and clinical data (traditional prognosticators) into a molecular integrated staging system. In the postgenomic era, new tumor-associated antigens and molecules can be identified at the protein level using proteomics, providing a major opportunity for screening and finding novel targets that are the basis of new emerging therapies for RCC. © 2008 Elsevier Inc. All rights reserved.

Keywords: Kidney neoplasm; Renal cell cancer; Pathological diagnosis; Prognosis; Targeted therapy

1. Introduction

Renal cell cancer (RCC) is a relatively uncommon solid tumor, accounting for about 2\% of all adult malignancies, but this rate of incidence is rising in Europe with the concomitant decrease of mortality rates [1]. In the 1980s, renal cancer was basically one disease: the higher the stage, the higher the grade, and the worse the prognosis. After the 1980s, molecular biologists and pathologists described new entities with totally different morphological and biological aspects, but always referring to the same renal cancer. The evidence of a distinct biological behavior and long-term prognosis among the histotypes of renal carcinoma makes the correct pathological diagnosis of a renal cancer critically important for the clinician. In this sense, the knowledge of specific chromosome alterations for each renal cancer histotype could provide the pathologist with an essential support. Currently, it is well accepted that RCC does not constitute a single biological entity but, rather, a varied group of malignancies.

We made a review of renal cell cancer malignancies and their clinical-pathological features related to the currently used histological classification; we evaluated the prognostic significance of pathological stage, tumor grade, microscopic
tumor necrosis, microscopic venous invasion, lymph node involvement. In addition, the relationship between RCC and new targeted approaches was taken into account.

2. Classification

In recent years, relevant advances in the understanding of the pathogenesis of renal neoplasia have been made, determining the proposal of increasingly complex classification systems. Before 1986 it was generally accepted that renal cell tumors could be subdivided into benign and malignant forms depending on whether the tumor diameter was smaller or greater than 3 cm. Malignant tumors were classified as renal adenocarcinoma or RCC, and while reports recognized that some morphotypes were associated with a more favorable prognosis, this was not generally considered in most classifications [2]. The Mainz classification diagnostic categories were defined at different consensus conferences held in 1986, 1996, and 1997, when the establishment of the Heidelberg/Rochester classification of renal parenchymal tumors provided a variety of subtypes of RCC, yielding a description of additional categories of renal cell neoplasia; RCC was thus seen not as a single tumor entity but, rather, as a group of tumors in which each subtype has unique morphologic and genotypic features. The studies on established subtypes of renal cell neoplasia are not confounded by the inclusion of atypical forms or hitherto unrecognized diagnostic categories. Molecular genetics had an impact on the classification of renal cell tumors. Genetic alterations affect the biology of the tumor cells, altering proliferation, cell death, differentiation, and cell adhesion; these different properties play a role in determining both the morphology and the behavior of tumors. In 2004, WHO published the new classification, which represents an extensive revision of the 1981 and 2002 classifications of renal cell cancer [3], with the five main histological subtypes of RCC (clear cell, papillary, chromophobe, collecting duct, and unclassified renal cell carcinomas) flanked by several new tumor entities. These have been described since the Heidelberg/Rochester consensus conference, considering morphological aspects and chromosome alterations, obtained by cytogenetic analysis of the neoplastic karyotypes. Most pathologists use recent classifications of renal tumors based on cytomorphologic and genetic characteristics (Table 1).

Nevertheless, the question of whether different histological variants of RCC portend different survival outcomes remains controversial.

3. Malignant renal cell tumors

3.1. Conventional renal cell carcinoma

Conventional cell renal carcinoma is the most frequently described histotype of RCC and accounts for 60% to 70% of cases. Sometimes, in the absence of clear cells, diagnosis of the tumor relies on the detection of a characteristic vascular network. In most cases, clear cytoplasm cells clustered in small groups are identified even in tumors composed predominantly of cells with an eosinophilic cytoplasm (Fig. 1). The term “conventional” is used to avoid the word clear, because not all types in this group present clear cells. At present, a peculiar set of chromosome alterations is known only for its conventional and papillary histotypes (accounting for about 85% to 90% of renal cancers), while these data are not yet available for the chromophobe nor for the collecting duct histotypes. The clear cell carcinoma type is

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<td>Chromophobe</td>
<td>BHD tumor suppressor</td>
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Genetic mechanisms are responsible for an inherited (germline) or acquired (somatic) mutations in tumor suppressors or oncogenes: loss of heterozigosity (LOH), homozygous deletion, point mutation, rearrangement, promoter hypermethylation.
associated mostly with germ line mutations of the von Hippel-Lindau suppressor gene, and some somatic mutations. Some of these sporadic tumors will also have a mutation in 3p, but basically these are the most common tumors to be recognized. Thus, approximately half of the cases of clear cell RCC show mutations of the VHL gene or hypermethylation of its promoter region. VHL-mutated RCC cases show a more favorable prognosis than those without VHL alterations [4]. Mutation of the VHL gene at 3p25–26, gene rearrangement, or promoter region hypermethylation is frequently associated with clear cell RCC [5]. Additional regions on 3p are associated with clear cell carcinogenesis, and loss of heterozygosity (LOH) studies have shown mutations at 3p14.2 and 3p21 to be early events in neoplastic transformation [6]. Recent studies have demonstrated that while a variety of chromosomal abnormalities are associated with advanced tumors, and LOH at 9p13, 14q and 10q (PTEN/MMAC1) are associated with poor prognosis, gains of 5q31-qter correlate with a more favorable outcome [7].

