Mototsugu Oya *Editor*

Renal Cell Carcinoma

Molecular Features and Treatment Updates

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ISBN 978-4-431-55530-8 ISBN 978-4-431-55531-5 (eBook)
DOI 10.1007/978-4-431-55531-5

Library of Congress Control Number: 2017932530

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer Japan KK
The registered company address is: Chiyoda First Bldg. East, 3-8-1 Nishi-Kanda, Chiyoda-ku, Tokyo 101-0065, Japan

Preface

Renal Cell Carcinoma: Molecular Features and Treatment Updates provides a comprehensive review of diagnosis and treatments of renal cell carcinoma (RCC) to practitioners and researchers with an interest in this disease. This book covers all topics from basic biology and pathology to clinical practice. Clinical topics include etiology, diagnosis, and all treatment options. The book also makes a strong effort to catch up with the key, most up-to-date advances in information about molecular bases and targeted therapy of this neoplasm. RCC therapy is the most advanced field in terms of molecular-targeted therapy. Medical oncologists as well as urologists have begun to be interested in this area. Basic researchers who focus on cancer metabolism, cancer genetics and epigenetics, vascular biology, and cancer immunotherapy may also be interested and inspired by this book.

In the past decade, treatment options for renal cell carcinoma have been expanding and moving quickly toward laboratory-based and molecular-targeted therapies. RCC is resistant to chemotherapy and radiotherapy and had been a rare cancer where immunotherapy including interleukin-2 or interferon-α were widely used before the introduction of targeted therapies. FDA approved one vascular endothelial growth factor (VEGF) antibody (bevacizumab); four tyrosine kinase inhibitors (TKIs) including sorafenib, sunitinib, pazopanib, and axitinib; two mammalian targets of rapamycin (mTOR) inhibitors including everolimus and temsirolimus; and the immune-checkpoint inhibitor nivolumab. The introduction of targeted therapy brought a paradigm shift to metastatic RCC therapy.

The pathological diversity of RCC should be noted. More than 80 % of RCC is clear cell (cc) RCC, followed by papillary and chromophobe RCC. With intense exploration of the linkage between pathological phenotype and molecular deterioration, several distinct subtypes of non-ccRCC have been clarified recently. Most of the clinical evidence for targeted agents was found in ccRCC patients. It is hoped that effective molecular-based therapeutic strategies can be developed for non-ccRCC.

The diagnosis of RCC is also unique. It is performed based on radiological findings by CT scans with contrast media, not by biopsy. Therefore, the quality of

radiography and precise interpretation of imaging is mandatory. Subtypes of RCC can be diagnosed before the operation through an understanding of definite patterns of each subtype by CT or MRI imaging.

RCC has been called an unpredictable and elusive cancer. I believe this is because of the heterogeneous nature of RCC biology. In addition, RCC has several peculiar characteristics that are not observed in other cancers: late recurrence (recurrence more than 5 years after the initial surgery), prominent hypervascularity, high frequency of paraneoplastic syndrome, and spontaneous regression of metastatic lesions after excision of the primary tumor. These observations in clinics suggest that cytokines or growth factors as well as immunological cells are important in the microenvironment where cancer cells grow. Accordingly, molecular-targeted therapy for RCC targets vascularization of RCC induced by VEGF. Furthermore, immune-checkpoint inhibitors including PD-1 or PD-L1 antibodies have been introduced recently to treat metastatic RCC. At present nivolumab is indicated for second-line therapy after TKIs. It is true that TKIs and mTOR inhibitors have prolonged the survival of metastatic RCC patients. However, lack of durable responses prompted us to continue searching for immune-modulating agents that can achieve a longer durable response. Translational research is underway to optimize this approach, but the significant heterogeneity in RCC makes the development of predictive biomarkers challenging.

RCC is also unique for its special characteristics in cancer behavior. Cancer cells are characterized by indefinite proliferation, invasiveness, and metastases. Usually these characteristics are related to one another. Namely, cancer cells that proliferate rapidly tend to invade and metastasize. Typically, RCC does not proliferate rapidly nor invade the surrounding tissues but does metastasize. Late recurrence of RCC might reflect these characteristics. Conceivably, metastatic lesions observed in late recurrence might exist as invisible cancer cells at the metastatic sites at the time of surgery. More than 5 years' growth is required for visualization by imaging. Different from cases of other cancers, lymph nodes are not the initial sites of metastases in general. Lung and bone are the preferable sites of metastases through vascular spreading.

The minimum contribution of cancer genes that are commonly mutated in other adult epithelial cancers has made RCC highly distinct from other types of solid neoplasms. The most dominant gene mutation is the *VHL* (von Hippel Lindau) tumor suppressor gene. By this mutation, hypoxia inducible factor (HIF) is constitutively activated leading to production of VEGF. Whole genome sequencing and integrated genomic analyses uncovered unique molecular deteriorations located at chromosome 3p. After *VHL*, *PBRM1* is the second most mutated gene in ccRCC followed by *BAP1* and *SETD2*. These three genes are chromatin-remodeling genes. Their exact roles have remained unclear, however. Elucidating the relevance to carcinogenesis of these genes could help determine meaningful therapeutic targets in RCC.

Additional topics include PK/PD in molecular-targeted agents and the putative mechanism of resistance to anti-angiogenic agents, such as intratumoral

heterogeneity or the cancer stem cell population. Intratumor heterogeneity and the evolutionary process were first demonstrated by RCC specimens.

I hope that this book will bring the most updated and helpful information to all of readers who are eager to cure this disease and are devoted to accomplishing that goal.

Tokyo, Japan Mototsugu Oya

Contents

Chapter 1
Epidemiology of Renal Cell Carcinoma

Xifeng Wu and Xiang Shu

Abstract The incidence of kidney cancer has been increasing for the past several decades. It ranks the second most common neoplasm found in the urinary system. The majority of kidney cancers are renal cell carcinomas (RCC, >85 %), and of these, clear cell tumors are the most common histologic type. Higher incidence is observed in industrialized western countries compared to less well-developed nations. Gender and ethnic disparity are observed for RCC. Current consensus is that cigarette smoking, hypertension, and obesity are three established risk factors for RCC. However, the exact biological mechanisms that underlie for these risk factors are still not fully elucidated. Energy balance that incorporates effects of energy intake and expenditure has attracted much attention in recent years. Diet/intake of nutrients, energy consumed, level of physical activity, and weight change could jointly affect RCC risk. Other risk factors that have been implicated include type II diabetes and other chronic medical conditions, occupational/environmental exposures, parity, and number of offspring. Recent genome-wide association studies (GWAS) have identified multiple chromosome regions harboring RCC susceptibility loci. A number of intermediate phenotypic markers, such as suboptimal DNA repair capacity, short telomere length, and low mitochondrial DNA copy number, have been shown to be associated with risk of RCC. The discovery of additional genetic susceptibility loci through next-generation sequencing (NGS) and intermediate biomarkers using various "omics" approaches, as well as the identification of gene–environment and gene–gene interactions, will all be important next steps to improve our understanding of RCC etiology.

Keywords RCC • Epidemiology • Risk factors • Genetic variants • Biomarker

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M. Oya (ed.), *Renal Cell Carcinoma*, DOI 10.1007/978-4-431-55531-5_1

1.1 Introduction

The kidney plays an essential role in maintaining body homeostasis. Essential functions include regulating electrolyte and fluid balance, filtering waste, and helping regulate blood pressure. The kidney is divided into the parenchyma and collection system. The parenchyma is composed of the renal cortex and renal medulla. The collection system includes calyces and renal pelvis. Renal cell carcinoma (RCC) accounts for more than 85 % of all kidney cancers with the remainder being renal pelvis carcinoma (approximately 10 %) and other rare malignancies [1]. The majority of RCC has clear cell histology (ccRCC), followed by papillary, chromophobe, and other rare histologic subtypes. It is speculated that RCC subtypes possess distinct etiologies.

The incidence of kidney cancer ranks 9th and 14th among all cancers diagnosed in men and women worldwide, respectively. The age-adjusted incidence rate is 6 per 100,000 in males and 3 per 100,000 in females. The age-adjusted mortality rate is around 2 per 100,000 and 1 per 100,000 in males and females, respectively. The incidence of kidney cancer varies considerably by region, with much higher rates in North America, Europe, and Oceania than in Asia, South America, and Africa (Table 1.1) [2]. For example, North America has the highest age-adjusted incidence (11.7 per 100,000), which is fourfold the rate reported for Asia (2.8 per 100,000).

In the United States, kidney cancer is the sixth most common cancer in men and eighth most common in women, with an estimated 63,920 new cases and 13,860 deaths in 2014 [3]. The age-adjusted incidence rate in men (21.0 per 100,000 person-years) is nearly twice as high as in women (10.6 per 100,000 person-years). Likewise, the mortality rate of men (5.8 per 100,000 person-years) is double that of women (2.6 per 100,000 person-years) [4]. During the past three decades, the incidence rate of kidney cancer has been steadily increasing at over 2 % per year (Fig. 1.1). The rise in incidence has been more rapid in blacks than in whites, especially in males. In contrast, mortality rates have been indiscriminate among blacks and whites since the early 1990s [5]. This raises the possibility that early-stage tumors with improved prognosis account for the excess in kidney cancer incidence among blacks. The etiology of RCC differs from that of other kidney cancers, and RCC is the major histologic type; thus, we only focus on epidemiology of RCC for the remainder of this chapter, and kidney cancer and RCC are used interchangeably.

Several modifiable risk factors have been established for RCC, including obesity, cigarette smoking, and hypertension [1]. There is a rapidly growing body of evidence showing that energy balance may play a critical role in the development of the disease. However, due to inconsistent results reported by different studies, more solid evidence is needed to establish the association of RCC risk with energy intake, diet/nutrients, physical inactivity, and weight change. Other potential modifiable risk factors for which association is inconclusive include alcohol consumption, type II diabetes, occupational or environmental exposures, etc.

Table 1.1 Age-adjusted incidence and mortality (per 100,000) of kidney cancer worldwide

Continents	Incidence	Mortality
North America		
Male	15.5	3.7
Female	8.3	1.6
Both	11.7	2.6
Europe		
Male	12.3	4.8
Female	5.9	1.9
Both	8.8	3.1
Oceania		
Male	11.0	2.8
Female	5.3	1.3
Both	8.0	2.0
Latin America & Caribbean		
Male	4.7	2.5
Female	2.5	1.2
Both	3.5	1.8
South America		
Male	5.1	2.8
Female	2.7	1.3
Both	3.8	2.0
Asia		
Male	3.8	1.7
Female	1.9	0.9
Both	2.8	1.3

Data is obtained from GLOBOCAN 2012, http://globocan.iarc.fr/Default.aspx

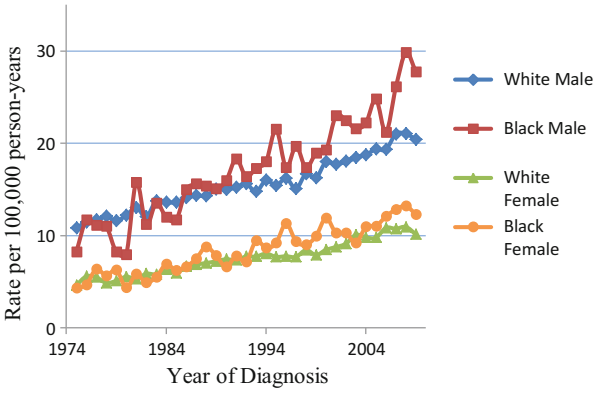

Fig. 1.1 Trends in age-adjusted incidence of kidney cancer by race and sex, 1974–2009 (Based on nine areas: San Francisco, Connecticut, Detroit, Hawaii, Iowa, New Mexico, Seattle, Utah, and Atlanta [3]. The rate is age-adjusted to the 2000 US Standard Population)

Systematic study of genetic variants is promising with the advent of genome-wide association study (GWAS) and next-generation sequencing (NGS). A few RCC susceptibility loci have been identified through recent GWASs which focus on common variants (minor allele frequency >0.05). Currently, four susceptibility loci

have been confirmed by large global consortiums. Although GWAS has been successful in identifying susceptibility loci that are replicable, the total heritability explained by these loci is small. Additionally, a large proportion of single nucleotide polymorphisms (SNPs) are located in noncoding areas. The functions of tagging SNPs or SNPs in high linkage disequilibrium in these regions are not fully understood. On the other hand, thanks to the advent of next-generation sequencing and lowering costs, we are now able to identify variants with low and rare frequency (minor allele frequency of 0.1–1 % and <0.1 %, respectively) but larger effect size. Furthermore, intermediate phenotypic biomarkers are also being evaluated for their utility in predicting the predisposition of the disease. A number of intermediate phenotypic biomarkers in peripheral blood leukocytes (PBLs) have been linked to RCC risk, such as mitochondrial copy number change, telomere length, and suboptimal DNA repair capacity. Less is known about the etiologic role played by gene–gene and gene–environment interactions that could explain some extent of missing heritability. In this chapter, we review the current state of knowledge on the epidemiology of RCC in detail.

1.2 Modifiable Risk Factors

1.2.1 Cigarette Smoking

Cigarette smoking has been recognized as a causal risk factor with moderate effect on RCC. As early in the 1960s, one hospital-based case-control study has linked tobacco use to RCC risk for both genders [6]. The association was significant in all types of tobacco users. Since then, various studies have confirmed the association in both case-control and prospective cohort studies. A large meta-analysis of 5 cohort and 19 case-control studies reported a 50 % increase in RCC risk among male smokers and a 20 % increase among female smokers. A dose–response relationship between the number of cigarettes smoked and RCC risk was reported for both genders. Smoking cessation should be promoted because an approximate 15–30 % reduced risk was observed in both men and women who stopped smoking for more than 10 years [7]. The attributable risk percentage (AR%) of smoking calculated from population-based case-control and cohort studies is comparable (21 vs. 23 %), which indicates that more than one fifth of RCC could be prevented in the general population if they had no exposure to smoking. Although smoking is primarily a lifestyle factor in ever smokers, it is an environmental exposure to never smokers. One study showed that patients who were never smokers were more likely to report exposure to environmental tobacco smoking (ETS) either at home or in public [8]. However, interpretation of these results requires caution due to the likelihood of recall bias and the relatively small sample size.

A number of potential mechanisms linking smoking to the development of RCC have been proposed [1]. Cigarette smoke contains more than 60 carcinogens and

most of them are formed during combustion [9]. One crucial mechanism of linking tobacco carcinogens with cancer is through the formation of DNA adducts. Benzo [α]pyrene (BaP), a polycyclic aromatic hydrocarbon (PAH), was the first carcinogen found in cigarette smoke, and its carcinogenicity has been confirmed in animals and humans. However, the level of BaP is much lower in cigarette products nowadays, and its carcinogenicity is weaker compared to some other PAH compounds [10]. Considerable evidence has linked cigarette-smoke nitrosamines such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N′-nitrosonornicotine (NNN), and aromatic amines such as 4-aminobiphenyl to several cancer types. However, little supporting evidence has been gained from studies of kidney cancer. Only two studies have been conducted, and these data suggest that peripheral lymphocytes derived from patients with RCC are more sensitive to NNK or benzo[α]pyrene diolepoxide (BPDE) than those derived from healthy controls [11, 12]. In addition, smoking-induced chronic hypoxia of renal tissue caused by carbon monoxide exposure, tubulotoxicity, increased oxidative stress, and endothelial cell dysfunction are all hypothesized to increase RCC risk [1].

Due to the great efforts in the campaign to target smoking cessation and changes of policy in public health, the overall prevalence of cigarette smoking has declined over the past several decades in the United States. By contrast, incidence rates of kidney cancer have steadily increased at more than 2 % per year since the 1970s. Thus, it is presumed that smoking is unlikely to be a major contributor to the rising RCC incidence trends observed in this country.

1.2.2 Hypertension

The prevalence of hypertension in US adults has remained quite stable since the 1990s [13]. Over this period, there has been considerable improvement in awareness and control of the disease, and this may translate into benefits of reduced comorbidity for hypertensive patients in the future. Hypertension is a confirmed risk factor for development of RCC. Although hypertension is highly correlated with obesity, sufficient data supports its independent effect. A cohort study found that elevated systolic and diastolic blood pressure may predispose to development of RCC in a clear dose–response manner [14, 15]. A meta-analysis of 18 studies showed a significant 1.6-fold increased risk of RCC associated with hypertension [16]. It is difficult to isolate the contribution of antihypertensive medication use from history of hypertension because of the solid correlation between the two. Many observational studies have reported the hazardous impact on kidney cancer risk by taking antihypertensive medicine [16–18]. However, results from other studies indicated that instead of medication use, the excess risk is attributable to history of hypertension. For example, both systolic and diastolic blood pressure were associated with elevated risk of RCC when the analyses were restricted to non-antihypertensive medication users [19]. Clinical trials also showed no effect of taking antihypertensive medicine on cancer incidence [20]. The biological

mechanisms that relate hypertension to RCC are still not fully understood. The renin–angiotensin system (RAS) plays an essential role in regulation of blood pressure and convincing evidence indicates that angiotensin II (Ang II) is involved in processes of cell proliferation, migration, angiogenesis, and inflammation [21]. Interestingly, slower development of tumor was observed in a xenograft mouse model of RCC when treated with captopril, an angiotensin-converting enzyme (ACE) inhibitor [22]. Furthermore, it is hypothesized that renal injury and metabolic or functional changes resulting from hypertension-induced chronic renal hypoxia and lipid peroxidation may subsequently raise renal susceptibility to carcinogens.

1.2.3 Energy Balance

1.2.3.1 Obesity

More than two thirds of US adults are overweight or obese [23]. Obesity has been linked to many chronic diseases including cancer. The association of obesity and breast cancer (postmenopausal), endometrial cancer, colon cancer, esophageal cancer, pancreatic cancer, and kidney cancer are well established [24, 25]. In the United States, about 40 % of RCCs are estimated to be attributable to being obese/overweight [5]. Interestingly, the effect of obesity has been reported to be specific for clear cell and chromophobe RCC histologies [26]. Although misclassification remains an inevitable limitation, body mass index (BMI) is widely accepted and used as a measure of obesity. Meta-analysis of prospective cohort studies has showed that each 5-unit increase in BMI contributes an approximate 24–34 % increase in risk of RCC for men and women [27]. From a statistical point of view, the effect size did not vary across ethnic groups, particularly in men. One interesting debate is at which period of life does being obese contribute most to the disease (i.e., childhood, adolescent, young adulthood, mid-age adulthood). One cohort study with more than 120,000 participants found association with BMI at baseline (aged 55–69 years) but not with BMI at age 20 years [28]. However, several studies have found that high BMI in adolescents may also confer excess risk [29–31]. These adolescent cohorts collected anthropometric measurements at baseline [29, 30]. BMI calculated from measured weight in the adolescent cohorts was more accurate than BMI calculated from recalled weight at their early ages by participants who are enrolled in most of the adult cohorts. It may provide more convincing evidence that obesity in early ages is associated with RCC risk.

Weight/BMI gain during adulthood is considered to be another risk factor for RCC that is independent of BMI per se, as reported in previous observational studies [1, 28, 31, 32]. The relationship is still inconclusive mainly due to confounding from obesity and inconsistent results. Similarly, weight/BMI gain relied on recalls from participants at enrollment for most of the studies that the misclassification remains an issue. A longitudinal study with multiple assessments

of weight and adequate adjustment of BMI would be valuable to disentangle the complicated interplay. However, such a study is difficult to conduct in practice. Likewise, the effects of other related factors, such as weight cycling and waist–hip ratio, are also tightly correlated with BMI so their independent influences are difficult to assess.

Various mechanisms have been proposed for the association between obesity and cancer. Increased mass of adipose tissue is not the only change that occurs in obese subjects. Levels of circulating adipokines and sexual hormones are altered as well. Additionally, it appears that higher concentrations of insulin and increased levels of insulin resistance were observed in subjects with abnormal BMI. Extensive data has linked leptin, adiponectin (inversely), hyperinsulinemia, as well as insulin-like growth factor (IGF) and its binding proteins to tumorigenesis through stimulation of cell proliferation, angiogenesis, and inhibition of apoptosis [24, 33]. Elevated circulating levels of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), chronic tissue hypoxia, lipid peroxidation, and increased oxidative stress have also been hypothesized to connect obesity to cancer. A limited number of studies have investigated the underlying mechanisms relating obesity to RCC. Two case-control studies showed contrasting results of association between serum leptin and RCC risk [34, 35]. Similarly, the association of adiponectin with RCC remains inconclusive [34, 36, 37]. Reverse causation may affect the validity of retrospectively designed studies. Prospective cohort studies with large sample size and nested case-control studies are warranted for the confirmation of previous findings.

1.2.3.2 Diet and Alcohol Consumption

On average, one third of cancers are estimated to be attributable to diet and nutrition [38]. Western diets are criticized for high intake of calories, meat and saturated fats, and their link to obesity and other chronic diseases. Incidence rates of RCC in the American Asian population are more than twofold that observed in Asian countries [2, 4]. Apparently, acculturation, such as changes in diet, is more likely to lead to the discrepancy rather than alterations in genetics.

High energy intake is a potential risk factor for RCC. However, due to its tight correlation with BMI, the association is still controversial. Several studies that evaluated energy intake and its association with RCC risk reported inconsistent results, even with adequate adjustment of BMI and other confounders [28, 39]. Various components of diet and nutrients in foods have been assessed for RCC risk. Although one recent study restricted to Caucasian male smokers showed no evidence of association [40], high fruit and vegetable consumption was associated with a >30 % reduction of RCC risk in a pooled analysis of 13 cohort studies [41]. It is interesting to further isolate the specific vegetable or fruit that drives the association. Suggestive inverse associations were found for highest quartile of cruciferous vegetables and whole citrus fruits consumption when compared to the lowest category [42, 43]. Furthermore, high intake of dietary fiber was also inversely

associated with RCC risk in a previous study [43]. Antioxidants may play a protective role in cancer development, although the reported associations of antioxidant nutrients including carotenoids and vitamins A, C, and E are inconsistent [41, 42, 44, 45]. Another speculation is that B vitamins and other components of the one-carbon metabolisms pathway which are important for DNA repair mechanisms in the body could partially explain the inverse association for fruits and vegetables. One cohort study reported that higher vitamin B6 level in plasma is in relation to both RCC incidence and survival [46].

Meat consumption may confer increased risk. A meta-analysis of cohort studies found that intake of fat and protein or their subtypes were not associated with RCC risk after adjusting for BMI, fruit and vegetable intake, and alcohol consumption [47]. In contrast, more recent studies have suggested a link and underscored the role of heterocyclic amines (HCAs), and polycyclic aromatic hydrocarbons (PAHs) found in meat cooked at high temperature [48, 49]. The intake of dietary acrylamide which is concentrated in baked and fried carbohydrate-rich foods was reported to confer a 60 % increased risk of RCC in a case cohort study in the Netherlands [50].

Regarding alcohol, moderate consumption was reported to be inversely associated with the risk of RCC [51]. A 28 % reduction in risk was observed in people who consumed >15 g per day of alcohol (equivalent to 1–2 drinks/day). No further reduction was observed for additional increases in consumption [52]. A recent large prospective cohort study has found a dose–response effect for alcohol consumption [53]. The protective effect is likely to be attributable to improved insulin sensitivity and antioxidant compounds contained in alcoholic beverages. The associations with intake of other beverages, such as coffee, milk, and tea, are inconsistent [1, 54].

Due to the high dimensions of nutrition data and collinearity among food items/nutrients, PCA (principal component analysis) and factor analysis are adopted for the purpose of data reduction. Interactions between different food items/nutrients are taken into account in this type of analysis. Suggestive evidence showed that patterns of “alcohol drinking” were inversely associated with RCC risk [55], and high-calorie, high-protein, and high-fat food patterns conferred an increased risk [56].

1.2.3.3 Physical Activity

Physical activity accounts for a large proportion of daily energy expenditure, which affects the status of energy balance of individuals. Most studies have focused on non-occupational or leisure-time physical activity and its association with cancer risk. Reduced RCC risk was reported by a number of observational studies for people with high level of physical activity [1]. Meta-analysis using the random effect model found an inverse association when comparing high levels to low levels of physical activity [57]. The heterogeneity seen across studies in the meta-analysis may be partially attributable to the differences in measures of physical activity between studies that included leisure-time physical activity during the past year, frequency of physical activity during a certain period, hours spent on physical

activity per day or per week, and metabolic equivalent task (MET) values. Although such differences were not statistically significant, the magnitude of estimations based on frequency or duration of physical activity appeared to be stronger than that estimated by METs or qualitative physical activity. Occupational physical activity studies are scarce and have mixed results [58, 59]. The beneficial effect of physical activity on RCC is likely to be mediated by reduction in body weight/ BMI, lowered blood pressure, changes to adipokines, alleviation of insulin resistance, and an improved profile of inflammation and oxidative stress.

1.2.4 Other Medical, Occupational, and Environmental Factors

Type II diabetes mellitus (DM) could be a risk factor for RCC independent of obesity/BMI. A meta-analysis that adjusted for obesity/BMI, alcohol consumption, and smoking has shown that a history of type II DM significantly confers higher predisposition of RCC [60]. However, only a few of the individual studies were further adjusted for history of hypertension that residual confounding may distort the association. In women, parity increases disease risk when compared to nulliparous, and a dose–response effect for parity number was significant in a meta-analysis [61]. Inflammation and elevated oxidative stress through pregnancy-induced physiologic changes, pregnancy-associated weight gain, and high levels of circulating estrogens have all been hypothesized as underlying mechanisms. There are other reported risk factors with inconclusive associations, such as end-stage renal disease, long-term hemodialysis, acquired renal cystic disease, use of statin and aspirin, and occupational and other environmental exposures [1, 62].

1.3 Genetic Susceptibility

1.3.1 Hereditary Kidney Cancer Syndrome

Compelling evidence supports genetic susceptibility to RCC. The risk of RCC is two to three times higher in individuals who have first-degree relatives with kidney cancer [63]. In addition, familial aggregation is seen in approximately 3 % of kidney cancer patients with inherited kidney cancer syndromes. Of these, the von Hippel–Lindau syndrome (VHL) is by far the most commonly recognized familial cancer syndrome associated with kidney cancer [64]. Other major familial kidney cancer syndromes include hereditary papillary renal cell carcinoma (HPRC), hereditary leiomyomatosis renal cell carcinoma (HLRCC), and Birt–Hogg–Dubé syndrome (BHD) [65], which are caused by mutations in the *c-Met* proto-oncogene,

FH (fumarate hydratase), and *FLCN* (folliculin), respectively. Interestingly, *VHL*, *FH*, *c-Met*, and *FLCN* are all involved in the pathways that respond to nutrient stimulation and cell metabolism, indicating kidney cancer is a metabolic disease [66].

1.3.2 Candidate Gene Approach

Most of the early candidate gene studies involved small numbers of cases and controls, and very few of the initially reported positive susceptibility alleles have been replicated in subsequent validation studies [67]. With regard to RCC, candidate gene studies reported many positive associations with single nucleotide polymorphisms (SNPs) in genes involved in xenobiotic metabolism, DNA repair, cell growth/apoptosis, inflammation, and other pathways [1]. None of the previously reported candidate SNPs has been replicated in large independent studies.

1.3.3 Genome-Wide Association Study (GWAS)

The advent of GWAS in recent years has revolutionized the study of cancer association. Unlike the hypothesis-driven candidate gene approach, GWAS is a discovery-driven, agnostic approach that does not depend on prior knowledge of SNPs and genes. It thoroughly screens up to millions of common SNPs across the entire genome. Due to the multiple testing of SNPs in the screening phase, to increase validity of the results, stringent Bonferroni correction (P-value $< 5\times10^{-8}$) and multistage follow-up validations with large sample sizes are required.

Three recent GWASs identified four novel genetic susceptibility loci that mapped to 2p21 (*EPAS1*), 11q13.3 (a *CCND1* transcriptional-enhancer site), 12p11.23 (*ITPR2*), and 2q22.3 (*ZEB2*) [68–70]. The observed effect size of each genetic locus is relatively small, which is expected for common variants. Two validated SNPs are located in intron 1 of *EPAS1* (endothelial PAS domain-containing protein 1) on chromosome 2p21. However, the putative function of these SNPs is still unclear. *EPAS1* encodes *HIF*-2α, which is a biologically plausible causal gene in the VHL/HIF pathway. Thus, GWAS results provide further support for the involvement of *EPAS1* in RCC etiology. Interestingly, although the SNP found in 11q13.3 is not close to any gene with known function (>50 kb), the locus is hypothesized to be a transcriptional-enhancer site of *CCND1* (encoding cyclin D1) [71]. Another locus maps to *ITPR2* (inositol 1,4,5-trisphosphate receptor, type 2) on 12p11.23. Interestingly, the same SNP has also been identified as being associated with waist–hip ratio in another GWAS [72]. Finally, the locus on 2q22.3 is mapped to *ZEB2* (zinc finger E-box-binding homeobox 2) that functions as a DNA-binding transcriptional repressor. Future pooled analysis of GWAS data with larger sample size will undoubtedly identify additional common RCC susceptibility SNPs.

1.3.4 Next-Generation Sequencing

The emergence of next-generation sequencing (NGS) provides a unique opportunity to discover rare variants that may explain some extent of the missing heritability in complex diseases. However, current NGS studies of kidney cancer have been mostly focused on profiling somatic mutations, while large-scale NGS studies using germline DNA have not been conducted. For example, a whole-exome sequencing (WES) study of tumor tissues has identified the SWI/SNF chromatin remodeling complex gene *PBRM1* (polybromo 1) as the second most frequently mutated gene in ccRCC [73]. Another study reported that the tumor suppressor gene *BAP1* (BRCA1 associated protein-1) could be used to define a new class of RCC [74]. With more than 400 tumor samples, The Cancer Genome Atlas (TCGA) identified 19 significantly mutated genes. Integrative analyses highlighted the importance of previously well-known pathways such as the VHL/HIF pathway, chromatin remodeling pathway, and PI3K/AKT/mTOR pathway [75]. Future NGS study using germline DNA may focus on identifying rare mutations, which can play critical role(s) in kidney cancer development.

1.3.5 Intermediate Phenotypic Assays for RCC Susceptibility

Intermediate phenotypic biomarkers have the advantage of measuring aggregated effects of genetic variations and have larger effect size than individual SNPs. The suboptimal DNA damage/repair capacity in PBLs that were challenged with different mutagens was shown to be associated with increased risk of RCC [11, 12, 76]. One study found BPDE (benzo[α]pyrene diol epoxide) induced lymphocytic chromosome 3p deletion was associated with RCC risk [11]. Two other studies used comet assays to show that the high sensitivity of PBLs to NNK (nicotine-derived nitrosamine ketone)- and BPDE-induced DNA damage was associated with increased risk of RCC [12, 76]. Another interesting phenotypic biomarker is telomere length. Telomeres protect chromosomes from degradation and end-to-end fusion. Short telomere length in PBLs was associated with increased risk of a variety of cancers [77]. However, the association in RCC is inconsistent. Two case-control studies found that shorter telomeres in PBLs to be associated with an increased risk of RCC [78, 79] and one prospective nested case-control study failed to confirm the association [80]. Mitochondrial DNA (mtDNA) copy number is recently attracting great interest as a potential cancer susceptibility marker. Decreased mtDNA copy number was shown to be associated with multiple types of cancer. In two studies, lower mtDNA copy number in PBLs conferred an increased risk of RCC [81, 82]. By contrast, a recently published prospective study showed that a high copy number of mtDNA increased risk [83].

Caution is required when interpreting the results from the retrospective case-control studies discussed above. Reverse causation is the major limitation for

biomarker research using case-control study. It is inevitable due to the study design. On the other hand, conducting a prospective cohort study is time consuming and costly. A case-control study nested in a cohort study is a good alternative due to its prospective design and cost efficiency.

1.4 Gene–Environment Interaction and Gene–Gene Interaction

Gene–environment and gene–gene interactions have been proposed to account for the missing heritability in chronic diseases such as cancer. However, convincing evidence is still sparse for kidney cancer. One study found smoking had a more pronounced effect on RCC risk in subjects with slow acetylator genotypes of N-acetyltransferase 2 (*NAT2*). Although smoking is an established risk factor for RCC, smokers with a genetically susceptible genotype in *NAT2* could be potentially targeted for primary prevention. Similarly, a significant interaction for occupational pesticide exposure and polymorphisms in glutathione S-transferase M1 and T1 (*GSTM1* and *GSTT1*) has been reported [84]. In addition, one recently published study found a link between the American/Western diet pattern and RCC risk, and the effect was modified by a previously reported GWAS risk locus [85]. The study further emphasized the synergistic effect of genetic variants involved in diet/nutrient metabolism and central obesity.

Evidence of gene–gene interaction is limited in RCC. In a meta-analysis, dual null genotypes of *GSTM1*/*GSTT1* were found to be significantly related to disease but not when considered individually [86]. Four out of five studies with dual null genotype data did not reach significance before pooling, demonstrating that small sample sizes with insufficient power impede discovery of significant associations. Importantly, further validation in prospective studies is required to consolidate the findings presented above before any public health implication can be made.

1.5 Novel Biomarkers for RCC Risk and Early Detection

The utility of novel biomarkers include circulating microRNAs (miRNAs), global/specific gene methylation, and metabolites in risk prediction, and early detection of RCC is an active area of investigation.

MiRNAs are a class of noncoding RNAs of 18–25 nucleotides. They regulate an estimated one third of all human genes. MiRNAs regulate gene transcription by binding to the 3′ UTR of target genes. They usually result in gene silencing by triggering degradation of pertinent mRNA. Circulating miRNAs are highly stable and protected from endogenous RNases and are promising biomarkers for cancer risk, diagnosis, and prognosis [87]. One study on circulating miRNAs in RCC

found an elevated level of miR-1233 in patients with this malignancy [88]. Although its upregulation was validated in an independent population, the discriminatory ability was lower than expected (area under the curve = 0.588). Two recent studies have also reported differential detection of several circulating miRNAs in RCC patients compared to controls [89, 90] but the associations remain controversial [91]. Thus, at present, circulating miRNAs as early diagnosis markers for RCC have not yet been consistently identified. Differences in methods used for detection and calculation may cause the inconsistencies. To confirm the utility of circulating miRNAs as cancer risk predictors and early detection markers, prospective studies are required with access to pre-diagnostic blood samples for marker evaluation.

Global DNA methylation levels have been repeatedly reported as biomarkers for risks of many cancers, including breast, bladder, colorectal gastric, lung, and ovarian cancer [92–96]. Hypermethylation found in CpG islands near promoter regions usually leads to gene inactivation, whereas hypomethylation confers higher gene expression. Until recently, few studies have focused on the association of methylation with RCC risk. One study reported that high LINE-1 (long interspersed nuclear elements) methylation levels in leukocyte DNA, which serves as a surrogate of global cytosine methylation (5MeC) levels, were associated with RCC risk [97]. The association was most pronounced in current smokers and the interaction was significant. However, as only one third of methylation is estimated to occur in repetitive elements across the genome, genome-wide methylation analysis in PBLs could lead to a better understanding of epigenetic alterations that occur during RCC development. Additionally, findings from tissue-based studies are also assisting in understanding the tumorigenesis. Frequently methylated promoter regions were found for nine genes in primary RCC tissues by genome-wide methylation analysis [98]. TCGA data also revealed that *VHL* was epigenetically silenced in 7 % of ccRCC samples [75].

Metabolomics is an "omics" approach that is attracting tremendous interest in cancer research and biomarker discovery. It allows for study of metabolic breakdown products derived from cellular processes. Analyses of urine samples from RCC patients and control subjects identified several potential biomarkers for diagnostics, including acylcarnitine, quinolinate, 4-hydroxybenzoate, and gentisate [99, 100]. Another study using serum samples also identified multiple potential biomarkers that belong to lysophosphatidylcholines (LPCs) and enriched in several pathways such as phospholipid catabolism, sphingolipid metabolism, and glycerophospholipid metabolism pathways [101]. For kidney and other urinary tract cancers, urinary markers may reflect the condition of the target organs directly and enhance the opportunity for discovery of promising diagnostic and prognostic markers specific to the urinary system. Once again, however, prospective studies are required for the establishment of valid diagnostic biomarkers based on the reports of previous studies.

1.6 Conclusions

The trend of increasing incidence rates of kidney cancer has been observed since the 1970s in the United States. The reason for this increase is not clear. The well-established risk factors for RCC are obesity, history of hypertension, and cigarette smoking. The increasing rate of obesity may partially explain the upward trend. Recent GWASs have identified four genetic susceptibility loci for RCC, and more common susceptibility SNPs are expected to be identified from pooled analysis of GWASs. The future directions in identifying genetic susceptibility include GWAS in different subtypes of RCC and in different races/ethnicities. Next-generation sequencing of the exome as well as the whole genome of germline DNA promises to identify rare variants for cancer susceptibility that could account for some of the missing heritability and provide significant biological insight into renal carcinogenesis. Integrative study design and analyses of multi-level "omics" data need to be emphasized in order to advance research. Prospective studies are needed for the discovery and validation of intermediate biomarkers. Additionally, conducting more research on gene–environment and gene–gene interactions is an important next step. Finally, a comprehensive risk assessment model integrating modifiable risk factors, genetic susceptibility loci, intermediate phenotypic biomarkers, circulating biomarkers, and gene–environment interaction will be needed to move toward personalized risk assessment and cancer prevention.

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Chapter 2
Hereditary Renal Cell Carcinoma

Masaya Baba, Laura S. Schmidt, and W. Marston Linehan

Abstract Hereditary renal cell carcinoma (RCC) is estimated to comprise 3% to 5% of all RCC. Since the manifestations that are associated with hereditary RCC syndromes are not well recognized by most clinicians, hereditary RCC may be underreported. Diagnostic criteria including multiple and/or bilateral renal tumors, a young age at diagnosis, a positive family history for RCC, a particular histological type of RCC, and extrarenal manifestations are suggestive of hereditary RCC. Hereditary RCC is a heterogeneous disorder comprised of a variety of hereditary syndromes caused by different gene alterations, including von Hippel-Lindau (VHL) disease, hereditary papillary renal carcinoma (HPRC), hereditary leiomyomatosis renal cell carcinoma (HLRCC), hereditary head and neck paragangliomas (HPGL) and pheochromocytomas (PCC) (SDH-RCC), Birt-Hogg-Dubé syndrome (BHDS), tuberous sclerosis complex (TSC), Cowden syndrome (CS), and BAP1 cancer susceptibility syndrome. All of these syndromes are associated with a germline mutation in a specific causative gene and are inherited in an autosomal dominant manner. In this chapter, clinical manifestations, genetics, and molecular functions of the responsible genes will be presented for each hereditary RCC susceptibility syndrome.

Keywords Renal cell carcinoma • Hereditary RCC • Familial RCC • Autosomal dominant • *VHL* • *MET* • *FH* • *SDH* • *FLCN* • *TSC1* • *TSC2* • *PTEN* • *BAP1*

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M. Oya (ed.), *Renal Cell Carcinoma*, DOI 10.1007/978-4-431-55531-5_2

2.1 Introduction

Hereditary renal cell carcinoma (RCC) is a heterogeneous disorder comprised of a variety of hereditary syndromes, each of which has a specific genetic/molecular basis, characteristic histology, and clinical features (Table 2.1). Hereditary RCC is estimated to account for 3% to 5% of all kidney cancers. However, the frequency of hereditary RCC is likely to be underestimated. Recognition and diagnosis of hereditary RCC susceptibility syndromes is important for patients and relatives at risk because of the medical consequences. Hereditary RCC tends to be bilateral and multifocal and has an early age of onset. Some hereditary RCCs display characteristic histologies. Furthermore, the presence of specific extrarenal manifestations is very useful for the proper diagnosis of hereditary RCC susceptibility syndromes (Table 2.1).

Through the study of hereditary RCC susceptibility syndromes, many important novel genes have been identified such as the *VHL (von Hippel-Lindau)* gene responsible for von Hippel-Lindau disease, and several previously known genes were rediscovered to have new essential functions including the *MET* proto-oncogene which is mutated in hereditary papillary renal cell carcinoma and *FH (fumarate hydratase)*, the gene responsible for hereditary leiomyomatosis renal cell carcinoma. Cloning of these genes and elucidating their molecular genetics have contributed to a better understanding of the pathogenesis of these hereditary RCC susceptibility syndromes and to the development of specific genetic tests, appropriate surveillance, and targeted therapies. These studies have also provided insight into the molecular basis of non-hereditary, sporadic RCC. In this chapter, clinical manifestations and the genetics of hereditary RCC susceptibility syndromes and the molecular function of the responsible genes will be presented.

2.2 Von Hippel-Lindau (VHL) Disease

Von Hippel-Lindau (VHL) disease is an autosomal dominant hereditary neoplastic disorder and the first described hereditary kidney cancer syndrome. The clarification of the molecular pathogenesis of VHL disease has made an enormous contribution toward understanding the molecular mechanism of sporadic clear cell renal cell carcinoma (ccRCC) development and provided the basis for developing molecular targeted therapies for ccRCC.