A recent report suggests that VHL status may have prognostic significance for patients with sporadic clear cell RCC. VHL gene, a key player in hypoxia-signaling pathway, is mutated or hypermethylated in 40% to 70% of sporadic clear RCC; it was detected in 108 of 187 RCC tumor samples, and VHL alterations were strongly associated with better cancer-free survival for 134 patients with stage I–III clear cell RCC treated by radical nephrectomy. When VHL suppressor protein function is lost or in hypoxia, the hypoxia-inducible factor (HIF) is accumulated at intracellular level and transactivates many genes responsible for the ability of the cancers to adapt to a hypoxic environment, and also for their resistance to radiation and chemotherapy. VHL can be considered a gate-keeper gene and remains an independent prognostic factor for patients with stage I–III tumors after adjustment for gender, age, stage, grading, and symptomatic presentation; furthermore it represents a molecular target for new therapies [8,9].

Although the histological grade and a variety of new molecular parameters have been shown to be of clinical utility, clear cell carcinoma prognosis is still based on evaluation of the clinical-pathological stage. The Robson staging system classifies all localized tumors as stage 1, while in the UICC/TNM system tumor size is still considered [10]. Patients with clear cell renal carcinoma have a poorer prognosis as compared with patients with papillary and chromophobe renal cell carcinoma, while there is no significant difference in cancer-specific survival between patients with papillary and chromophobe renal cell carcinoma.

3.2. Multilocular cystic renal cell carcinoma

Unlike clear cell carcinoma, multilocular cystic RCC seems to have a favorable course as, in more than 50 cases reported in literature to date, no metastatic disease has been described. These tumors are composed of cystic structures with fibrous tissue septae lined by a single layer of epithelial cells with a clear cytoplasm, and usually exhibit a low nuclear grade [11]. Clear cells are also present individually within the septal wall. If epithelial nests are visible microscopically, then the tumor should be diagnosed as a clear cell carcinoma. The true nature of multilocular cystic RCC and its relationship to clear cell RCC remain to be elucidated [12].

3.3. Papillary renal cell carcinoma

In 1989, Kovacs et al. reported that clear cell carcinomas have abnormalities of 3p, and that there is an entity called papillary carcinoma that has no abnormalities of 3p, but only polysomies of chromosomes 7 or 17 and a deficiency of Y. So he classified the tumors as papillary and nonpapillary; papillary tumors that only had the genetic abnormality of trisomy 7 were benign, a hypothesis that has never been tested. Papillary RCC account for approximately 10% of RCC in large surgical series [13].

This tumor typically consists of a central fibrovascular core with epithelial covered papillae, although a compact
tubular architecture or sheets of short papillae resembling glomeruli may be found. It is possible to distinguish two varieties: type 1 tumors, with papillae covered by small, scanty cytoplasm cells arranged in a single layer on the papillary basement membrane, often contain aggregates of foamy macrophages and scattered psammoma bodies (Fig. 2); type 2 tumors, with pseudostratification of voluminous epithelial cells, show an eosinophilic cytoplasm and a higher nuclear grade (Fig. 3). These tumors are usually multifocal and are frequently associated with sclerosis of adjacent non-neoplastic renal tissue. Immunohistochemically, papillary RCCs coexpress vimentin and epithelial markers and are also often positive for CD-10 (93%) RCC antibody (93%) and S-100 protein (55%). Cytokeratin 7 and MUC1 immunohistochemical expression is more frequently seen in type 1 than type 2 papillary RCC [12].

Zbar et al. [14] showed the hereditary nature of papillary carcinoma: these forms had mutations of the MET proto-oncogene, and now we divide tumors into type 1 (small cells forming a single layer or the pure variant) and type 2 (large cells with pseudostratification and a solid architecture). Type 2 tumors tend to show a more unfavorable clinical outcome. Immunohistochemical and lectin histochemical studies have revealed both proximal and distal nephron phenotypes. Renshaw et al. [15] showed that, using in situ hybridization, patients also with trisomy 7, trisomy 17, and with a normal chromosome 3 presented a solid variant of papillary carcinoma (glomeruloid variant). And again, looking at a large cohort of patients, the pure variants of papillary carcinoma are really the minority, at least in this sporadic group.

Types 1 and 2 tumors differ in terms of genotype and clinical outcome. Type 1 tumors show gains of chromosomes 7p and 17p, and differing patterns of allelic imbalance at 17q and 9p have been noted between the two tumor types. Type 1 tumors are usually of lower nuclear grade and clinical stage than type 2 tumors, while longer post-treatment survival for patients with type 1 tumors has been shown on multivariate analysis [16].

Amin et al. published a study showing that in univariate analysis the grade seems to have a prognostic value [17] but as soon as the grade is inserted in multivariate analysis, as in clear cell carcinoma, the grade and histotype seem to lose their prognostic significance. This induced Delahunt et al. to
suggest a different type of grading scheme for papillary cancers [16].

Unfortunately, rather than using the terms low grade–high grade, they also chose type 1, type 2, complicating the diagnosis. Some data suggest the type was actually more powerful, as the \( P \)-value showed in Delahunt’s study, than traditional histotype and grade. The limit of this study, of course, is the small size and lack of statistics, as far as the mortality rate is concerned. Neither using the Fuhrman classification nor this particular type of classification really helps in grading these tumors.