2.2.1 Clinical Manifestations of Von Hippel-Lindau Disease

VHL disease is a rare disease which occurs about 1 in 36,000 [1, 2, 3], which is characterized by a predisposition to develop ccRCC, pheochromocytomas, central

Table 2.1 Hereditary RCC susceptibility syndromes

Syndrome	Gene	Chromosome location	Mutation rate in sporadic RCC	Prevalence	% de novo	Renal tumor histology	Multiple/ solitary	Penetrance of RCC	Age (years) at RCC diagnosis	Extrarenal manifestations
VHL disease	*VHL*	3p25	92% in sporadic ccRCC	1/36,000	20%	ccRCC	BMF	25–45%	45 (mean)	Pheochromocytoma
										CNS hemangioblastoma
										Retinal angioma
										Endolymphatic sac tumor
										Pancreatic tumor/cyst
										Epididymal cystadenoma
										Broad ligament cystadenoma
HPRC	*MET*	7q31	13% in papillary type 1 RCC	Very rare, around 30 families	47%	Papillary type 1 RCC	BMF	67%	46~63 (median)	None
HLRCC	*FH*	1q42	Not found in sporadic RCC	TBD	TBD	Papillary type 2 RCC (62.5%)	Mostly solitary	14–18%	39–46 (median)	Skin leiomyoma
						Tubulopapillary (20%)				
						Tubular (5%)				
						Solid (1.5%)				Uterine leiomyoma
						Mixed patterns (10%)				
SDH-RCC	*SDHB/C/D*	SDHB:1p35–36 SDHC: 1q23 SDHD:11q23	Not found in sporadic RCC	TBD	TBD	Unique form of oncocytic RCC, ccRCC, chromophobe RCC	BMF	SDHB:14% SDHD:8%	SDHB:33 (mean) SDHC:47 (mean), SDHD:48 (median)	Pheochromocytoma
						Papillary type 2 RCC, oncocytoma				Paraganglioma
BHDS	*FLCN*	17p11	Controversial (0~5% in chromophobe RCC)	TBD (report of 500 families worldwide)	TBD	Oncocytic hybrid tumor (50%), chromophobe RCC (35%), ccRCC (9%), oncocytoma (5%)	BMF	12–34%	48 (median)	Fibrofolliculoma, lung cyst, spontaneous pneumothorax
TSC	*TSC1/TSC2*	TSC1:9q34 TSC2:16p13	6% in chromophobe RCC, 2% in ccRCC	1/6000 to 1/10,000	66%–83%	Renal angiomyoadenomatous tumors (RAT)-like RCC	BMF	2–3%	30–42 (mean)	Angiofibroma fibrous cephalic plaque, ungual fibroma, shagreen patches, hypomelanotic macule, confetti-like macule, cortical dysplasia, subependymal nodule (SEN), subependymal giant cell astrocytoma (SEGA), cardiac rhabdomyoma, lymphangioleiomyomatosis (LAM), uterine PEComa
						RCC with smooth muscle stroma, chromophobe-like RCC, unique granular eosinophilic-macrocystic RCC, TSC-associated papillary RCC, hybrid oncocytic/chromophobe tumor (HOCT), unclassified RCC, angiomyolipoma, epithelioid angiomyolipoma				

(continued)

Table 2.1 (continued)

Syndrome	Gene	Chromosome location	Mutation rate in sporadic RCC	Prevalence	% de novo	Renal tumor histology	Multiple/ solitary	Penetrance of RCC	Age (years) at RCC diagnosis	Extrarenal manifestations
Cowden syndrome	*PTEN*	10q23	7.5% in sporadic RCC, 9% in chromophobe RCC, 2–4% in ccRCC	At least 1/200,000	10.7%–47.6%	Papillary RCC, ccRCC, chromophobe RCC	Mostly solitary	34%	49 (median) 45 (mean)	Trichilemmoma (hair follicle hamartoma), papillomatous papule, acral/plantar keratoses, macrocephaly, dolichocephaly dysplastic gangliocytoma of the cerebellum, benign tumors(colorectal polyposis, thyroid goiter/nodule, lipoma, fibroma, and proliferative breast change), lifetime risk for cancer (breast cancer, thyroid cancer, endometrial cancer, colorectal cancer)
BAP1 cancer syndrome	*BAP1*	3p21	7.5 to 14% in ccRCC	TBD	TBD	ccRCC	BMF	TBD	45 (mean)	Malignant mesothelioma, uveal melanoma, cutaneous melanoma, melanocytic BAP-1 mutated atypical intradermal tumors (MBAITTs), lifetime risk for other cancers (breast cancer, lung cancer, neuroendocrine carcinoma, basal cell carcinoma, meningioma)

BMF bilateral multifocal, *TBD* to be determined

nervous system (CNS) hemangioblastomas, retinal angiomas, endolymphatic sac tumors, pancreatic tumors, cysts in the kidney and pancreas, epididymal cystadenomas, and broad ligament cystadenomas (Fig. 2.1a–i) [4–7]. VHL disease is classified generally into two subtypes, type 1 without pheochromocytoma and type 2 with pheochromocytoma. Type 2 is further subclassified into type 2A without RCC, type 2B with RCC, and type 2C with pheochromocytoma only without any other manifestations [8, 9]. Twenty-five to 45% of affected members of VHL families have bilateral, multifocal ccRCC [3]. Since the biological behavior of ccRCC in VHL disease is known to be mild and VHL patients have a lifetime risk for recurring ccRCC development, active surveillance is recommended until the size of the largest tumor reaches 3 cm in diameter. To conserve kidney function,

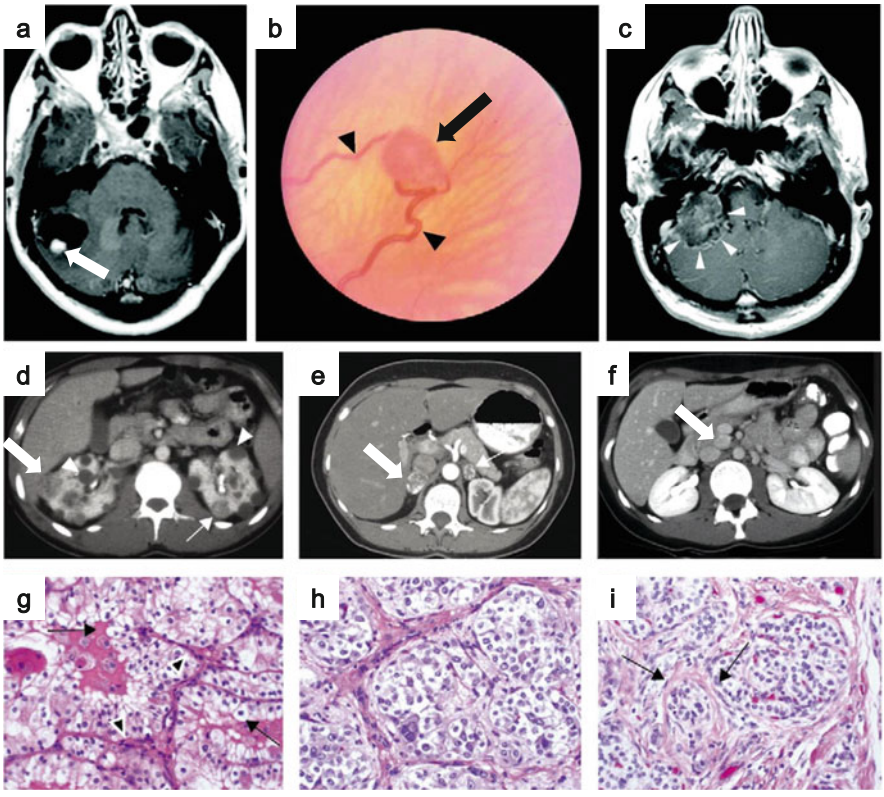

Fig. 2.1 Clinical manifestations of VHL disease. (**a**) MRI image of a cerebellar hemangioblastoma (*arrow*) with an associated cyst. (**b**) Ophthalmoscopic view of retinal hemangioblastoma (*arrow*) with an enlarged vessel (*arrowheads*). (**c**) MRI image of an endolymphatic sac tumor (*arrowheads*). (**d**) Postcontrast CT imaging shows bilateral multifocal RCC with solid (*arrows*) and cystic (*arrowheads*) disease. (**e**) Postcontrast CT image of bilateral pheochromocytomas (*arrows*). (**f**) Pancreatic neuroendocrine tumor (*arrow*). (**g**) Histology of clear cell RCC. (**h**) Histology of pheochromocytomas. (**i**) Histology of pancreatic neuroendocrine tumors with trabecular architecture (Images from Lonser et al. [6])

nephron-sparing surgery including enucleation of the tumor is preferred as a surgical intervention [10, 11]. Pheochromocytomas develop in 10 to 20% of individuals with VHL disease, which can be multiple and bilateral. Extra-adrenal paragangliomas can arise in the carotid body and sympathetic paraganglia. Minor populations of pheochromocytoma in VHL disease can be malignant [3, 6]. CNS hemangioblastomas are the most common manifestations seen in 60–80% of affected patients. Although these are benign tumors, they are a major cause of morbidity in VHL disease because of their localization in the cerebellum, brainstem, and spinal cord [6, 12, 13].

2.2.2 Genetics of Von Hippel-Lindau Disease

Loss of heterozygosity (LOH) on chromosome 3p was first found in sporadic RCC [14]. The study of age incidence for sporadic ccRCC and for RCC in VHL disease suggested that the chance of developing RCC in VHL disease was compatible with a "one-hit" model, while the chance of developing RCC in a sporadic setting was compatible with a "two-hit" model [15]. Based on these findings, a tumor suppressor for ccRCC was predicted to be located on chromosome 3p, and a novel *VHL* gene was isolated on chromosome 3p25–26 by positional cloning in VHL kindreds [16]. Individuals affected with VHL disease harbor a germline mutation in the *VHL* gene. LOH or somatic inactivation of the second allele was observed in VHL-associated RCC, indicating a classical tumor suppressor function for *VHL* [17, 18]. Germline *VHL* mutations in VHL disease encompass a broad spectrum of mutations, including frameshift mutations, nonsense mutations, large deletions, splicing defects, and missense mutations substituting an amino acid in the VHL protein. Over 945 VHL families worldwide have been analyzed for *VHL* germline mutations, and more than 700 different VHL mutations have been found throughout the entire *VHL* gene with the exception of the first 35 amino acids, which are not conserved across species [3]. *VHL* germline mutations were identified in nearly 100% of VHL families facilitated by the development of new methods to detect large deletions, confirming that VHL disease is caused solely by germline mutations in the *VHL* gene [19].

One of the major findings that has come from studying VHL disease to understand the molecular mechanism of RCC is that somatic mutations of the *VHL* gene accompanied by loss of the wild-type *VHL* allele are found in most sporadic ccRCC [20, 21]. Ninety-two percent of sporadic ccRCC are reported to have somatic mutations in or methylation of the *VHL* gene, indicating that loss of the *VHL* gene function is the fundamental initial step in most sporadic ccRCC development [22]. Insights gained from studies of families with VHL disease serve as a model for how discoveries obtained from study of a familial cancer may be applied to sporadic cancers.

2.2.3 Molecular Function of VHL Protein

The protein encoded by the *VHL* gene, pVHL, was a novel protein with no known functional domains, when it was isolated. Extensive research has clarified that pVHL functions as a substrate recognition component of an E3 ubiquitin ligase protein complex composed of elongin C, elongin B, Cul2, and Rbx1 [23–30]. Under normoxic conditions, transcription factors hypoxia-inducible factor 1α (HIF1α) and hypoxia-inducible factor 2α (HIF2α) are hydroxylated on their N-terminal transactivation domain (NTAD) by the EglN family of prolyl hydroxylases (PHDs), which require α-ketoglutarate, oxygen, ascorbic acid, and iron. Prolyl hydroxylated HIF1α and HIF2α are bound by the β-domain of pVHL, ubiquitinated and degraded by the proteasome [30–37]. Under conditions when oxygen or iron is insufficient, HIF1α/HIF2α is not hydroxylated, escapes from pVHL-mediated ubiquitination, and accumulates, driving transcription of hypoxia-responsive genes through their binding to hypoxia-responsive elements (HREs). Thus, in *VHL*-deficient cells, HIF1α/HIF2α is not ubiquitinated, and hypoxia-responsive genes, which are important for cell proliferation, including *VEGF*, *PDGFB*, *TGFα*, *GLUT1*, and *CCND1*, are upregulated even under normoxic conditions [38]. The fact that germline *VHL* mutations are frequently found in the α-domain of pVHL that interacts with elongin C and the pVHL β-domain, which interacts with prolyl hydroxylated HIFα, emphasizes the physiological importance of HIFα degradation for pVHL tumor suppressor function. HIF1α and HIF2α are similar in their structure, form heterodimers with HIFβ (ARNT) to bind to HREs, and share many hypoxia-responsive gene targets. However, their target genes are not identical and differ in a context-dependent manner. For example, glycolysis-related genes are mainly regulated by HIF1α, and *CCND1* is regulated by HIF2α in RCC cells [38–43]. In terms of kidney cancer development, many in vitro and in vivo studies support the idea that HIF2α is a renal oncoprotein and HIF1α is a renal tumor suppressor [43–46]. Chromosome 14, where the HIF1α gene is located, is frequently deleted in ccRCC, and loss of 14q is associated with poor prognosis of ccRCC patients [47].

2.2.4 VHL Research: Bench to Bedside

As mentioned above, VHL disease research has made invaluable contributions to the clarification of the molecular mechanisms of ccRCC development and to the development of molecular target therapies for RCC [48]. Many drugs targeting the VHL-HIFα axis that have been approved by the FDA as therapeutic agents for advanced ccRCC patients have proven efficacy and superseded conventional immunotherapies. The details of targeted therapies for RCC will be discussed in other chapters.

2.2.5 Additional Gene Alterations in ccRCC

High throughput sequencing analysis of sporadic ccRCC has identified a number of gene alterations in addition to *VHL* mutations [49–51]. Genes mutated in sporadic ccRCC are involved in chromatin remodeling (*PBRM1*) [52], or histone modification, which regulates chromatin structure (*SETD2*, BAP1, *JARID1C*, and *UTX*, also known as *KDM5C* and *KDM6A*) [49, 53, 54]. Interestingly, *PBRM1*, *SETD2*, and *BAP1* are located on chromosome 3p and could be deleted with *VHL* as a result of chromosome 3p loss. These gene alterations could contribute to ccRCC development and progression, which is initiated by loss of *VHL*. In fact, *BAP1* mutation is associated with poor prognosis of ccRCC [51, 54]. Further analysis of the physiological consequence of alterations in these genes will provide a better understanding of the nature of ccRCC and might lead to the development of next-generation therapeutic agents for ccRCC.

2.3 Hereditary Papillary Renal Cell Carcinoma Type 1 (HPRC)

Hereditary papillary renal cell carcinoma type 1 (HPRC) is an autosomal dominant hereditary cancer syndrome (Fig. 2.2a), which was first described by Zbar et al. in 1994 [55, 56]. HPRC is a very rare type of hereditary RCC syndrome that predisposes affected individuals to develop bilateral multifocal papillary type 1 RCC (Fig. 2.2b) [57]. Causative germline mutations have been identified in the *MET* gene, which has an essential role in cancer cell proliferation, survival, invasion, and metastasis. Molecular genetic studies of HPRC have also contributed to our understanding of the molecular basis of RCC and provided the basis for development of targeted therapies for papillary RCC.

2.3.1 Clinical Manifestations of HPRC

Distinct from other hereditary RCC syndromes, no manifestations other than RCC have been reported in HPRC. The patients have a lifelong risk for the development of multiple papillary type 1 RCC with age-dependent penetrance, which is estimated to be 67% by 60 years of age [58]. However, there are rare cases of HPRC kindreds presenting with earlier-onset RCC [59, 60]. RCC in HPRC tends to grow slowly, but is malignant and may metastasize when the tumor size becomes large. Since patients have a lifelong risk of developing multiple renal tumors, active surveillance is recommended until the largest tumor size reaches 3 cm when nephron-sparing surgery should be considered [11]. Histologically papillary type 1 RCC exhibits a characteristic papillary/tubulopapillary architecture lined by a

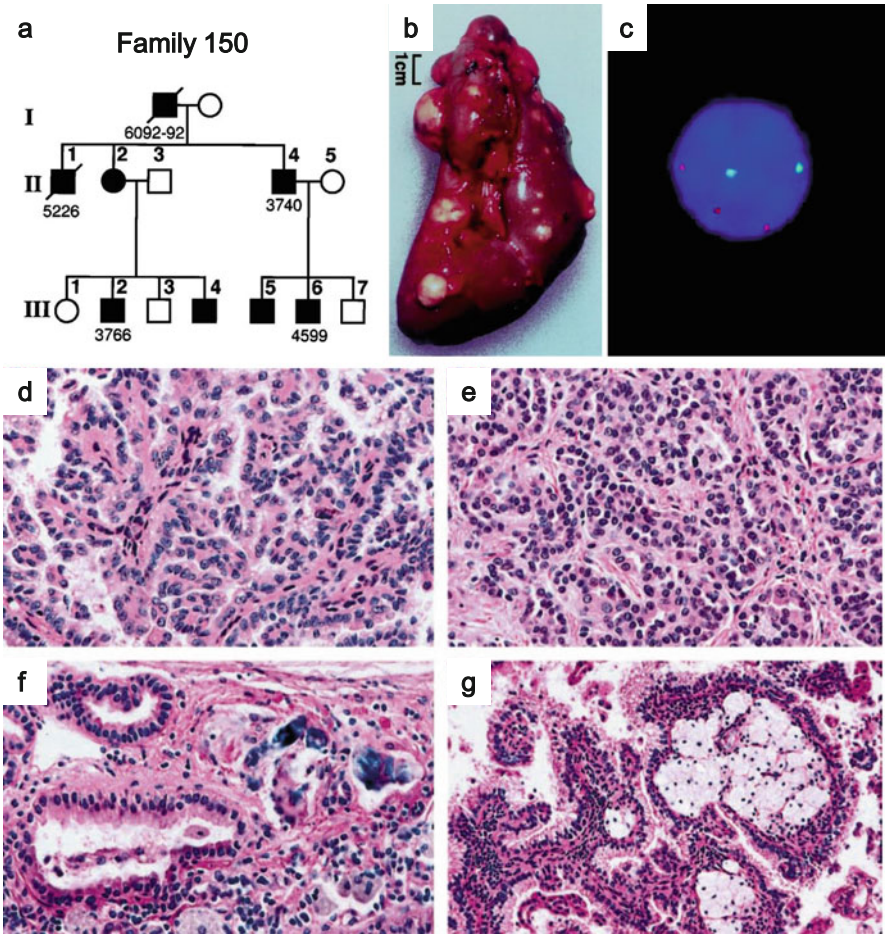

Fig. 2.2 Clinical manifestations of HPRC. (**a**) Pedigree of HPRC family. Solid symbols indicate individuals with RCC. (**b**) Gross image of a nephrectomized kidney from an HPRC patient. (**c**) Fluorescent in situ hybridization (FISH) on an RCC touch preparation shows trisomy of chromosome 7 (red chromosome 7, green chromosome 17). (**d**) Histology of RCC showing papillary architecture characterized by thin interstitium. (**e**) Tubulopapillary architecture composed of small RCC cells with basophilic nuclei and amphophilic cytoplasm. (**f**) Psammoma bodies are prominent histological features. (**g**) Most tumors in HPRC demonstrate foamy macrophages in fibrovascular cores. Focal clear cells can be seen occasionally (Images from Lubensky et al. [61])

single layer of small cells having small basophilic nuclei and amphophilic cytoplasm (Fig. 2.2d, e). Occasionally focal areas of cells with eosinophilic cytoplasm can be seen [61]. Fuhrman nuclear grade is predominantly 1–2. In some tumors, focal areas of Fuhrman nuclear grade 3 can be seen. Most tumors in HPRC exhibit foamy macrophages in fibrovascular cores. Psammoma bodies are frequently seen. There can be focal areas of clear cells (Fig. 2.2f, g). Multiple adenomas and microscopic papillary lesions can be seen in the renal parenchyma surrounding

the tumors [61]. HPRC-associated RCC is hypovascular, and computed tomography (CT) imaging shows hypoenhancement with a contrast agent [57].

2.3.2 Genetics of HPRC

Trisomy of chromosome 7 was identified as a characteristic feature of papillary RCC, which suggested the localization of an oncogene on chromosome 7 [62, 63]. Through genetic linkage analysis in HPRC families, the responsible locus for HPRC was narrowed to chromosome 7q31.1–34, where the *MET* proto-oncogene was located. Schmidt et al. identified germline missense mutations in the tyrosine kinase domain of *MET* on chromosome 7q31 in affected individuals of HPRC kindreds [64]. Subsequently, somatic mutations of *MET* were identified in 13% of sporadic papillary type 1 RCC [64, 65]. These *MET* mutations were located in codons homologous to codons in *KIT* and *RET*, which were mutated in systemic mastocytosis and multiple endocrine neoplasia (MEN) type 2B, respectively. These findings support the idea that these missense mutations in *MET* are gain of function mutations, acquiring oncogenic activity.

2.3.3 Molecular Consequence of MET Mutation in HPRC

The *MET* proto-oncogene encodes c-Met, the hepatocyte growth factor/scatter factor (HGF/SF) receptor tyrosine kinase. HGF/SF, the ligand of c-Met, is produced by mesenchymal cells and stimulates a variety of neighboring cells including epithelial, endothelial, hematopoietic, and neuronal cells during normal embryonic development and throughout adulthood [66]. HGF/SF/c-Met signaling induces multiple biological activities, which include proliferation, survival, motility, epithelial-mesenchymal transition, and branching morphogenesis. Upon ligand binding, two tyrosine residues (Y1234 and Y1235) of c-Met in the activation loop of the tyrosine kinase domain are autophosphorylated and enhance c-Met kinase activity. Subsequent phosphorylation on two tyrosine residues (Y1349 and Y1356) near the carboxy terminus of c-Met form a multifunctional docking site which recruits a variety of signaling molecules, transmitting the signals further downstream for a variety of biological outputs [67, 68]. The pathological significance of *MET* missense mutations found in HPRC or PRC was investigated in NIH3T3 transfectants [69, 70]. Mutant c-Met showed increased autophosphorylation on tyrosine residues compared to wild-type c-Met. NIH3T3 cells expressing mutant c-Met are able to make foci on monolayer culture and form larger tumors in nude mice than cells expressing wild-type c-Met. In addition these cells displayed increased motility and increased activation of the Ras-Raf-MEK-ERK signaling pathway without HGF. Furthermore, the fact that a transgenic mouse model expressing mutant c-Met developed metastatic mammary carcinoma solidified the

idea that mutant *MET* functioned as an oncogene [69, 70]. However, the data that support ligand-independent activation of mutant c-Met should be considered with caution. Most of the initial functional experiments with mutant c-Met were done in NIH3T3 cells, which express HGF/SF endogenously, but epithelial cells including renal tubular cells do not express HGF/SF. In fact, MDCK kidney epithelial cells reconstituted with c-Met mutants require the addition of exogenous HGF/SF for colony formation in soft agar. Deletion of the extracellular domain of mutant c-Met abrogates its transformation ability. In addition, expression of the soluble c-Met extracellular domain was able to block colony formation of NIH3T3 cells expressing mutant c-Met. Taken together, these data suggest that the availability of HGF/SF may contribute greatly to the oncogenesis of *MET* mutations in HPRC [71]. Mutant *MET* appears to have a lower threshold for kinase activation by HGF/SF, stabilizes the active conformation of the kinase, and exhibits a reduced susceptibility to inactivation by phosphatases in some cases [57]. It is noteworthy that most (95%) sporadic papillary type 1 RCC (PRC) exhibit chromosome 7 trisomy, while only 13% of PRC have somatic mutations in *MET*. Importantly, both *MET* and *HGF*/*SF* localize on chromosome 7. So trisomy 7 causes increased dosage of both HGF/SF and c-Met thereby driving HGF/c-Met signaling, which might be important for PRC development. Zhuang et al. precisely analyzed 16 RCCs in HPRC and found trisomy 7 in all tumors. Importantly, duplication of the specific chromosome 7 that harbors the mutant *MET* was seen in all 16 RCCs (Fig. 2.2c) [72]. This selective duplication of the mutant *MET* allele may function as a second hit event for RCC development in HPRC. These findings may suggest that the increased dosage of HGF/SF and c-Met and enhanced signaling through this axis is the essential factor for RCC development for both sporadic PRC and HPRC. Together with ligand dependency of mutant c-Met activation, these findings suggest an attractive hypothesis to explain why affected family members of HPRC develop cancer only in the kidney. The kidney produces large amounts of HGF/SF, as well as urokinase, which is necessary to activate the secreted immature form of HGF/SF [72].

2.3.4 HPRC Research: Bench to Bedside

These studies to understand the molecular pathogenesis of HPRC have provided significant insights into the development of targeted therapeutics [73]. Based on basic research, there are three possible strategies to target c-Met for HPRC and PRC: (1) direct inhibition of c-Met tyrosine kinase activity, (2) blockage of HGF/SF and c-Met interaction, and (3) inhibition of the molecular interaction between the cytoplasmic docking motif of c-Met and the effector downstream molecules. To date several humanized anti-HGF/SF monoclonal antibody drugs have been developed and are being tested in clinical trials for a variety of cancers [74]. An anti-c-Met humanized monoclonal antibody drug has also been developed and is being tested in a clinical trial for non-RCC cancers [75]. Small molecules targeting c-Met

kinase activity are also being tested for efficacy in treating PRC and HPRC. The presence of germline mutations in *MET* is a factor well correlated with a positive response [75–77]. Since c-Met is activated in *VHL*-deficient ccRCC cells, c-Met could also be a target molecule for advanced ccRCC therapy [78].

2.4 Hereditary Leiomyomatosis and Renal Cell Carcinoma (HLRCC)

HLRCC is an autosomal dominant hereditary kidney cancer syndrome that was first reported in 2001 [79] as an inherited susceptibility to uterine leiomyomas and papillary RCC. HLRCC is caused by germline mutations of the *fumarate hydratase* (*FH*) gene encoding the TCA cycle enzyme [80]. RCC in HLRCC is very aggressive and has to be managed totally differently from RCCs that develop in other types of hereditary kidney cancer syndromes.

2.4.1 Clinical Manifestations of HLRCC

HLRCC is characterized by three manifestations: cutaneous leiomyomas, uterine leiomyomas (fibroids), and renal tumors and benign renal cysts. Originally HLRCC was reported in 1973 as Reed's disease, in which patients presented with cutaneous leiomyomas and uterine leiomyomas. Subsequently cosegregation of kidney cancer with skin and uterine leiomyomas was identified, and Reed's disease was renamed HLRCC (Fig. 2.3a). Skin leiomyomas are the most common manifestations seen in 76–100% of affected individuals with HLRCC, which present as multiple, firm, skin-colored to light-brown-colored papules and nodules (Fig. 2.3b) [81–83]. The number of lesions range from 10 to 100, and the size ranges from 0.4 to 2.5 cm in diameter. They develop on the trunk and extremities increasing over time with a disseminated pattern or a combination of disseminated and segmental distribution. Most of the lesions are symptomatic with pain and paresthesias. Mean age of onset of cutaneous lesions is 25 years (range 10–47 years old) [81]. The other common manifestations are uterine leiomyomas (fibroids), developing in most women affected with HLRCC (Fig. 2.3c) [81–85]. Multiple leiomyomas with diameters ranging from 1.5 cm to 10 cm develop very early (median 28–31 years) in HLRCC patients [84, 86]. In one study, 91% of affected women with skin and uterine leiomyomas had a myomectomy or hysterectomy, and 57% of affected women with skin and uterine leiomyomas had a hysterectomy before 30 years of age [81]. Uterine leiomyomas in HLRCC show characteristic histology including increased cellularity, large single nuclei, or multiple nuclei with large orangiophilic nucleoli surrounded by a halo [86].

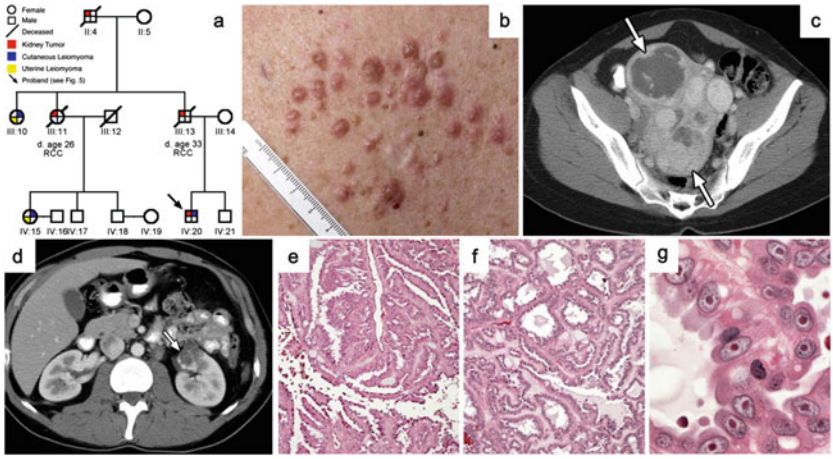

Fig. 2.3 Clinical manifestations of HLRCC. (**a**) Pedigree of an HLRCC family. *Red quadrants* indicate individuals with RCC. (**b**) Multiple skin leiomyomas on the trunk. (**c**) CT image of multiple large uterine leiomyomas (*arrows*). (**d**) CT image of a solitary RCC. (**e**) Histology of RCC showing papillary type 2 RCC architecture with thick and elongated collagen-abundant stalks. (**f**) Other architectural patterns including tubulopapillary are seen. (**g**) The characteristic large nucleus with a prominent large inclusion-like eosinophilic or orangiophilic nucleolus surrounded by a clear halo (Images from Grubb et al. [89])

HLRCC patients have an increased risk for developing RCC compared to unaffected family members. However, the penetrance for RCC in HLRCC is much lower than for leiomyomas, ranging from 14 to 18% in North American and French studies, and even lower in a Dutch study [87, 88]. In contrast to other hereditary kidney cancer syndromes, most RCCs in HLRCC are solitary and unilateral (Fig. 2.3d). However, there are reports of two cases of bilateral or bilateral, multifocal RCC among 38 HLRCC patients [89, 90]. RCC can develop in HLRCC at a young age (10–44 years) [79, 81, 89] with a reported median age at diagnosis of 39–46 years [81, 89]. The histology of HLRCC-related RCC is classified typically as papillary type 2 RCC, in which papillae are thick and elongated with fibrovascular cores (Fig. 2.3e). Many RCCs in HLRCC show this papillary pattern (62.5%), which is composed of characteristic cells with abundant amphophilic cytoplasm and large nuclei (Fig. 2.3g). However, it should be noted that they may also display other architectural patterns including tubulopapillary (20%) (Fig. 2.3f), tubular (5%), solid (1.5%), and mixed patterns (10%) [90]. Many cases of RCC in HLRCC have no cystic component (47.5%), but some cases have cystic areas (40%) or are predominantly cystic (12.5%). The hallmark of the HLRCC tumors is the presence of a characteristic large nucleus with a prominent large inclusion-like eosinophilic or orangiophilic nucleolus surrounded by a clear halo (Fig. 2.3g). Although the histology can be variable in HLRCC tumors, the characteristic feature of a macronucleus and prominent nucleolus with clear halo is commonly seen in all RCC in HLRCC [90]. Based on the nucleolus size, Fuhrman

nuclear grade is classified as high grade in all cases. To date, there is no specific immunohistochemical marker for HLRCC-associated RCC. HLRCC-related RCC reveals an extremely malignant character, which differentiates HLRCC from other hereditary kidney cancer syndromes. More than 70% of RCC patients with HLRCC present with advanced stage III or stage IV disease [79, 81, 89]. Importantly, HLRCC-related RCC tends to metastasize to lymph nodes very early, when the primary tumors are T1. Even if patients initially present with localized disease, 50% will eventually develop lethally metastatic disease [89]. Based on this malignant nature, HLRCC-related RCC must be treated differently from other inherited forms of RCC. HLRCC tumors should be surgically treated immediately upon detection regardless of size rather than management by active surveillance until the largest tumor size reaches 3 cm, which is recommended for most other inherited RCC syndromes [81, 91, 92].

Ten percent of affected individuals with HLRCC have been reported to have adrenal cortical adenomas [83, 93].

2.4.2 Genetics of HLRCC

Genetic linkage analysis in HLRCC families localized the disease locus on chromosome1q42, and germline mutations were identified in a gene encoding fumarate hydratase (*FH*), an enzyme of the tricarboxylic acid (TCA) cycle, which catalyzes the conversion of fumarate to malate [80, 81]. These mutations are predicted to cause absence or truncation of the FH protein or substitutions or deletions of conserved amino acids. Missense mutations are most common. Moreover FH enzyme activity was shown to be absent or reduced in tumors, lymphoblastoid cells, and fibroblasts from HLRCC patients [80, 82, 94, 95]. LOH studies show loss of the wild-type *FH* allele in skin and uterine leiomyomas and RCC, indicating *FH* is a classical tumor suppressor gene in HLRCC. More than 150 unique *FH* mutations, which may be pathogenic, have been reported in the Leiden Open Variant Database [96]. The mutation detection rate in affected individuals with HLRCC is reaching 90% [81, 82, 95]. There is a missense mutational hot spot at Arg190, which is mutated to histidine, leucine, or cysteine [81, 82, 95]. Kiuru et al. searched for *FH* mutations in sporadic skin and uterine leiomyomas and sporadic RCC and found few mutations [97].

2.4.3 Molecular Consequence of Mutation in FH

FH functions as a tetramer. Reduced FH enzymatic activity in lymphoblastoid cells and fibroblasts from HLRCC patients indicates that mutant FH may function as a dominant negative form to disturb normal function of the FH tetramer

[98, 99]. Loss of FH activity impairs oxidative phosphorylation enabling a cell metabolism shift to aerobic glycolysis [100, 101]. Due to a blockage in the TCA cycle, accumulated fumarate and succinate are transported out of the mitochondria into the cytoplasm and compete with α-ketoglutarate, which is a cosubstrate of the EglN family of prolyl hydroxylases (PHDs) that target HIFα , resulting in inhibition of PHD activity and accumulation of HIFα and thereby evading pVHL-mediated ubiquitination and proteasomal degradation [102, 103]. This pseudo-hypoxic condition results in elevation of HIF-target genes such as *VEGF* and *GLUT1*, which leads to upregulated angiogenesis and glucose uptake [104]. More evidence supporting the oncometabolite function of fumarate and succinate is accumulating. Elevated fumarate and succinate in *FH*-mutated RCC can inhibit multiple α-ketoglutarate-dependent dioxygenases, which include histone demethylases (KDMs, JMJDs), prolyl hydroxylases, and the ten-eleven translocation (TET) family of DNA hydroxylases. As a consequence of dioxygenase impairment, genome-wide epigenetic alterations could occur which may contribute to kidney cancer development in HLRCC patients [105]. RCCs in HLRCC have increased levels of ROS, leading to HIFα stabilization [101]. This suggests another mechanism of tumor suppression by FH as well as the possible involvement of the antioxidant response in RCC development in HLRCC. S-(2-succinyl) cysteine (2SC) has been identified as an endogenous chemical modification of proteins. Fumarate is an electrophile and reacts with cysteine sulfhydryl groups to form 2SC under physiological conditions [106]. This reaction is termed succination, which could be detected endogenously, and modifies the activity of many proteins [107]. One of the significant proteins that is modified by succination is KEAP1. KEAP1 is the substrate recognition subunit of an E3 ubiquitin ligase complex, which is composed of KEAP1, Cul3, and Rbx1. This complex ubiquitinates a transcription factor, nuclear factor erythroid 2-related factor 2 (Nrf2) for proteasome mediated degradation [108, 109]. Nrf2 transcriptionally upregulates target genes containing antioxidant-response elements (ARE), in response to oxidative and electrophilic stress [110]. In *FH*-mutated cells, elevated fumarate causes succination of critical cysteine residues in KEAP1, resulting in a conformational change of the KEAP1 containing E3 complex and an abrogation of Nrf2 recognition by KEAP1. As a result, Nrf2 is stabilized, accumulates, and upregulates ARE-containing genes leading to overactivation of the Nrf2-dependent antioxidant pathway [111–113]. In fact, somatic mutations in *NRF2* and *CUL3* are found in sporadic type 2 papillary RCC, which result in Nrf2 activation. Consistent with this model, loss of function mutations in *KEAP1* are frequently seen in sporadic cancers [114–119]. Although Nrf2 is activated to transcriptionally upregulate antioxidant genes, ROS levels are still high in FH-deficient RCC cells. Sullivan et al. found that accumulated fumarate directly binds the antioxidant glutathione (GSF), which works as an alternative substrate to glutathione reductase, resulting in decreased NADPH and increased ROS [120].

2.4.4 HLRCC Research: Bench to Bedside

HLRCC-associated RCC has very malignant features and metastasizes even when the primary tumor is small in size. Advanced RCC in HLRCC is refractory to conventional immunotherapy and lethal. However, results from many research studies have been reported in the decade since *FH* germline mutations were identified in HLRCC, which may provide a foundation for the development of rational targeted therapies. Based on basic research, a phase II clinical trial to evaluate combination therapy of bevacizumab (anti-VEGF monoclonal antibody) and erlotinib (EGFR inhibitor) is currently under way at the National Cancer Institute, NIH (https://clinicaltrials.gov/ct2/show/NCT01130519?term=HLRCC&rank=1). The recent findings that define the KEAP1-Nrf2 axis in HLRCC may provide another basis for developing new targeted therapies for advanced HLRCC-associated RCC.

2.5 Hereditary Head and Neck Paragangliomas (HPGL) and Pheochromocytomas (PCC): SDH-RCC

Hereditary head and neck paragangliomas (HPGL), extra-adrenal pheochromocytomas (paragangliomas), and hereditary pheochromocytomas (PCC) are caused by germline mutations in genes encoding the three subunits (*SDHB*, *SDHC*, and *SDHD*) of the mitochondrial TCA cycle enzyme succinate dehydrogenase (*SDH*) [121, 122]. Bilateral multifocal RCC was reported as a novel manifestation of *SDHB*-mutated HPGL in 2004 [123]. A unique form of oncocytic RCC is seen most frequently in SDH-RCC. However, a variety of histologies including clear cell RCC, chromophobe RCC, papillary type 2 RCC, and oncocytoma have been reported [124–127]. SDH-RCC also can be very aggressive, similar to HLRCC.

2.5.1 Clinical Manifestations of SDH-RCC

Fifty-four percent of affected individuals with *SDHB* germline mutations developed HPGL or PCC and 79% of *SDHC* mutation carriers presented with HPGL or PCC. PCC and HPGL can be bilateral and/or multifocal [125, 128]. The mean age of diagnosis is younger for PCC (42.3 and 40.1 years of age) than for HPGL (27.4 and 20.7 years of age) in *SDHB* and *SDHD* mutation carriers, respectively. Approximately 13.6% of *SDHB* mutation carriers and 3.2% of *SDHD* mutation carriers developed malignant PCC or HPGL [126]. The frequency of RCC development is not very high. The lifetime risk of developing a renal tumor at the age of 70 was 14% in *SDHB* and 8% in *SDHD* mutation carriers, respectively [126]. RCC associated with SDH-RCC can have bilateral, multifocal, and early-onset characteristics.