3.4. Chromophobe renal cell carcinoma

Before 1986, chromophobe RCCs were included in the class of clear cell RCC, assuming them to have a low histological grade and so a favorable outcome. The publication of detailed descriptions of similar tumors in nitrosamine-induced animal models led to the realization that this was a new group of RCCs, later shown to have a low malignancy potential. As in carcinogen-induced chromophobe RCC in rodents, human chromophobe RCCs exhibit a wide histological spectrum, with typical balloon cells with an abundant granular pale cytoplasm, or sometimes tumors composed of smaller cells with a deeply eosinophilic cytoplasm, resembling those commonly associated with oncocytoma [18].

It may be difficult to differentiate between the eosinophilic variant of chromophobe RCC and oncocytoma, particularly in larger tumors, and a hybrid form of the tumor has been suggested to exist. This is supported by the fact that chromophobe RCC and oncocytoma may coexist in the kidney, in the form of multiple tumors in the same kidney (oncocytomatosis).

Histologically, chromophobe RCC shows mostly a solid pattern of growth with abundant granular eosinophilia in the cytoplasm (in 1982 these forms were called “granular renal cell carcinomas,” before Stephan Strokel and Wolfgang Tunis from Germany defined them as separate entities) (Fig 4). Pathologists have learned to ignore the aggressive appearance of the nuclei of chromophobe cells because these tumors have a much better prognosis. About 10% of chromophobe RCC show eosinophilic cells and in such cases features in favor of carcinoma over oncocytoma are: (1) cellular discohesion in paraffin-embedded sections, (2) wrinkling of the nuclear margin with an inconspicuous nucleolus, (3) perinuclear cytoplasmic clearing (perinuclear halo), (4) hyalinization of the walls of larger vessels, (5) diffuse Hale’s colloidal iron staining, (6) the presence of classical “balloon” chromophobe cells elsewhere in the tumor (sample widely), and (7) the presence of amorphous calcific deposits but not psammoma bodies within the tumor interstitium [12].

Immunohistochemistry provides limited information for distinguishing chromophobe RCC from oncocytoma. About 10% of oncocytoma, 28% of classic chromophobe RCC, and 100% of eosinophilic chromophobe RCC show CD10 expression. Both types of chromophobe RCC are CK7 positive in >80% of tumors, while 100% of oncocytoma present CK7 positivity. Recent reports suggest that CD74 expression may differentiate chromophobe RCC from oncocytoma, positive staining being reported in 4 of 6 carcinomas, compared with negative staining in all of 8 oncocytomas studied [19].

Molecular biology has demonstrated that these tumors are not characterized by polysomy 7s or 17s or losses of 3p. These tumors are hypodiploid; they typically show loss of heterozygosity involving numerous chromosomes and chromosome regions, 1, 2, 6, 10, 13, 17, and 21 monosomy and loss of X or Y being most frequently reported [20]. Oncocytomas are characterized by a variety of LOH in chromosome 1, 6p, 14, and/or 21 in some tumors, while 5:11 translocation has also been reported. Recent studies have shown that loss of two or more among chromosomes 1, 2, 6, 10, and 17, as detected by FISH, differentiates classic/eosinophilic chromophobe RCC from oncocytoma, having 90% sensitivity and 100% specificity [21]. Bugert et al. analyzed 42 chromophobe renal cell carcinomas for allelic
losses by employing microsatellite markers. Loss of chromosomes 1, 2, 6, 10, 13, and 17 was detected in between 75% and 95% of tumors, and loss of chromosome 21 was observed in 54% of cases. All but one tumor showed a combination of monosomes at the specific chromosomes [22]. According to the molecular pattern, chromophobe RCC is believed to originate from the intercalated cells of the collecting duct.

So despite the fact that many of these tumors appear to be extracapsular (25%), larger than other RCC (about 9 cm) and that these tumors represent an extremely large group, they seem to have much better prognosis than clear cells, and also much better prognosis than papillary carcinomas.

Oncocytomas present hypovascular stroma in the background, and a nest of eosinophilic cells. They look mahogany-brown, because they contain abundant amounts of mitochondria. Unfortunately, chromophobe carcinomas can do the same. Based on the following facts, oncocytoma and chromophobe RCC are considered to be intimately related. First, both are considered to be derived from the intercalated cell of the collecting duct. Second, both are expected to have alterations of mitochondria, i.e., rearrangements of mitochondrial DNA and increased mitochondria in oncocytoma and numerous mitochondria-derived microvesicles in chromophobe RCC. Third, both are frequently observed in oncycytosis, with or without Birt-Hogg-Dubé (BHD) syndrome. In addition, there are several reports of a hybrid tumor-composed mixture of oncocytic and chromophobe elements. Therefore, oncocytoma could be the benign counterpart of chromophobe RCC.

The identification of hybrid lesions between oncocytomas and hybrid tumors and chromophobe tumors, have raised the hypothesis that there might be hybrid diseases, and so we have used the term oncycytosis. It is a rare pathological condition in which renal parenchyma is replaced by numerous oncocytic nodules. Recently, the candidate gene responsible for BHD syndrome has been cloned (BHD gene), a cancer suppressor gene for chromophobe RCC [23]. The BHD gene product is named folliculin, which is conserved among and between species. Nevertheless, the protein does not belong to any known protein family and its function remains to be elucidated. Could there be a missing link, or could this family teach us what the initiating events of renal carcinogenesis really are, and what steps lead it to take one pathway or the other? It is a very exciting area. Recently, the morphologic manifestations of renal tumors in the BHD syndrome have been published [23]. The BHD syndrome is a familial hamartomatous syndrome, which is characterized by the triad composed of fibrofolliculoma, spontaneous pneumothorax, and renal neoplasm. Considerable numbers of renal neoplasms are chromophobe RCC and oncocytomas, as are clear RCC with a background of oncycytosis.

Several studies have confirmed the favorable prognosis of chromophobe RCC with metastatic spread, which is seen in less than 10% of tumors, regardless of the size of the primary malignancy. Early indications were, however, that chromophobe RCC was associated with a higher rate of sarcomatoid progression than other RCC morphotypes and recent studies have confirmed these early observations, reporting tumors showing sarcomatoid morphology in more than one high power field in approximately 10% of cases [24].

3.5. Collecting duct carcinoma

The last, and most dangerous, type is collecting duct carcinoma (Bellini’s carcinoma), a rare histotype of renal cancer. Its low incidence (<1% of RCC) makes the natural history difficult to define; however, the clinical behavior is often aggressive with an inauspicious prognosis. Advanced and metastatic disease is present in about 35% to 40% of patients and two thirds of them die within 2 years of diagnosis, because it is refractory to chemotherapy and immunotherapy. In accordance with the Heidelberg classification, after 1997, collecting duct carcinoma underwent cytogenetic analysis, like all the renal tumors treated in the same period. There are not yet any definitive diagnostic criteria of this subtype, and diagnostic confusion reigns among pathologists because of the low incidence and lack of reliable diagnostic criteria; moreover, there are few case studies.

This rare tumor is characterized by pleomorphic cells arranged in irregular tubules within a desmoplastic stroma. A cytological atypia has often been described in residual Bellini ducts near the tumor. These tumors originate in the renal medulla but the site of origin is often unclear due to the advanced stage of the tumor at diagnosis. Immunoreexpression of *Ulex europaeus* agglutinin lectin is an important diagnostic feature. The nature of so-called low grade collecting duct carcinoma is unresolved and its relationship to true collecting duct carcinoma remains in doubt. Unlike true collecting duct tumors, low grade carcinomas have been found confined to the renal cortex. Their designation as low grade carcinoma is also debated, as they do have a metastatic potential and in the main they exhibit Fuhrman grades 2 and 3 morphology.

No peculiar set of chromosome alterations for collecting duct carcinoma has yet been identified due to its low incidence; this fact has likely led to an over-diagnosis of unclassifiable renal cancers, particularly in cases with complex morphological aspects. Monosomes of chromosomes 18 and 21, and the loss of chromosome Y have been described by Cavazzana et al. [25] and Schoenberg et al. [26]. These authors also reported the gain of chromosomes 7, 12, 17, and 20. Using polymorphic microsatellite markers, other investigators have described the loss of heterozygosity (LOH) on chromosome 1q in 57% to 69% of cases, especially in the region of 1q32. LOH has also been observed on chromosomes 8p (48%), 6p (45%), 21q (40%), and 13q (50%), while alterations of chromosome 3 have rarely been described. Monosomy of chromosomes 1, 6, 14, 15, and 22 was observed by Fuzesi et al. [27]. Molecular biology and cytogenetic studies are very limited. To elucidate the char-
acteristics of collecting duct RCC, reliable diagnostic criteria should be established, helping to make a differential diagnosis from papillary RCC. Antonelli et al. [28] indicate that collecting duct carcinomas are cytogenetically characterized by: (1) a hypodiploid stemline; (2) a high number of numerical and structural chromosome aberrations, and (3) a common involvement of chromosome 1 and the autosomes.

In our series of 521 patients who underwent renal surgery for neoplasia from 1979 to 2005, a histological diagnosis of collecting duct carcinoma was made in 5 cases (1%). Mean age was 46.8 years (range 28–65, 4 males and 1 female) and mean follow-up was 36.4 months (range 10–65).

Histological features included neoplastic large masses (mean weight 788 g, range 210–2,800), involving the whole organ (mean size: 8.24 cm, range 4–23), often infiltrating the perirenal cellular fat and pyelocaliceal system, with central necrosis, signs of vascular permeation and perineoplastic inflammation. Microscopic pattern was typical as shown in Fig. 5. The grade (Fuhrman grading system) was G3–4, except for one G1 case with better prognosis. Immunohistochemical analysis did not suggest any common specific markers in the five cases, as already found in the previous cytological study. The disease presented at an advanced stage (multiple metastases) in 3 cases out of 5; in the other 2 cases (still alive after more than 5 years) the neoplasia (G1 and G3) was localized in the kidney with no evidence of postoperative recurrence, and no adjuvant therapy was performed. Early detection and immediate tumor removal remain the best option.

### 3.6. Renal medullary carcinoma

Renal medullary carcinoma is recognized as a distinct entity from collecting duct carcinoma, as indicated in the WHO 2004 classification of renal neoplasia. It is almost exclusively diagnosed in young black men with sickle cell traits or sickle cell disease and it has poor prognosis. The cell origin is from the renal medulla. Intrarenal infiltration and satellitosis are common, and many cases show intravascular invasion by the tumor. The histological pattern consists of highly pleomorphic cell nests with a focal papillary or cystic architecture. The tumor cell cytoplasm frequently contains eosinophilic hyaline globules [29]. Immunohistochemical studies are limited because of the rarity of these tumors; positivity for EMA, cytokeratin AE1/AE2, and CEA has been reported.