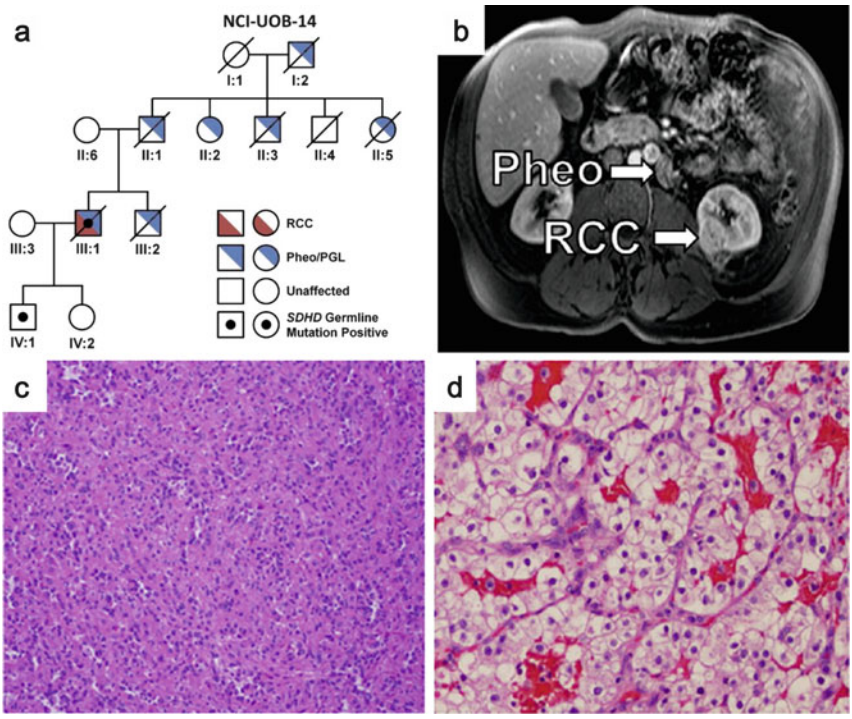

Fig. 2.4 Clinical manifestations of SDH-RCC. (**a**) Pedigree of an *SDHD* mutation-associated SDH-RCC family. Patient III:1 had advanced ccRCC. (**b**) MRI image of an *SDHB* mutation-associated SDH-RCC patient showing a pheochromocytoma and an RCC. (**c**) A unique form of oncocytic RCC is seen most frequently in SDH-RCC. (**d**) Histology of ccRCC seen in an *SDHC* mutation-associated SDH-RCC patient (Images from Ricketts et al. [127])

2.5.2 Genetics of SDH-RCC

Germline mutations in *SDHD* were first identified in HPGL families in 2000 [121]. An *SDHD* germline mutation was found in a kindred with familial PCC as well [129]. Subsequently, germline mutations in *SDHB* and *SDHD* were also identified as causes of susceptibility to familial PCC and HPGL [130, 131]. In 2004, two young affected family members with HPGL and germline *SDHB* mutations were diagnosed with clear cell RCC [123]. Subsequently RCCs with a variety of histologies have been identified in family members inheriting germline mutations in *SDHB*, *SDHC*, and *SDHD* (Fig. 2.4a–d) [124–128]. All types of loss of function germline mutations including missense, frameshift, and nonsense are seen in SDH-RCC kindreds.

2.5.3 Molecular Consequence of Mutations in SDHB, SDHC, and SDHD

SDH enzymatic activity is impaired in *SDH*-mutated cells, resulting in the accumulation of succinate. Similar to increased fumarate in FH-deficient cells in HLRCC, accumulating succinate is exported into the cytoplasm and can compete with α-ketoglutarate, resulting in inhibition of enzymes which utilize α-ketoglutarate as a cosubstrate, including prolyl hydroxylases (PHDs) [102, 103, 105]. Upon PHD inactivation, HIFα evades ubiquitination by the pVHL E3 complex, accumulates, and transcriptionally activates expression of HIFα target genes that support cell proliferation through neovascularization, glucose uptake, or cell proliferation. Analogous to FH-mutated HLRCC, RCCs in SDH-RCC tend to have malignant features [127]. Based on the molecular consequence of *SDH* inactivation that drives upregulation of HIFα target genes, targeted therapies such as anti-VEGF antibodies or VEGFR inhibitors are expected to be effective for advanced RCC associated with SDH-RCC. In fact, there is a case report of advanced RCC in SDH-RCC, which shows nearly complete remission in response to a standard regimen of sunitinib [132].

2.6 Birt-Hogg-Dubé Syndrome (BHDS)

Birt-Hogg-Dubé syndrome (BHDS) is an autosomal dominant hereditary kidney cancer syndrome, which predisposes affected individuals to develop benign tumors of the hair follicle (fibrofolliculomas), pulmonary cysts, spontaneous pneumothorax, and kidney tumors (RCC and/or oncocytoma) (Fig. 2.5a–d). Causative germline mutations were identified in a novel gene *FLCN* in affected BHDS family members. In contrast to other hereditary kidney cancer syndromes, a variety of histologies including chromophobe RCC, oncocytoma, ccRCC, papillary RCC, and hybrid tumors consisting of features of both chromophobe RCC and oncocytoma can be seen in BHDS.

2.6.1 Clinical Manifestations of BHDS

BHDS was first described in 1977 by three dermatologists, Birt, Hogg, and Dubé, as a hereditary cutaneous disorder in which patients presented with fibrofolliculomas [133]. A case report of a BHD patient having bilateral multifocal chromophobe RCC in 1993 raised the question of whether kidney tumors might be part of the manifestations of BHDS. One hundred fifty-two patients from 49 familial renal tumor families were analyzed for cutaneous lesions at the National Institutes of Health in the U.S. The cosegregation of fibrofolliculomas and kidney tumors was

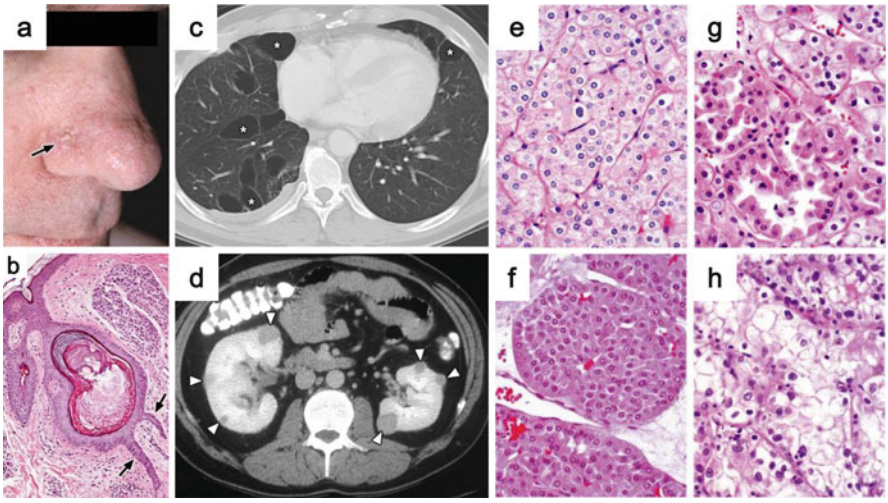

Fig. 2.5 Clinical manifestations of BHDS. (**a**) Fibrofolliculomas on the face (*arrow*). (**b**) Histology of fibrofolliculoma showing epithelial strands with thick connective tissue stroma (*arrows*). (**c**) CT image indicating multiple lung cysts. (**d**) CT image of bilateral multifocal renal tumors (*arrowheads*). (**e**–**h**) BHDS-associated renal tumors show multiple histological types: chromophobe RCC (**e**), oncocytoma (**f**), hybrid oncocytic tumor (**g**), and ccRCC (**h**) (Images from Pavlovich et al. [146])

seen in three families in an autosomal dominant manner, which established BHDS as a hereditary kidney cancer syndrome [134]. BHDS is a rare syndrome with roughly 500 families reported worldwide to date. However, this number could be underestimated because BHDS is a newly categorized syndrome and not widely known yet.

Fibrofolliculomas are the most common clinical manifestations of BHDS, which are seen in 82–92% of affected individuals with BHDS who are older than 25 years old. It is a benign tumor, so-called hamartoma, seen as flesh-colored papules with a smooth surface, 2–4 mm in diameter, and frequently seen on the face, neck, and upper trunk singly or coalescing into a plaque (Fig. 2.5a) [135–138]. Histologically, fibrofolliculomas show anastomosing epithelial strands emanating from an aberrant hair follicle, surrounded by a thick fibrous tissue and mucin-rich stroma (Fig. 2.5b) [133, 134]. Other than a cosmetic issue, fibrofolliculomas exhibit no symptoms.

Lung cysts are the second most common manifestations of BHDS. Multiple bilateral thin-walled lung cysts can be observed on thin-section chest CT scans in 70–84% of affected individuals with BHDS (Fig. 2.5c) [135, 136, 138] Tobino et al. precisely described the characteristic features of pulmonary cysts in BHDS seen on CT scans. The cysts vary in their numbers (29–407/person), sizes (a few mm–2 cm or larger), and shape (76.6% of cysts are irregular-shaped). Cysts are predominantly distributed to the lower, medial, and subpleural regions of the lung, abutting or involving the proximal portion of lower pulmonary arteries or veins [139]. Respiratory function tests generally exhibit normal lung function [139, 140]. Affected

family members with BHDS have a 50-fold higher risk for having spontaneous pneumothorax than unaffected siblings [141]. An analysis of 198 patients from 89 BHDS families evaluated for risk of pneumothorax revealed that 24% of BHDS patients had a history of pneumothorax. The presence of lung cysts, total lung cyst volume, and largest cyst size were significantly associated with pneumothorax. The median age of onset for pneumothorax in BHDS is 38 years old [142].

Zbar et al. performed a risk assessment study of a large cohort of BHDS families and concluded that affected family members of BHDS have a sevenfold greater risk of developing renal tumors than unaffected siblings [141]. The penetrance of renal tumors in BHDS ranges from 12 to 34% [135, 136, 143]. Kidney neoplasia present in BHDS patients can be bilateral, multifocal, or solitary (Fig. 2.5d). Kidney tumors in BHDS exhibit a wide spectrum of histological subtypes both in the single kidney of a BHDS patient and in multiple affected individuals from the same BHDS kindred, differentiating this syndrome from other hereditary kidney cancer syndromes (Fig. 2.5e–h). The unique kidney tumors, hybrid oncocytic/chromophobe tumors, which contain features of chromophobe RCC and renal oncocytoma [144] are the most common kidney tumors in BHDS (Fig. 2.5g). Pavlovich et al. have reported the frequency of histologies seen in BHDS-related kidney tumors as follows: hybrid oncocytic/chromophobe tumors (50%), chromophobe RCC (35%), clear cell RCC (9%), and renal oncocytoma (5%) [145, 146]. Multiple microscopic foci of eosinophilic dysplastic cells, so-called oncocytosis, can be seen frequently in the normal parenchyma of kidneys from BHDS patients [145]. Kidney tumors in BHDS tend to grow slowly and less aggressively, although they have the potential to metastasize. Affected family members without renal masses are recommended to be screened for kidney tumors by MRI every 36 months starting at the age of 21. If a renal mass less than 3 cm is detected, annual or semiannual imaging, depending on the size, location, and growth rate, should be considered. When the diameter of the largest tumor reaches 3 cm, surgical intervention is recommended. Since BHDS patients have a lifelong risk for developing multiple bilateral renal tumors, nephron-sparing surgery should always be considered to conserve renal function as much as possible, to prepare for multiple surgeries. During the nephron-sparing surgery of the largest tumor, all of the detectable small tumors should be removed with the aid of intraoperative ultrasound [146, 147]. So far there is no report of metastatic RCC developing in BHDS patients with primary tumors less than 3 cm in diameter [11]. However, BHDS-associated large RCC can metastasize and cause mortality [146, 148]. Appropriate regular follow-up has to be performed for BHDS-related kidney tumors.

Although it is not clear whether or not they are real BHDS manifestations, there are many reports of neoplasms in BHDS patients. Parotid oncocytomas have been identified in many BHDS patients [135, 136, 149–151]. It is controversial whether BHDS patients are at risk of developing colon polyps and/or colorectal carcinoma. There are several case reports describing colorectal manifestations in BHDS patients [152–154]. However, Zbar et al. have conducted a risk assessment study of BHD families who were evaluated by colonoscopy and showed no increased risk for colon polyps and/or carcinomas in affected members of BHDS families

compared to unaffected members. On the other hand, Nahorski et al. and Khoo et al. have described increased risk of colorectal neoplasia in a large European BHD cohort and in a large French BHD family, respectively [155, 156]. Further analysis will be required to conclude if colon neoplasia should be included as a manifestation of BHDS.

2.6.2 Genetics of BHDS

The causative gene for BHDS was localized on the short arm of chromosome 17 by genetic linkage analysis in BHDS kindreds [157–159]. Subsequently in 2002, germline mutations were identified in a novel gene in affected family members with BHDS, which was named *FLCN* [160]. Since the cloning of *FLCN*, many germline mutations have been reported [136, 137, 144, 162, 163]. To date more than 100 unique germline mutations have been reported in the Leiden Open Variation Database (LOVD) for *FLCN* [163] (https://grenada.lumc.nl/LOVD2/shared1/home.php?select_db=FLCN). Lim et al. have reported analysis of 70 unique germline mutations based on this database in 2010. Germline mutations are found in all coding exons (4–14). Deletion mutations are most frequently seen (31/70, 40%), followed by single base substitutions (25/70, 35.7%), duplications (10/70, 14.3%), and deletion/insertions (4/70, 5.7%). Most of these germline mutations are predicted to cause loss of function of the encoded protein FLCN. Frameshifts, causing a premature termination codon, are the most frequent mutational consequences (37/70, 52.9%), followed by splice site mutations (14/70, 20%), nonsense mutations (10/70, 14.3%), missense mutations (6/70, 8.6%), and deletion mutations (3/70, 4.3%) [163]. Partial gene deletions of *FLCN* have been seen in the germline of affected BHDS family members, which are also predicted to cause loss of function [143, 161, 164]. Benhammou et al. have reported germline intragenic deletion of the noncoding exon 1 causing loss of promoter activity of the *FLCN* gene [164]. The mutation detection rate of *FLCN* in affected BHDS patients is reaching 90% with advanced technologies for identifying gene deletions and accurate sequencing [135, 136, 143]. There is no report of a clear genotype-phenotype correlation in BHDS [135, 136, 142].

The majority of tumor suppressor genes that are causative for hereditary cancer syndromes follow the Knudson two-hit theory. Tumors have germline loss of function mutations in one allele and additional inactivation of the other allele by LOH, somatic mutation, or methylation [165]. A second hit somatic inactivation of *FLCN* is seen in BHD associated renal tumors [156, 166]. Vocke et al. analyzed 77 renal tumors from 12 individuals with BHDS who were confirmed to carry germline mutations in *FLCN*. The majority of renal tumors (41/77, 53%) showed somatic mutations in *FLCN*, most of which resulted in frameshifts and loss of function. LOH at the *FLCN* locus was also seen at a relatively lower frequency (14/77, 17%). Interestingly, each tumor within a group of multifocal tumors from a single kidney of a BHDS patient showed a distinct second hit inactivation

[166]. These observations support the idea that *FLCN* is a classical tumor suppressor gene, which follows the Knudson two-hit theory.

FLCN somatic mutations are seen infrequently in sporadic RCC. Multiple losses of whole chromosomes are a characteristic of sporadic chromophobe RCC. Chromosome 17, where *FLCN* is located, is frequently lost in chromophobe RCC [167]. This motivated Gad et al. to look at the somatic mutations of *FLCN* in sporadic renal tumors including 46 samples of chromophobe RCC, 19 ccRCC, 18 renal oncocytoma, and 9 papillary RCC. After five samples of chromophobe RCCs having mutations in normal tissues were excluded, somatic *FLCN* mutations were seen in 4.9% of sporadic chromophobe RCCs and in 5.6% of sporadic renal oncocytoma. No *FLCN* mutations were seen in ccRCC or papillary RCC. Methylation status of the *FLCN* promoter was analyzed in 61 of 92 samples, and no *FLCN* promoter methylation was found [168]. Khoo et al. analyzed 39 renal tumors, 7 samples of renal oncocytomas, 9 chromophobe RCC, 11 papillary RCC, and 12 ccRCC. Only one papillary RCC exhibited a somatic frameshift mutation in *FLCN*. However, LOH on chromosome 17p was observed in 36% of sporadic renal tumors (33% of all chromophobe RCC), and *FLCN* promoter methylation was detected in 28% of sporadic renal tumors (36% of all chromophobe RCC). Interestingly, 11% of chromophobe RCC, 27% of papillary RCC, and 8% of ccRCC showed both LOH and promoter methylation [169]. On the other hand, da Silva et al. found no evidence of *FLCN* CpG island methylation in 20 RCC tumors and 6 RCC cell lines. Nagy et al. did not find *FLCN* somatic mutations in any of 8 sporadic chromophobe RCC or 8 sporadic renal oncocytoma. They saw LOH on chromosome 17p in 100% of chromophobe RCC and 0% of oncocytoma [170]. The latest publication from the Cancer Genome Atlas project describing whole-exome sequencing of 66 sporadic chromophobe RCCs reports no mutations in *FLCN* [171].

2.6.3 Molecular Function of FLCN

FLCN gene encodes a novel 579 amino acid protein FLCN, which does not share any homology or known functional domains with other proteins at the level of amino acid sequence or secondary structure prediction [160]. However, FLCN is well conserved across species, suggesting its fundamental role for organisms. Baba et al. have identified a novel FLCN-binding protein, FNIP1, which is also conserved across species and has no known functional domains to suggest its function. FNIP1 binds to the C-terminus of FLCN, which is sometimes the target of protein truncating germline *FLCN* mutations in BHDS families, and interacts with 5′-AMP-activated protein kinase (AMPK), which has an important role as an energy sensor and metabolic switch to maintain energy homeostasis in cells and organisms [172, 173]. AMPK negatively regulates mechanistic target of rapamycin (mTOR) [174], the master regulator of protein translation and cell growth [175]. The significant role of the AMPK-mTORC1 signaling axis is well documented in hereditary

cancer syndromes [176] including Cowden syndrome which is caused by *PTEN* inactivation [177], Peutz-Jeghers syndrome caused by *LKB1* inactivation [178, 179], and tuberous sclerosis complex caused by *TSC1* or *TSC2* inactivation [180]. Indeed, there are several lines of evidence supporting FLCN/FNIP1 involvement in the AMPK-mTORC1 signaling pathway. FLCN is phosphorylated on multiple serines and threonines, which are differently inhibited by mTORC1 inhibition or AMPK inhibition. FNIP1 expression facilitates FLCN phosphorylation in an mTORC1 dependent manner [181, 182]. Regulation of mTORC1 activity by FLCN/FNIP1 seems to be context dependent. For example, a *FLCN*-null RCC cell line showed higher mTORC1 activity than the *FLCN*-restored RCC cell line under serum-starved conditions. On the other hand, serum stimulation activated mTORC1 inefficiently in the *FLCN*-null RCC cell line under amino acid-starved conditions, while the *FLCN*-restored RCC cell line demonstrated efficient activation of mTORC1 [181]. Recently, Tsun et al. have shown that FLCN functions as a RagC/D GTPase-activating protein (GAP) to facilitate mTOR recruitment to the lysosome for amino acid-dependent mTORC1 activation. Petit et al. have also shown that FLCN is required for mTOR to be recruited to lysosome by Rags upon amino acid stimulation. In this case they showed that FLCN functions as a RagA guanine nucleotide exchange factor (GEF) [183]. A crystal structure of the C-terminal half of FLCN was solved in 2012 and found to be structurally similar to a Rab-GEF family of proteins [184]. Animal models also support a complex FLCN role in mTOR regulation. Kidney-targeted *Flcn* deletion causes acute cell proliferation in kidney epithelial cells of the distal nephron, accompanied by mTORC1 activation. Kidney epithelial cells aberrantly proliferate in monolayer, resulting in a polycystic kidney-like morphology and lethal renal failure by 4 weeks of age. This phenotype is suppressed by rapamycin treatment, supporting the involvement of mTORC1 activation in the pathogenesis of BHDS [185, 186]. *Flcn* heterozygous knockout mice, mimicking affected BHDS patients, develop solid tumors, which demonstrate LOH of the remaining *Flcn* allele and similar histologies to human BHDS-associated tumors. mTOR activity, evaluated by western blotting, was high in these solid tumors [187]. On the other hand, another *Flcn* heterozygous mouse model showed suppressed mTORC1 activity in solid tumors and cysts which were evaluated by immunohistochemistry of phosphorylated S6 ribosomal protein on paraffin-embedded samples [188]. A third *Flcn* heterozygous mouse model exhibited increased phospho-S6 staining in large cysts and suppressed phospho-S6 staining in small cysts on paraffin-embedded samples [189]. In addition to these in vivo data, mTORC1 regulation by FLCN is shown to be cell type dependent [189–191].

A second FLCN-binding protein, FNIP2 (which is also known as FNIPL [192] or MAPO1 [193]), was identified by bioinformatics search [194]. FNIP2 is very similar to FNIP1 (identity, 49%; similarity, 74%) and shares the same characteristics as FNIP1 in binding to FLCN and AMPK. Hasumi et al. have shown that FNIP1 and FNIP2 can make hetero- or homomultimers, which can complex with FLCN and AMPK. This finding suggests that FLCN/FNIP1/FNIP2 may function as a tumor suppressor in a complex. *Fnip1* homozygous knockout mice have B cell developmental defects and show no obvious phenotype in kidneys

[195, 196]. *Fnip2* homozygous knockout mice show no phenotype at all. However, kidney-targeted *Fnip1* and *Fnip2* double knockout mice exhibit completely identical phenotypes to kidney-targeted *Flcn* knockout mice [197]. This finding indicates that Fnip1 and Fnip2 may have redundant function and that FLCN and FNIP1/FNIP2 function coordinately as a tumor suppressor complex.

Other signaling pathways are also regulated by FLCN. Klomp et al. have compared gene expression profiles between BHDS-associated renal tumors and sporadic chromophobe RCC/oncocytoma and found that mitochondrial genes which are regulated by PPAR-γ coactivator 1α (PPARGC1A) were expressed significantly higher in BHDS-associated renal tumors [198]. Hasumi et al. have demonstrated that Flcn regulates Ppargc1a in vivo by analyzing muscle-targeted *Flcn* knockout mice. *Flcn*-deficient muscle shows increased mitochondrial biogenesis accompanied by increased Ppargc1a expression and a metabolic shift to oxidative phosphorylation, which is completely neutralized by the additional deletion of *Ppargc1a* [199]. It remains to be determined whether regulation of PPARGC1A activity by FLCN serves an essential role in FLCN tumor suppressor function. Hasumi et al. have shown suggestive data indicating that deletion of *Ppargc1a* in kidney-targeted *Flcn* knockout mice results in complete loss of hyperplastic cells, although aberrant kidney epithelial cell proliferation is seen in these animals and eventually causes lethal renal failure [199]. *Flcn* inactivation in murine cardiac muscle led to ATP overproduction, caused by aberrant mitochondrial biogenesis, AMPK suppression followed by mTORC1 activation, and cardiac hypertrophy, which was suppressed by rapamycin treatment or inactivation of Ppargc1a [200].

Recent evidence suggests that FLCN is a multifunctional protein. One of the important functions for FLCN is regulation of transcriptional activity of the basic-helix-loop-helix leucine zipper transcription factor, TFE3, a member of the microphthalmia-associated transcription factor (MiT) family. Under *FLCN*-deficient conditions, TFE3 translocates into the nucleus and has increased transcriptional activity [191, 201]. TFE3 regulation by FLCN might be essential for the role of FLCN as a tumor suppressor for the following reason. There is a rare subset of sporadic RCC, Xp11.2 translocation RCC, with translocations between *TFE3* at Xp11.2 and a variety of genes, including *ASPL*, *PRCC*, *NonO*, *PSF*, and *CLTC* [202–204]. All of the proteins encoded by these *TFE3* fusion genes maintain the C-terminal half of TFE3, where the basic-helix-loop-helix leucine zipper domain is located, and show nuclear TFE3 immunostaining in the corresponding Xp11.2 translocation RCC [205], suggesting that TFE3 constitutive activation leads to RCC development.

Moreover, FLCN is involved in the TGF-β signaling pathway [206, 207], ciliogenesis [208], and autophagy [209, 210]. The pathogenesis of lung cysts in BHDS has been uncertain for a long time. Identification of a FLCN-binding protein, plakophilin-4 (p0071), shed light on the molecular role of FLCN in cell-cell adhesion and cell polarity, which might be involved in the lung manifestations of BHDS [190, 211, 212]. Rho A signaling, which is regulated through p0071, is disordered under *FLCN*-deficient conditions. FLCN regulates cell-cell adhesions,

and defects in this process may cause lung cyst formation [190]. Goncharova et al. have developed lung-targeted *Flcn* knockout mice and showed increased apoptosis in lung epithelium, which was caused by a dysregulated E-cadherin-LKB1-AMPK axis [213]. The multifunctionality of FLCN might explain the broad phenotype seen in *Flcn* knockout mice as well as the distinct manifestations of BHDS.

2.6.4 BHDS Research: Bench to Bedside

Currently there is no approved targeted therapy for BHDS. Part of the reason for this may be the rarity of BHDS and indolent nature of most BHDS-associated RCC. Based on kidney-targeted *Flcn* knockout mouse model results [185], mTORC1 inhibition might be a promising targeted strategy. In fact, Nakamura et al. treated advanced BHDS-related RCC with the mTORC1 inhibitor, everolimus, as a sixth-line therapy after disease was refractory to IL-2 (3 month, progressive disease (PD)), IFNα (3 month, PD), S-1(28 month, PD), sorafenib (1 month, PD), and sunitinib (4 month, PD). Even though everolimus was used as a sixth-line systemic therapy, it displayed a relatively long-term effect (SD for 7 month). Further progress in both basic research and translational research will be necessary for developing successful treatments for advanced RCC in BHDS.

2.7 Tuberous Sclerosis Complex (TSC)

Tuberous sclerosis complex (TSC) is an autosomal dominant hereditary hamartoma syndrome, which is caused by germline loss of function mutations in *TSC1* or *TSC2* genes. Disease manifestations are seen in multiple organs, including the skin, brain, heart, lung, eye, and kidney, with widely variable clinical presentations even among relatives (Fig. 2.6a–h) [214, 215]. Affected individuals are highly predisposed to develop renal angiomyolipomas, which are benign tumors in most cases. It should also be noted that TSC patients can develop renal epithelioid angiomyolipomas with malignant potential and, in rare cases, RCC with a characteristic histology. Since epithelioid angiomyolipoma is sometimes misdiagnosed for RCC, it is important to correctly distinguish these renal lesions in TSC patients.

2.7.1 Clinical Manifestations of TSC

TSC has been underdiagnosed because of the variable severity of manifestations among affected individuals [216]. Through the discoveries of the causative genes and establishment of diagnostic criteria, significant advancements have been made in the management of TSC. Currently its prevalence is estimated at 1/6000 to

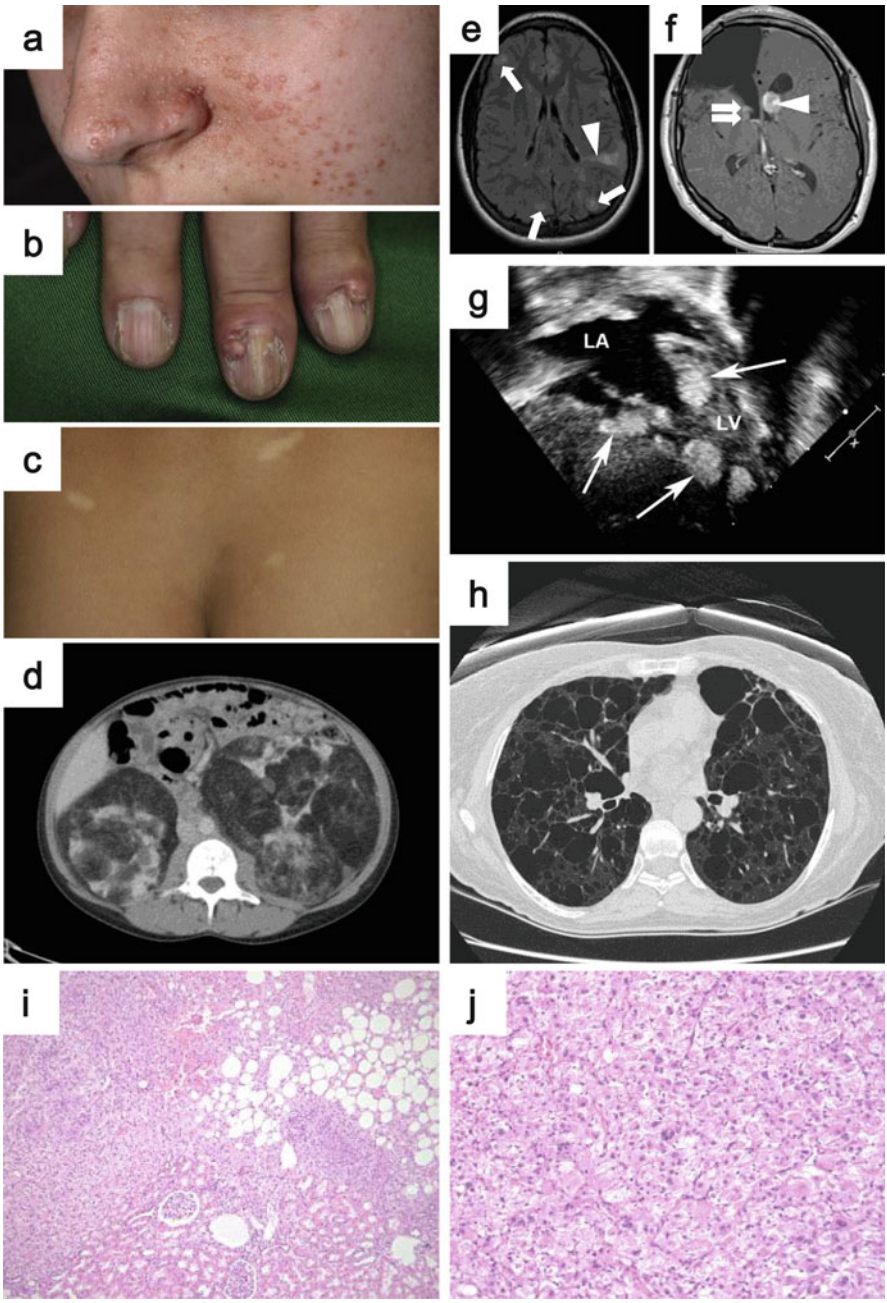

Fig. 2.6 Clinical manifestations of TSC. (**a**) Angiofibromas on the centrofacial area. (**b**) Ungual fibromas arising from the nail bed. (**c**) Hypomelanotic macules are observed frequently in TSC patients. (**d**) CT image showing bilateral multifocal angiomyolipomas. (**e**) MRI image demonstrating cortical dysplasia, which is observed very frequently in TSC patients (*arrows*: tubers, *arrowhead*: radial migration line). (**f**) MRI image indicating subependymal nodules (SEN) with *arrows* and subependymal

1/10,000 of live births [215, 217]. The second International TSC Consensus Conference was held in 2012 and revised the clinical diagnostic criteria published in 1998. The identification of a pathogenic mutation in *TSC1* or *TSC2* in genomic DNA is sufficient for a definitive diagnosis of TSC. Since conventional genetic testing does not identify germline mutations in TSC1/2 in a significant population (10–25%) of TSC patients, a negative outcome of a genetic test does not exclude TSC. Here, an outline of TSC manifestations will be described. For details of the clinical diagnostic criteria, the reader is referred to the literature [216].

2.7.1.1 Extrarenal Manifestations of TSC

Dermatologic features are seen in almost 100% of TSC-affected individuals, which can be easily recognized by physical examination (Fig. 2.6a–c). The prominent manifestations are skin hamartomas, which include angiofibromas, fibrous cephalic plaques, ungual fibromas, and shagreen patches. Facial angiofibromas are seen in 75% to 93% of affected individuals [218–220]. Angiofibromas are red to pink papules with smooth surface, which distribute over the centrofacial area (Fig. 2.6a). Histologically, dermal fibrosis, coarse collagen bundles, stellate fibroblasts in the upper dermis, and capillary dilation are seen with atrophic sebaceous glands [221, 222]. Second-hit somatic *TSC1* or *TCS2* mutations were identified in cultured fibroblasts isolated from angiofibromas of TSC patients, supporting the idea that UV-induced DNA damage caused second-hit mutations in skin fibroblasts resulting in hamartoma formation [223]. There are two reports, suggesting a possible phenotypic overlap of skin hamartoma between TSC and Birt-Hogg-Dubé syndrome (BHDS). One publication reports angiofibromas in BHDS, while the other reports fibrofolliculomas in TSC [222, 224]. Clinicians should be aware that these overlapping clinical manifestations can sometimes make the differential diagnosis of TSC and BHDS challenging. In addition, angiofibromas are also seen frequently in another hereditary neoplastic syndrome, multiple endocrine neoplasia type 1 (MEN1), which do not develop kidney neoplasia [225]. Fibrous cephalic plaques (forehead fibrous plaques) are seen in around 25 to 46% of TSC-affected individuals [215, 220]. Fibrous cephalic plaques are histologically similar to angiofibromas, with remarkably sclerotic collagen tissue [221]. Ungual fibromas show later onset and are seen in 20 to 80% of patients in an age-dependent manner (Fig. 2.6b) [226, 227]. They are skin-colored or red nodules, arising from the nail bed of fingers or toes. Histologically they are similar to angiofibromas or fibrous cephalic plaques [215, 218, 220, 228]. Another proliferative skin manifestation is

Fig. 2.6 (continued) giant cell astrocytoma (SEGA) with *arrowhead*. (**g**) Echocardiogram of cardiac rhabdomyomas. (**h**) Chest CT image demonstrating lymphangioleiomyomatosis (LAM). (**i**) Histology of angiomyolipoma. (**j**) Histology of epithelioid angiomyolipoma composed of pleomorphic cells with large hyperchromatic nuclei and abundant eosinophilic cytoplasm (Images from Northrup et al. [215] (**a–h**), and Kato et al. (**i**, **j**) [254])

the shagreen patch, which is specific for TSC and seen in 50 to 80% of TSC-affected individuals in their first decade of life [219, 220, 227¸ 228]. They usually appear as large plaques on the lower back with the rough surface resembling an orange peel. Histologically it is a connective tissue hamartoma composed of vascular structures, adipose tissue, collagen, smooth muscle, and cutaneous appendages [229]. Another set of skin manifestations are large hypomelanotic macules and tiny confetti-like macules. Hypomelanotic macules are observed frequently in 65 to 90% of TSC patients (Fig. 2.6c) [218, 220, 227]. Confetti-like macules are numerous scattered tiny white hypomelanotic macules usually covering the arms and legs, which are seen in about 50% of affected patients [218, 226].

Central nervous system features are also very common in TSC. Cortical dysplasia, including cortical tuber and cerebral white matter radial migration lines, which can be diagnosed by MRI, are observed in 90% of patients (Fig. 2.6e). Cortical dysplasia is associated with intractable epilepsy and learning difficulties in TSC [215]. Subependymal nodules (SEN) and subependymal giant cell astrocytomas (SEGA) are observed in 80% of TSC patients (Fig. 2.6f) [215]. They are basically benign and slow growing, but can cause serious neurological morbidity. Cardiac rhabdomyomas can occur in 50% of cases (Fig. 2.6g) [218–220], which are rarely observed in non-TSC patients.

Lymphangioleiomyomatosis (LAM) is one of the major manifestations of TSC. Histologically, benign-appearing smooth muscle cells (LAM cell) are infiltrating into lymphatics, airways, blood vessels, and alveolar septa, and thin-walled lung cystic changes, which are the cause of destruction of alveolar structures, are observed [230, 231]. Upon high-resolution CT scanning, at least 30 to 40% of TSC-affected females present cystic pulmonary parenchymal changes, which are consistent with LAM (Fig. 2.6h) [232, 233]. The cystic changes of lung, consistent with LAM, are observed in about 10% of male TSC individuals, but symptomatic LAM in males is rare [234]. The risk of LAM is age dependent, increasing by 8% each year. The prevalence of LAM in females reaches 80% by 40 years of age [233]. Cudzilo et al. have reported that 12.5% of TSC patients with LAM eventually die from LAM. The origin of LAM cells is unknown. Ninety-three percent of TSC patients with LAM have concurrent renal angiomyolipomas, and 100% have uterine PEComas (tumors showing perivascular epithelioid cell differentiation) [235], suggesting that these extra lung manifestations might be the source of LAM cells.

2.7.1.2 Kidney Manifestations of TSC

Angiomyolipoma is the major kidney manifestation of TSC, which can cause the most severe clinical symptoms. Angiomyolipomas are frequently seen bilaterally and multifocally in kidneys of nearly 80% of TSC-affected individuals (Fig. 2.6d) [236]. Angiomyolipomas can also develop in other organs including the liver [237]. Renal angiomyolipoma is a benign mesenchymal clonal neoplasm composed of variable proportions of hyalinized thick-walled dysmorphic blood vessels, immature spindle-shaped smooth muscle-like cells, and mature adipose tissue

(Fig. 2.6i) [238, 239]. Karbowniczek et al. have microdissected each component of sporadic angiomyolipomas and demonstrated that all three components have LOH at the *TSC2* locus and shown that all cell components have immunoreactivity to anti-phospho-S6 antibody, supporting mTORC1 activation presumably caused by loss of *TSC2* [240]. This may support the idea that all the components of angiomyolipomas are derived from a common progenitor cell [241]. It has been postulated that the origin of angiomyolipoma is a renal mesenchymal precursor cell or a neural crest lineage cell [242, 243]. Most renal angiomyolipomas behave biologically as a benign lesion and show favorable prognosis, although there have been reports of nodal involvement and extensions into the renal vein and inferior vena cava [244, 245]. On the other hand, angiomyolipomas confer a risk to TSC-affected patients by causing chronic kidney disease (CKD) [246] and hemorrhage [247]. The abnormal vascular components of larger angiomyolipomas have a tendency to develop aneurysms, which can rupture and cause patients to go into shock [248, 249].

Angiomyolipomas contain a subset of the smooth muscle-like cells, which appear epithelioid with clear to pale eosinophilic granular cytoplasm and focally associate with the blood vessels. This distinctive cell type is called a perivascular epithelioid cell or PEC, which is shared among a family of mesenchymal neoplasms known as "PEComas" (tumors showing perivascular epithelioid cell differentiation). PEComas include angiomyolipomas, lymphangiomyomatoses, and clear cell "sugar" tumor of the lung [250]. As mentioned above, angiomyolipomas can display extremely variable proportions of each component. Angiomyolipomas, which display dominantly or exclusively epithelioid cells, are classified as epithelioid angiomyolipomas or epithelioid PEComas (Fig. 2.6j) [235]. It is important to note that epithelioid angiomyolipomas could be misdiagnosed as RCC. Epithelioid angiomyolipoma is histologically characterized by polygonal cells with eosinophilic to clear cytoplasm, prominent nucleoli, occasional marked nuclear atypia, and pleomorphic forms (Fig. 2.6j), forming solid arrangements [235, 251–254]. More importantly, epithelioid angiomyolipomas can be malignant neoplasms, which metastasize and cause death, especially in cases that show malignant histology [252, 253, 255, 256]. In rare cases, typical angiomyolipomas can become malignant with epithelioid or sarcomatous transformation [257, 258]. It is important to consider the possibility of epithelioid angiomyolipoma when a high-grade epithelioid renal neoplasm is observed in a TSC patient or is found coexisting with a conventional AML [254]. Immunohistochemistry is extremely useful for differential diagnosis of epithelioid angiomyolipoma. PEC are positive for melanocytic antigens (HMB-45 and melan-A) as well as smooth muscle-specific actin and negative for epithelial markers, EMA, and cytokeratin [254].

The incidence of RCC in TSC-affected individuals is thought to be very rare and estimated to be 2 to 3%, which is comparable to the incidence of sporadic RCC in the general population [259, 260]. There have been many case reports of TSC-associated RCC with a variety of histologies. But there has not been any systematic evaluation and/or classification of these TSC-associated RCC. Recently, two groups have evaluated and classified TSC-associated RCC independently

[261, 262]. Both groups have concluded that the RCCs in TSC show distinct histology and character, which differ from sporadic RCC in non-TSC general populations. Guo et al. have analyzed 57 RCCs from 18 TSC-affected patients. They describe unique clinicopathologic features of TSC-associated RCC including female predominance, younger age at diagnosis, multiplicity, association with angiomyolipoma, favorable clinical course, and three distinct histologic patterns as follows: (1) carcinoma resembling renal angiomyoadenomatous tumors (RAT-like) or RCC with smooth muscle stroma (30%), (2) carcinoma resembling sporadic chromophobe-type RCC (chromophobe-like) (59%), and (3) a unique granular eosinophilic-macrocystic histology (11%) [261]. Yang et al., have analyzed 46 RCC from 19 TSC patients and classified them into three categories based on morphologic, immunologic, and molecular profiles as follows: (1) "TSC-associated papillary RCC" with prominent papillary architecture and loss of SDHB expression (52%), (2) hybrid oncocytic/chromophobe tumor (HOCT) (33%), and (3) unclassified (15%) [262]. In both studies, HMB-45 negativity and Pax8 positivity were tested to exclude epithelioid angiomyolipoma. Both studies share distinct clinicopathologic characteristics of TSC-associated RCC.