### 3.7. RCC associated with Xp11.2 translocation/TFE3 gene fusion

A group of apparent clear cell RCC is associated with translocation involving Xp11.2, known as the transcription...
factor gene, as indicated by sporadic reports dating back to 1986 and named “clear cell papillary RCC with voluminous cytoplasm.” In many cases these tumors are found in children and young adults. Initially, these cases were proven by cytogenetics or the detection of fusion transcripts by RT-PCR; the latest studies showed these tumors to have a similar morphology. Initially, these tumors showed ASPL-TFE3 gene fusion and it was recognised that these fusion transcripts were comparable to those seen in alveolar soft part sarcoma although, unlike sarcomas, the translocation was balanced \( [t(X; 17) (p11.2;q25)] \) [30]. In this series patient age ranged from 17 months to 17 years and the tumors were mostly in a high clinical stage at presentation.

Histological findings show acini, trabeculae, or papillary structures composed of cells with a clear cytoplasm, rich glycogen content and prominent cell borders. Small nuclei and an eosinophilic nucleolus are usually present, even if mitotic figures are rare. It is possible to identify cytoplasmic eosinophilic proteinaceous hyaline aggregates and psammoma bodies. The ultrastructural profile is typical of an epithelial tumor. Immunohistochemical analysis shows diffuse staining for CD10 and RCC; vimentin and cytokeratin/EMA expression is either absent or focal. On the basis of published case reports some tumors show similar features to the ASPL-TFE3 tumor, but these were characterized by translocation of TFE3 and the PRCC gene at 1q 21.2 [31]. Like ASPL-TFE3 tumors, PRCC-TFE3 carcinomas are more common in younger patients, with a mean age of 21.3 years at diagnosis having been reported. They have no specific gross morphology, although they usually have a pronounced pseudocapsule, often calcified. Histologically, the tumors show large cells with a clear cytoplasm (like those with the ASPL-TFE3 translocation) and psammoma bodies and aggregates of foam cells are occasionally present, while mitotic figures are rare. An alveolar architecture is most frequently present although acinar, tubular, and papillary areas may also be seen. Immunohistochemistry is comparable to that of ASPL-TFE3 tumors and the ultrastructure is similar to that of clear cell RCC. Assessment of the prognosis of Xp11 translocation tumors is difficult because of the reduced numbers of reported cases; ASPL-TFE3 tumors are thought to be associated with a more favorable prognosis than clear cell RCC, despite an apparently higher stage of presentation of most of these tumors. Two other translocation tumors involving Xp11.2 have been reported; these TFE3 tumors showed a fusion with splicing factor genes PSF (1p34) or NonO (Xq12) [32]. Further morphological studies are needed to clarify these genetic rearrangements.

### 3.8. Renal cell carcinoma associated with neuroblastoma

Recently included in the WHO 2004 classification, this heterogeneous group is associated with childhood neuroblastoma. Histologically, it presents oncocytoid features and genetically it shows allelic imbalances at the 20q13 locus. It has a similar prognosis to other RCC [33].

#### 3.9. Mucinous, tubular, and spindle cell carcinoma

Usually asymptomatic, this is a low-grade carcinoma recently included in the WHO 2004 classification. It is composed of tubular aggregates, spindle cell components, and mucinous stroma.

It seems to derive from distal nephrons. Genetically, it presents both losses of chromosome 1, 4, 6, 8, 13, 14, and gains of chromosomes 7, 11, 16, 17 [33].

### 3.10. Unclassified renal cancer

It is still difficult to know how to classify some renal tumors. In these unclassified cases it is not possible to refer to traditional morphology electron microscopy; this group accounts for approximately 7% of tumors. Why is it not possible to classify these unclassified RCC? Are they all high grade tumors? Modern molecular techniques will be able to make the pathologist a better diagnostician, and make the urologist a better predictor of how this form might behave.

#### 3.11. Sarcomatoid variant

The sarcomatoid variant represents a relatively rare, high grade form of RCC typified by a spindle cell growth pattern, evident in less than 5% of RCC cases, and associated with a poor outcome. A sarcomatoid component can occur in all histologic subtypes of RCC and indicates an aggressive tumor [34].

Initially called carcinosarcoma [35], mixed renal tumor [36], and subsequently renal sarcomatoid tumor according to Farrow et al. [37], the sarcomatoid differentiation is not a distinct histological entity but confers a higher aggressiveness to any of the different subtypes of RCC; it has a frequency ranging from 1% to 8% in the described series [38]. Studies indicate that the presence of sarcomatoid component makes the disease locally aggressive; it typically presents at an advanced grade that is associated with fast progression and a fatal outcome in a vast proportion of cases, with a shorter median survival than 1 year in many series [39,40]. Jones et al. support the hypothesis that the sarcomatoid elements seen in conventional renal cell carcinomas arise from the clear cell population as a consequence of clonal expansions of neoplastic cells with progressively greater genetic alterations [41]. The sarcomatoid pattern is a specific variant associated with a poor clinical outcome. A similarly poor prognosis has been described for tumors showing “rhabdoid” differentiation (about 3% of clear cell tumors); it usually presents at a high clinical stage, and has a median survival of 8 months [42].

There is controversy as to whether the amount of sarcomatoid tumor present is really relevant when analyzing the disease’s potential for recurrence.
4. Benign renal cell tumors

4.1. Papillary adenoma

This represents the most common epithelial tumor of renal tubules and is found in 10% to 40% of specimens. Histological alterations are similar to those of papillary RCC; it is usually solitary, small, with regular borders and a tubulopapillary architecture in the renal cortical parenchyma.

Previous studies have indicated that a combined trisomy of chromosomes 7 and 17 is a constant finding in these benign tumors, while papillary RCC are marked by additional trisomies such as trisomy 12, 16, and 20 [43].

4.2. Oncocytoma

Oncocytomas are benign tumors deriving from the epithelial intercalated cells of the kidney. They are usually diagnosed postoperatively due to differential diagnostic problems with renal cell carcinoma. Renal oncocytoma has several features that overlap those of other renal neoplasms with a preponderance of granular cytoplasm, such as chromophobe, granular, and papillary renal cell carcinomas. The poor knowledge of this entire spectrum of eosinophilic renal cell neoplasms has led to several misconceptions in the literature regarding grading and metastasis, as well as the impression that renal oncocytoma is usually low grade and lacks prominent nucleoli.

Histologically, renal oncocytoma is composed of an exclusive or predominant component of acidophilic cells with three architectural patterns: (1) the “classic” pattern, composed of a characteristic nested or organoid arrangement of cells, each surrounded by a distinct reticulin framework; (2) a “tubulocystic pattern” with numerous closely packed, cystically dilated tubular structures; and (3) a “mixed pattern,” which has both the organoid and the tubulocystic patterns. A gross or microscopic scar may be present and histologically, a distinctive myxoid and/or hyalinized stroma separated nests of cells.

Cytologically, the neoplasms also showed a mixture of cell types, the most common being the classic oncocyte, which consisted of round or polygonal cells with a moderate to abundant granular, eosinophilic cytoplasm, and small round nuclei with evenly dispersed granular chromatin. Generally, in renal oncocytoma the nuclei are round with uniform nuclear contours and nearly half of such tumors may show prominent basophilic nucleoli (equivalent to Fuhrman’s Grades III or IV). Obviously, oncocytomas are not graded because they are, by definition, a benign tumor. Loss of chromosomes 1 and/or 14 and alterations of mitochondrial DNA are frequent.

In conclusion, renal oncocytoma is a benign neoplasm and therefore does not merit a nuclear grading scheme. The overwhelming majority of such cases behave in a benign fashion, although in rare cases they can metastasize. The presence of atypical morphologic features does not alter the excellent prognosis associated with oncocytomas and does not predict an aggressive clinical course [44].

5. Prognosis

5.1. Pathological stage

Recently, increasing use of nephron sparing surgery has led to detailed assessment of the prognostic significance of tumor size. In 1997, the UICC/AJCC staging system subdivided pT1 and pT2 tumors according to size, although the sensitivity of this limit as a predictor of cases suitable for partial nephrectomy has been debated and, for this reason, in 2002 a further subdivision with pT1a < 4.0 cm, pT1b > 4.0 cm was added [45]. Clinical studies have confirmed that any of the size cut-off points defined in the various editions of the TNM classification are significantly associated with survival.

Size is a constant variable and in many series any cut-off point will correlate with survival, so the predictive value of these data in individual cases is limited [46]. Current staging for renal cancer does not directly rely on tumor size; it partly defines pathological stage, namely pT1a (less than 4 cm), pT1b (>4 and <7 cm) and pT2 (>7 cm), as shown in the 2002 TNM categories.

However, tumor size is important by itself as a prognostic factor, independent of pathological stage. Small tumors may have a high pathological stage due to invasion of the fat or renal vein (pT3a) or the inferior vena cava (pT3b and pT3c), but this does not necessarily decrease patient survival. These findings seem to demonstrate that the prognostic relevance of the pathological stage can be markedly decreased by the presence or absence of other prognostic factors. The T3a classification is a heterogeneous mix of lesions, including those that involve the ipsilateral adrenal as well as lesions that invade through the renal capsule into the perinephric fat. In a recent study, Han et al. challenged the appropriateness of grouping these various lesions in a single T3a category [47]. They compared survival in 27 patients with pT3a disease with direct adrenal invasion to 187 patients with pT3a disease without adrenal invasion. Median survival of patients with adrenal invasion was significantly worse compared with patients without (12.5 months vs. 36 months, P < 0.001) and was similar to patients with pT4 disease (11 months). Similar results were reported by Thompson et al., who recommended that tumors with direct ipsilateral adrenal invasion should be classified in the pT4 category [48]. Another study indicates that tumors invading the renal sinus fat are more aggressive than tumors with perinephric fat involvement [49].

Karakiewicz et al. [50] examined whether inclusion of pathologically determined tumor size in the prediction of nodal metastases (N+), distant metastases (M+), and cancer-specific survival resulted in increased accuracy. They analyzed 2,245 patients who underwent partial or radical
nephrectomy, with clear cell histology. The pathologic stages were T1a in 566, T1b in 490, T2 in 303, T3 in 831, and T4 in 55 patients. Multivariate models relied on 1997 and 2002 TNM variables and addressed N+ and M+ disease, and cancer-specific survival. Their accuracy was compared according to the inclusion of tumor size among the factors. In all univariate and multivariate models, tumor size was a statistically significant predictor of all outcomes (P < 0.001).

In all multivariate models, tumor size added between 3.7% and 0.8% to the predictive accuracy of both the 1997 and 2002 TNM categories. Tumor size represents a highly significant, multivariate, and informative predictor of RCC outcomes and may warrant inclusion in future TNM revisions.