2.7.2 Genetics of TSC

Through linkage analysis of TSC families, causative germline mutations in *TSC1* and *TSC* genes were identified [263–265]. *TSC1* localizes on chromosome 9q34, encoding an 1164 amino acid 140kD protein, hamartin. *TSC2* localizes on chromosome 16p13, encoding an 1807 amino acid 200kD protein, tuberin. Seventy-five to 90% of TSC patients diagnosed through clinical criteria exhibit pathogenic germline mutations in either *TSC1* or *TSC2*. Extensive genetic analysis of the *TSC1* and *TSC2* genes in TSC patients have identified a broad spectrum of mutations [219, 266–269]. To date, more than 500 unique *TSC1* sequence variants and 1400 unique *TSC2* sequence variants, which do not include nonpathogenic variants, have been reported (http://chromium.lovd.nl/LOVD2/TSC/home.php?select_db=TSC1, http://chromium.lovd.nl/LOVD2/TSC/home.php?select_db=TSC2). Missense mutations, large genomic deletions, and in-frame deletions are very rare in *TSC1*. The germline mutation frequency in *TSC2* is higher than *TSC1*. Especially in de novo cases, mutation frequency in *TSC2* is reported to be two to ten times higher than in *TSC1* [219, 268–273]. On the other hand, the mutation frequency in TSC pedigrees which segregate across multiple generations is approximately equal in *TSC1* and *TSC2* [180]. This might be explained by the fact that *TSC1* mutations are associated with a less severe phenotype in TSC patients [219, 268]. LOH in *TSC1* or *TSC2* is consistently observed in most TSC-associated neoplastic lesions including angiomyolipomas, but rarely observed in cerebral cortical tubers [274, 275]. This indicates that *TSC1* and *TSC2* are classical tumor suppressor genes which follow the Knudson two-hit theory [276]. Although TSC is an autosomal hereditary syndrome, the sporadic cases, which have acquired de novo

mutations without family history, are predominant. It has been estimated that about 66% to 83% of all TSC patients are sporadic cases [219, 268, 273, 277]. Therefore, although TSC is a hereditary syndrome, one should notice that lack of family history does not exclude TSC from the differential diagnosis.

2.7.3 Molecular Function of TSC1/TSC2

Both *TSC1* and *TSC2* are confirmed to function as tumor suppressor genes by in vitro and in vivo experiments [278–280]. *TSC1* encodes a 140kD protein, TSC1 (hamartin), which does not have any known functional domains. *TSC2* encodes a 200kD protein, TSC2 (tuberin), which has a GAP (GTPase-activating protein) domain in its c-terminal region. TSC1 and TSC2 share no homology and form a heterodimer [281, 282] to function as a GAP toward the small G-protein Rheb (Ras homolog enriched in the brain). As expected from the fact that both mutations in *TSC1* and *TSC2* cause a single disease, TSC1 and TSC2 function as a complex. TSC1 binds to TSC2 and stabilizes it by preventing ubiquitin-mediated degradation [283, 284]. The GAP activity is essential for TSC1/TSC2 tumor suppressor function [285]. Indeed, missense germline mutations are frequently found in TSC patients in the GAP coding regions of *TSC2*, underscoring the importance of GAP activity for TSC2 tumor suppressor function [286]. The TSC1/TSC2 complex activates Rheb GTPase and accelerates the conversion of GTP-bound Rheb to GDP-bound Rheb, resulting in inhibition of mTORC1 (composed of mTOR, RAPTOR, mLST8, and PRAS40) activity [287–289]. The TSC1/TSC2 complex receives upstream signals from many canonical signaling molecules including AKT, AMPK, Ras-ERK-RSK, Wnt-GSK3β, and HIF1α-REDD1 and works as a central hub of signaling transduction, which regulates mTORC1 activity [290]. Inactivation of TSC1 or TSC2 causes aberrant accumulation of GTP-bound Rheb resulting in constitutive activation of mTORC1 [291]. mTORC1 has a pivotal role in regulation of cell growth and proliferation and is activated in a majority of cancers [292].

Therapies that target mTORC1 using rapalogues have shown a very dramatic effect on angiomyolipoma and LAM in TSC patients. The problem is that the mTORC1 effect is cytostatic and termination of rapalogue treatment causes regrowth of tumors [293]. Although there were two advanced cases reported that did not respond to rapalogue treatment [294, 295], there are several case reports of advanced epithelioid angiomyolipomas treated with rapalogues with dramatic responses [296–298]. One thing to be considered is that constitutive activation of mTORC1 by loss of TSC1/2 function suppresses insulin signaling-mediated PI3K/AKT activation through a feedback loop [299]. So mTORC1 inhibition by rapalogues might release this feedback loop and reactivate PI3K/AKT signaling.

2.8 Cowden Syndrome (CS)/PTEN Hamartoma Tumor Syndrome (PHTS)

Cowden syndrome (CS)/PTEN hamartoma tumor syndrome (PHTS) is an autosomal dominant hereditary cancer syndrome, which is caused by germline mutations in a tumor suppressor gene PTEN. CS/PHTS predisposes patients to develop breast, thyroid, kidney, uterine, and other types of cancers as well as benign neoplasia and neurodevelopmental disorders. Because of its rareness and difficulty to diagnose due to the wide spectrum of manifestations, CS tends to be underestimated as a cause of kidney cancer. PTEN hamartoma tumor syndrome (PHTS) was defined to describe patients having germline mutations in *PTEN* [300]. In this chapter the term CS will be used to represent CS/PHTS.

2.8.1 Clinical Manifestations of CS/PHTS

CS was first reported in 1962 describing a case with a family history and was named after the first patient's name [301]. This rare syndrome is inherited in an autosomal dominant manner with an estimated prevalence of at least 1 in 200,000 individuals [302].

CS displays a wide range of clinical characteristics including benign neoplasia, malignancies, central nervous system anomalies, and dysmorphic characteristics [303]. Mucocutaneous manifestations are the most common manifestations of CS, which include trichilemmomas (hair follicle hamartoma), papillomatous papules, and acral/plantar keratoses, and are present in 99% of CS patients by their third decade of life (Fig. 2.7b, c) [304]. Other commonly observed features seen in CS patients are macrocephaly (Fig. 2.7a), dolicocephaly, and dysplastic gangliocytoma of the cerebellum (Lhermitte-Duclos) [304]. In addition, affected patients can develop benign tumors that include colorectal polyposis, thyroid goiter/nodules, lipomas, fibromas, and proliferative breast changes [303].

Individuals affected with CS are at risk throughout their lifetime to develop a variety of cancers, which can be bilateral and multifocal, similar to other inherited cancer syndromes. Affected women have the lifetime risk for breast cancer ranging from 67% to 85% [305–307], which is even higher than the lifetime risk of hereditary breast and ovarian cancer (HBOC) syndrome caused by germline mutations of *BRCA1* or *BRCA2* [308]. CS patients can have a variety of benign breast lesions, which are difficult to differentiate from cancers [309]. Careful and close follow-up of breast lesions is required. The lifetime risk for thyroid cancer is from 7.8% to 38% [305–307]. Among the thyroid cancers, the papillary type is the most common histology (52%), followed by a follicular variant of papillary (28%) and follicular (14%) [310]. Since most CS patients have multinodular thyroids, goiter (73%), and Hashimoto's disease (27%), careful differential diagnosis and close follow-up are also necessary [310]. Affected women have an increased risk of

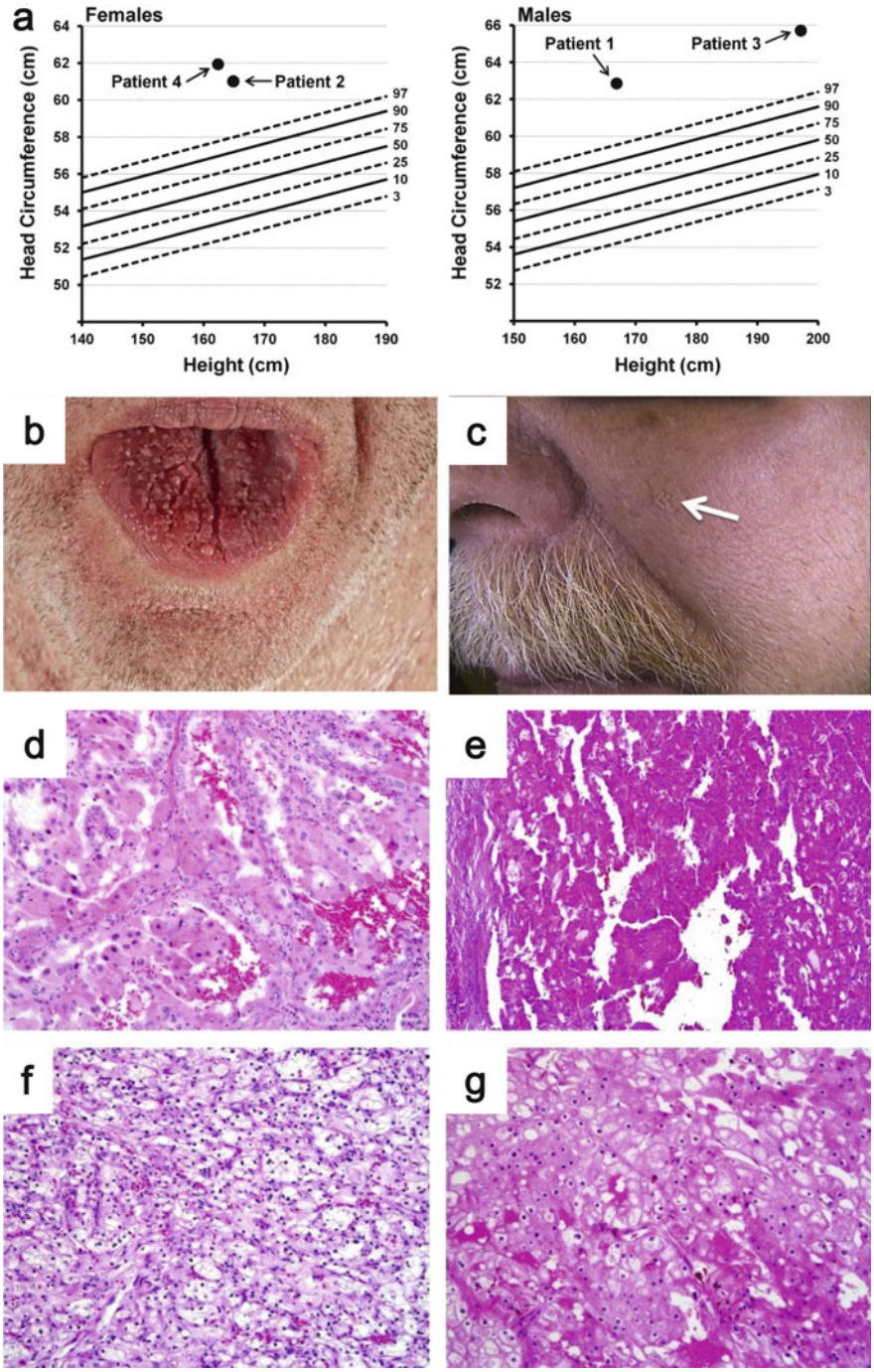

Fig. 2.7 Clinical manifestations of CS. (**a**) Macrocephaly is commonly observed in CS patients. (**b**) Mucocutaneous manifestations are the most common in CS patients. Image shows papillomatous papules on dorsum of the tongue. (**c**) Cutaneous verrucous papule over the centrofacial area.

endometrial cancer with a lifetime risk of 21%–28% [305, 307]. The lifetime risk for colorectal cancer is 9%, while 93% of affected patients who had a GI tract endoscopy were found to have polyps [311].

Mester et al. have analyzed the prevalence and histology of RCC among 219 CS patients who were confirmed to have pathogenic germline mutations in *PTEN* [312]. Nine of the 219 patients had a medical history of RCC, which means the age-adjusted standardized incidence ratio (SIR) is 31.7. Differently from sporadic RCC, the SIR is higher for females (46.7) than males (21.6). The lifetime risk of RCC for CS affected patients is calculated as 34% [305]. Shuch et al. have reported a higher incidence of RCC cases among the CS patients (4 in 24 patients) and have pointed out that RCC is an underappreciated feature of CS [313]. A wide variety of histologies have been reported in CS-associated RCC (Fig. 2.7d–g). Mester et al. have reported that 75% of cases are papillary RCC and 25% of cases are chromophobe RCC [312]. Shuch et al. have reported 50% papillary RCC (Fig. 2.7d, e), 25% clear cell RCC (Fig. 2.7f), and 25% chromophobe RCC in CS (Fig. 2.7g) [313]. Clearly CS-associated RCCs have different characteristics from other types of hereditary kidney cancers. Further analysis with a larger cohort will be required to define the histological spectrum of CS-related RCC. Although it has to be confirmed in a larger cohort, CS-associated RCC seems to be less malignant. To date there are no reports of metastatic RCC in CS [303, 313].

Another characteristic of RCC in CS is the absence of family history of RCC, although the total number of reported CS-associated RCCs is limited [303, 313]. Shuch et al. discuss that this is probably because of low disease penetrance and a high rate of de novo germline mutations in *PTEN*, which is estimated to be between 10.7% and 47.6% [313, 314]. Therefore, lack of RCC family history does not exclude a diagnosis of CS in a patient. Recognition of pathognomonic characteristics like mucocutaneous lesions, medical history of other type of cancers, GI hamartomas, and neurodevelopmental disorders would be important for clinicians to diagnose CS patients with RCC.

2.8.2 Genetics of CS/PHTS

Genetic linkage analysis of 12 CS families identified a responsible genetic locus on chromosome 10q22–23 in 1996 [315]. *PTEN*, a candidate tumor suppressor gene located on 10q23, was found to be mutated in cell lines of glioblastomas, prostate cancers, and breast cancers as well as in primary glioblastomas and other cancers [316, 317]. Subsequently, loss of function mutations of *PTEN* were found in the germline of CS kindreds [318–320]. The germline *PTEN* mutation spectrum

Fig. 2.7 (continued) (**d**–**g**) Renal tumor histology in CS patients showing papillary type 1 RCC (**d**, **e**), ccRCC (**f**), and chromophobe RCC (**g**) (Images from Shuch et al. [313])

includes all types of mutations located throughout the gene. Although the physiological meaning is unknown, there are significant correlations between promoter mutations and breast cancer incidence and between nonsense mutations and colorectal cancers [305]. There is no clear correlation between germline mutations in *PTEN* and a specific histology of RCC in CS [313]. LOH of *PTEN* has been analyzed in five cases of CS-associated RCC and was found in four cases, indicating that LOH might be the major mechanism for second-hit *PTEN* alterations driving RCC development in CS [313]. Mester et al. have reported negative PTEN immunohistochemistry staining in all 5 cases of analyzed CS-associated RCC. Negative PTEN staining might be a useful marker to suggest the possibility of CS-associated RCC, because PTEN expression is mostly positive in sporadic RCC [312]. Kondo et al. have reported that 5 of 68 (7.5%) cases of sporadic RCC exhibit somatic loss of function mutations and 25% of cases show LOH of *PTEN*, including 3 of the cases with somatic mutations in *PTEN*. Among the five somatic *PTEN* mutation cases, four cases were high-grade advanced ccRCC with poor prognosis. The other case was low-grade papillary RCC [321]. The biological behavior of CS-associated RCC and *PTEN*-inactivated sporadic RCC appears to be different. Recent exome sequencing studies have identified *PTEN* loss of function mutations in sporadic ccRCC, papillary RCC, and chromophobe RCC [50, 51, 171, 322].

2.8.3 Molecular Function of PTEN

PTEN is a 403 amino acid multifunctional protein, which has phosphatase activity both on lipid and protein [323–326]. The main tumor suppressor function of PTEN is maintaining the homeostasis of the phosphatidylinositol 3 kinase (PI3K)/AKT cascade [327–329]. In response to extracellular signaling, receptor tyrosine kinases, G-protein-coupled receptors, and RAS can activate PI3K, which converts phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-triphosphate (PIP3) [330]. Increased local PIP3 recruits many signaling molecules, including phosphatidylinositol-dependent kinase 1(PDK1) and AKT together, to the plasma membrane, where AKT is activated by PDK1 [331]. Activated AKT regulates many downstream biological effects, including proliferation, survival, cell polarity, motility, cell cycle, metabolism, and angiogenesis [332]. PTEN dephosphorylates PIP3 to PIP2, resulting in reduced AKT activity and antagonizes PI3K/AKT signaling pathways. One of the most important signaling molecules downstream of PI3K/AKT is mTOR. AKT activates mTORC1 (composed of mTOR, RAPTOR, mLST8, PRAS40) by phosphorylating TSC2 [333, 334] and PRAS40 [335], causing phosphorylation of p70 ribosomal protein S6 kinase and 4EBP1 to promote protein translation. mTORC1 regulates many cellular processes, including protein synthesis, lipid synthesis, autophagy, cell cycle, growth, and metabolism [336, 337]. Among them, the PI3K/AKT/mTOR/HIF1α axis has an important role in cancer development by regulating glucose metabolism as well as angiogenesis [338, 339]. Apart from its tumor suppressor role in the PI3K/AKT

axis, PTEN has a phosphatase independent role in the nucleus to regulate chromosomal stability, double-strand DNA break repair, and the cell cycle [327, 340, 341]. These findings suggest that targeting the PI3K/AKT/mTOR axis itself may not be sufficient to treat *PTEN*-deficient cancers. Targeting the loss of function effect of PTEN in the nucleus might be useful in combinatorial therapy or next-generation targeted therapy for *PTEN*-deficient cancers.

2.9 *BAP1* Germline Mutations (BAP1 Cancer Syndrome and BAP1 Tumor Predisposition Syndrome)

BAP1 (BRCA1-associated protein 1) is a tumor suppressor gene [342, 343] which resides on chromosome 3p21 and is frequently deleted in ccRCC. Recently a novel autosomal dominant tumor predisposition syndrome, associated with loss of function germline mutations in *BAP1*, has been proposed. [344, 345] *BAP1* germline mutations predispose patients in a familial setting to develop a variety of tumors including ccRCC (Fig. 2.8a) [344, 346, 347]. *BAP1* inactivation also contributes to the development and progression of sporadic ccRCC, which underscores the importance of gaining a better understanding of this emerging cancer syndrome [50–54].

2.9.1 Clinical Manifestations of BAP1 Tumor Predisposition Syndrome

BAP1 germline mutations predispose patients to develop malignant mesothelioma, uveal melanoma, cutaneous melanoma, and new category of tumor "melanocytic *BAP1*-mutated atypical intradermal tumors" (MBAITs) [344]. MBAIT is a newly proposed term to describe atypical melanocytic tumors that were previously diagnosed using various terminologies [348–351]. Carbone et al. have performed meta-analysis of published families with *BAP1* germline mutations [348–351] and have shown that MBAITs are the most highly penetrant manifestation of the BAP1 cancer syndrome, seen in 66.7% of affected individuals. MBAITs are often associated with a compound nevus or intradermal nevus, grow very slowly, and are thought to be benign tumors. MBAITs are characterized histologically as intradermal lesions with large epithelioid and spindle-shaped melanocytes (MBAITs cells), which show cellular atypia and pleomorphic/hyperchromatic nuclei, but no mitotic figures or Ki67 staining. Through meta-analysis, Carbone et al. have reported the prevalence of other tumors in *BAP1*-mutated individuals as follows: malignant mesothelioma (MM, 21%), uveal melanoma (UM, 17.7%), and cutaneous melanoma (CM, 12.9%). None of these tumors has been observed in non-affected family members, suggesting that these manifestations are significant features of the BAP1

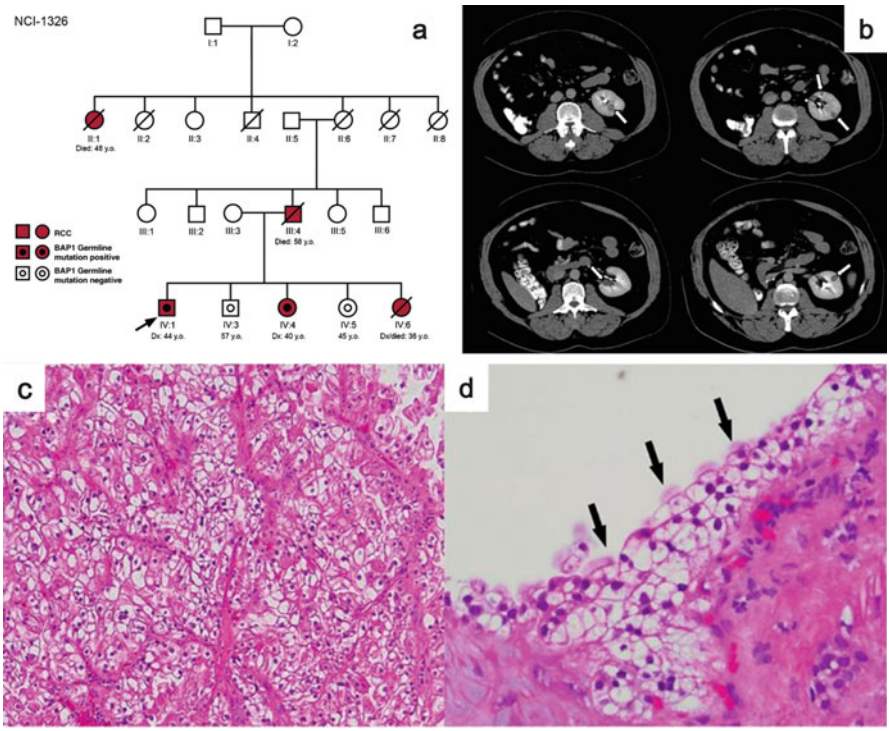

Fig. 2.8 Clinical manifestations of BAP1 tumor predisposition syndrome. (**a**) Pedigree of BAP1 tumor predisposition syndrome family. *Red symbols* indicate individuals with RCC. (**b**) CT image of affected individual following *right* radical nephrectomy demonstrating multifocal *left* renal lesions. (**c**) Histology of solid ccRCC in affected individual. (**d**) Histology of atypical renal cyst with clear cell lining (Images from Farley et al. [347])

cancer syndrome [344]. Popova et al. have reported that among 6 of the 11 families with BAP1 cancer syndrome, 9 affected individuals presented with RCC [346]. Farley et al. also have reported a novel germline mutation in *BAP1*, which predisposes to familial ccRCC [347]. These findings strongly support RCC as a manifestation of the BAP1 cancer syndrome. To date, there is no report regarding the pathological analysis of BAP1 cancer syndrome-associated RCC and no consensus of histological features, which would be useful for diagnosis of BAP1 cancer syndrome-associated RCC. However, based on two reports, bilateral multifocal early-onset ccRCC with high Fuhrman grade might be characteristic of BAP1 cancer syndrome-associated RCC (Fig. 2.8b–d) [346, 347]. There are many reports suggesting the involvement of other types of cancers in BAP1 cancer syndrome, i.e., breast cancer, meningioma, lung cancer, neuroendocrine carcinoma, and basal cell carcinoma [350, 352–355]. To define the true tumor spectrum of BAP1 cancer syndrome, a large-scale recruitment of affected families and intensive analysis would be required.

2.9.2 Genetics of BAP1 Tumor Predisposition Syndrome

BAP1 inactivating somatic mutations were first identified by whole-exome sequencing of metastatic uveal melanomas, which had chromosome 3 monosomy [356]. Additional Sanger sequencing of all *BAP1* exons revealed the frequent loss of function mutations of *BAP1* in metastasizing uveal melanomas (26/31; 84%). Subsequently, somatic inactivation mutations were found in 23% of malignant pleural mesothelioma [357]. Following these findings, germline mutations in *BAP1* were reported as predisposing to malignant mesothelioma, melanocytic tumors, uveal melanoma [348–352], and RCC [346, 347]. From 7.5 to 14% of cases of sporadic ccRCC are reported to have somatic inactivating mutations in *BAP1*, which underscores the significance of loss of BAP1 function in developing ccRCC [50–54]. Most of the germline mutations reported to date are nonsense or insertion/deletion mutations causing frameshift and premature terminations [358]. To date, there is no report describing distinct genotype-phenotype correlations in BAP1 cancer syndrome.

2.9.3 Molecular Function of BAP1

The precise molecular function of BAP1 as a tumor suppressor for RCC remains to be clarified. BAP1 is a 729 amino acid nuclear protein, which is a deubiquitinase belonging to the ubiquitin carboxyl-terminal hydrolase (UCH) family. It was originally identified as a BRCA1 interacting protein and a deubiquitinase of BRCA1, which activates the tumor suppressor function of BRCA1 [342]. Later BRCA1 was reported to form an E3 ubiquitin ligase heterodimeric complex with BRCA1/BARD1, whose E3 ligase activity is dramatically increased by auto-ubiquitination [359]. BAP1 was shown to interact with BARD1 and inhibit the E3 ligase activity of the BRCA1/BARD1 complex by interfering with the BRCA1/BARD1 association, instead of deubiquitinating BRCA1 [360]. Deubiquitinase enzymatic activity of BAP1 seems to be necessary for its tumor suppressor function, because missense mutations, which abrogate deubiquitinase activity, are frequently found in the catalytic domains of BAP1 in RCC [54, 347]. Drosophila BAP1 (Calypso), which is a polycomb repressive deubiquitinase, deubiquitinates H2A and regulates the expression of genes involved in body patterning [361]. Likewise, mammalian BAP1 is able to deubiquitinate the ubiquitinated H2A [361], suggesting the involvement of BAP1 in gene expression regulation.

BAP1 binds to host cell factor (HCF-1) through its HCF-1 binding motif (HBM), which is absent in Drosophila BAP1 [362–364]. HCF-1 is a 2035 amino acid nuclear scaffold protein, which regulates the transcription of a variety of genes by recruiting chromatin remodeling complexes to transcription factors

[365–367]. HCF-1 recruits H3K4 histone methyltransferases to the E2F transcription factors to transcribe genes for S phase initiation and promote cell cycle progression [368]. Since BAP1 regulates the ubiquitination status of HCF-1 [362, 364] and is involved in cell cycle regulation [362], it would be an attractive idea to test if BAP1 regulates the E2F transcription activity through deubiquitination of HCF-1.

As mentioned above, *BAP1* somatic mutations are found in approximately 10% of sporadic ccRCC. Since most of the sporadic ccRCC have lost 3p and *BAP1* resides on 3p21, *BAP1*-mutated ccRCC do not have functional BAP1. The *BAP1*-mutated sporadic ccRCC show higher Fuhrman grade and significantly shorter median overall survival [54, 369, 370]. In addition, BAP1 protein expression can be an independent prognostic marker for ccRCC patients [371, 372]. Kidney-targeted $Vhl^{f/f}$, $Bap1^{f/+}$ double knockout mice develop kidney cancers, which are not seen in $Vhl^{f/f}$ mice, indicating that inactivation of both *Vhl* and *Bap1* synergizes toward the kidney cancer development [373]. Clarification of the BAP1 molecular function would shed light on our understanding of the molecular pathogenesis of sporadic ccRCC as well as the BAP1 tumor predisposition syndrome.

2.10 Conclusion

Although hereditary RCC accounts for only a small portion of all RCC, the medical consequences for patients and their affected family members can be serious. Detailed medical history, family history, and careful physical examination are of great importance for their proper diagnosis.

Studies of patients with hereditary RCC susceptibility syndromes and their families have made tremendous contributions toward the clarification of the molecular pathogenesis of sporadic RCC as well as hereditary forms of RCC. These findings have led to improved clinical outcomes for patients with hereditary and non-hereditary forms of RCC and provided the foundation for developing new targeted therapies.

Acknowledgments This work was supported by JSPS KAKENHI; Grant-in-Aid for Scientific Research (B), Grant Number 15H04975; and Grant-in-Aid for Challenging Exploratory Research, Grant Number 15 K14370. This work was also supported by the Intramural Research Program of the National Institutes of Health (NIH), National Cancer Institute (NCI), and Center for Cancer Research and funded in part with Federal funds from the Frederick National Laboratory for Cancer Research, NIH, under Contract HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsements by the US Government.

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Chapter 3
Molecular Genetics of Renal Cell Carcinoma

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M. Oya (ed.), *Renal Cell Carcinoma*, DOI 10.1007/978-4-431-55531-5_3

Abstract In the last decade, from the early large-scale multigene profiling using traditional Sanger sequencing to the more recent next-generation whole-exome and whole-genome sequencing, the genomic landscapes of renal cell carcinoma (RCC), consisting mainly of clear-cell, papillary (1 and 2), and chromophobe subtypes, have been characterized. This genomic information, coupled with DNA methylation, has shed light on the molecular biology of RCC and created tremendous opportunities for future research that hopefully will lead to improvement in diagnosis, prognosis, treatment, and prevention of RCC. This chapter will summarize the most recent genomic and DNA methylation profiles of these three subtypes of RCC and highlight the major biological pathways involved and their clinical relevance.

Keywords RCC • Cancer • Genomics

3.1 Introduction

Renal cell carcinomas, arising from the renal epithelium, are responsible for nearly 4 % of cancer incidence and 2 % of cancer mortality in the United States [95]. It has three major histological subtypes, i.e., clear cell, papillary (1 and 2), and chromophobe, and each subtype is broadly associated with its own clinical behavior, biology, and molecular genetics. Interestingly, each subtype is also associated with a hereditary cancer syndrome, and to date, our knowledge of their underlying molecular basis have mainly emanated from the studies of the predisposition genes of these inherited RCC syndromes.

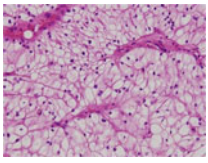
Because RCCs are often radiation and chemotherapy resistant, surgery remains the primary treatment. However, one third of patients who undergo surgical resection have recurrence, and up to 20 % of patients are diagnosed with metastatic disease [41, 68]. Since 2005, seven targeted agents, bevacizumab, sorafenib, sunitinib, pazopanib, axitinib, temsirolimus, and everolimus, have been approved by the US Food and Drug Administration (FDA) for the treatment of advanced RCC. With the advent of these targeted agents, overall survival for RCC has improved, and patients are being treated continuously for increasingly long periods of time; however, these treatments rarely yield complete responses and are not curative. Recently, the development of next-generation sequencing (NGS) has allowed systematic and comprehensive profiling of all the genomic and DNA methylation alterations. Although most of the key findings and pathways identified through these comprehensive profiling efforts are previously known, such as the VHL-HIF pathway in clear-cell RCC (ccRCC) and the MET pathway in type 1 papillary RCC (pRCC), these recently generated global genomic and epigenetic landscapes have revealed novel alterations and their associated molecular mechanisms such as frequent mutations in chromatin regulators. This advancement has provided unprecedented understanding of the complexity of RCC tumorigenesis and progression, offering new dimensions for basic, translational, and clinical research in the field.

Consequently, better therapeutic agents can hopefully be developed, and the drugs, old and new, can be more effectively administered in optimal combination and sequence.

3.2 Molecular Characterization of Clear-Cell RCC (Fig. 3.1)

3.2.1 VHL Alterations and HIF Pathway

Seventy percent of RCCs are ccRCC which forms part of the autosomal dominant von Hippel-Lindau disease, characterized also by cerebellar hemangioblastoma, retinal angioma, and pheochromocytoma. Both *VHL*-related and sporadic ccRCCs are most commonly characterized by biallelic inactivation of the *VHL* gene: inactivating mutations (germline in *VHL*-related tumors or somatic in sporadic cases) or *VHL* promoter hypermethylation, coupled with deletion of chromosome 3p harboring the wild-type *VHL*. The VHL protein is the recognition component of an ubiquitin ligase complex that facilitates degradation of cellular proteins, including the α-subunits of hypoxia-inducible factor (HIF) which dimerize with HIFβ to form an oxygen-responsive transcription factor. When VHL is inactivated, the HIF proteins accumulate and induce the transcription of a multitude of metabolic and angiogenesis factors such as *GLUT1* or *VEGFA*. The very importance of the VHL E3 ubiquitin ligase complex in ccRCC tumorigenesis was further accentuated by the recent finding of *TCEB1* mutations in the elongin C component of the complex in *VHL*-wild-type ccRCC [88]. Furthermore, what has transpired recently in the field is the appreciation of the divergent roles of HIF1α and HIF2α in RCC tumorigenesis. *HIF1α* appears to serve as a tumor suppressor gene, and accumulating evidence has supported this conclusion: (1) frequent loss of chromosome 14q harboring *HIF1α*, especially in aggressive ccRCC, (2) its knockdown leads to



Hereditary disease	von Hippel-Lindau disease, hereditary ccRCC: Germline mutations of *VHL, BAP1, PBRM1.*
Chromosome alterations	Losses of chromosome 3p and 14q. Gain of chromosome 5q. Focal deletions of chromosome 9p21 and10q23. Focal amplifications of chromosome 1q32, 3p26, 5q35, 8q24 and 9p24.
Gene alterations	Mutations of *VHL* (55%). Mutations in SWI/SNF complex: *PBRM1* (32-41%), *ARID1A* (2-4%). Mutations in chromatin modifiers: *SETD2* (3-12%), *BAP1* (8-11%), *JARID1C* (5-9%). Other mutations: *MTOR* (6%), *PTEN* (3%), *PIK3CA* (2%), *TP53* (2%).
Notes	Divergent roles of HIF1α and HIF2α. Poor prognosis in *BAP1*-mutant or *ARID1A*-mutant tumors.

Fig. 3.1 Molecular characterization of clear-cell renal cell carcinoma *PBRM1*

increased *VHL*-defective RCC cell proliferation [94], and (3) the overexpression of HIF1α results in a decreased tumor mass [83, 95]. On the other hand, *HIF2α* is considered as an oncogene based on several observations: (1) the SNP located in the *HIF2α* locus (called *EPAS*) in chromosome 2q has been associated with RCC predisposition, (2) its knockdown leads to reduced tumor growth [49, 50], and (3) in the ccRCC xenograft models, the overexpression of HIF2α with intact DNA binding domain can promote tumor growth [50]. It is becoming increasingly evident that each isoform may regulate its specific transcriptome that separately contributes to RCC tumorigenesis. Studies looking at the expression of both isoforms in normal kidney and tumor tissues have found differential patterns: HIF1α, and not HIF2α, is expressed in normal tubular cells, whereas both α-subunits of HIF are identified in precancerous VHL-defective lesions, pointing to the tumorigenic role, especially of HIF2α [64, 83]. These results suggest that de novo HIF2α expression is induced by the lack of VHL functions accompanied by induction of its target genes [64]. It is therefore important to identify these RCC-specific downstream drivers that may be potentially targetable since currently approved drugs, as described above, mainly target VEGF-related angiogenesis or mTOR-related pathway. One approach to achieve this is to identify the *cis*- and *trans*-regulatory elements specific to both isoforms in the primary tumor context using epigenomic profiling coupled with RNA sequencing with reference to recently established epigenome databases. Already, efforts have been underway to identify, for example, noncoding RNA (e.g., miR-17-5p and miR-224) that are involved in regulating VHL-HIF pathway [57], and to examine histone marks that signify nucleosome occupancy [96]. In addition, previous GWAS have discovered a susceptibility locus on chromosome 11q13.3 in RCC [81, 9, 100], and chromatin immunoprecipitation (ChIP) analysis shows that this locus corresponds to HIF-binding enhancer of the well-known oncogene, *CCND1* [92], which appeared to be regulated by HIF2α in *VHL*-defective RCC. Furthermore, another recent study [111] has shown that epigenetic alterations to the VHL-HIF pathway in a subpopulation of RCC cancer cells enable metastasis through activation of CXCR4 (C-X-C motif receptor 4), a well-known mediator of metastatic colonization [99], and CYTIP (cytohesin 1 interacting protein), an intracellular signal modulator that protects cancer cells from death cytokine signals and promotes metastasis [111]. These observations apparently result from loss of polycomb-repressive complex 2 (PRC2)-dependent H3K27Me3 and DNA methylation, activating CXCR4 and CYTIP, respectively.

3.2.2 Mutations of Chromatin Enzymes

The importance of chromatin dynamics and its dysregulation in tumorigenesis is increasingly appreciated since the discovery of frequent mutations in chromatin regulators, including in RCC. Earlier studies of hereditary and sporadic ccRCC have pointed to the existence of ccRCC-related genes other than *VHL*

[63, 101]. Furthermore, in vitro studies using mouse embryo fibroblast cells or human tubular cells have shown that *VHL* inactivation alone paradoxically induces senescence [123] and $VHL^{+/-}$ mice fail to form RCC in mice model [27], whereas tubule-specific inactivation of *VHL* only shows cystic degeneration instead of renal tumorigenesis [115]. All these suggest that loss of VHL function alone is not sufficient to cause renal tumorigenesis. Recent high-throughput profiling has identified recurrent mutations including chromatin modifiers such as polybromo 1 (*PBRM1*) [112], SET domain containing 2 (*SETD2* or *KMT3A*), lysine (K)-specific demethylase 5C (*KDM5C* or *JARID1C*), lysine (K)-specific methyltransferase 2D (*KMT2D* or *MLL2*) [17], and AT-rich interactive domain-containing protein 1A (*ARID1A*) [112] and also BRCA1-associated protein-1 (*BAP1*) in ubiquitin-mediated proteolysis pathway [31, 76]. Subsequent whole-exome and whole-genome studies further confirm these findings ([88], 15). Several key chromatin regulators involved are highlighted and further discussed below.

3.2.2.1 PBRM1 and SWI/SNF Complex

PBRM1 is the second most frequent mutated gene in ccRCC, ranging from 32 to 41 % [15, 88, 112]. The vast majority of *PBRM1* mutations are indels or nonsense, typical of tumor suppressors including *VHL*. Furthermore, a family with four cases of ccRCC has recently been shown to be associated with germline *PBRM1* mutation and loss of wild-type copy of chromosome 3p in the tumors [6]. *PBRM1* encodes the BAF180 protein, an accessory subunit of the SWI/SNF (PBAF) chromatin remodeling complex [85] which is implicated in replication, transcription, DNA repair, and control of proliferation and differentiation [85]. The SWI/SNF subunits can be grouped into three categories of proteins: (1) enzymatic, (2) core, and (3) accessory. Distinct SWI/SNF complexes, consisting of PBAF and BAF variants, are formed by combinatorial assembly of a central ATPase, the core subunits, and selected accessory proteins. The latter dictates the specificity of the SWI/SNF complex function [85, 118], and BAF180 falls into the accessory group of the PBAF variant. Interestingly, the *PBRM1* counterpart in the BAF variant, *ARID1A* which encodes BAF250a, has been previously linked to ovarian clear-cell carcinoma, mutated in 50 % of the tumors with similar pattern like *PBRM1* in ccRCC [43, 117]. The fact that both are clear-cell tumors, which reflects the high glycogen and lipid contents in their cytoplasm, may point to a common biological link between the functions of these two SWI/SNF complexes. In ccRCC, although the mutations of *ARID1A* are significantly lower (around 2–4 %), 60–70 % of ccRCC tumors exhibit significantly lower *ARID1A* mRNA and protein expression according to one study [58], suggesting that decreased expression of BAF250a may contribute significantly to ccRCC tumorigenesis. Interestingly, one study has shown association of *PBRM1* mutations with significant increase in ccRCC recurrence [16] and tumor-related death, while the other association of decreased BAF250a expression with higher tumor stage and grade [58]. Obviously further

studies are warranted to address the role(s) of SWI/SNF complexes in ccRCC tumorigenesis.

3.2.2.2 BAP1 and Ubiquitin-Mediated Proteolysis Pathway

By whole-exome sequencing, Guo et al. identify several mutated genes from the ubiquitin-mediated proteolysis pathway (UMPP), a pathway that includes *VHL* along with 135 other genes [31]. A positive correlation found between the alterations in the UMPP and overexpression of HIF1α and HIF2α in ccRCC tumors suggests that alterations in the ubiquitin-mediated proteolysis pathway may contribute to ccRCC via the α-subunits of HIF [31]. Besides *VHL*, notable among the mutated genes in the UMPP is *BAP1*, which is located in the short arm of chromosome 3. Its mutations are mainly nonsense, frameshift, or splicing [76] accompanied by the loss of heterozygosity in ccRCC. Its germline mutations have also been associated with hereditary ccRCC predisposition. [80]. Originally discovered as an interaction partner of BRCA1, it regulates DNA damage response, and loss of *BAP1* sensitizes RCC cell lines to γ-irradiation treatment [76]. Similar to *PBRM1*, *BAP1* also plays a role in chromatin biology. It binds to transcription factor host cell factor 1 (HCF1) via UCH37-like domain. This interaction may sustain the formation of complexes between histone modifiers and transcription factors and regulate cell-cycle progression [67, 109]. Besides that, it can also form part of polycomb-repressive deubiquitinase complex (PR-DUB) by interacting with ASXL1. This complex deubiquitylates H2A and represses *HOX* gene expression [89]. Importantly, unlike the germline mutations found in melanoma and mesothelioma of which most mutants have intact UCH domain, the *BAP1* mutations in ccRCC are evenly disrupted throughout the whole open reading frame (ORF). Studies have shown that its loss correlates with higher tumor grade associated with mammalian target of rapamycin (mTOR) activation [44, 76]. This correlation may have potential predictive value for prognosis and drug response.

Interestingly, another tumor suppressor gene that is inactively mutated in ccRCC and located in the frequently deleted chromosome 3p is *SETD2*, which encodes a histone H3 lysine-36 methyltransferase associated with methylation of active chromatin. Mutations found in either *SET2* or *JARID1C*, a chromatin modifier, lead to decreased expression of the respective gene compared to normal kidney tissue. Furthermore, multiregional genetic analysis reveals that *SETD2* and *JARID1C* harbors multiple distinct and spatially separated inactivating mutations within a single tumor, supporting their potential tumor suppressor roles in ccRCC [24]. *SETD2* mutations found in RCC result in changes to chromatin accessibility and RNA processing defects [96].

Indeed, the relationship between the four chromosome 3p genes, *VHL*, *SETD2*, *BAP1*, and *PBRM1*, in ccRCC tumorigenesis is intriguing. First, *BAP1* and *PBRM1* tend to be mutually exclusive and anticorrelate in ccRCC. Using both a local database and publically available database, Kapur et al. showed overall survival to be significantly shorter for patients with *BAP1*-mutant tumors than for patients

with *PBRM1*-mutant tumors [44]. A small subset of patients who have both *PBRM1*- and *BAP1*-mutant tumors appear to have worse overall survival. Interestingly, Wang et al. show that even though knockout of *BAP1* fails to develop tumor just like the *VHL* inactivation models, combined inactivation of the *VHL* and *BAP1* leads to RCC development [115]. These results imply that additional mutations are needed to drive ccRCC development in *VHL*-mutant cases, a concept that has been proposed by several lines of studies. Obviously, further functional studies to parse the biological relevance of the four genes and their interactive roles in ccRCC tumorigenesis are scientifically interesting, and tumors harboring different mutations or combination of mutation may have distinct clinical phenotypes and therapeutic implications.

3.2.2.3 Mutations of Other Chromatin Modifiers

Besides the four recurrently mutated genes in chromosome 3p, there are several other chromatin modifier genes which are mutated at a frequency of 1–4 % of ccRCC [15, 17, 110]. These include the histone methylases (i.e., *MLL2*) and the histone demethylases (i.e., *JARID1C*, *JARID1D*, and *UTX*). The size of *MLL* genes is large and it is possible that mutations in these genes are "passenger" mutations that arise due to random background mutation frequency. However, arguing against the random selection hypothesis is that the family of *MLL* genes is frequently mutated in many tumor subtypes [7, 87, 124]. In addition, JARID1C regulates histone H3 lysine 4 trimethylation (H3K4Me3) levels of HIF target genes in *VHL*-mutant ccRCC cells. *JARID1C* expression is also regulated by HIF, and depletion of *JARID1C* in these cells significantly promotes tumor growth, suggesting that these mutations contribute to ccRCC tumorigenesis by dysregulation of HIF-related pathway [71]. Overall, the discovery of frequent chromatin modifier mutations in ccRCC presents ample of opportunities for further research in the field of tumorigenesis and hopefully will lead to another level of understanding of these cancers.

3.2.3 Intra-tumor Heterogeneity

The traditional view of cancer development is that of clonal expansions of cells that acquire selective advantage through multiple genetic alterations acquired during its development [75]. Subsequently many studies have demonstrated that accumulation of mutations leads to divergent development of subclonal cancer cells in same tumor [26, 29, 65, 122]. Gerlinger et al. recently establish intra-tumor heterogeneity in ccRCC by multiregion exome sequencing in primary metastatic disease [24, 25]. In the study, nonsynonymous nucleotide alterations considered as potential driver mutations and found in at least one region are used to construct phylogenic trees divided into trunk, internal branches, and terminal branches. Gene inactivation of *VHL* is present in each case and mapped onto the trunks of

phylogenetic trees. Mutations of *PBRM1* are located on the trunks, suggesting that inactivation of *PBRM1* also occurs at an early stage of tumor development. Overall the other driver mutations are located on the branches of the phylogenetic trees. These results demonstrate a branched rather than linear evolution in ccRCCs, similar to the branched evolution described in other types of cancers [3, 5, 8, 70, 98, 102]. An important implication of these results is that in designing drug treatment strategies, targeting mutations mapped on the trunk of phylogenetic tree may provide more preferable results than those targeting subclonal driver events [122].

The results of Gerlinger et al. also show that when multiple regions are considered for each case, the prevalence of most driver mutations is higher than single case as a whole. For instance, *TP53* gene mutations are found in 2–6 % of single cases or biopsies, but up to 40 % of cases harbor the mutations when multiple regions are assessed. These data suggest that a single biopsy is unlikely to represent the full set of mutations present in a particular cancer leading to underestimation of their associated alterations. Furthermore, the relevance of subclonal driver mutations may contribute to failure of therapies [23]. Overall the evidence surrounding intra-tumor heterogeneity demonstrates the complexity and challenges in implementing precision oncology. Further understanding of the underlying biology and mechanisms of tumor heterogeneity may provide insights and help guide appropriate therapeutic strategies.

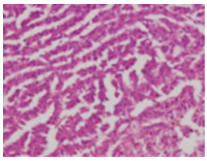
3.3 Molecular Characterization of Papillary RCC

Papillary RCC constitutes about 10–15 % of all RCC cases. It is further divided into two subtypes, type 1 and type 2, based on histological criteria [2, 19, 42]. Type 1 tumors have small cuboid cells that are arranged in a single or double layer. Type 2 tumors consist of large eosinophilic cells that are arranged in an irregular or pseudostratified manner. Type 1 pRCCs are relatively indolent and are associated with patient survival rates of approximately 90 %. In contrast, upward of 50 % of individuals with type 2 pRCC succumb to the disease within 10 years [30, 47, 78, 114]. Although the histological classification requires expert evaluation, the classification is supported by cytogenetic, gene expression, and mutational profiles that exist between these two subtypes [20, 30, 47, 61, 120].

3.3.1 Type 1 Papillary RCC (Fig. 3.2)

3.3.1.1 Copy Number Alterations

Type 1 pRCC is characterized by frequent gains of chromosomes 3q 7, 12, 16, 17, and 20 [20, 30] demonstrated by cytogenetic studies and gene expression-based deduction of chromosome changes. These findings are further confirmed by cluster



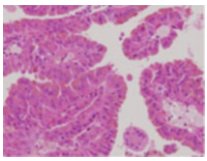
Hereditary disease	Hereditary pRCC: Germline mutations of *MET.*
Chromosome alterations	Frequent gains of chromosome 7 and 17. Less frequent gains of chromosome 2, 3, 12, 16 and 20.
Gene alterations	Mutations of *MET* (10-20%). Mutations in SWI/SNF complex: *ARID1A* (5%), *SMARCB1* (3%). Mutations in chromatin modifiers: *KDM6A* (5%).
Notes	Frequent overexpression of *MET* (chromosome 7) and *LRRK2* (chromosome 12).

Fig. 3.2 Molecular characterization of type 1 papillary renal cell carcinoma

analysis of SNP array data which shows nearly universal gain of chromosomes 7 and 17 and less frequent gain of chromosomes 2, 3, 12, 16, and 20 [61].

3.3.1.2 MET Pathway

The molecular genetics of type 1 pRCC is based on studies of hereditary pRCC that is associated with germline mutations of *MET* tyrosine kinase receptor, and somatic mutations in *MET* are also observed in up to 20 % of sporadic type 1 pRCC [61, 90, 91]. However, amplification of chromosome 7, which contains the locus of *MET*, and overexpression of *MET* are found in most of sporadic type 1 papillary tumors [1, 61]. The majority of the *MET* mutations are in the tyrosine kinase domain, but recently an alternate *MET* RNA transcript lacking its canonical exons 1 and 2, which may result in ligand-independent MET activation, is also found in some of the cases [61]. Besides the amplification of the *MET* locus, a member of the leucine-rich repeat kinase family, leucine-rich repeat kinase 2 (*LRRK2*) located in chromosome 12, is also frequently amplified and overexpressed in type 1 papillary tumors [62]. Mutations of *LRRK2* are well characterized as a cause of autosomal dominant Parkinson's disease, whereas upregulation of LRRK2 is observed in inflammatory diseases such as leprosy [125] and Crohn's disease [4]. MET and LRRK2 cooperate during tumor growth via the mTOR and STAT3 pathway to promote cell growth and survival, and ablation of LRRK2 reduces downstream MET signaling in pRCC [62]. The central role of MET in type 1 pRCC indicates the targeted use of MET inhibitor such as foretinib, a multikinase inhibitor that targets VEGFR-2 and MET, as well as other receptors. Clinical trial of advanced stage hereditary and sporadic pRCC has seen an improvement in disease-stabilization rate and progression-free survival in patients treated with foretinib with minimal toxicity [12].



Hereditary disease	Hereditary leiomyomatosis and renal cell cancer: Germline mutations of *FH.*
Chromosome alterations	No specific pattern. Loss of chromosome 9p (*CDKN2A*) associated with poor prognosis.
Gene alterations	Mutations in NRF2 pathway: *CUL3* (5%), *FH* (3%), *NFE2L2* (3%), *KEAP1* (2%). Mutations in SWI/SNF complex: *PBRM1* (8%), *ARID1A* (7%). Mutations in chromatin modifiers: *SETD2* (17%), *BAP1* (7%). *TFE3* and *TFEB* fusion genes (12%). CpG Island Methylator Phenotype (CIMP) (6%).
Notes	Poor prognosis in CIMP phenotype.

Fig. 3.3 Molecular characterization of type 2 papillary renal cell carcinoma

3.3.2 Type 2 Papillary RCC (Fig. 3.3)

3.3.2.1 Copy Number Alterations

Compared with type 1 pRCC, type 2 tumors harbor variable chromosome abnormalities but at different frequency. It has less gains of chromosomes 7, 12, and 17p but more frequent losses of chromosomes 8p and 9p associated with poorer survival [30, 61].

3.3.2.2 FH and NRF Pathways

Besides chromosome abnormalities described above, it often contains additional ones of no specific pattern, and this cytogenetic complexity may be a reflection of the more aggressive nature of this cancer type. Again, much of our understanding of type 2 pRCC comes from studies of hereditary leiomyomatosis and renal cell cancer (HLRCC) syndrome, which is caused by germline mutations of *fumarate hydratase* (*FH*), a member of the Krebs cycle. Individuals afflicted with HLRCC develop type 2 pRCC, uterine fibroids, and cutaneous leiomyomatosis (fibroid skin tumors) at high frequencies [56, 84, 104, 107]. These tumors were found to favor the Warburg-like metabolic shift to glycolysis-dependent metabolism and increased expression of hypoxia-related genes [105, 121]. Inactivation of *FH* leads to accumulation of fumarate that can compete against 2-oxyglutarate and inhibit PHD-mediated hydroxylation of HIFα proteins [40]. This results in stabilization of HIFα as in the case of ccRCC, but when examining their gene expression pattern, it is very obvious that the molecular signature of type 2 pRCC is rather different from that of ccRCC: the latter is predominantly of angiogenesis and metabolism while the former NRF2 pathway [72]. This leads to the demonstration that excessive fumarate, due to the inactivation of *FH*, is translocated into the cytosol where it reacts with cysteines of KEAP1 altering its conformation and subsequently releasing NRF1 and NRF2 from the cytoskeleton. Free NRF1 and NRF2 can then be translocated to the nucleus, where they can bind to antioxidant response element

(ARE) and drive the expansion of genes such as *AKR1B10* and *NQO1* [72]. This pathway is further confirmed by pathway analysis of both microRNA and mRNA signatures, which clearly identifies the NRF2 pathway as a distinguishing feature of type 2 tumors [61]. Indeed, high expression of NRF2-regulated or ARE-controlled genes can be used as biomarkers with *KRIB10* as a useful diagnostic biomarker [72] and *NQO1*, a prognostic biomarker signifying worse prognosis [61]. As inactivating mutations are uncommon in sporadic cases, it is hypothesized that members of the NRF2 pathways may be involved which is borne out by the discovery of mutations in members of the NRF2 pathways including *NFE2L2*, *CUL3*, *KEAP1*, and *SIRT1* [51, 61, 73].

3.3.2.3 *CDKN2A* Alterations

High-throughput profiling has identified *CDKN2A* alterations in mainly type 2 pRCC (focal loss of 9p21, mutations or hypermethylation). As expected from its function, *CDKN2A* alterations lead to both increased levels of phosphorylated Rb and increased expression of cell-cycle-related genes. The overall survival in the patients with *CDKN2A* altered tumors is significantly shorter than those without *CDKN2A* alterations [61].

3.3.2.4 *TFE3* and *TFEB* Fusion Genes

In a subset of type 2 pRCC, recent TCGA network has identified gene fusions involving *TFE3* or *TFEB*, which are known to be associated with pRCC in young patients [45]. But the mean age in this TCGA study is 54 years suggesting that these fusions should be taken into account in any type 2 pRCCs [61]. In all cases with these fusions, increased mRNA expressions of known TFE3 or TFEB transcriptional targets such as *CTSK*, *BIRC7*, *DIAPH1*, and *HIF1*α are confirmed suggesting that these fusions are probably driver alterations that contribute to their tumorigenesis.

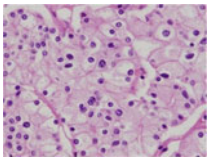
3.3.2.5 DNA Methylations and Mutations of Chromatin Enzymes

Epigenetic aberrations have been identified as important contributors of human carcinogenesis. One of them is global genome hypermethylation, resulting in suppression of tumor suppressor genes, described as CIMP [108]. DNA methylation analysis has identified CIMP, including hypermethylation of the *CDKN2A* promoter, in a subset of tumors with decreased *FH* mRNA expression. The tumors are predominantly type 2 pRCC and harbor either germline or somatic mutation of *FH*. These CIMP-associated tumors are associated with worst survival and increased expression of glycolysis-related, pentose phosphate pathway-related, fatty-acid synthesis-related genes [61].

Just like in ccRCC, several multiple recurrently mutated genes involved in the chromatin remodeling process have been identified in type 2 pRCC. These include *SMARCB1*, *PBRM1*, and *ARID1A* in the SWI/SNF complex and *SETD2*, *KDM6A*, and *BAP1* in chromatin modifier pathways [51, 61]. Unlike the ccRCC, only a portion of cases with *PBRM1*, *SETD2*, and *BAP1* mutations show loss of chromosome 3p where all three genes are located. The mutual exclusivity of *PBRM1* and *BAP1* mutations and the frequent co-occurrence of *PBRM1* and *SETD2* point to the intricacy and complexity of their roles in this tumor type. Further investigation is warranted to understand how mutations of these chromatin regulators impact cancer-specific gene expression in this tumor type.

3.4 Molecular Characterization of Chromophobe RCC (Fig. 3.4)

Chromophobe RCC (chRCC) comprises approximately 5 % of all renal cancers. The most distinctive and defining feature of this subtype is a perinuclear clearing (i.e., halo). The tumor cells typically show a relatively transparent cytoplasm with a fine reticular pattern – this chromophobic feature therefore gives rise to its name as chRCC [53, 69]. However, about 30 % of chRCC show eosinophilic pattern [53, 55, 103] with mitochondrial accumulation. Prognosis in localized chRCC is better than in clear-cell or papillary RCC, but metastatic disease still carries a poor prognosis without any obvious targeted therapy [46, 86]. It forms part of Birt-Hogg-Dube (BHD) syndrome characterized by cutaneous fibrofolliculomas, pulmonary cysts, and sometimes papillary RCC [106]. It is associated with germline mutations of *folliculin* gene (*FLCN*) in chromosome 17p11.2, and several reports suggested that this gene plays a tumor suppressor role in a number of cellular pathways, including PGC1α-related mitochondrial biogenesis [33, 48], TFE3/TFEB transcriptional regulation [35, 36, 77], and TGF-β signaling pathway [35, 36]. We have recently generated a mouse model with conditional knockout of proximal tubule-specific *Flcn*, and the mice develop multiple types of kidney tumors including chRCC which are associated with activation of mTOR and TGF-β signaling pathway



Hereditary disease	Birt-Hogg-Dubé syndrome: Germline mutations of *folliculin* gene (*FLCN*).
Chromosome alterations	Losses of whole chromosome 1, 2, 6, 10, 13, 17 and 21.
Gene alterations	Mutations of *TP53* (32%) and *PTEN* (9%). Mutations in mitochondrial complex I (18%) including *MT-ND5* (10%). Recurrent genomic structural rearrangements involving *TERT* promoter.
Notes	Metabolic dysregulation through changes in mitochondrial function. *TERT* up-regulation.

Fig. 3.4 Molecular characterization of chromophobe renal cell carcinoma

[11]. Furthermore, inhibition of mTOR pathway with rapamycin can suppress the tumor growth in these knockout mice suggesting that AKT-mTOR signaling pathway plays an important role in tumorigenesis of BHD syndrome and may potentially be targeted therapeutically [11, 32, 119].

3.4.1 Copy Number Alterations

Several studies have previously reported multiple chromosomal aberrations, including the loss of whole chromosomes 1, 2, 6, 10, 13, 17, and 21 [39, 79]. Consistent with those data, SNP array analysis conducted by TCGA network shows that in 66 sporadic primary chRCC, the vast majority of tumors have loss of at least one copy of chromosomes 1, 2, 6, 10, 13, and 17 [18]. They also find losses of chromosomes 3, 5, 8, 9, 11, 18, and 21 at lower but significant frequencies. Because there are relatively few somatic mutations identified in chRCC, whether some of these altered chromosomal regions harboring key cancer genes may contribute to its tumorigenesis remains to be investigated.

3.4.2 Genomic and Mitochondrial Alterations

Interestingly, no mutations of *FLCN* gene are found in sporadic chRCC, and only *TP53* (32 %) and *PTEN* (9 %) were identified as significantly mutated genes [18]. Besides *PTEN*, other members of the MTOR pathway that are mutated, at a frequency of <5 %, include *MTOR*, *TSC1*, and *TSC2* pointing to the involvement of this pathway. Previously, frequent mutations of *TP53* and mitochondrial genome have been reported in chRCC [21], and its benign counterparts, oncocytoma, are known mitochondriopathies, which are diseases related to abnormal function or mutations of mitochondria [52, 93]. Indeed, mutations of mitochondria DNA are identified in complex I of the electron transport chain (18 % of cases), especially in *MT-ND5* (10 %) [18], and chRCCs harboring these mutations are linked to feature of mitochondria accumulation in cytosol and eosinophilic histology [18] similar to oncocytoma. Since these mutations are associated with loss of complex I activity [22, 66], the mitochondria accumulation has been thought as results of compensatory mechanisms for inefficient oxidative phosphorylation [97]. However, the expression levels of genes in Krebs cycle and electron transport chain are upregulated in tumors harboring these mutations [18, 22, 93]. Furthermore, the expression of *PPARGC1A* which encodes a regulator of mitochondrial biogenesis, PGC1α, is also increased [18]. These results suggest the existence of a metabolic shift, and these cancer cells generate much of ATP through increased mitochondria biogenesis to support the tumor growth [18].

3.4.3 *Kataegis and* TERT *Promoter Alterations*

Whole-genome sequencing conducted by TCGA network reveals ketaegis pattern, a phenomenon of localized hypermutation, in a subset of chRCC [18]. From the comparison between chRCC with and without a strong ketaegis pattern, they find very high expression of *TERT* which encodes the catalytic subunit of telomerase whose role is to maintain telomere ends by addition of the telomere repeat TTAGGG. Previously, deregulation of telomerase has been identified in a wide variety of cancers with upregulation of the enzyme TERT [34] which appear to play an important role in tumor progression. In these cancers, increased TERT activity is associated with point mutations [37, 38], gene amplification [13, 116], and germline polymorphisms [82]. Interestingly, in chRCC, it is the structural rearrangements involving the *TERT* promoter region that are associated with its strong upregulation [18], even though the underlying mechanism of how this upregulation occurs remains to be elucidated. The spectrum of events involved in the structural rearrangements includes tandem duplication, inversion, deletion, and inter-chromosomal translocation.

3.5 Molecular Characterization of Sarcomatoid RCC

Sarcomatoid RCC is not considered a distinct subtype as sarcomatoid features can be seen in any histological subtype and is associated with poorer prognosis. It is characterized by the appearance of spindle-shaped mesenchymal cells in histology specimens, considered as an example of epithelial-to-mesenchymal transition (EMT) with increased expression of markers of EMT such as N-cadherin and Snail [10, 14, 54]. Recently RNA-seq of sarcomatoid RCC compared to adjacent clear-cell components has demonstrated increased expression of M phase and cell-cycle-related genes [74]. One gene, aurora kinase A (*AURKA)*, is highlighted to have increased expression associated with activation of the mTOR pathway, which may indicate the use of temsirolimus and everolimus in this group of tumors [113]. Interestingly, we have also demonstrated the therapeutic efficacy of aurora kinase inhibitors in in vitro and in vivo RCC models [59, 60]), although there are concerns about the toxicity-related side effects related to this group of drugs. In addition, studies have suggested that the sarcomatoid component of RCC has an impact on response to the VEGF TKIs. In a study comparing VEGF-targeted therapy, partial responses are limited to patients who have underlying clear-cell histology and less than 20 % sarcomatoid elements [28]). As sarcomatoid RCC is well established as more aggressive tumors with poorer prognosis, further investigations are warranted to study their genomic and epigenomic changes that may shed light on their underlying molecular mechanism and potential novel treatment options. One of the greatest challenges in the genetic classification of RCC tumors is the high heterogeneity among individual tumors. Mutations may not be present in

every region of the tumors, and thus, biopsies may not capture all genetic aberrations. Thus, a single biopsy result may be inaccurate to predict a prognostic profile. These challenges need to be carefully considered when clinical validation studies are performed. However, despite the challenges, breakthroughs in genetic mining and the evolving treatment options are accelerating progress that hopefully will be reflected in improved RCC survival rates.

3.6 Future Direction

Discovery of novel genomic and epigenomic alterations in RCC has opened up tremendous opportunities for research, both basic and translational. Based on these advances, more translational studies can be carried out to improve molecular classification with clinical relevance such as prognostication and drug response. Clinicians can now conduct clinicopathological correlation with these novel alterations to explore if some of them may serve as effective therapy-related biomarkers. The latter may also include immune-related genomic profiles associated with the cancers (e.g., neoantigens) and infiltrating immune cells that may be indicative of efficacy for immunotherapy. For sure the next decade will witness the outcomes of many of these exciting studies in the field of RCC. In the meantime, it is imperative to understand the functional roles of the genomic and epigenomic alterations in RCC tumorigenesis. Key pathways such as the HIF pathway in ccRCC are well established, but whether the new genomic findings (e.g., frequent *PBRM1* mutations in ccRCC) signify involvement of a novel biological pathway or actually complementing known pathways remains to be established. Since the major group of novel mutated genes found in both ccRCC and pRCC are involved in chromatin biology and gene regulation, new investigative approaches need to be undertaken to study the changes of chromatin marks, *cis*- and *trans*-regulatory elements, and 3D chromatin. All these require advanced chromatin technology and extensive bioinformatics analyses. Hopefully, new functional data from these studies will not only comprehensively parse biological pathways involved in RCC tumorigenesis and progression but also can be translated into novel therapeutic regimens. For example, with the prominent involvement of chromatin enzymes in RCC tumorigenesis, it is natural to imagine that future chromatin or epigenetic drugs, either as single or combined therapy, may be useful for treatment of RCC. Similarly, with better understanding of the biology, we will improve our chance to generate animal models with phenotypes that mimic human cancers, which can be used for drug development. To date, there is a strong epidemiological evidence to show that the incidence of RCC is associated with common modern health problems such as obesity and hypertension, but to date no biological rationale is known. Further functional studies may hopefully shed light on this association leading to better preventive strategies of RCC.

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Chapter 4
Pathology of Renal Cell Carcinoma

Shuji Mikami, Naoto Kuroda, and Yoji Nagashima

Abstract Renal cell carcinoma (RCC) is defined as a group of malignancies arising from the epithelium of the renal tubules (WHO) (Moch H, Humphrey P, Ulbright T, Reuter V, WHO classification of tumours of the urinary system and male genital organs, 4th edn. IARC Press, Lyon, 2016). Although RCCs can be completely removed surgically in many cases, distant metastasis is common and may be observed even at an early stage of the disease. The classification of renal tumor had been traditionally determined according to the morphological features, such as the cytological appearance and architecture of tumor cells, and the application of immunohistochemistry, electron microscopy, and cytogenetics resulted in significant advances in the classification of RCC. Recently, many genetic aberrations of kidney tumors have been elucidated, and the genetic features have also become major criteria for the classification of some tumors. RCCs also occur in several inherited cancer syndromes, such as von Hippel-Lindau disease. Therefore, the current classification of kidney tumors is based on genetic difference as well as morphological characteristics (Moch H, Humphrey P, Ulbright T, Reuter V, WHO classification of tumours of the urinary system and male genital organs, 4th edn. IARC Press, Lyon, 2016). Furthermore, some new disease entities have been proposed recently. This chapter aims to describe the histological, immunohistochemical, and genetic characteristics of RCCs that are useful for the differential diagnosis.

Keywords Renal cell carcinoma (RCC) • Pathology • Classification • Immunohistochemistry • Differential diagnosis

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M. Oya (ed.), *Renal Cell Carcinoma*, DOI 10.1007/978-4-431-55531-5_4

4.1 Clear Cell RCC (ccRCC)

4.1.1 General Features

ccRCCs are defined as a morphologically heterogeneous malignant neoplasms composed of cells with clear or eosinophilic cytoplasm, and they are associated with a delicate vascular network and inactivation of *von Hippel-Lindau (VHL)* gene and upregulation of hypoxia-inducible factor (HIF) [1]. The term "granular cell RCC" had been used for many years for renal tumors with eosinophilic cytoplasm [2]. However, the genetic abnormalities of many tumors classified as "granular cell RCC" were identical to those of ccRCC. Therefore, the term "granular cell RCC" should no longer be used [3]. ccRCC is the most common type of RCC and it accounts for about 80 % of all RCCs. Many patients are diagnosed as having ccRCC when they are aged in their sixth to seventh decades. The male-to-female ratio is 2–3:1. The classical triad of presenting symptoms is composed of hematuria, flank pain, and abdominal mass, but recently the initial presentation has generally been demonstrated during routine health checkups with abdominal ultrasonography as well as microscopic hematuria.

4.1.2 Genetic and Molecular Biological Characteristics

Although most clear cell RCCs are not related to VHL disease, mutations of the *VHL* gene on chromosome 3p have been reported in about 30–60 % of ccRCCs [4–6]. Many ccRCCs demonstrate loss of heterozygosity (LOH) of chromosome 3p. DNA methylation was detected in 19 % of ccRCCs [7]. Therefore, somatic inactivation of the *VHL* gene may occur by allelic deletion, mutation, or epigenetic silencing in about 70 % of sporadic ccRCCs [4, 7, 8], suggesting that the *VHL* gene is the most likely candidate for a tumor suppressor gene in sporadic ccRCC. Recently, the SWI/SNF chromatin remodeling complex gene *PBRM1* was reported as a second major ccRCC cancer gene with truncating mutations in 41 % of ccRCCs [9]. Deletions of chromosome 3p have been reported in very small-sized ccRCC, and they are regarded as the initial event in ccRCC development [10, 11]. The VHL protein negatively regulates HIF, which is a transcription factor that activates genes involved in cell proliferation, neovascularization, and extracellular matrix formation [12]. In cases of *VHL*-mutated RCC, HIF excessively accumulates to induce the expression of downstream genes, such as *VEGF*, *Glut1*, and *carbonic anhydrase 9 (CA9)*. VEGF protein is a potent mitogen of vascular endothelial cells, which is believed to play an important role in forming the sinusoid-like vasculature. Because CA9 is overexpressed in diffuse membranous distribution in ccRCC, its immunostaining is very useful in the differential diagnosis of RCC.

4.1.3 Morphological Features

Typically, the gross appearance of ccRCC is whitish yellow in color, and it tends to form a well-circumscribed tumor (Fig. 4.1). Necrosis, hemorrhage, cysts, and calcification are commonly observed, especially in large tumors (Fig. 4.1). Histologically, ccRCC is composed of alveolar architecture tumor cells with clear to eosinophilic cytoplasm associated with rich vascular stroma (Fig. 4.2a). A regular and delicate network of small thin-walled blood vessels is a diagnostically helpful characteristic of ccRCC. Generally, cancer cells possess clear cytoplasm containing abundant lipid droplets and glycogen (clear cells). However, ccRCCs often contain tumor cells with eosinophilic cytoplasm, which is often observed in high-grade ccRCCs and cancer cells adjacent to areas with necrosis or hemorrhage (Fig. 4.2b). Focal sarcomatoid change, which is not a distinct histological entity but the malignant transformation of RCC, occurs in about 5 % of ccRCCs and is associated with worse prognosis (arrows in Fig. 4.3) [13, 14].

4.1.4 Grading

Nuclear grade is an important prognostic factor of ccRCC [15, 16]. There are 4-tiered and 3-tiered grading systems, and Fuhrman nuclear grade was most widely used (Fig. 4.4) [17]. However, the criteria for nucleolar prominence and nuclear pleomorphism are poorly defined in Fuhrman nuclear grade, and there is no indication regarding the relative importance of each feature. Therefore, the recently updated World Health Organization (WHO) classification recommends the use of

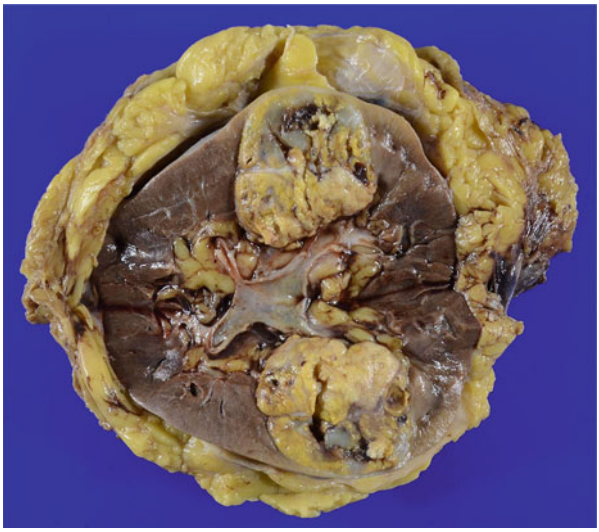

Fig. 4.1 Gross findings of ccRCC**.** The tumor is *whitish yellow* and well circumscribed, in which degeneration and hemorrhage are observed

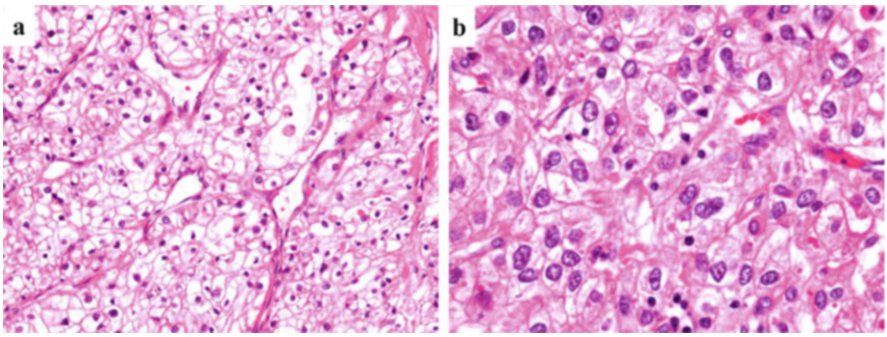

Fig. 4.2 Microscopic findings of ccRCC. Low-grade ccRCC has clear cytoplasm with a regular and delicate network of small thin-walled blood vessels (**a**), whereas high-grade ccRCC tends to have eosinophilic (granular) cytoplasm (**b**)

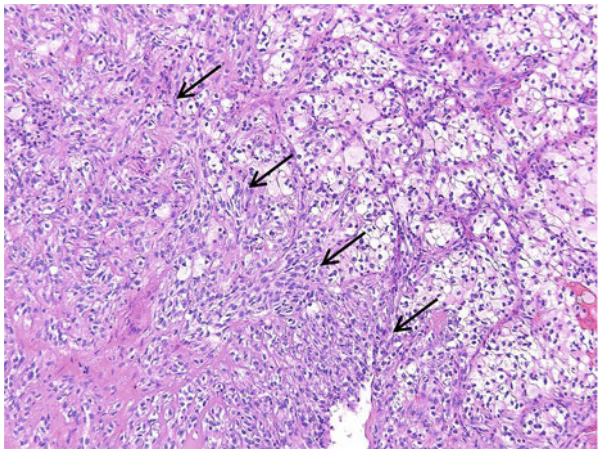

Fig. 4.3 Sarcomatoid carcinoma (*arrows*) in ccRCC

WHO/International Society of Urological Pathology (ISUP) grading system [1]. According to WHO/ISUP grading system, nuclear grade 1 cells have small nuclei like mature lymphocytes, and nucleoli are absent or inconspicuous and basophilic at x400 magnification. Nucleoli of grade 2 cells are conspicuous and eosinophilic at x400 magnification and visible but not prominent at x100 magnification. The nucleoli of grade 3 cells are conspicuous and eosinophilic at x100 magnification. Extreme nuclear pleomorphism, multinucleated giant cells, and/or rhabdoid and/or sarcomatoid differentiation are observed in nuclear grade 4 cells. Generally, nuclear grade is assigned based on the highest grade present in ccRCC tissues.

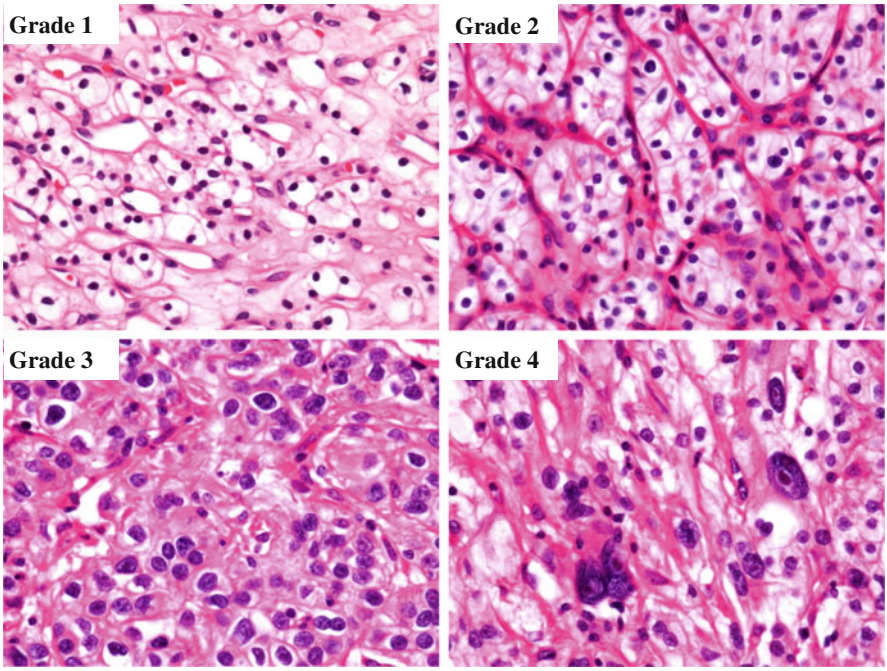

Fig. 4.4 WHO/ISUP grade**.** Grade 1 cells have small hyperchromatic nuclei resembling mature lymphocytes, and grade 2 cells have finely granular chromatin. The nucleoli can be easily recognized in grade 3 cells, and nuclear pleomorphism, hyperchromasia, and macronucleoli are observed in grade 4 cells

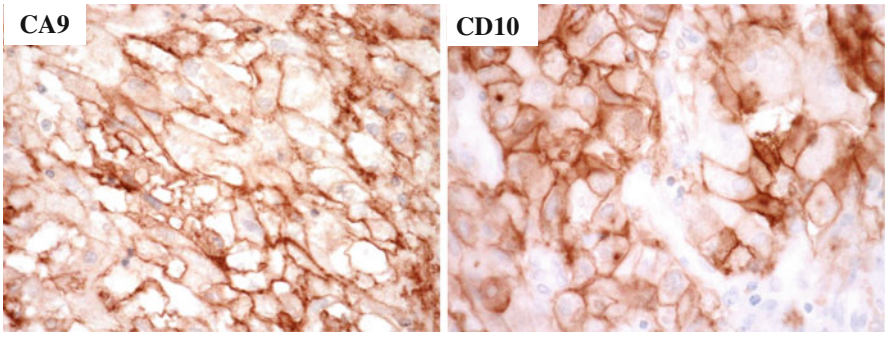

Fig. 4.5 CA9 and CD10 immunostaining in ccRCC

4.1.5 Immunophenotype and Differential Diagnosis

ccRCCs are diffusely positive for CA9 (Fig. 4.5), which is usually negative or focally positive in other renal tumors such as papillary and chromophobe RCCs and oncocytoma. Therefore, CA9 staining is helpful in the differential diagnosis of

ccRCCs from other renal neoplasms. ccRCC is positive for low-molecular-weight cytokeratin (clone CAM5.2, CK18) and negative for high-molecular-weight cytokeratin (clone 34βE12, CK5/6). It is also positive for vimentin, EMA, RCC-Ma, CD10 (Fig. 4.5), PAX2, and PAX8 but usually negative for CK7, AMACR (α-methylacyl-CoA racemase), E-cadherin, and kidney-specific cadherin [18].

4.2 Multilocular Cystic Renal Neoplasm of Low Malignant Potential

4.2.1 General Features

Multilocular cystic renal neoplasm of low malignant potential is defined as a tumor composed of many cysts and fibrous septa containing small groups of clear cells without expansile growth, and these clear cells are indistinguishable from those of low-grade ccRCC [1]. Loss of heterozygosity of chromosome 3p was identified in most multilocular cystic renal neoplasm of low malignant potential by FISH analysis. *VHL* gene mutation was found in 25 % of multilocular cystic renal neoplasm of low malignant potential [19]. Recurrence or metastasis has not been reported [14].

4.2.2 Morphological Features

Grossly, multilocular cystic renal neoplasm of low malignant potential is a well-circumscribed tumor that is entirely composed of fibrous septa and numerous cysts (Fig. 4.6). RCCs containing a cystic structure and expansive nodules of tumor cells with clear cytoplasm must be diagnosed as ccRCC with cysts, not multilocular

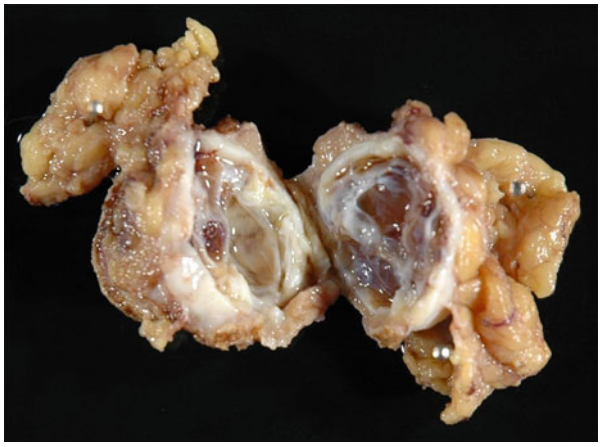

Fig. 4.6 Gross appearance of multilocular cystic renal neoplasm of low malignant potential

cystic renal neoplasm of low malignant potential (Fig. 4.7). This is because the patients with multilocular cystic renal neoplasm of low malignant potential showed excellent outcomes compared with those with ccRCC [20]. Microscopically, this tumor is composed of multiple cysts lined with tumor cells and fibrous septa (Fig. 4.8). The lining tumor cells have small dark nuclei and clear to pale cytoplasm, and they are flat or plump. By definition, the tumor cells do not form expansive nodules.