In our series of 521 patients who underwent renal surgery for neoplasia from 1979 to 2005, we established the predictive role of tumor size in the recurrence rate, compared to other known prognostic factors, particularly pathological TNM in early cases. We retrospectively studied a selected group of 284 patients with localized or locally advanced disease (stages T1, T2, T3a, b). Mean age was 59 years (range 22–84), mean follow-up 32.8 months (range 3–144), mean tumor size 6.8 cm (range 1–20). Our primary end point was recurrence rate during follow-up, by abdominal CT scan or ultrasound and chest X-ray every 6 months.

The selected group was stratified according to pathological stage, Fuhrman grade, time of surgery, incidental diagnosis, surgical approach, clinical group. Basically, we created 6 clinical groups of patients on the basis of tumor size and pathological stage: Group 1, T1 stage; Group 2, T2 stage; Group 3, T3a stage and size <7 cm; Group 4, T3a stage and size >7 cm; Group 5, T3b stage and size <7 cm; Group 6, T3b stage and size >7 cm (Table 2). Disease recurrence occurred in 35 patients out of 284 (12.3%); there were 25 cancer-related deaths (8.8%). Disease-free survival curves (Kaplan-Meier statistical analysis) evidenced significant P-values for stage, grade, and clinical group (P = 0.0001; P = 0.017; P = 0.0001, respectively). Patient distribution based on pathological stage and tumor size showed a similar tumor behavior in Groups 1 and 2 (recurrence rate 5.8% and 8.8%, with no significant P-value, Fig. 6), with a minor effect of tumor size on prognosis in organ-localized disease. Tumor size had prognostic relevance in stage pT3 (clinical Groups 3 and 4), P-value 0.03 and recurrence rates of 7.8% and 21.4%, respectively (Fig. 7). Renal vein invasion was not related to size. Perirenal fat infiltration did not affect disease evolution in small tumors (Groups 1 vs. 3, = 0.5; Fig. 8); in size >7 cm, in T3 stage, the recurrence rate increased as compared to stage T2 (21.4% vs. 8.8%). Our analysis concluded that histological grade, pathological stage and tumor size represent relevant prognosticators in early stage renal cell carcinoma patients; in small size tumors the pathological stage (perirenal fat infiltration) has a minor effect on survival and so a conservative approach in <7 cm masses appears to be justified.

5.2. Histological grade

Apart from the controversy over changes to criteria within the TNM staging system, there has been even greater debate over the need for prognostic indicators not included in the mainstream staging system. One such factor is histological grade. In 1971, Skinner et al. noted a correlation between nuclear features of RCC tumors and survival and

<table>
<thead>
<tr>
<th>Group</th>
<th>Stage</th>
<th>Size (cm)</th>
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<tbody>
<tr>
<td>1</td>
<td>T1</td>
<td>&lt;7</td>
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<tr>
<td>2</td>
<td>T2</td>
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<tr>
<td>3</td>
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<td>4</td>
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<tr>
<td>6</td>
<td>T3b</td>
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The group of 284 studied patients with localized and locally advanced RCC, stratified by size and stage.

Fig. 6. Prognosis. Disease-free survival by 2002 primary tumor classification between Groups 1 vs. 3 (P = 0.5).

Fig. 7. Prognosis. Disease-free survival by 2002 primary tumor classification between Groups 3 vs. 4 (P = 0.03).
5.3. Microscopic tumor necrosis and microscopic venous invasion

Histological tumor necrosis was significantly associated with death from clear cell and chromophobe renal cell carcinoma. Patients with necrosis in the pathology specimen had a 3-fold higher risk of death from RCC compared with patients without histological necrosis. Notably, necrosis in papillary renal cell carcinoma is not associated with a decreased cancer-specific survival. The finding of extensive necrosis in renal cell carcinoma specimens does not seem to be related to tumor biology but may rather reflect the relation between size and vascularity of the tumor [57].

The presence or absence of microscopic tumor necrosis should be noted in the pathology report in cases of conventional renal cell carcinoma. Some studies have shown the relevance of microscopic venous invasion (MVI) as an independent prognostic factor of specific and overall survival; MVI has a significant influence on disease-free, specific, and overall survival at univariate analysis. It is strongly correlated to size and renal vein extension and to the appearance of metastases. The pathologist could include MVI in the evaluation in order to help the clinician to choose the best strategy. According to Lang et al. [58], hematoxylin and eosin staining is sufficient in routine examinations, using immunohistochemistry with anti-factor VIII antibodies or anti-CD34 antibodies in doubtful cases. The increased number of samples obtained per tumor, in accordance with the guidelines, has improved the identification of MVI.

5.4. Lymph node involvement

The clinical detection of lymph node involvement depends on an increase in the number or size of the nodes. At present there is no imaging method that can confidently be used to differentiate between metastases and hyperplastic nodes. Thus, lymph nodes harboring malignant disease are not always identified on CT and, conversely, enlarged nodes do not necessarily indicate metastatic disease. Lymph node dissection is essential in the correct staging of RCC, it provides a knowledge of the true status of regional stations and consequently a correct prognosis and permits an adequate follow-up and adjuvant therapy to be programmed, while its therapeutic value remains controversial [59].

The rate of regional lymph node involvement in RCC depends on several factors (i.e., stage of the disease, extent of the surgical resection, accuracy of the pathological examination) and reportedly ranges between 3.3% to 24% [60]. Quality criteria for assessing regional lymph nodes are lacking and there is no published information about the correlation between the number of lymph nodes removed and the incidence of nodal involvement in RCC.