Immunohistochemically, cancer cells show positive staining for CA9 (Fig. 4.7), PAX2, PAX8, CD10, EMA, and CK7 but are negative for AMACR [19]. This tumor should be distinguished from other benign cystic renal neoplasms such as cystic nephroma, adult cystic nephroma/mixed epithelial and stromal tumor

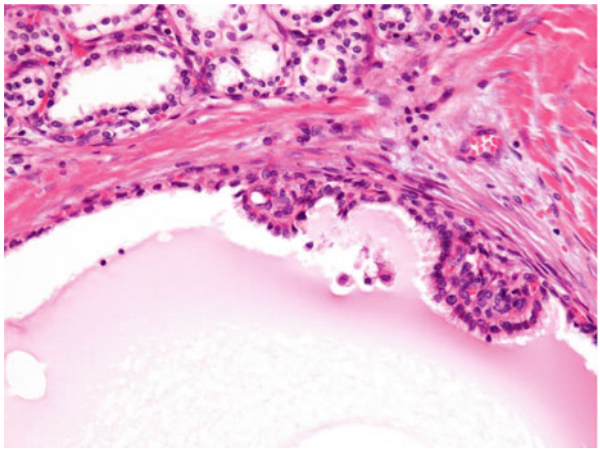

Fig. 4.7 Microscopic appearance of ccRCC with cysts. Cysts are observed in this case. However, this case should be diagnosed as cystic RCC with cysts, not multilocular cystic renal neoplasm of low malignant potential, because there are expansive nodules of tumor cells with clear cytoplasm

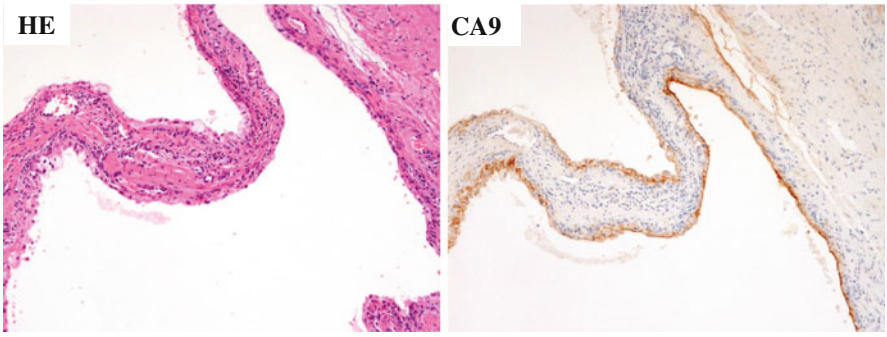

Fig. 4.8 Microscopic appearance of multilocular cystic renal neoplasm of low malignant potential. The tumor is composed of fibrous septa and tumor cells lining multiple cystic spaces without expansive nodules (**a**). Immunohistologically, tumor cells are positive for CA9 (**b**)

(MEST), and angiomyolipoma with epithelial cysts (AMLEC). Multilocular cystic RCC is positive for CA9, but other cystic tumors are negative for CA9 staining. Estrogen receptor (ER) and progesterone receptor (PgR) are negative in multilocular cystic RCC, whereas they are positive in adult cystic nephroma/MEST and AMLEC. Only AMLEC is reactive with antibodies for melanosome-associated antigen such as clone HMB45 and clone A103 (Melan-A).

4.3 Papillary RCC (pRCC)

4.3.1 General Features

pRCC is defined as a malignant renal tumor derived from renal tubular epithelium, and it has papillary or tubulo-papillary architecture [1]. Previously, any tumor with papillary and/or tubular architecture larger than 0.5 cm was classified as pRCC, while tumors 5 mm in diameter or smaller are defined as papillary adenomas [14]. However, unencapsulated WHO/ISUP low-grade (G1–2) tumors of papillary renal tumors $\leq$15 mm have virtually no capacity to metastasize and been classified as papillary adenoma, according to the recently updated WHO classification [1]. This tumor is the second most commonly encountered subtype of RCC and accounts for about 10 % of all renal tumors [21, 22], and the age, sex distribution, and signs of pRCC are similar to those of ccRCC [23, 24].

4.3.2 Morphological Features

Grossly, pRCC is well circumscribed and frequently contains areas of hemorrhage, necrosis, and cystic degeneration (Fig. 4.9) [23, 24]. Microscopically, pRCC is usually surrounded by a fibrous capsule and characterized by cancer cells forming papillary and papillo-tubular structures (Fig. 4.10). Foamy macrophages and cholesterol crystals in the fibrovascular core of the papillo-tubular structure are also characteristic features of pRCC. Necrosis and hemorrhage are frequently observed. Hemosiderin granules may be present in macrophages, stroma, and tumor cell cytoplasm [24]. pRCC has been subclassified into two categories, type 1 and type 2 pRCC, based on the morphologic features. In type 1 pRCC, cancer cells are small, and they have scanty basophilic or pale cytoplasm and low-grade nuclei without nuclear pseudostratification (Fig. 4.10a). Type 2 pRCC is composed of cancer cells having abundant eosinophilic cytoplasm and high-grade nuclei with pseudostratification (Fig. 4.10b). Sarcomatoid carcinoma is observed in about 5 % of pRCCs [25]. Importantly, papillary renal tumors showing features of other recognized morphological subtypes of RCC (i.e., MiT family translocation RCC,

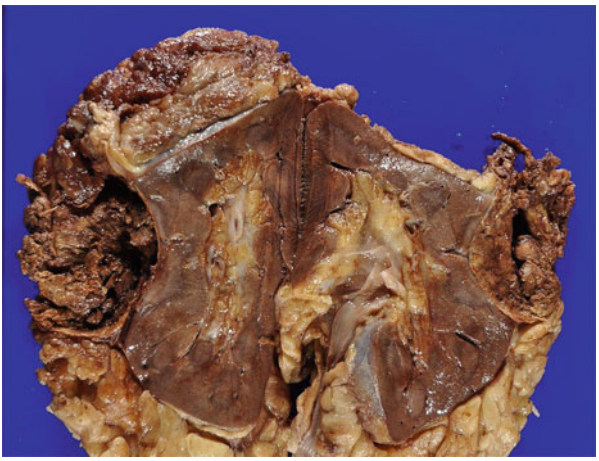

Fig. 4.9 Gross appearance of pRCC. The tumor is well circumscribed, and marked hemorrhage and necrosis are observed

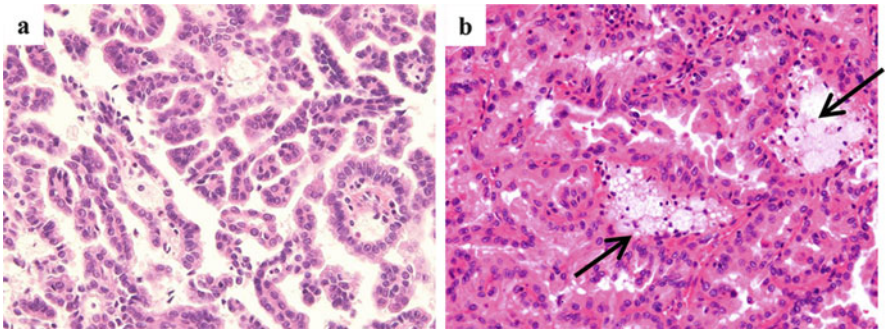

Fig. 4.10 Microscopic appearance of pRCC type 1 (**a**) and type 2 (**b**). *Arrows* indicate foamy macrophages

collecting duct carcinoma, mucinous tubular, and spindle cell carcinoma) should not be diagnosed as pRCC [1].

Many pRCCs are positive for RCC-Ma, AMACR, PAX8, CD10, cytokeratin (CK) AE1/AE3, CK (CAM5.2), and CK7 (Fig. 4.11). pRCC is usually negative for high-molecular-weight CKs such as CK (34βE12), CK5/6, and TFE3 (Fig. 4.11). CA9 staining is negative or only focally positive in pRCC, in contrast to that in ccRCC, which shows diffuse CA9 staining. pRCC is associated with a more favorable prognosis compared to ccRCC and collecting duct carcinoma [1]. Generally, the prognosis of type 1 papillary RCC is better than that of type 2 tumors [26]. Sarcomatoid and rhabdoid differentiation is associated with poor survival, and the WHO/ISUP grading system is an important predictor for the patients with pRCC [1].

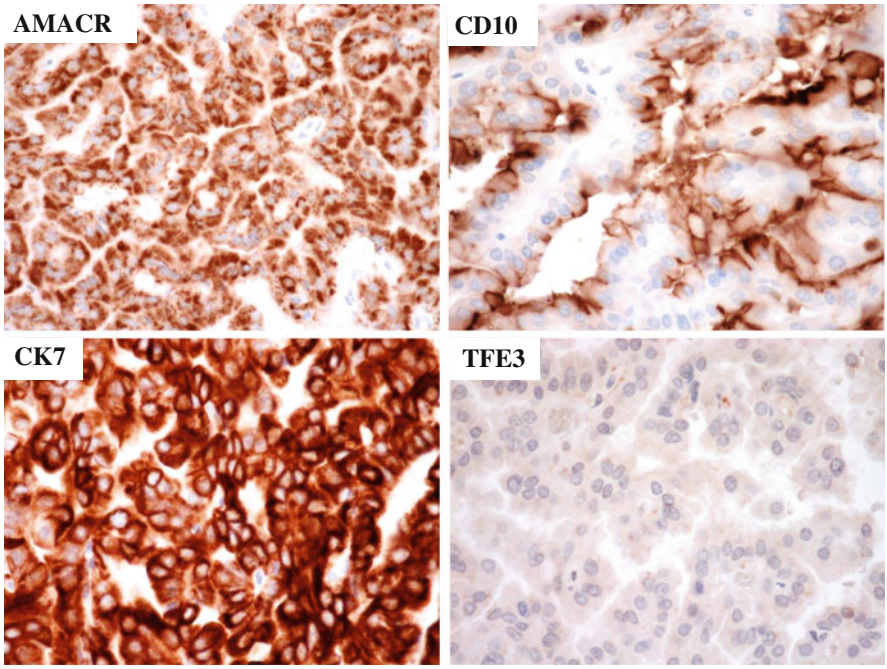

Fig. 4.11 Immunostaining of pRCC. Cancer cells are positive for AMACR, CK7, and CD10 but negative for TFE3

4.4 Chromophobe RCC (chRCC)

4.4.1 General Features

chRCC is defined as an RCC characterized by tumor cells with prominent cell membranes, wrinkled nuclei with perinuclear halos, and pale to eosinophilic cytoplasm, and it comprises about 5 % of all renal tumors [1]. The average patient age is in the sixth decade (range, 27–86 years), and both genders are equally affected. Many chRCCs are in stage T1 and T2 (86 %), and relatively few cases with metastatic disease have been reported [27].

4.4.2 Morphological Features

Grossly, chRCCs are well-circumscribed and solid tumors with slightly lobulated surfaces (Fig. 4.12). The cut surface of the tumor is homogeneously light brown or tan, and it turns gray after formalin fixation [14]. Microscopically, tumor cells tend to grow in solid or glandular patterns, with focal calcifications and broad fibrotic

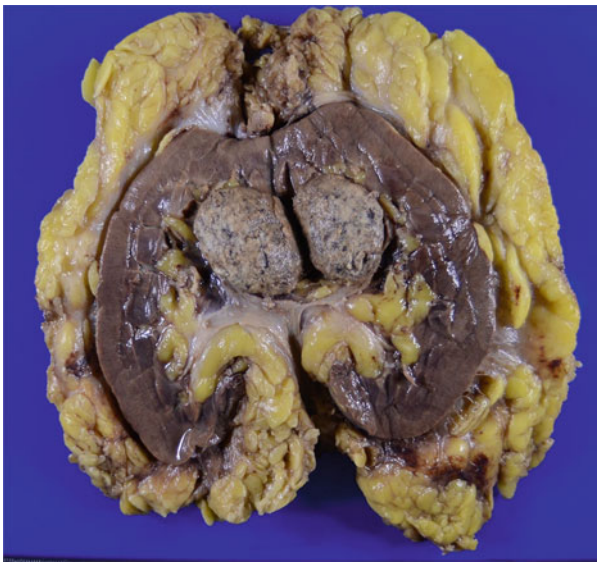

Fig. 4.12 Macroscopic appearance of chRCC. The gray tumor is solid and well circumscribed

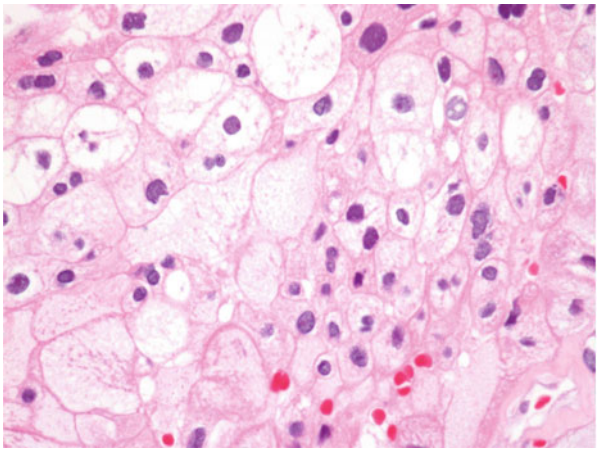

Fig. 4.13 Microscopic appearance of chRCC. Cancer cells have a distinct cell border and perinuclear halo, and there are large polygonal cells with pale cytoplasm and small cells

septa. The cell border is distinct, and wrinkled nucleus or binucleation is common. Perinuclear halo is also a characteristic (Fig. 4.13). Cancer cells are of two types, that is, (1) large polygonal cells with prominent cell membranes and pale cytoplasm (pale cells) and (2) small cells with eosinophilic granular cytoplasm (eosinophilic cells). According to the proportion of pale and eosinophilic tumor cells, chRCC can be divided into classical, eosinophilic, and mixed subtypes [28]. Oncocytoma also shows similar morphology to eosinophilic subtype of chRCC, and there is a small subset of renal tumors showing overlapping histology between oncocytoma and chRCC (hybrid oncocytic/chromophobe tumors). A diffuse cytoplasmic staining reaction with Hale's colloidal iron stain is a diagnostic hallmark and characteristic

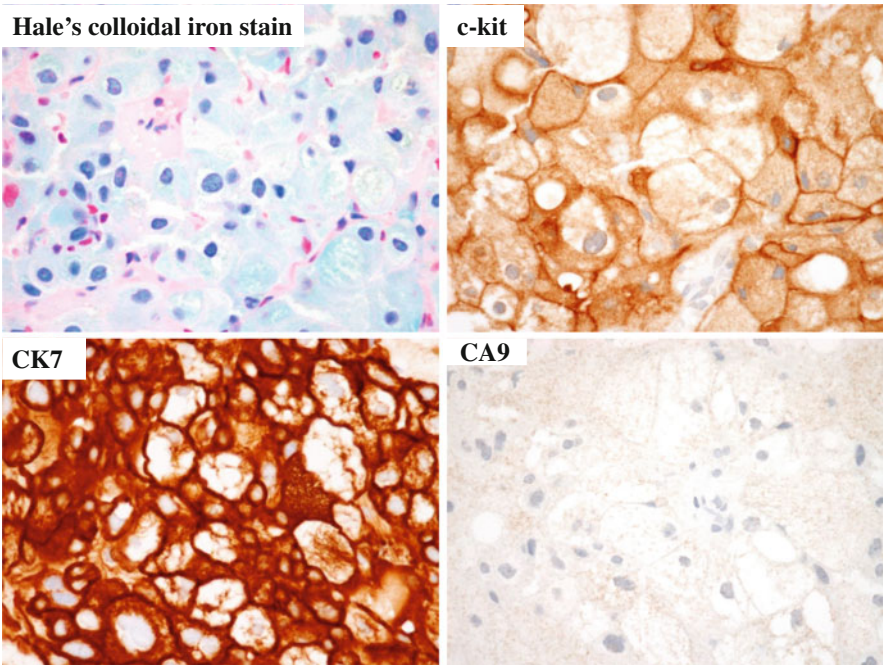

Fig. 4.14 chRCC is positive for Hale's colloidal iron stain, CD117, and CK7 and negative for CA9

feature (Fig. 4.14). Immunohistologically, cancer cells are positive for CK7, CD82, ERA (MOC31), parvalbumin, CD117 (c-kit), E-cadherin, and kidney-specific cadherin, and they are usually negative for CA9, CD10, AMACR, and RCC-Ma (Fig. 4.14) [18]. Strong cytoplasmic CK7 staining may be a characteristic feature of this tumor, whereas it is negative or focally positive in renal oncocytoma (Fig. 4.15). Many chRCCs show distinct and peripheral cytoplasmic accentuation of CD117 staining (Fig. 4.14), whereas oncocytoma shows cytoplasmic positivity (Fig. 4.15) [18, 29]. Generally, chRCC has a favorable prognosis and tumor state, and sarcomatoid change, necrosis, and microvessel invasion are the predictors of the patients with chRCC [1]. WHO/ISUP grading system should not be applied to chRCC, because chRCC has its innate nuclear atypia [1].

4.5 Collecting Duct Carcinoma of Bellini

4.5.1 General Features

CDC is a subtype of RCC, which is considered to be derived from the principal cells of the collecting duct of Bellini, and it accounts for about 1 % of all renal tumors

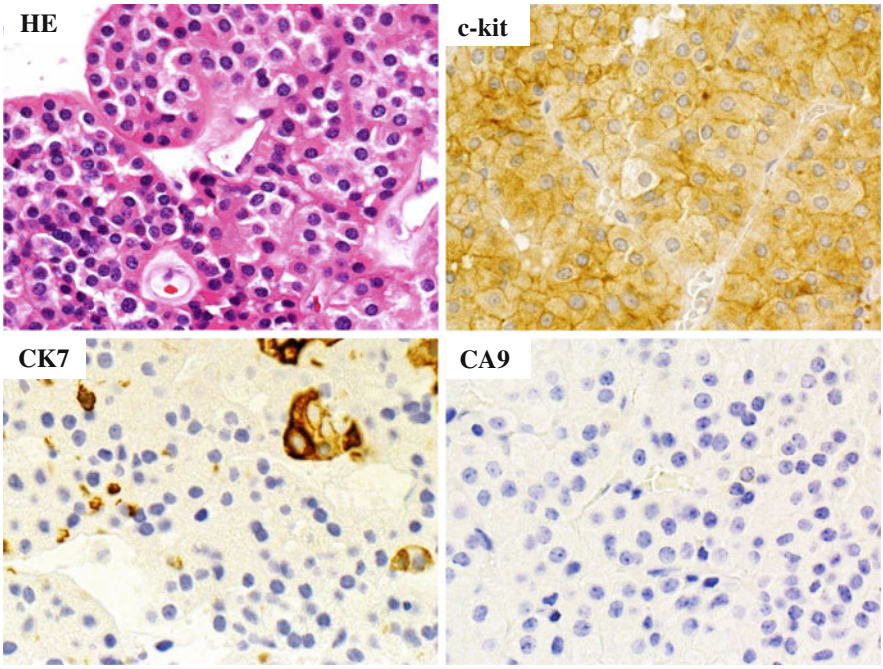

Fig. 4.15 Microscopic appearance and immunostaining of oncocytoma. Microscopically, oncocytoma is composed of small eosinophilic cells, and it is weakly positive for CD117 staining and negative for CA9. Many tumor cells are negative for CK7, but a small number of tumor cells are positive

[1]. The mean age of the patients is about 50 years (range, 13–83 years) with a male-to-female ratio of 2–3:1 [30]. Abdominal pain, flank mass, and hematuria are frequently observed symptoms of patients with CDC. About one-third of patients have metastases at the time of diagnosis.

4.5.2 Morphological Features

Grossly, small CDCs are predominantly located in the medulla of the kidney. However, many CDCs are advanced tumors, and they diffusely involve the renal cortex (Fig. 4.16). Their cut surfaces show a firm gray-white appearance with irregular borders [30]. The diagnosis of CDC is difficult in many cases, and an exclusive diagnosis of other histological subtypes of RCC, urothelial carcinoma, and metastatic carcinoma using immunohistochemistry is mandatory. Microscopically, CDC has a tubular to papillary growth pattern with diffuse infiltration to renal parenchyma accompanied by a desmoplastic stroma, and the boundary of the tumor is usually poorly defined (Fig. 4.17) [31]. A sarcomatoid or rhabdoid

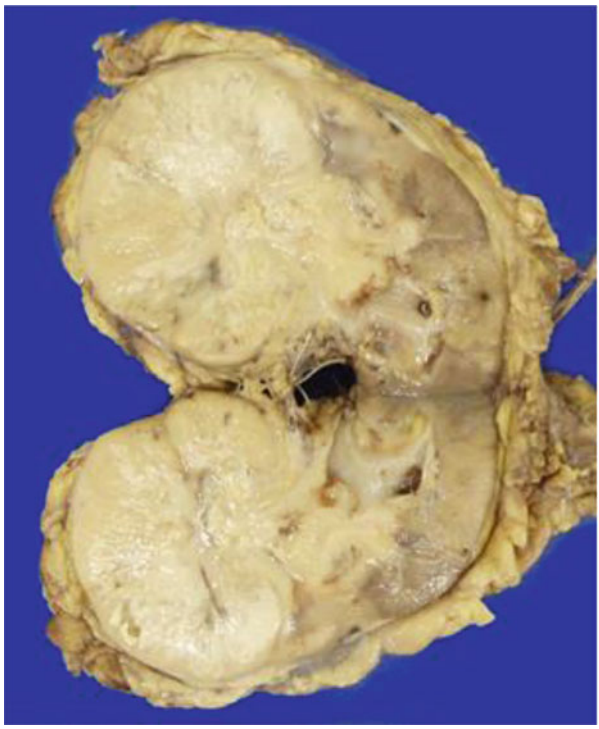

Fig. 4.16 Gross appearance of CDC. The cut surfaces of the tumor have a firm gray-white appearance with irregular borders

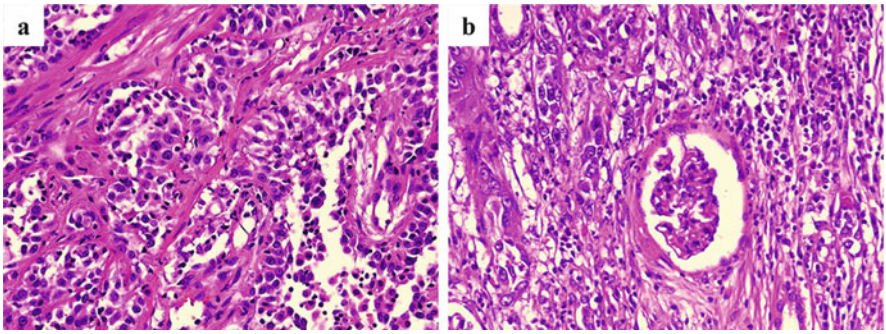

Fig. 4.17 Microscopic appearance of CDC. Cancer cells proliferate in a tubular to papillary growth pattern (**a**), and they diffusely infiltrate to nonneoplastic renal tissues with inflammation and fibrosis (**b**)

transformation, which is similar to that seen in other RCCs, has been also reported [32]. Cancer cells of CDCs usually show high-grade (Fuhrman nuclear grades 3 and 4) nuclear features with eosinophilic cytoplasm. The surrounding collecting ducts sometimes show dysplastic change.

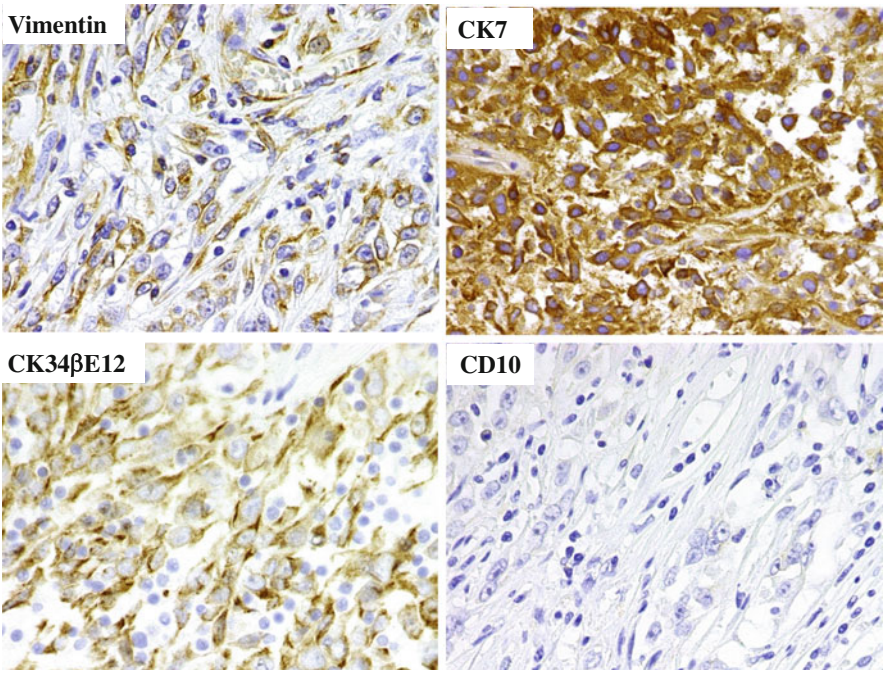

Fig. 4.18 Immunostaining of CDC. The tumor cells are positive for vimentin, CK (34βE12), and CK7 and negative for CD10

Immunohistochemically, tumor cells are positive for vimentin, CK7, CK19, PAX2, and PAX8 but negative for CK5/6, CK17, kidney-specific cadherin, CD10, RCC-Ma, CD117, p63, and CK20 (Fig. 4.18) [18]. Positive staining for CK (34βE12) and *Ulex europaeus* agglutinin 1 (UEA-1) was reported as one of the diagnostic criteria in the previous WHO classification [14], but immunohistochemistry is not included in the criteria of updated WHO classification. Recently proposed six histological diagnostic criteria are medullary involvement, predominantly tubular morphology, desmoplastic stromal reaction, cytological high grade, invasive growth pattern, and absence of renal cell carcinoma subtypes or urothelial carcinoma [1]. CDC is usually negative for the markers of ccRCC (CA9), pRCC (AMACR), and urothelial carcinoma (p63, GATA3, and uroplakins). Metastases are found in many patients at presentation, and about two-thirds of the patients die of the disease within 2 years of diagnosis [30].

4.6 Mucinous Tubular and Spindle Cell Carcinoma (MTSCC)

4.6.1 General Features

MTSCC is defined as a renal epithelial neoplasm composed of tubular formations merging with bland spindle cells and mucinous stroma [1]. The age of the patients ranges from 13 to 81 years (mean: 58), and marked female predominance is noted (1:3) [1].

4.6.2 Morphological Features

Grossly, MTSCCs are well-circumscribed solid tumors, and their cut surfaces are gray or light tan (Fig. 4.19). Microscopically, cuboidal tumor cells proliferate in cord-like, tubular, and papillary structures, and there are also small spindle cells with low nuclear grade (Fig. 4.20). Another characteristic feature of MTSCC is mucinous stroma, which is demonstrated by Alcian blue staining (Fig. 4.21). Cancer cells are positive for vimentin, EMA, CK (AE1/AE3), CK7, CK8, CK18, CD15, and AMACR (Fig. 4.21), and there is considerable similarity of

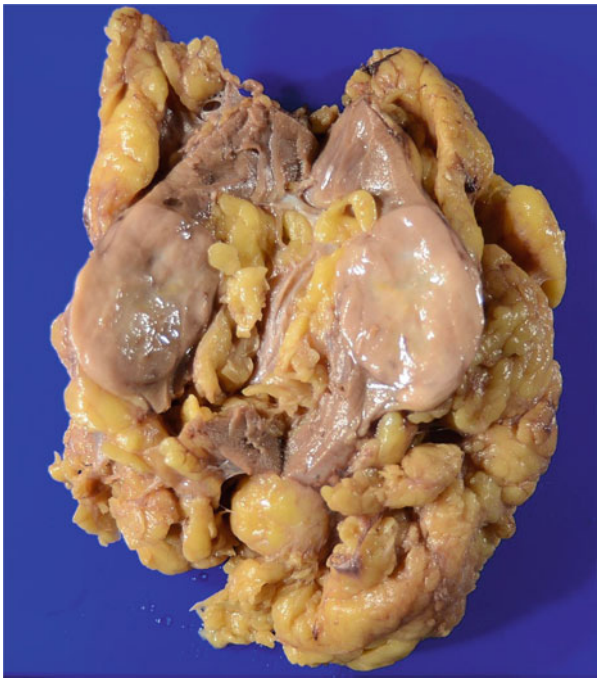

Fig. 4.19 Macroscopic appearance of MTSCC. The tumor with a light tan color is solid and well circumscribed

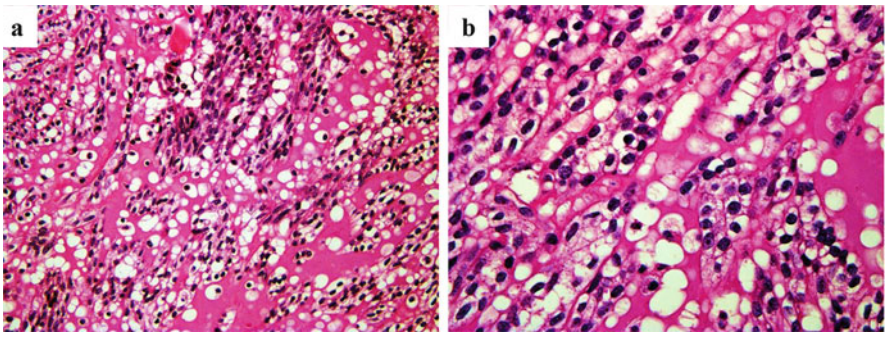

Fig. 4.20 Microscopic appearance of MTSCC. Cuboidal tumor cells proliferate in tubular and papillary structures (**a**), and there are also small spindle cells with low nuclear grade (**b**)

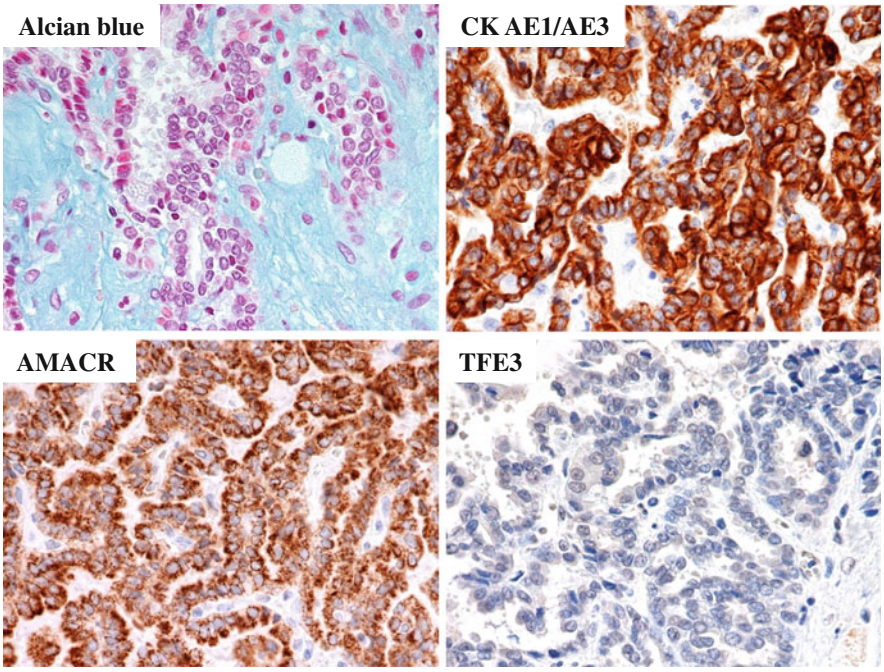

Fig. 4.21 Alcian blue staining and immunostaining of MTSCC. Alcian blue staining reveals mucinous stroma. Tumor cells are positive for AMACR and CK (AE1/AE3) and negative for TFE3

immunostaining between MTSCC and pRCC [18]. Therefore, the differential diagnosis of MTSCC and pRCC is often challenging. Although many MTSCCs were considered as low-grade RCC at first, tumors with poor prognosis have been reported recently [33, 34]. Sarcomatoid change is also observed [35]. Although

most MTSCCs have an indolent course, tumors with high-grade transformation may show distant metastasis and can be fetal [1].

4.7 MiT Family Translocation RCCs

4.7.1 General Features

MiT family translocation RCCs are defined as renal tumors harboring gene fusions involving *TFE3* or *TFEB* of the MiT family translocation factors [1]. The gene fusions of RCC associated with Xp11 (Xp11 RCC) include *ASPCR1*/*ASPL-TFE3* (*alveolar soft part sarcoma critical region 1*/*alveolar soft part sarcoma locus-TFE3*), *PRCC-TFE3* (*papillary renal cell carcinoma-TFE3*), and other minor ones such as *PSF-TFE3* (*PTB-associated splicing factor-TFE3*), *NONO-TFE3*, and *CLTC-TFE3*. Xp11 RCCs tend to affect children and young adults, but some older patients with this tumor have also been reported [36]. RCC with t(6; 11) (6p21 RCC) also occurs predominantly in children and young adults and has a translocation between the *TFEB* gene located on chromosome 6q21 and the *Alpha* gene located on chromosome 11q12. 6p21 RCCs are less common than Xp11 RCC [1].

4.7.2 Morphological Features

No distinctive gross appearance has been known in MiT translocation RCCs. A case of Xp11 RCCs shows tan-yellow and solid tumors (Fig. 4.22). Microscopically, many Xp11 RCCs are composed of a carcinoma with papillary, alveolar, or solid architecture comprised of clear to eosinophilic cells. The *ASPCR1*/*ASPL-TFE3* tumors have abundant clear to eosinophilic cytoplasm, discrete cell borders, vesicular chromatin, and prominent nucleoli. Psammoma bodies and hyaline nodules are often observed in the stroma [37]. The *PRCC-TFE3* tumor cells have less abundant cytoplasm and fewer psammoma and hyaline nodules, and the tumor cells form more nested, compact architecture [36]. 6p21 RCC is composed of nests of large and small cells. Rosette-like structures surrounding basement membrane-like materials are an important diagnostic clue.

Because *TFE3* gene fusions lead to overexpression and retention of fusion protein compared with native TFE3, strong immunohistochemical nuclear staining for TFE3 is a sensitive and specific marker for Xp11 RCC (Fig. 4.23) [38]. However, as TFE3 is ubiquitously distributed in the normal human body, immunohistochemical factors such as excessive antigen retrieval, high antibody concentration, and excessive signal amplification could lead to false-positive results [38]. Appropriate positive (alveolar soft part sarcoma or Xp11 RCC diagnosed by genetic analysis) and negative controls should be simultaneously stained for TFE3

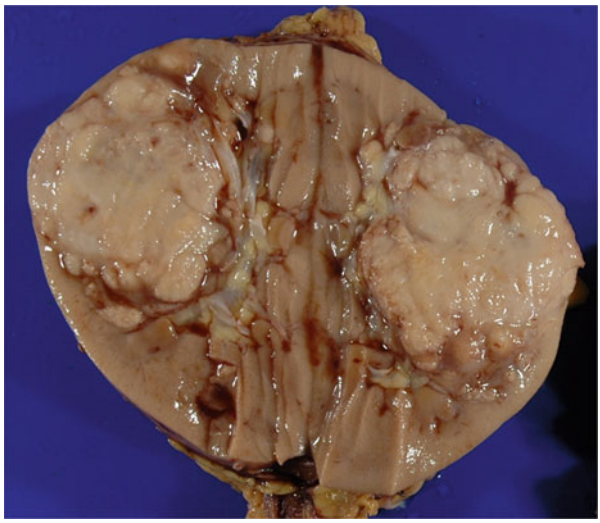

Fig. 4.22 Gross appearance of Xp11 RCC. The tumor is tan yellow and solid

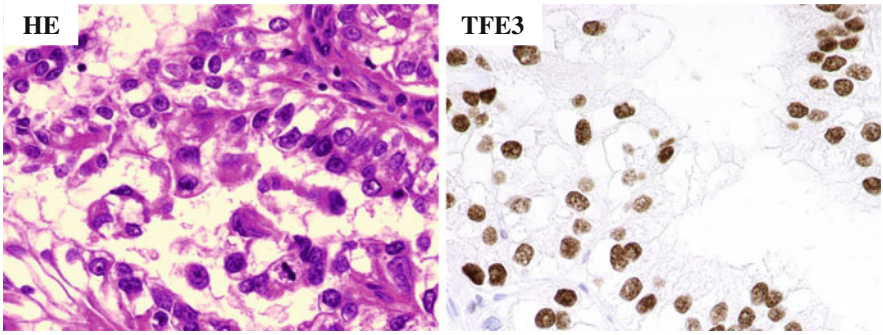

Fig. 4.23 Microscopic appearance and TFE3 immunostaining of Xp11 RCC. The tumor cells proliferate in a papillary pattern and their nuclei are positive for TFE3

immunostaining. Therefore, the detection of *TFE3* gene translocation by RT-PCR or FISH is necessary for the definitive diagnosis of this tumor. Only about 50 % express epithelial markers such as CK and EMA, and Xp11 RCC is positive for RCC-Ma and CD10 [36, 37]. Nuclear staining for TFEB is a highly sensitive and specific marker for 6p21 RCC, and this tumor is frequently positive for melanoma markers and usually negative for epithelial antigen markers.

Prognosis of the patients with Xp11 RCC is significantly worse than that of the patients with pRCC, and it is similar to those with ccRCC [1]. 6p21 RCCs generally have more indolent clinical course than Xp11 RCC [1].

4.8 Dialysis-Related RCC

Long-term hemodialysis causes atrophy and cystic change of the renal parenchyma, which is called acquired cystic disease (ACD) of the kidney. About 3–7 % of patients with ACD develop RCC [39, 40]. Although ccRCC, pRCC, and chRCC may occur, there are unique subtypes of RCC associated with long-term dialysis, such as ACD-associated RCC and clear cell papillary RCC.

4.8.1 ACD-Associated RCC

This tumor is common in the patients receiving long-term dialysis and occurs with the background of ACD. Grossly, it is well circumscribed with hemorrhage and necrosis (Fig. 4.24). Microscopically, this tumor is characterized by cribriform architecture containing numerous microcysts with eosinophilic cytoplasm, prominent nucleoli, and calcium oxalate crystals (Fig. 4.25) [1]. Immunohistochemically, cancer cells are positive for AMACR, RCC-Ma, CK (AE1/AE3), CK (CAM5.2), and CD10 but negative for cytokeratin CK7, EMA, CK (34βE12), CD117, and TFE3. This immunophenotype seems to resemble that of type 2 pRCC. This tumor generally shows a favorable prognosis, but patients having tumor with metastasis and/or sarcomatoid change show worse clinical outcomes [41].

4.8.2 Clear Cell Papillary RCC

Clear cell papillary RCC is defined as indolent tumor composed of clear epithelia cells arranged in tubular and papillary patterns, and it is characterized by linear

Fig. 4.24 Gross appearance of ACD-associated RCC. The tumor is well circumscribed with hemorrhage and necrosis (*arrows*). Nonneoplastic renal tissue is occupied by numerous cysts

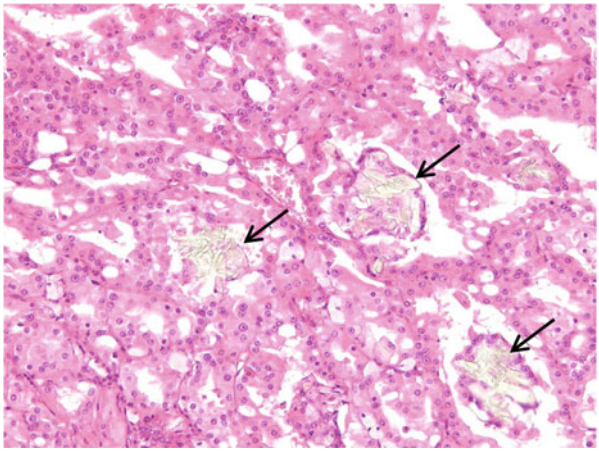

Fig. 4.25 Microscopic appearance of ACD-associated RCC. The tumor shows cribriform architecture containing numerous microcysts with eosinophilic cytoplasm, prominent nucleoli, and calcium oxalate crystals (*arrows*)

nuclear alignment away from the basement membrane and distinct immunophenotype [1]. This tumor occurs in both non-cystic end-stage renal disease including ACD and also affects patients without underlying disease. Grossly, this tumor is well circumscribed and histologically features cancer cells with clear cytoplasm and low-grade nuclei with a papillary growth pattern [41, 42]. Immunohistochemically, cancer cells are positive for CK7, CA9, vimentin, and CK (34βE12) but usually negative for RCC-Ma, CD10, AMACR, and TFE3 [42]. This immunohistochemical phenotype seems to be different from those of ccRCC and pRCC. The prognosis of this tumor is considered to be indolent, and no local recurrence or metastasis has been reported.

4.9 Familial RCC

Although most RCCs are sporadic, 2–4 % of tumors have a familial cause [1]. There are five major types of inherited syndromes that predispose individuals to distinct subtypes of RCC (Table 4.1). Affected patients often develop bilateral and multifocal RCCs except for hereditary leiomyomatosis and renal cell cancer.

4.9.1 Von Hippel-Lindau Disease (VHL)

This syndrome is caused by germline mutations of the von Hippel-Lindau (*VHL*) tumor suppressor gene, which is located on chromosome 3p25–26 [1]. VHL disease is inherited in an autosomal dominant fashion and is characterized by the development of capillary hemangioblastomas of the central nervous system and retina, ccRCC, clear cell papillary RCC, pheochromocytoma, pancreatic cysts, and inner

Table 4.1 Major hereditary RCCs and their responsible genes

Syndrome	Responsible gene/ localization	Histological subtypes
VHL disease	VHL/3p25–26	ccRCC
HPRC	c-MET/7q31	pRCC, type 1
HLRCC	FH/1q42.1	pRCC, type 2
BHD syndrome	BHD (FLCN)/17p11.2	ccRCC, pRCC, chRCC, oncocytoma, hybrid tumor
Tuberous sclerosis	TSC1/9q32, TSC2/ 16p13.3	AML, ccRCC

ear tumors. Germline *VHL* mutations can be identified in all VHL patients [43], and typically there are multiple renal cysts and ccRCCs in the kidney. However, histological subtypes other than ccRCC and clear cell papillary RCC do not develop in VHL patients [44].

4.9.2 Hereditary Papillary Renal Carcinoma (HPRC)

HPRC is an autosomal dominantly inherited tumor syndrome characterized by late-onset, multiple, bilateral pRCCs [14]; its diagnosis is based on multiple and bilateral papillary renal tumors. About 50 % of affected family members may develop the disease by the age of 55 years, and no extrarenal manifestations of HPRC have been identified [45]. Macroscopically, patients develop multiple papillary tumors ranging from microscopic lesions to clinically symptomatic carcinomas [46]. Histologically, they are characterized by papillary or tubulo-papillary architecture very similar to sporadic pRCC, type 1. The genetic alterations responsible for the disease are activating mutations of the *MET* oncogene located on chromosome 7q31. The *MET* gene codes for a receptor tyrosine kinase [45, 47], and its ligand is hepatocyte growth factor (HGF). Therefore, HGF-MET signal pathway might be a therapeutic target of HPRC.

4.9.3 Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC)

HLRCC is an autosomal dominant tumor syndrome caused by germline mutations in the *fumarate hydratase* (*FH*) gene [14]; it is characterized by a predisposition to benign leiomyomas of the skin and uterus. Some HLRCC families have a predisposition to RCC and uterine leiomyosarcoma. Multiple leiomyomas of the skin and the uterus, pRCC type 2, and early-onset uterine leiomyosarcoma are suggestive of HLRCC [48, 49]. However, the detection of *FH* mutation is necessary for the

definitive diagnosis of HLRCC. Histologically, HLRCCs show pRCC, type 2 histology, composed of large cells with abundant eosinophilic cytoplasm, large nuclei, and prominent eosinophilic nucleoli.

4.9.4 Birt-Hogg-Dubé (BHD) Syndrome

BHD syndrome is defined as a syndrome characterized by benign skin tumors, specifically fibrofolliculomas, trichodiscomas, and acrochordons [14], and its responsible gene (*BHD* gene) is located on 17p11 [50]. Multiple renal tumors and pneumothoraces are common in patients with BHD syndrome. Patients with this syndrome may develop a variety of renal tumors, such as ccRCC, pRCC, chRCC, oncocytoma, and hybrid tumor, and the tumors can be multiple and bilateral.

4.9.5 Tuberous Sclerosis (TS)

TS is an autosomal dominant hereditary disease, which is characterized by systemic hamartomas. Its responsible genes are identified as *TSC1* and *TSC2*, coding hamartin and tuberin proteins, respectively [51]. The prognosis of the patients mainly depends on whether there is a lung lesion (lymphangioleiomyomatosis) or a kidney lesion. Renal tumors of this syndrome are mainly angiomyolipoma and epithelioid angiomyolipoma, but some patients develop ccRCC and peculiar histological subtypes.

4.10 Other Rare RCCs

4.10.1 Renal Medullary Carcinoma

This rare tumor is a rapidly growing neoplasm of the renal medulla occurring in young black patients with sickle cell disease [1]. High-grade RCCs showing similar morphological and immunohistochemical features to medullar carcinoma of the patients with no evidence of sickle cell trait disease should be diagnosed as unclassified RCC with renal medullary phenotype. This tumor is very aggressive and most patients exhibit metastasis at the time of diagnosis [52]. Macroscopically, it is located in the renal medulla and poorly circumscribed. Histologically, the tumor is composed of an area of reticular architecture and of poor differentiation and desmoplastic stroma. Cancer cells are positive for CEA, CK7, CK20, CK (CAM5.2), CK (AE1/AE3), and vimentin [53].

4.10.2 RCC associated with Neuroblastoma

RCC associated with neuroblastoma occurs in long-term survivors of neuroblastoma, and males and females are equally affected [14]. At first, this tumor is reported to be characterized histologically by tumor cells with rich eosinophilic cytoplasm. However, other RCCs such as ccRCC and Xp11.2 RCC may develop in patients treated for neuroblastoma. Prognosis may be dependent on the histological subtypes of RCC.

4.10.3 Tubulocystic RCC

Macroscopically, this tumor has a well-circumscribed, spongy, and "bubbly wrap" appearance. Histologically, there are variably sized cysts and tubules lined by a single layer of flat, hobnail, cuboidal, and columnar cells with eosinophilic cytoplasm and Fuhrman nuclear grade 3 nuclei. Immunohistochemically, cancer cells are positive for CK7, AMACR, and CD10 [54, 55]. The prognosis of the majority of the tumor is reported to be favorable [56]. However, tubulocystic RCC is often associated with pRCC, which worsens the clinical outcomes.

4.10.4 Renal Carcinoma with ALK Gene Rearrangement

This tumor has been recently identified and seems to affect relatively young adults [57]. Histologically, this tumor displays a cribriform pattern or papillary architecture with stromal mucin deposition [58]. Fusion of the *ALK* gene on chromosome 2p23 and other genes has been identified [57–59]. Positive staining for ALK protein is a characteristic feature of this tumor. However, the intercalated antibody-enhanced polymer method is necessary in the immunohistochemical screening of this tumor because conventional immunostaining may generate negative or weakly positive findings in this tumor. The clinical behavior of this tumor remains almost completely unknown.

4.11 Differential Diagnosis of Renal Cell Tumors Using Immunohistochemistry

As shown in Table 4.2, immunohistochemistry is very useful for the differential diagnosis of renal tumors. In this section, useful staining is described corresponding to the morphological features.

Table 4.2 Immunostaining for differential diagnosis of renal tumors

Histological type	CK	CK7	HMWCK	Vim	CD10	AMACR	Other useful staining
Clear cell RCC	+	−	−	+	+	−*	CA9
Papillary RCC	+	+	−/+	+	+	+	
Chromophobe RCC	+	+	−*	−	−*	−	CD117
MTSCC	+	+	−/+	+	−/+	+	Alcian blue
Xp11 RCC	−*	−	−*	−/+	+	+	TFE3
CDC	+	+	+/−	+	−	−	
Oncocytoma	+	−*	−	−	+	−	
EAML	−	−	−	+	−	−	HMB45

CK, cytokeratin clone AE1/AE3; CK7, cytokeratin 7; HMWCK, high-molecular-weight cytokeratin (clone 34βE12); Vim, vimentin; AMACR, α-methylacyl-CoA racemase; CA9, carbonic anhydrase 9; MTSCC, mucinous tubular and spindle cell carcinoma; Xp11 RCC, RCC associated with Xp11 translocations/TFE3 gene fusions; TFE3, transcription factor enhancer 3; CDC, collecting duct carcinoma; EAML, epithelioid angiomyolipoma; +, positive; +/−, frequently positive; −/+, frequently negative; −, negative; − *, rarely positive

4.11.1 Solid Renal Tumors with Clear Cytoplasm

In these tumors, the candidates for differential diagnosis include ccRCC, chRCC (typical variant), and Xp11 RCC. Because ccRCCs are diffusely positive for CA9, its staining is very helpful for the differential diagnosis of clear cell tumors (Fig. 4.26). Although most cases of solid renal tumor with clear cytoplasm are ccRCC, there are rare cases of Xp11 RCC showing similar histology to ccRCC (Fig. 4.26). Xp11 RCC predominantly affects children and young adults, and calcification and lymph node metastasis are often observed. Therefore, clinical information is very important in the differential diagnosis, and TFE3 staining is useful for the identification of Xp11 RCC. Morphologically, chRCC (typical variant) shows characteristic pale cells with perinuclear halo, and it is usually positive for CK7 and CD117.

4.11.2 Renal Tumors with Eosinophilic or Granular Cytoplasm

In these tumors, the candidates for differential diagnosis include ccRCC (previously called "granular cell RCC"), chromophobe RCC (eosinophilic variant), oncocytoma, and epithelioid angiomyolipoma (EAML). If the typical histology of ccRCC (clear cell tumors and rich and fine vascular network) is identified in some part of the tumor tissue, it suggests ccRCC. CA9 staining can confirm the diagnosis (Fig. 4.27). chRCC (eosinophilic variant) has a perinuclear halo, and CD117 and CK7 staining is useful for the differential diagnosis. Cytoplasmic CD117 staining is

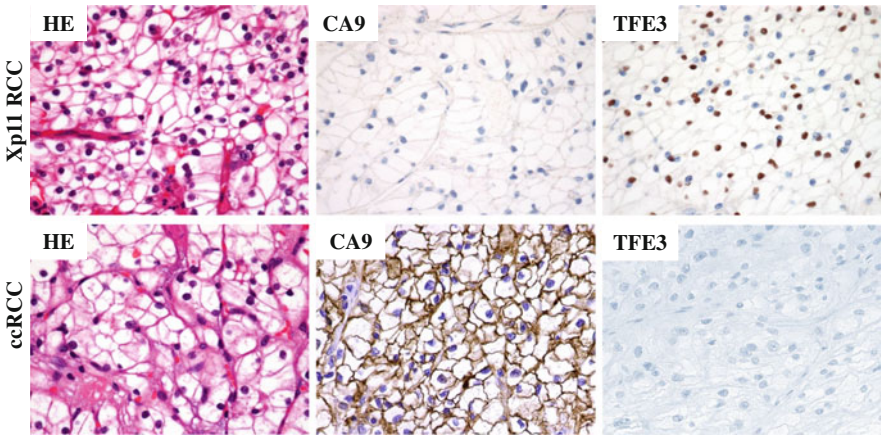

Fig. 4.26 Immunostaining of solid renal tumors with clear cytoplasm (Xp11 RCC and ccRCC)

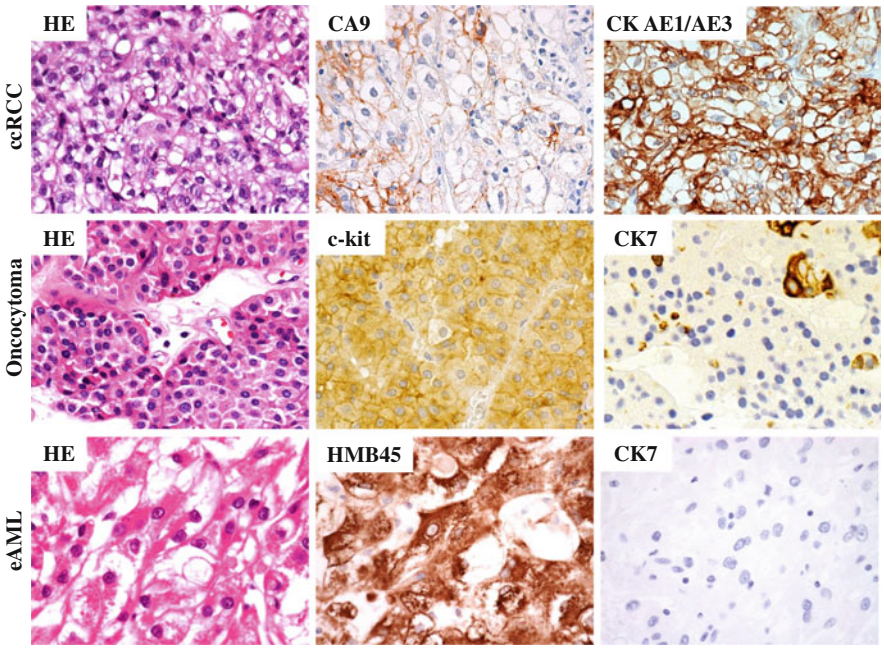

Fig. 4.27 Immunostaining of solid renal tumors with eosinophilic or granular cytoplasm (ccRCC, oncocytoma, EAML). Although morphological findings on H&E staining are similar in these tumors, the differential diagnosis can be made using immunostaining

also observed in oncocytoma, but CK7 staining is negative or only focally positive (Fig. 4.27). EAML is sometimes misdiagnosed as high-grade ccRCC or sarcomatoid RCC because adipose tissue is usually not observed. When there are no characteristic features of ccRCC, chRCC, and oncocytoma, immunostaining of

melanosome-associated antigen (clone, HMB45) and Melan-A as well as epithelial markers such as CK and EMA should be performed to differentiate EAML from high-grade RCC (Fig. 4.27).

4.11.3 High-Grade Infiltrating Renal Tumors

In these tumors, the differential diagnosis includes CDC, urothelial carcinoma (UC), and other high-grade RCCs. Morphologically, squamous differentiation and micropapillary growth pattern within the tumor tissue suggest UC. Immunohistochemically, UC is positive for CK7, CK20, GATA3, uroplakin 2, p63, thrombomodulin, and CK5/6, whereas CDC expresses vimentin, PAX2, and PAX8 [60]. Most RCCs express CD10, RCC-Ma, and vimentin [61, 62].

4.11.4 Renal Tumors with Spindle Cell Morphology

In these tumors, the candidates of differential diagnosis include sarcomatoid carcinoma, sarcoma, MTSCC, and AML. To differentiate sarcomatoid carcinoma from sarcoma, the identification of an epithelial component by thorough tissue sampling and immunohistochemistry for epithelial markers is very important. However, some sarcomas such as synovial sarcoma can express epithelial markers. Alcian blue-positive mucinous stroma is a characteristic feature of MTSCC, and it expresses CK7, CK19, and AMACR and generally possesses low-grade nuclei [63].

4.11.5 Papillary Renal Tumors

In papillary renal tumors, the major differential diagnosis includes pRCC, MTSCC, and Xp11.2 RCC (Fig. 4.28). If mucinous stroma and a small spindle cell component exist within the tumor tissue, Alcian blue staining plays a key role in the differential diagnosis of MTSCC (Fig. 4.28). The possibility of Xp11.2 RCC should be considered in papillary renal tumor with calcification, clear to eosinophilic cytoplasm, and/or lymph node metastasis. TFE3 immunostaining plays an essential role in these tumors (Fig. 4.28).

4.11.6 Cystic Renal Tumors

In cystic renal tumors, CA9 staining is very important for the differential diagnosis. Basically, CA9-positive cystic renal neoplasms are multilocular cystic renal

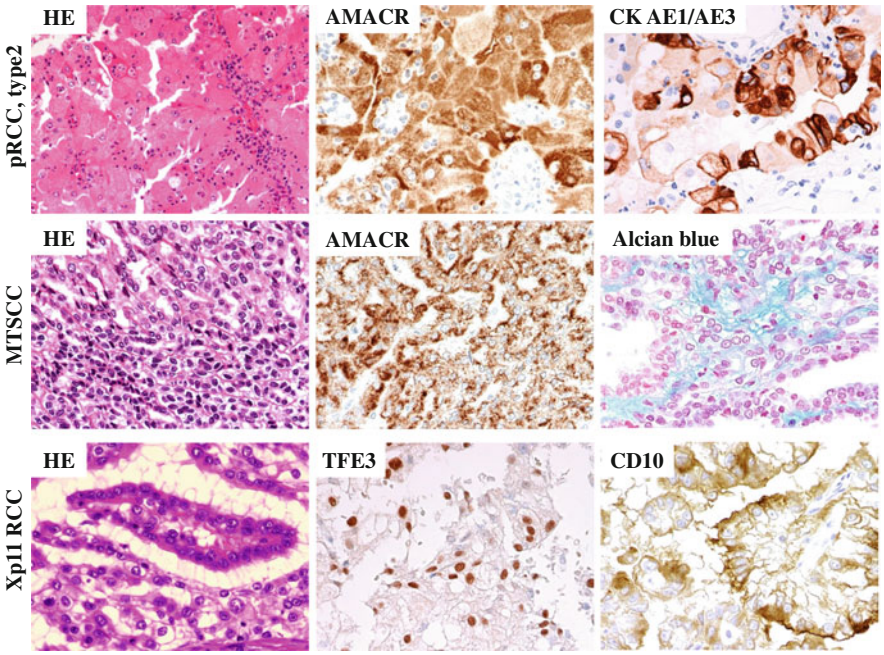

Fig. 4.28 Immunostaining and special staining of papillary renal tumors (pRCC, MTSCC, Xp11 RCC). Although morphological findings on H&E staining are similar in these tumors, the differential diagnosis can be made using immunostaining

neoplasm of low malignant potential. However, CA9-positive tumors with focally solid tumor growth should be diagnosed as ccRCC with cystic change not multilocular cystic neoplasm of low malignant potential. By contrast, tubulocystic RCC possesses tall lining cells with higher-grade nuclei and eosinophilic cytoplasm. The fibrous septa are thicker and rather fibrotic. Immunohistochemically, the tumor cells are positive for AMACR, but not for CA9. In CA9-negative cystic tumors, immunostaining for estrogen receptor (ER) and progesterone receptor (PgR) is important because these are positive markers for adult cystic nephroma/MEST family. Although angiomyolipoma with epithelial cysts (AMLEC) also shows a similar immunostaining pattern, AMLEC is also positive for HMB45 and Melan-A [64].

4.12 Conclusions

Recent advances in our knowledge of its molecular characteristics have resulted in improvement of the classification of RCC. Multiple therapeutic options for RCC are now available for advanced and metastatic tumors, and especially tyrosine kinase inhibitors targeting the HIF-VEGF pathway are effective against ccRCC.

Therefore, pathologists should render correct diagnoses, employing immunohistochemistry using antibody panels and molecular biological analyses.

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Chapter 5
Imaging Features of Renal Cell Carcinoma Differential Diagnosis, Staging, and Posttreatment Evaluation

Masahiro Jinzaki, Hirotaka Akita, and Mototsugu Oya

Abstract With the widespread use of ultrasonography (US), computed tomography (CT), and magnetic resonance imaging (MRI), there has been an increase in the detection of various subtypes of renal cell carcinomas (RCCs) and benign renal tumors. The differentiation of RCCs from benign tumors such as fat-poor angiomyolipoma and oncocytoma is very important to prevent unnecessary surgery. In addition, the recent progress of therapies to treat RCCs, including nephron-sparing surgery, percutaneous ablative procedures, and targeted antiangiogenic therapies, has increased the need for the accurate determination of subtypes and staging. With the development of imaging modalities, there have been significant improvements in correlating preoperative imaging with pathologic characteristics. Many studies performed within the last two decades have helped to clarify diagnostic imaging clues for both cystic and solid renal tumors.

In this chapter, we discuss the current status of US, CT, and MRI for the detection and differential diagnosis of renal tumors as well as staging, postprocedural imaging, and imaging for the targeted antiangiogenic treatment of RCC.

Keywords Differential diagnosis • Staging • Cystic renal tumor • Solid renal tumor

5.1 Introduction

With the widespread use of ultrasonography (US) and computed tomography (CT), there has been an increase in the detection of various renal tumors [1–3]. The characterization and staging of renal tumors during imaging are very important for

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M. Oya (ed.), *Renal Cell Carcinoma*, DOI 10.1007/978-4-431-55531-5_5

selecting an optimal management strategy. Much progress has been made in the technology used for radiological examinations and in interpreting images of renal tumors. Actually, remarkable progress in US, CT, and magnetic resonance imaging (MRI) has been made over the last two decades. Many studies have evaluated the imaging findings of various subtypes of renal tumors and have clarified the possibilities and limitations of characterizing renal tumors [4–10]. Furthermore, the recent progress of therapies to treat renal tumors, including laparoscopic nephrectomy, percutaneous ablative procedures, and targeted antiangiogenic therapies, has increased the need for accurate preoperative radiological planning.

In this review, we discuss the current status of US, CT, and MRI for the detection and differential diagnosis of renal tumors as well as the staging, postprocedural imaging, and imaging for the targeted antiangiogenic treatment of RCC.

5.2 Imaging Methods

In general, US has been the most commonly used imaging technique for renal imaging. With the advancement of CT, however, the role of US has decreased, and today it is mainly used during the incidental detection of renal tumors. CT is now the first choice for imaging and has been the mainstay imaging modality in cases of suspected renal tumors for the past several decades because of its high resolution, reproducibility, reasonable acquisition time, and acceptable cost. MRI can be considered as an alternative modality in cases where patients are allergic to iodine contrast materials or are pregnant. It is also useful when patients require further imaging after having already undergone CT examination or surveillance.

5.2.1 Ultrasonography

US is performed using a 3–6 MHz transducer in the tissue harmonic mode. US has been used as the primary method for the detection and characterization of renal tumors because of its noninvasive nature, lack of a need for potentially nephrotoxic iodinated contrast agents, lack of radiation exposure, and cost-effectiveness. In fact, US plays an important role in the incidental detection of RCC during health checkups [11, 12]. However, US is an operator experience-dependent modality, and the kidneys cannot always be imaged satisfactorily, especially in large patients. Furthermore, US has been shown to be inferior compared with CT imaging for the detection of renal tumors [13, 14]. The sensitivity of US for the detection of RCC is dependent upon the size. In lesions that are less than 5 mm, US has a detection rate of 0%, whereas CT has a detection rate of 47%. For larger lesions (10–35 mm), the reported detection rates are 82% for US and 80% for CT [13]. Especially, smaller isoechoic intraparenchymal lesions are difficult to detect [14]. Thus, ultrasound is not the primary imaging modality used for kidney imaging in patients suspected of

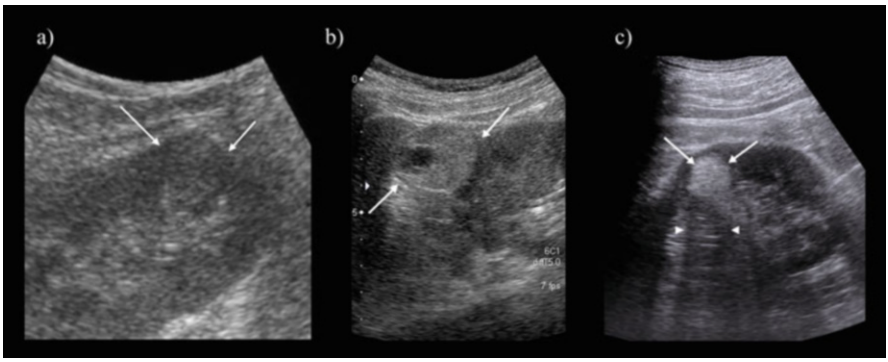

Fig. 5.1 RCC and AML as visualized using gray-scale ultrasonography. (**a**) Isoechoic lesion relative to the renal parenchyma (*arrows*). This lesion was surgically resected and diagnosed as an RCC. (**b**) Markedly hyperechoic lesion with an anechoic rim (*arrows*) and intratumoral cysts. This lesion was surgically resected and diagnosed as an RCC. (**c**) Markedly hyperechoic lesion (*arrows*) with acoustic shadowing (*arrow heads*). This lesion was diagnosed as an AML based on unenhanced CT findings

having cancer. Despite its limited sensitivity for small lesions, US may be useful for determining whether a lesion is likely to be cystic or solid in nature if a hyperattenuation appears on a CT scan in patients in whom contrast agents are contraindicated [15, 16].

On gray-scale US, RCC can be hypoechoic or hyperechoic, compared with normal renal parenchyma, but is often similar in echogenicity to normal parenchyma (Fig. 5.1a, b). RCC can show both cystic and solid natures. Solid-type RCC is characterized by the existence of an anechoic rim or intratumoral cysts, reportedly appearing in 73% and 31% of RCC, respectively, in one study [4]. A representative benign tumor, angiomyolipoma (AML), is usually solid and markedly hyperechoic relative to the renal parenchyma or as hyperechoic as the renal sinus fat [4, 5]. However, echogenicity is not pathognomonic, because RCCs also appear as hyperechoic masses. AML is characterized by the existence of shading posterior to the tumor resulting from the existence of a fat component. However, this finding is seen in only 21–33% of AML [4, 5] (Fig. 5.1c). Thus, the ability to detect fat within a lesion is less robust using US compared with other cross-sectional imaging methods.

Color Doppler or power Doppler US can be used to distinguish solid renal tumors from pseudotumors, allowing the possibility of renal pseudotumors including prominent column of Bertin, dromedary hump, and compensatory hypertrophy to be excluded [4] by depicting blood flow similar to that in normal renal parenchyma. Kitamura et al. reported that approximately 90% of clear cell RCCs as seen on CT were identifiable as hypervascular lesions on color Doppler US [14]. Power Doppler US is useful for describing the vascular distribution of renal tumors. Jinzaki et al. proposed a classification for the vascular distribution of renal tumors, classifying them into pattern 0 to pattern 4 [4] (Fig. 5.2). They found that RCC tended to exhibit a basket pattern (pattern 4), while AML tended to exhibit no signal

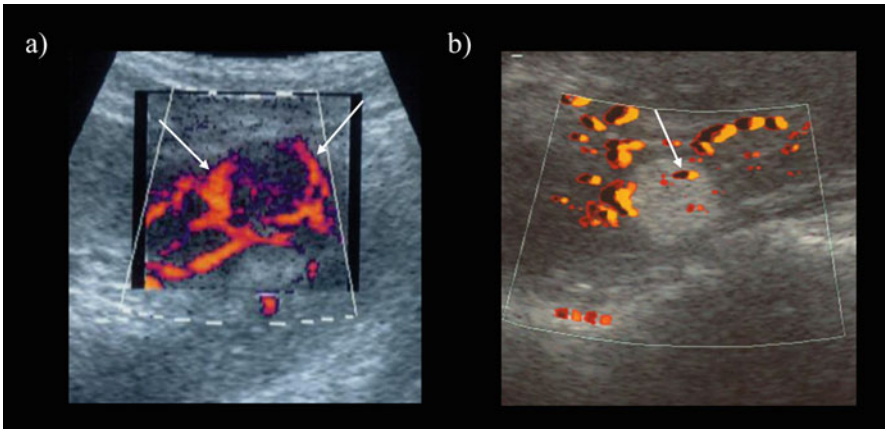

Fig. 5.2 RCC and AML as visualized using power Doppler ultrasonography. (**a**) Isoechoic lesion with a basket pattern (pattern 4, mixed penetrating and peripheral flow) (*arrows*). This lesion was diagnosed as an RCC. (**b**) Hyperechoic lesion with intratumoral focal signals (pattern 1) (*arrow*). This lesion was diagnosed as an AML

or an intratumoral focal signal pattern (pattern 1); however, some overlap existed between the vascular patterns of RCC and benign lesions.

Contrast-enhanced ultrasonography (CEUS) can depict renal vessels and show enhancement using microbubbles, which do not affect renal function and have a minimal effect on allergic reactions. These agents, therefore, may be especially useful in patients with renal insufficiency or asthma. The sensitivity of US for the detection of renal tumors appears to be improved with the use of intravenous microbubble contrast agents; in one study, the sensitivity was 97%, while that for gray-scale US alone was 70% [17]. Contrast agents can be used to improve the characterization of complex renal cysts [18–21]. A strong enhancement of the vascularity in septations and mural nodules improves lesion classification [18]. Furthermore, positive enhancement on CEUS is useful for the detection of RCC in acquired cystic disease of the kidney, since the degree of enhancement of this disease is often low on dynamic CT [22]. However, US contrast agents are not covered by health insurance in many countries, including the USA and Japan.

5.2.2 Computed Tomography

Computed tomography is the gold standard for the detection and characterization of renal masses, as well as for RCC staging and preoperative planning. Multiphase CT scanning is considered to be the optimal technique [23–26]. There are two widely used protocols for multiphase CT scanning: a three-phase scan and a four-phase scan. A three-phase scan includes an unenhanced scan, a corticomedullary phase (CMP) scan, and a late nephrographic phase (late NP) scan, while a four-phase scan

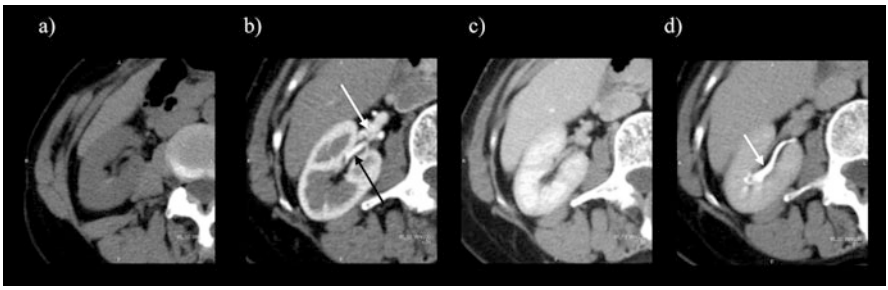

Fig. 5.3 Multidetector CT images of four-phase scan**. (a)** Unenhanced CT image. (**b**) Corticomedullary phase image. This phase shows a densely enhanced cortex with minimal enhancement of the renal medulla. The renal artery (*black arrow*) and renal vein (*white arrow*) are well depicted. (**c**) Nephrographic phase image. The renal parenchyma is homogeneously enhanced. (**d**) Excretory phase image. The attenuation of the renal parenchyma has decreased progressively, and the collecting system shows contrast (*arrow*)

includes an unenhanced scan, a CMP scan, a NP scan, and an excretory phase (EP) scan (Fig. 5.3). An unenhanced scan is very important for the detection of fat attenuation for classic AMLs, small calcifications for RCCs, and hyperattenuation corresponding to a smooth muscle component in fat-poor AML or an intratumoral hemorrhage. An unenhanced scan must also be included as part of the baseline data when assessing the degree of enhancement. After the administration of contrast material at an injection rate of more than 3 mL/s, the optimal time until kidney imaging is approximately 30~40 s for the CMP scan, 90–120 s for the NP scan, 150–180 s for the late NP scan, and 180 s or more for the EP scan. The CMP is useful for assessing the renal vasculature anatomy, and enhancement seen during this phase can also be useful for characterizing lesions [27]. However, since the renal cortex, but not the renal medulla, is strongly enhanced during the CMP, hypovascular lesions located in the renal medulla may be missed, resulting in false-negative CMP findings [23–26]. The NP is the most sensitive for the detection of renal tumors, since both the renal cortex and the renal medulla are homogeneously enhanced. The EP is useful for imaging the collecting system and for detecting the obstruction or displacement of the renal pelvis.

One advantage of CT over US and MRI is that quantitative evaluations are possible through the measurement of the CT attenuation value (Hounsfield units), which enables tumor components such as fat components, simple cystic components, and smooth muscle components to be defined using CT attenuation values. A fat component is defined as a region of less than −10 HU [28–30], a simple cyst as a region of 0~20 HU [31], and a smooth muscle component as a region of 40~50 HU [8] (Fig. 5.4). The degree of enhancement can also be quantitatively measured using the CT attenuation value. A change of 20 HU between unenhanced and enhanced scans is the threshold for determining enhancement, while a 10–20 HU change is considered an indeterminate finding and indicative of a need for other imaging modalities, such as MRI [32]. The degree of enhancement on the CMP and the enhancement pattern during multiphase CT are very important for the characterization of renal tumors or the determination of RCC subtypes. In fact, the three main

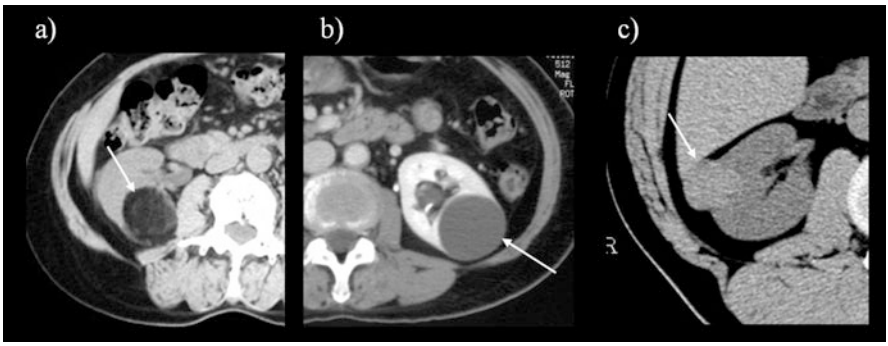

Fig. 5.4 Importance of unenhanced CT. (**a**) The lesion is less than −10 HU, corresponding to fat attenuation (*arrow*). (**b**) The lesion is between 0 and 20 HU, corresponding to water attenuation (*arrow*). (**c**) The lesion is hyperattenuating (40~50 HU), corresponding to a smooth muscle component (*arrow*)

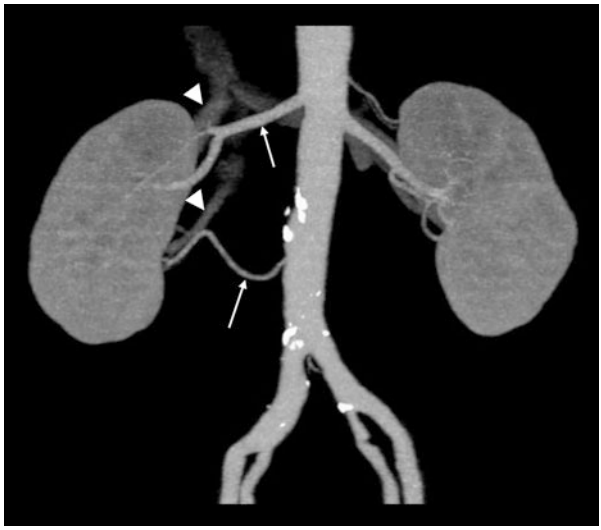

Fig. 5.5 Renal vasculature anatomy as assessed using CMP images. A maximum intensity projection image generated from CMP images shows two right renal arteries (*arrows*) and two right renal veins (*arrowheads*)

subtypes of RCC, clear cell RCC, papillary RCC, and chromophobe RCC, show differing enhancement patterns [6].

Another advantage of CT over US and MRI is that the development of multidetector CT technology has enabled the acquisition of images with thinner slice thickness. As a result, two-dimensional (2D) multiplanar reformation images or three-dimensional (3D) post-processing images such as maximum intensity projection and volume rendering have become available using CT [33–35] (Fig. 5.5). These images are very useful for preoperative planning.

5.2.3 *Magnetic Resonance Imaging*

MRI is comparable to CT for RCC staging, for posttreatment follow-up, and for the evaluation of indeterminate renal masses [36]. It is the test of choice for patients with contrast allergies or those who are pregnant. Because of its lack of ionizing radiation exposure, MRI is also an attractive option for serial radiographic monitoring of patients with indeterminate renal masses or hereditary syndromes, such as tuberous sclerosis and von Hippel–Lindau disease [37, 38]. Similar to CT, MR imaging is performed before and after the administration of contrast material.

Before the administration of contrast material, T2-weighted imaging (T2WI) and T1-weighted imaging (T1WI), frequency-selective (FS) fat suppression imaging, and chemical-selective fat suppression imaging are usually performed. T2WI is useful for characterizing subcentimeter cysts, for detecting a low-intensity rim (corresponding to a pseudocapsule, which is a characteristic of RCC) [39] (Fig. 5.6), and for characterizing the muscle component of fat-poor AML as a low-intensity area [8]. Frequency-selective (FS) fat suppression generally indicates the presence of bulky fat cells, while chemical-selective fat suppression (in- and opposed-phase imaging) is used to detect rather small amounts of intracellular lipid or fat cells in the tumor [40, 41] (Fig. 5.7). A signal loss on opposed-phase images, compared with in-phase images, is seen when a small amount of fat is present, and this is often useful for the diagnosis of fat-poor AML [41].

After the administration of contrast material, T1WI with a fat-suppressed breath-hold sequence is obtained during several phases in both the axial and coronal planes [42, 43]. In patients with normal renal function, gadolinium-based MRI contrast agents do not appear to have substantial nephrotoxic effects [44]. Recently,

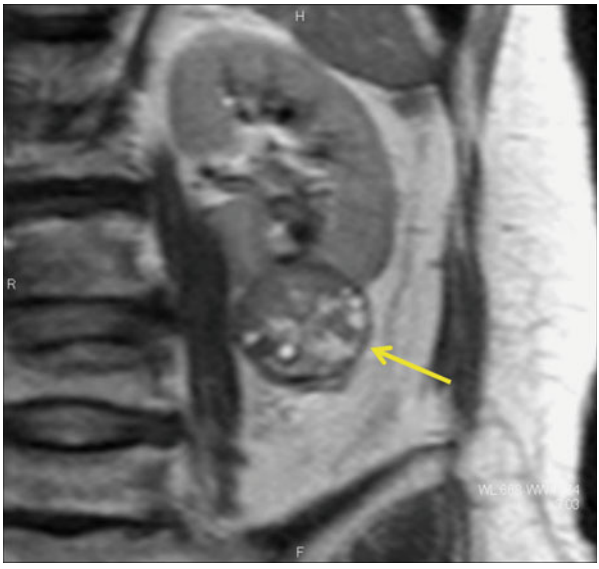

Fig. 5.6 RCC on a T2-weighted image. The low-intensity rim corresponds to a pseudocapsule (*arrow*)

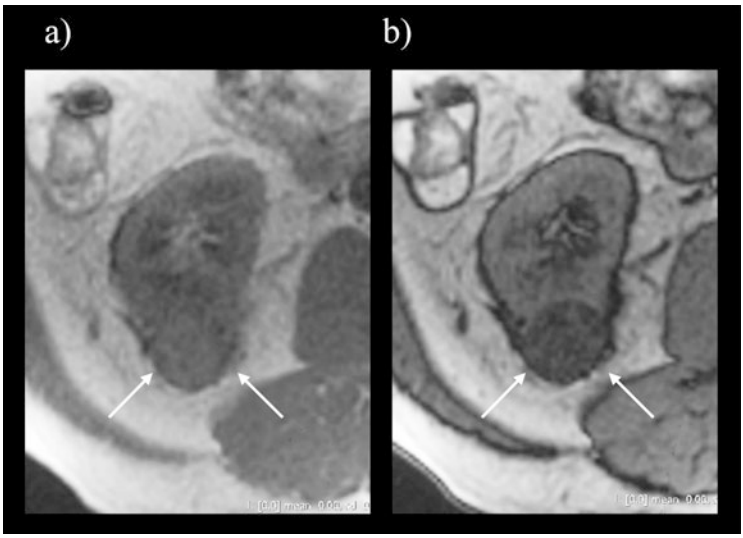

Fig. 5.7 Clear cell RCC on chemical-selective fat suppression images. In-phase (**a**) and opposed-phase (**b**) images demonstrate dropout on the opposed-phase image, compatible with the presence of intracytoplasmic fat in clear cell RCC

nephrogenic systemic fibrosis (NSF) has been reported as an adverse effect specific to gadolinium contrast media in patients with compromised renal function [45–47]. NSF is a severe, usually progressive, and potentially fatal systemic fibrotic disease that affects the dermis, subcutaneous fasciae, and striated muscles. The prevailing theory regarding gadolinium and NSF is that gadolinium (Gd^{3+}) ions are released from the Gd-chelate complex of MRI contrast agents and accumulate in tissues such as the skin, thereby initiating what some have described as a "toxic" reaction. Currently, NSF is considered to be correlated with the administration of relatively high doses (e.g., >0.2 mM/kg) and with agents in which gadolinium is least strongly chelated. Many guidelines recommend that gadolinium contrast agents should not be administered to patients with an estimated GFR <30 mL/min/1.73 m, who have recently received a liver or kidney transplant or who have hepatorenal syndrome [46, 48]. In general, MRI is sensitive to contrast enhancement and is better at detecting enhancement and characterizing solid or cystic lesions that are indeterminate on CT. However, the signal intensity in MRI is based on a relative scale, not an absolute quantitative scale such as HU for CT [43]. Therefore, the difference between pre-contrast and post-contrast MRI sequences is often used to determine the enhancement after contrast on MR images. A threshold of a 15% increase in signal intensity has also been advocated to identify enhancement in renal tumors [49, 50]. The identification of calcium in renal masses on MRI is limited because calcium appears as a signal void. The sensitivity for the detection of renal tumors is similar between MRI and CT, and both are more sensitive than that of ultrasound [32, 51].

5.3 Differential Diagnosis of Renal Tumors

RCC can have cystic growth patterns and solid growth patterns. The differential diagnosis of cystic renal tumors has been based on the Bosniak classification. This classification was first proposed in 1986 [52] and is now widely used to describe and manage cystic renal tumors. The differential diagnosis of solid renal tumors is often difficult, as the disease spectrum is quite wide. Before the mid-1990s, any solid renal tumor in which a fat component was not detected was considered to be an RCC. Thus, many benign tumors were misdiagnosed as RCC and, consequently, were unnecessarily resected [53]. Actually, a systemic review of 19 studies showed that the frequency of the surgical removal of benign renal masses because of suspicions of RCC was generally proportional to the lesion size and was approximately 20% for masses larger than 1 cm but smaller than 3 cm and 40.4% for masses smaller than 1 cm [54]. Over the last 20 years, many researchers have tried to establish additional diagnostic criteria for solid renal tumors, other than the detection of a fat component alone. Here, we review the diagnostic criteria of the Bosniak classification for cystic tumors and the most recently proposed diagnostic criteria for solid tumors.