The latest two editions of the TNM system simply consider the absolute number of metastatic lymph nodes (pN0 = none, pN1 = 1, pN2 = > 2), assuming that regional lymphadenectomy will include at least 8 lymph nodes [61], but this suggestion is not always adopted [62]. Few reports in literature deal with the pN1/pN2 classification [63,64], and none considers the clinical impact. It has still to be established whether such a subdivision reflects real differences in terms of prognosis and survival. Of course, lymph node involve-
ment is often associated with poor prognosis [61]. The therapeutic role of lymphadenectomy in RCC remains controversial, but its importance in correct staging is not in doubt [65]. Terrone C et al. [66] did not find any difference in cause-specific survival between pN1 and pN2 patients and most long-term surviving patients had pN2 disease. These results could be influenced by the different extent of lymphadenectomy in the two groups of patients, although a threshold of 4 positive lymph nodes stratifies patients in two categories, with different clinical outcomes. The authors considered lymph node density, in terms of the ratio of the number of positive lymph nodes to the total number of lymph nodes removed; this better defines the surgical outcome than conventional lymph node staging. Lymph node density provides a better discrimination between pN1 and pN2 and it is a significant risk factor for cause-specific survival, maintaining a correlation with the outcome in multivariate analysis after adjustment for the number of positive lymph nodes (< 4 vs. > 4), metastases, tumor stage, and grade [66].

Could the number of lymph nodes retrieved vary, regardless of the dissection technique used and the accuracy of the histopathological examination? Is molecular analysis of metastatic lymph node tissue reliable? These issues need to be solved.

6. New directions

Advances in understanding of the pathogenesis, behavior, and importance of prognostic factors for RCC have paved the way for a revision of its classification and staging. In the past, a lack of consistent classification and terminology for RCC histology and staging complicated the comparability of clinical studies investigating patient prognosis and response to treatment.

In summary, all renal cancers arise from different cells in the nephron. What matters is that these tumors are different genetic entities with a variable morphologic pattern. The importance to the pathologist and to the urologist as the treating physician is that nowadays there are biologic parameters correlated to these RCC types. It is not only a question of how big the tumor is or what the grade of the tumor is and, clearly, all these factors are type-specific. There are the symptoms, the grade, the morphologic criteria, and they all matter. In future studies, urologists as well as other specialists will try to address those areas that might be of interest, such as genes identification, and then to develop markers that are able to predict those patients with the worst outcome, or markers able to predict those patients that might or might not respond to compounds, especially the many new targeted agents.

The following new directions seem to stand out:

(1) A contemporary classification (which the pathological report should refer to) taking into account pathologic and molecular data and defining clinically distinct tumor types, particularly the rarest; this is the new way the disease should be looked at now, taking into account that renal cancer is not a single entity, but may present as papillary cancer, conventional renal cancer, chromophobe cancer with specific features.

(2) The clinical relevance of the traditional morphologic criteria really are all tumor-type dependent; for example, stage and grade or microscopic venous invasion and tumor necrosis must be given different weight according to each tumor type.

(3) Molecular tumor markers are used to assist differential diagnosis of renal cell carcinoma and to monitor disease progression and recurrence; new defined pathologic and genetic markers within tumor types and within tumor stages can improve cancer prognostication and confirm each tumor type as a single clinical entity; genetic changes detected by molecular and cytogenetic methods provide specific markers for each neoplasm. High-throughput DNA and RNA tissue microarray techniques promise to revolutionize the discovery and validation of novel molecular markers (genomics, proteomics, and metabolomics).

(4) The surgeon is going to need to revise the concept of how to handle solid renal masses because they are a heterogeneous group of lesions. Soon, within the next decade, the surgeon will take into account the host, the patient, and will be able to ask the pathologist to evaluate the solid mass in situ, with the collaboration also of the radiologist (interdisciplinary approach) and, in addition, to consider minimally invasive surgery and new approaches (cryosurgery and others).

(5) In the postgenomic era, the identification of tumor-associated antigens (TAA) that induce humoral and cellular responses can be accomplished at the protein level using proteomics.

Proteomics provides a major opportunity for screening and identifying novel TAA that can form the basis of prospective targeted therapies, such as monoclonal antibodies or dendritic cell-based vaccines for RCC. Moreover, several studies on genetic alterations of RCC have shown that specific types and stages of renal cancer can be characterized by specific genetic alterations. To date, however, there are no accepted powerful prognostic parameters that allow an individual risk assessment to be performed. In order to optimize the management of patients with RCC it is necessary to develop new prognostic parameters at molecular and cellular levels. There are many efforts to integrate molecular data (from tissue microarrays) and clinical data (traditional parameters) into a molecular integrated staging system.

Currently, many tumor markers relating to proliferation, growth, angiogenesis, loss of cell adhesion, and immune
regulation are being evaluated to provide not only prognostic information to aid in the identification of patients at risk for recurrence or metastasis but could also hold the key to targeted therapeutic interventions (kinase-inhibitors sunitinib and sorafenib seem to work). New trials are ongoing, applying state-of-the-art technologies to undertake a systematic profiling of RCC gene and protein expression patterns, in order to identify novel TAA multiple cancer biomarkers that will likely prove useful for the development of innovative, specific, targeted therapies and for the early detection and monitoring of RCC development/progression that is so important for the clinical management of patients.

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References