5.3.1 Cystic Renal Tumors

Cystic RCC can be unilocular or multilocular, and RCC can develop within the wall of an otherwise benign cyst. The classification of complex renal cysts based on imaging was originally proposed by Bosniak in 1986 to help predict the likelihood of a cystic lesion being malignant [52]. This classification has since been modified. Bosniak originally proposed four categories; more recently, the classification was modified to include a fifth category [55] (Fig. 5.8). The diagnostic clues for the differential diagnosis of cystic renal tumors are CT attenuation on unenhanced CT, the existence of calcification, the number of septa, the irregularity and thickness of walls and septa, the enhancement of walls and septa, and the existence of a solid component.

Category I cysts are simple cysts with thin, noncalcified walls (Fig. 5.8a). These cysts contain simple serous fluid measuring less than 20 HU on CT images. Category II cysts are minimally complicated cysts with either high attenuation lesions (<3 cm) because of an increased protein content or hemorrhage or are multilocular with few septa (Fig. 5.8b) or thin peripheral calcifications. Category II cysts do not show enhancement after the injection of intravenous contrast material and are almost always benign. Category IIF cysts are complex lesions that have a high attenuation (>3 cm) or multiple septa or calcifications and a relatively benign appearance (Fig. 5.8c). Category III cysts are complicated cystic lesions that have some features suggesting malignancy that have multiple septa, thick or irregular rims, or heterogeneity suggesting necrosis (Fig. 5.8d). Category IV cysts contain

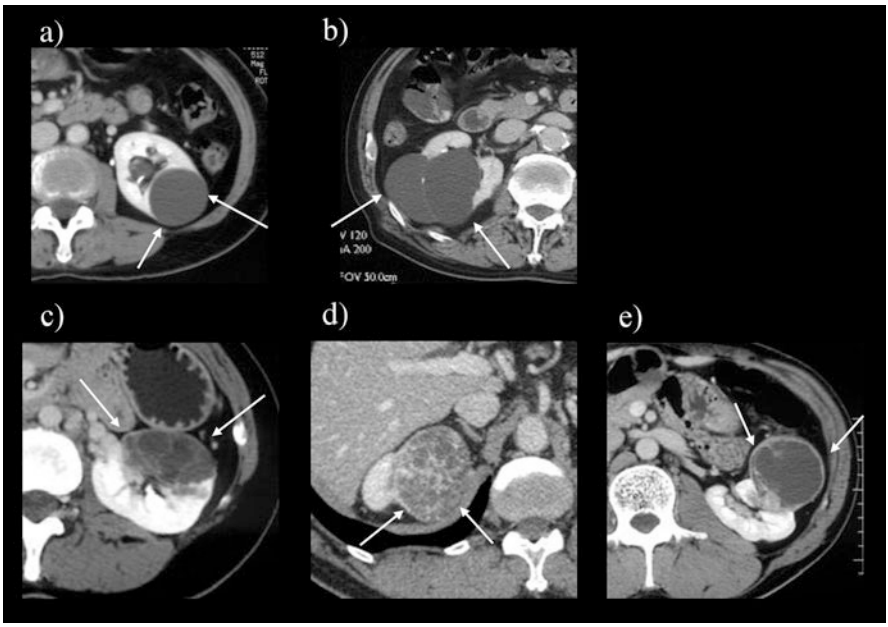

Fig. 5.8 CT images of Bosniak classifications. (**a**) Bosniak I: Thin-walled simple cyst (*arrows*) with homogenous low attenuation content. (**b**) Bosniak II: Cystic lesion with thin (<−1 mm) partially calcified internal septa (*arrows*). (**c**) Bosniak IIF: Slightly thicker wall cystic lesion with peripheral calcifications (*arrows*). (**d**) Bosniak III: Exophytic cystic renal lesion with multiple thick (>1 mm) enhancing septa (*arrows*). (**e**) Bosniak IV: Cystic renal lesion with a solid enhanced mural nodule (*arrows*)

solid, soft tissue-enhancing elements seen either within the cyst or as part of a complex cystic mass (Fig. 5.8e).

In general, Category I and II lesions are benign and so do not need further evaluation. Category IIF cysts have a less than 25% chance of malignancy and, therefore, may be followed to document stability over time [57, 58]. Concerning the intervals of follow-up examinations, it is recommended to perform the first follow-up after half a year and, if there is no change, to continue the examination annually at least for 5 years [58]. Category III cysts have an approximately 54–84% chance of a cystic RCC [56, 57]. Typically, these lesions are surgically explored. Category IV lesions are almost always cystic RCC and should be treated as such.

While one typically expects Bosniak III or IV lesions to represent cystic RCC, there are a number of benign cystic neoplasms that mimic this diagnosis. These include cystic nephroma (CN) and mixed epithelial and stromal tumor (MEST) of the kidney. In one series, 70% of 22 CN were characterized as Bosniak III lesions, and 70% of 10 MEST had enhanced solid elements [59]. These tumors are much more commonly seen in women or men receiving exogenous hormone treatment.

Biopsies for indeterminate cystic renal masses, such as Category IIF or III, can also be considered. Some studies [60, 61] have reported that a "definitive" diagnosis could be made for 61–88% of lesions, and negative results were supported by interval follow-up.

5.3.2 Solid Renal Tumors

Solid RCC can show an expansive growth pattern and an infiltrative growth pattern. Renal tumors with an infiltrative growth pattern are almost definitely malignant. On the other hand, the differential diagnosis of benign and malignant lesions is often challenging for renal tumors with an expansive growth pattern. The diagnostic clues for the differential diagnosis of solid renal tumors are CT attenuation on unenhanced CT images, the existence of calcification, the degree of enhancement on the CMP and the enhancement patterns during multiphase CT, the heterogeneity of the tumor, the signal intensity on T2WI images, and the presence or absence of cystic degeneration.

Renal tumors with an expansive growth pattern are well marginated but show various enhancements, depending on the tumor. The major subtypes of RCCs, such as clear cell RCC, papillary RCC, and chromophobe RCC, are included among renal tumors with an expansive growth pattern. This type of tumor also includes almost all benign tumors, such as AML, leiomyoma, or metanephric adenoma. For the diagnosis of renal tumors with an expansive growth pattern, there are three key criteria: the first criterion is that when fat attenuation (attenuations less than −10 HU) is detected in the tumor on an unenhanced CT image, the lesion is a classic AML [28–30, 62] (Fig. 5.9); the second criterion is that when the tumor exhibits marked heterogeneous enhancement almost equal to or greater than that in the renal cortex on the CMP of multiphase CT, the lesion is a clear cell RCC [6, 63] (Fig. 5.10); and the third criterion is that when the tumor appears as a hyperattenuated lesion (>45 HU) on an unenhanced CT image with homogeneous enhancement and T2W hypointensity, the lesion is a benign tumor, such as a fat-poor AML, leiomyoma, or metanephric adenoma [8–10] (Fig. 5.11). For the first criterion, AML with a fat component detectable on imaging is called classic AML. On US, classic AML is almost always markedly hyperechoic relative to the renal parenchyma [4, 5], but RCC can also be hyperechoic. Shadowing is a characteristic finding of AML on US, but this characteristic is only seen in 21–33% of AMLs [4, 5]. Thus, it is often difficult to diagnose a classic AML using US alone, and further CT examination is usually necessary. When evaluating AMLs with CT, the acquisition of thin (1.5–3 mm) sections and the use of attenuation measurements for small ROIs or even pixel values might be necessary to detect small amounts of fat [63, 64]. For the second criterion, no renal tumors other than clear cell RCC exhibit marked enhancement equal to or greater than that of the renal cortex during the CMP. Heterogeneity is caused by intratumoral hemorrhage or necrosis, which is frequently seen in clear cell RCC. For the third

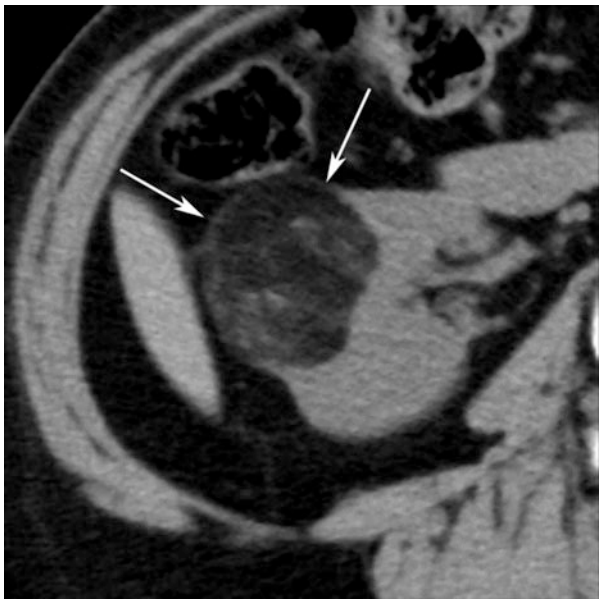

Fig. 5.9 Classic AML. A transverse, unenhanced CT image shows a right renal mass (*arrows*) with fat attenuation (−60 HU)

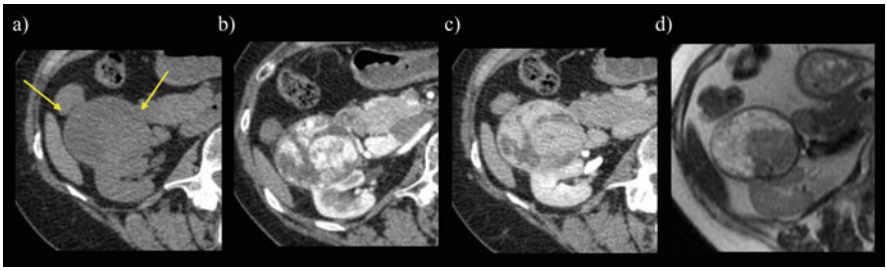

Fig. 5.10 Clear cell RCC. A transverse, unenhanced CT image (**a**) shows an isoattenuating mass. The mass shows a heterogeneously marked enhancement on enhanced CT during CMP (**b**) and rapid washout during late NP (**c**), while it appears hypointense on a transverse T2-weighted image (**d**)

criterion, the findings of both hyperattenuation (>45 HU) on unenhanced CT and T2 hypointensity correspond to a smooth muscle component in fat-poor AML and leiomyoma and to psammomatous calcifications in metanephric adenoma [6, 8–10, 65].

Renal tumors with an infiltrative growth pattern are poorly marginated and show relatively decreased and heterogeneous enhancement [66] (Fig. 5.12). The renal contour is maintained, but the involved portion of the kidney is often enlarged. Rare subtypes of renal carcinomas such as collecting duct carcinoma, renal medullary carcinoma, type 2 papillary RCC, transitional cell carcinoma infiltrating the renal parenchyma, and sarcomatoid carcinoma are included in this type. It is often

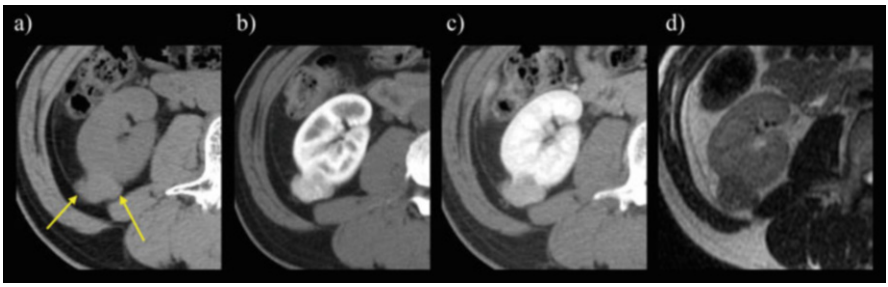

Fig. 5.11 Fat-poor AML. A transverse, unenhanced CT image (**a**) shows a hyperattenuating (47 HU) mass (*arrows*). The mass appears as a homogeneous enhancement on enhanced CT during CMP (**b**) and late NP (**c**) and was hypointense on a transverse T2-weighted image (**d**)

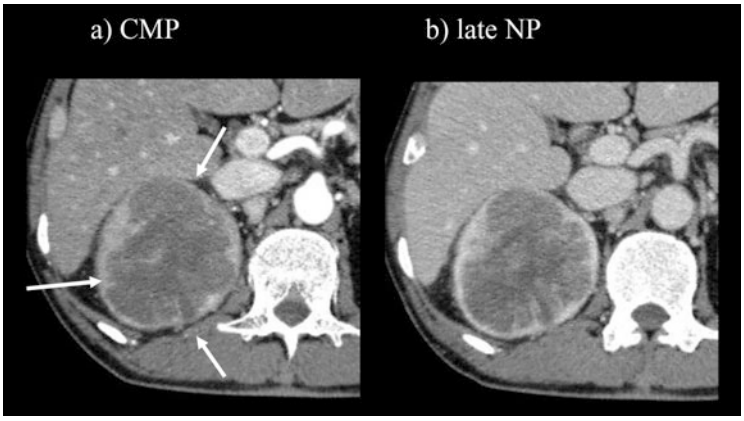

Fig. 5.12 Collecting duct carcinoma. The mass appears as an ill-defined infiltrative hypoenhancement on enhanced CT during CMP (**a**) and late NP (**b**)

difficult to differentiate these diseases from each other, but most require active management, including surgery. Primary renal non-Hodgkin's lymphoma (NHL) can also appear as either a focal mass or a lesion with an infiltrative appearance. Lymphoma is typically homogeneous, with less enhancement than the normal renal parenchyma, and is often present as multiple lesions [67]. This disease should be considered when splenomegaly or bulky retroperitoneal or mesenteric lymphadenopathy is present. Perinephric confluent tissue is more suggestive of NHL than RCC. Metastatic disease to the kidneys also appears as multiple, bilateral, poorly marginated solid lesions that can occasionally demonstrate an infiltrative pattern [68, 69]. Metastatic disease to the kidneys is particularly common with lung and breast carcinoma as well as melanoma. Metastases should be considered when infiltrative renal tumor is accompanied by nonrenal tumors. A biopsy should be considered to differentiate from RCC when other imaging findings are suggestive of lymphoma or metastases. It is important to recognize that some nonmalignant

conditions can exhibit an infiltrating pattern with decreased enhancement on imaging. Acute pyelonephritis appears as wedge-shaped areas of decreased enhancement that extend from the papilla to the cortex. This appearance should be distinguished from tumor infiltration, especially in patients with a history of fever, flank pain, and pyuria.

5.4 Imaging Findings for RCCs and Benign Tumors

The representative classic subtypes of RCCs are clear cell type, chromophobe type, and papillary type, followed by collecting duct type, medullary carcinoma, and multilocular cystic type. Recently, new subtypes of RCCs have been proposed: the Xp11.2 translocation–*TFE3* gene fusion type and the mucinous spindle and tubular type. The main benign tumors that need to be differentiated from RCCs are fat-poor AML, oncocytoma, and metanephric adenoma. Each of these tumors has characteristic findings, although there are still some overlaps among the findings for these tumors. The ability to identify the imaging features of each type is very important for the improved diagnosis of renal tumors.

5.4.1 Clear Cell RCC

Clear cell RCC, the most common type of RCC, originates from the proximal convoluted tubule and accounts for 70–80% of all RCCs. This tumor is seen in patients with von Hippel–Lindau (VHL) disease.

Clear cell RCCs typically show marked heterogeneous enhancement almost equal to that of the renal cortex during the CMP (more than 100 HU) and rapid washout during the NP or EP (80 HU) of multiphase CT (Fig. 5.10). The degree of enhancement is more avid than those of other RCC subtypes because of the deregulated angiogenesis of clear cell RCC (Fig. 4.2) [6, 7, 70]. Clear cell RCC also usually shows a heterogeneous enhancement because it is often accompanied by intratumoral hemorrhage or necrosis [6]. Cystic degeneration is also more common (15%) in the clear cell subtype than in the other subtypes irrespective of tumor size. On MRI, clear cell RCC is typically isointense on T1-weighted images and isointense to hyperintense on T2-weighted images, compared with the normal renal parenchyma (Fig. 4.13) [71, 72]. Clear cell RCC contains intracellular lipids in the tumor that cannot be detected on conventional fat suppression MR images but can be detected as a signal loss on chemical shift suppression images [72, 73]. This finding can help distinguish clear cell RCC from other RCC subtypes; however, this characteristic is also seen in fat-poor AML (isoattenuating type). The apparent diffusion coefficient calculated from diffusion-weighted images is reportedly lower among high-grade clear cell RCCs than among low-grade clear cell RCCs [74].

Clear cell RCCs tend to be more aggressive than other cell types, and they may directly involve and invade the renal collecting system [75]. Intratumoral necrosis and discontinuity of the capsule are correlated with higher-grade clear cell RCC [76]. Clear cell RCC may contain calcification, but less frequently than that seen in papillary and chromophobe subtypes [77]. Venous invasion is more commonly associated with clear cell RCCs [76].

5.4.2 Papillary RCC

Papillary RCC comprises 10–15% of all RCCs. An important feature of papillary RCC is that it is more commonly bilateral and multifocal than other RCC subtypes. Papillary RCC occurs in familial papillary RCC syndrome. Papillary RCC has a greater tendency to be of a lower stage and to have a better prognosis than clear cell RCC. There are two different histologic types of papillary RCC: those with small basophilic cells (type 1) and those with eosinophilic cells (type 2) [78]. Type 2 tumors have less distinct margins, are more heterogeneous, generally present at more advanced stages, frequently grow centripetally, and are associated with a poorer outcome [79].

Papillary RCC typically shows mild enhancement less than that of the adjacent cortex during the CMP (50–60 HU) and gradual enhancement during the NP or EP (65–75 HU) of multiphase CT (Fig. 5.13). Type 1 tumors are well marginated and exhibit homogeneous enhancement because they have lower frequencies of intratumoral necrosis and hemorrhage than clear cell RCC [6, 79, 80]. Type 2 tumors are poorly marginated and usually exhibit heterogeneous enhancement, but they can exhibit homogeneous enhancement when they are relatively small [79]. On MRI, papillary RCC can be visualized as a decreased signal intensity on T2-weighted images, compared with the normal renal parenchyma, possibly because of iron-containing hemosiderin, which can be found in the cytoplasm of tumor cells [71]. The imaging findings of type 1 papillary RCC are usually similar

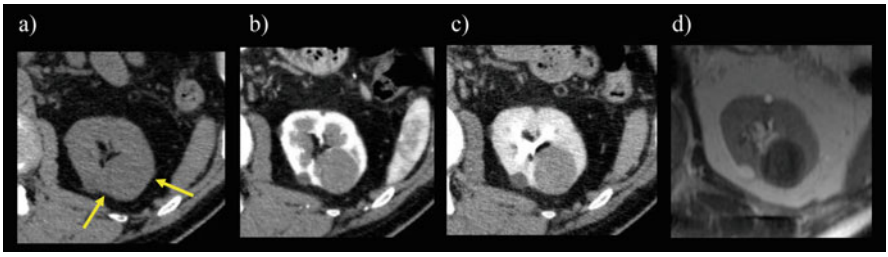

Fig. 5.13 Papillary RCC. Transverse, unenhanced CT shows an isoattenuating mass on unenhanced CT (*arrows*) (**a**). The mass exhibits a homogeneous mild enhancement on enhanced CT during CMP (**b**) and gradual enhancement during late NP (**c**), while it was hypointense on a transverse T2-weighted image (**d**)

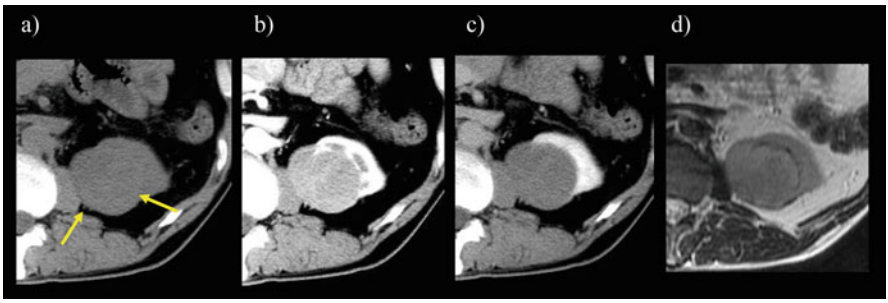

Fig. 5.14 Chromophobe RCC. Transverse, unenhanced CT (**a**) shows an isoattenuating mass on unenhanced CT (*arrows*). The mass shows a homogeneously moderate enhancement on enhanced CT during CMP (**b**) and washout during late NP (**c**), while it was isointense on a transverse T2-weighted image (**d**)

to those of metanephric adenoma [6], while the imaging findings of type 2 papillary RCC are similar to those of collecting duct carcinoma, spindle cell carcinoma, and urothelial carcinoma infiltrating the renal parenchyma.

5.4.3 Chromophobe RCC

Chromophobe RCC accounts for only 5% of all RCCs. Chromophobe RCC and hybrid oncocytic/chromophobe tumors are associated with Birt–Hogg–Dubé syndrome.

Chromophobe RCC shows a moderate homogeneous enhancement during the CMP and washout during the NP and EP of multiphase CT (Fig. 5.14). The degree of enhancement during the CMP is intermediate between that of clear cell and papillary RCC [6, 81]. This tumor usually exhibits homogeneous enhancement. On MRI, chromophobe RCC is typically isointense on T1-weighted images and isointense to hyperintense on T2-weighted images, compared with normal renal parenchyma. However, this enhancement pattern is also seen in oncocytoma [6, 81]. Thus, differentiating between these two tumors is difficult. One study reported the presence of a spoke-like enhancement pattern with a central stellate form [82]. This pattern can be seen for both chromophobe RCC and oncocytoma and is, therefore, not specific for either tumor. Hale's colloidal iron stain has been used to differentiate between the two pathologically.

5.4.4 *Collecting Duct Carcinoma*

Collecting duct (Bellini duct) carcinomas are uncommon, accounting for 1–2% of renal tumors. Collecting duct carcinoma is an aggressive tumor, with most patients presenting with high-stage disease.

Collecting duct RCC are usually located in the medullary portion or infiltrating the central sinus and have only rarely been reported in the renal cortex [83–85]. They exhibit an infiltrating growth pattern, rather than showing expansile growth, and preserve the reniform shape of the kidney (Fig. 5.12). They are commonly hypovascular with heterogeneous enhancement and may be difficult to differentiate from infiltrating urothelial carcinoma infiltrating the renal parenchyma and sarcomatoid variants of RCC [86]. They have variable signal intensities on T1-weighted images and typically have a low signal intensity on T2-weighted images [86, 87].

Metastases are more common at presentation than with other types of RCC, occurring in 35–40% of patients [84]. When bone metastases occur, they are frequently osteoblastic, unlike metastases from clear cell RCC, which are osteolytic.

5.4.5 *Xp11.2 Translocation–*TFE3 *Gene Fusion Carcinoma*

Xp11.2/TFE RCC is a rare subtype of RCC characterized by Xp11.2 chromosome translocations and fusion with the transcription factor E3 and is now accepted as a distinct entity according to the 2004 World Health Organization renal tumor classification [88]. It primarily affects children and adolescents. In the adult population, it is associated with a poor prognosis, presenting at an advanced stage and more frequently with lymph node metastasis [88, 89].

Xp11.2/TFE RCC appears as a heterogeneous mass that is frequently accompanied by cystic and necrotic portions. Calcification is frequently seen, especially in younger patients, and is often distributed within the marginal area of the tumor (eggshell calcification). The lesion appears as a hyperattenuation on unenhanced CT and exhibits moderate enhancement during the CMP and gradual enhancement during the NP and EP [90, 91] (Fig. 5.15). The gradual enhancement pattern is similar to that seen for papillary RCC. However, Xp11.2/TFE RCC appears as a hyperattenuation on unenhanced CT, unlike papillary RCC, and has a higher attenuation value during the CMP than papillary RCC. Cystic change, calcification, and lymph node metastasis are more frequent in Xp11.2/TFE RCCs than papillary RCC [91].

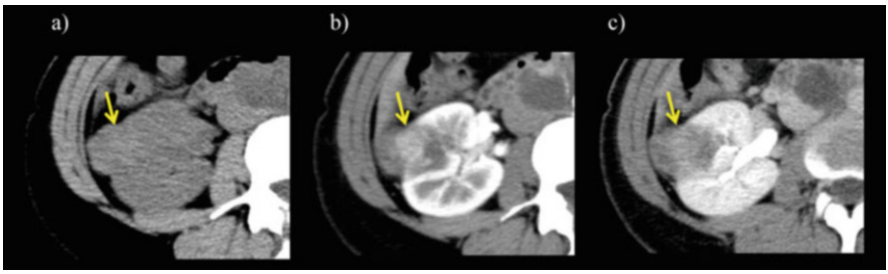

Fig. 5.15 Xp11.2 translocation–*TFE3* gene fusion carcinoma. Transverse, unenhanced CT (**a**) shows a hyperattenuating (47 HU) mass (*arrows*). The mass exhibited heterogeneously moderate enhancement on enhanced CT during CMP (**b**) and persistent enhancement during late NP (**c**)

5.4.6 Mucinous Tubular and Spindle Cell Carcinoma

Mucinous tubular and spindle cell carcinoma (MTSCC) is a low-grade polymorphic epithelial carcinoma associated with a favorable prognosis.

MTSCC exhibits mild enhancement less than the adjacent cortex during the CMP (50–60 HU) and gradual enhancement during the NP or EP (65–75 HU) of multiphase CT, similar to findings for papillary RCC [92, 93]. Unlike papillary RCC, however, it shows an intermediate to high signal intensity on T2-weighted images corresponding to a mucinous component within the tumor [94]. An enhancement pattern similar to that of papillary RCC but with an intermediate- to high-intensity area on T2WI is suggestive of MTSCC.

5.4.7 Angiomyolipoma

Angiomyolipoma is typically a solid tumor composed of varying amounts of three elements: dysmorphic blood vessels, smooth muscle components, and mature adipose tissue. Once thought to be a hamartoma, AMLs are now considered to belong to the family of perivascular epithelioid cell tumors (PEComa) [62]. While 80% of AMLs are sporadic and most of them inconsequential, approximately 20% are associated with tuberous sclerosis complex (TSC).

Because most AMLs contain substantial amounts of adipose tissue, they are usually diagnosed using CT or MRI by identifying the imaging features of fat cells in the mass [28, 29]. Those that can be diagnosed using imaging have been called "classic AMLs" [28–30, 62] (Fig. 5.9). The presence of regions of attenuation less than −10 HU on unenhanced CT or frequency-selective (FS) fat suppression or chemical shift suppression on MRI enables fat to be identified with confidence [28–30, 40, 41]. Intratumoral hemorrhage can occur, particularly in tumors larger than 4 cm; the high attenuation of blood can mask fat, particularly if only a small amount is present, and lead to the misdiagnosis of a classic AML as RCC [95]. On

ultrasound, a classic AML is almost always markedly hyperechoic relative to the renal parenchyma and is often as hyperechoic as renal sinus fat [4, 5]. Acoustic shadowing is a characteristic finding of AML but seen only in 21–33% of AMLs smaller than 3 cm [4, 5] (Fig. 5.1). Thus, a confident diagnosis of a classic AML requires the identification of fat using CT or MRI.

Some AMLs, however, have small amounts of fat that cannot be identified preoperatively using unenhanced CT (1.5–3 mm) and rich amounts of smooth muscle component [8]. These subtypes are now collectively referred to as "fat-poor AMLs," which pathologically contain no more than 25% fat cells [96]. - Fat-poor AMLs are divided into mainly two subtypes—hyperattenuating and isoattenuating AMLs—depending on the relationship of the amount of fat cells and their distribution in the mass. Hyperattenuating AMLs represent approximately 4.5% of all AMLs. These lesions are hyperattenuating relative to the renal parenchyma on unenhanced CT (usually more than 45 HU) and T2 hypointese, corresponding to smooth muscle components, and typically show homogeneous enhancement on CT [8, 62] (Fig. 5.11). Pathological examination generally reveals a fat cell content of only 4% (range, 3–10%) and a composition consisting mostly of a smooth muscle component [97]. Signal loss on fat-suppressed pulse sequences and chemical shift suppression are not observed. On ultrasound, they are usually homogeneously isoechoic, similar to smooth muscle components elsewhere [8, 62]. Because only 2% of RCCs show these findings, a percutaneous biopsy is recommended to avoid unnecessary surgery when encountering a renal mass that is hyperattenuating on unenhanced CT, T2 hypointense, and homogeneously enhancing [5, 6, 59, 60]. Isoattenuating AMLs show close attenuation to the renal parenchyma (−10 and 45 HU) on unenhanced CT and slightly hyperechogenicity on US [62]. This type of AML contains diffuse, scattered fat cells (theoretical fat cell content of 10–25%) among the smooth muscle component. Because there are more fat cells than hyperattenuating AMLs, isoattenuating AMLs typically show chemical shift suppression [62, 98]. At the same time, because of the predominance of the smooth muscle component, the lesion exhibits T2 hypointensity [62, 98]. Thus, T2 hypointensity in combination with the signal loss of opposed-phase imaging and homogeneous enhancement is suggestive of an isoattenuating AML, and a percutaneous biopsy is reasonable in such cases.

When calcification is seen in a lesion with no or minimal fat, it is most likely an RCC, and not an AML [99, 100].

5.4.8 Oncocytoma

Oncocytoma is a common benign renal tumor that accounts for 9% of all renal cell neoplasms. Bilateral and/or multifocal oncocytomas, oncocytosis, and hybrid oncocytic/chromophobe tumors are associated with Birt–Hogg–Dubé syndrome.

Oncocytoma appears as a solid, well-circumscribed tumor with marked or moderate enhancement during CMP and washout during the NP and EP of

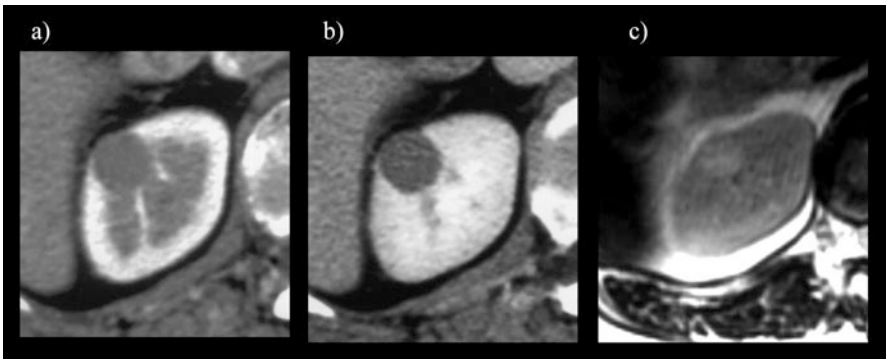

Fig. 5.16 Small oncocytoma. The mass appeared as a homogeneously moderate enhancement on enhanced CT during CMP (**a**) with washout during late NP (**b**), while it was hyperintense on a transverse T2-weighted image (**c**)

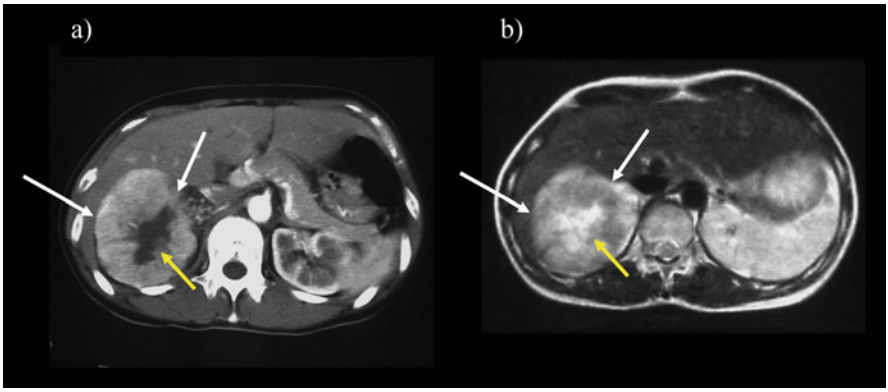

Fig. 5.17 Large oncocytoma with central stellate scar. A large right renal mass (*arrows*) with a central stellate-shaped scar (*yellow arrow*) appears as a low attenuation area on enhanced CT during CMP (**a**) and as a hyperintense area on a T2-weighted image (**b**)

multiphase CT (Fig. 5.16). The degree of enhancement during the CMP is intermediate between that of clear cell and chromophobe RCC [6, 7]. When the tumor is small in size, there is a homogeneous enhancement pattern with an absence of internal necrosis and hemorrhage [6, 101, 102]. Oncocytoma is typically hypointense to hyperintense on T1-weighted images and isointense to hyperintense on T2-weighted images [103]. When the tumor size is larger, oncocytomas often have a central stellate scar that appears as a low attenuation area during the CMP and an area with delayed enhancement during the NP or EP; such lesions have a low T1 signal and a high T2 signal when observed using MRI [72, 104] (Fig. 5.17). Although this feature is suggestive of oncocytoma, it is nonspecific and can been seen in both chromophobe and clear cell RCCs [101, 102]. Oncocytomas, therefore,

present the greatest diagnostic challenge because of the overlap in their appearance with RCC. Recently, the appearance of segmental enhancement inversion during CMP and EP was reported to be a possible characteristic enhancement pattern of small renal oncocytoma when observed using multiphasic CT [105]. This finding consists of two distinct regions of enhancement in which the degree of enhancement reverses during the CMP and EP. The highly enhanced segments during the CMP develop a lower attenuation during the EP (corresponding to the tumor cells), while less-enhanced segments during the CMP develop a higher attenuation during the EP (corresponding to abundant hypocellular hyalinized stroma). Although chromophobe RCC also exhibits this finding, it seems to be more common in oncocytoma and may be helpful for differentiating small oncocytoma from RCC [106, 107].

5.4.9 Metanephric Adenoma

Metanephric adenoma (MA) is a rare benign tumor of the kidney, accounting for 0.2% of adult renal epithelial tumors [108]. They predominantly occur in women and commonly occur in the fifth decade. Most cases are asymptomatic and are detected incidentally during imaging performed for other indications. For patients with symptoms, the most common are hematuria, palpable mass, and flank pain [108, 109]. The incidence of polycythemia in patients with MA is higher than in those with other renal tumors [108].

MA are typically hyperattenuating on unenhanced CT and hyperechoic on US because of the presence of psammomatous calcifications [6, 7]. After the administration of contrast material during multiphase CT, MA exhibit mild enhancement during the CMP (50–60 HU) and gradual enhancement during the NP or EP (65–75 HU) [6, 110, 111]. MA is usually homogeneous but is often accompanied by cystic changes, necrosis, or hemorrhage [110]. This enhancement pattern is similar to that of papillary RCC [6]. When the patient is young and female, MA is more likely than papillary RCC. On MRI, MA can appear as hypointense or hyperintense on T2-weighted images, compared with the normal renal parenchyma, depending on the existence of degenerative changes (necrosis or hemorrhage) and the degree of psammomatous calcifications [109, 111, 112].

5.5 Staging and Preoperative Planning for RCC

The preoperative staging of renal cell carcinoma is indispensable for planning treatment. RCC staging is usually based on the TNM system. CT plays a primary role in preoperative staging. Overall, the accuracies of CT and MRI for RCC staging appear to be similar [113–115]. The accuracy of contrast-enhanced CT and MRI for RCC staging ranges from 72% to 98% [32, 116, 117]. However, the role of MRI in staging is limited, as MDCT enables a wider area scan in a shorter

examination time for the diagnosis of distant metastases. MRI is indicated for patients in whom contrast-enhanced CT is contraindicated. US is not generally recommended for RCC staging, since it is inferior to CT and MRI, mainly because of poor lymph node visibility [118]. Bone scintigraphy is recommended in patients who are strongly suspected of having bone metastasis [119, 120]. PET is considered to play a complementary role for lesions suspected of being metastases based on the results of other examinations [121, 122]. While the sensitivity and negative predictive value of PET for primary lesions, lymph node, and distant metastases are low, its specificity and positive predictive value are relatively high.

5.5.1 Primary Tumor

Multiphase dynamic CT is considered to be useful not only for qualitative diagnosis but also for RCC staging. The accuracy of staging of RCCs is reported to be 91% using a three-phase scan (non-contrast, corticomedullary, and nephrographic phases) [123]. The major limitation of imaging for T staging is its low diagnostic ability for the detection of invasion to perirenal fat (distinction between T1/T2 and T3a) [123–127]. The typical findings of T3a are a discrete nodule (>3 mm) or the thickening of a septum (also > 3 mm) in the perinephric space (Fig. 5.18). However, these criteria are neither sensitive nor specific for spreading beyond the renal capsule, since discriminating between tumor invasion to fat tissue and benign changes, such as those associated with inflammation, is difficult. The accuracy of

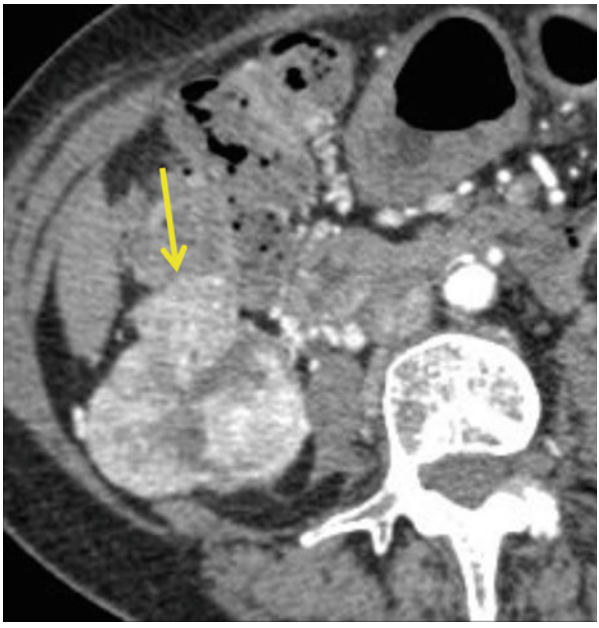

Fig. 5.18 RCC with perirenal fat invasion (T3a). An enhanced CT image shows a discrete nodule invading the perirenal fat, compatible with T3a

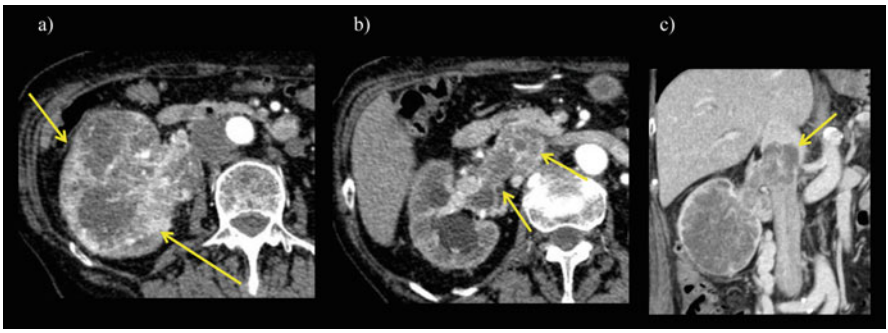

Fig. 5.19 RCC with tumor thrombus (T3b). A right large renal mass (**a**: *arrows*) is accompanied by an enhanced tumor thrombus invading the right renal vein on transverse enhanced CT (**b**: *arrows*) and the IVC on a coronal-oblique enhanced CT image (**c**: *arrow*)

the diagnosis of perirenal fat invasion using MDCT has been reported to be 64% [127]. However, this understaging using CT does not appear to affect a patient's overall prognosis. Patients with pT3a RCC that was underestimated as T1 because of preoperative CT findings did not exhibit a statistically significant difference in the 5-year survival rate when compared with that of patients with pT1 RCC [125]. MRI is thought to be advantageous for determining whether nephron-sparing surgery is possible (i.e., discriminating between T1a and T3a lesions). Especially, a high diagnostic ability (accuracy, 91%) can be obtained for the identification of perirenal fat invasion based on a combination of pseudocapsule rupture and changes in the surrounding adipose tissue on T2-weighted imaging [128]. The lack of perinephric fat infiltration on MRI has been shown to have a high negative predictive value for extracapsular tumor invasion [129].

The identification of tumor thrombosis in the renal vein or inferior vena cava (IVC) and its precise localization are critical for proper staging. Before the era of MDCT, CT was not as sensitive as MRI; however, the ability of MDCT to detect tumor thrombi has been improved through the use of multiplanar reconstruction (Fig. 5.19). The sensitivity and specificity of this modality are 93% and 80%, respectively [130], which is almost equivalent to the accuracy of MRI. One advantage of MRI is that tumor thrombi can be evaluated using a non-contrast, steady-state free-precession technique (True FISP, FIESTA, balanced FFE, and True SSFP) [131], the diagnostic performance of which is similar to that of dynamic MRI. US may be accurate for assessing renal vein involvement and can be used as an adjunct to CT or MRI if the findings are otherwise equivocal or limited.

5.5.2 Regional Lymph Nodes

The most widely used diagnostic criteria for lymph node metastasis are a short-axis diameter of 1 cm or greater and the loss of a horseshoe shape, but these criteria have

long been considered insufficient. While reports on the diagnostic ability of MDCT for lymph node metastasis are few, one study reported that the accuracy, false-positive rate, and false-negative rate were 74, 19, and 7%, respectively, using MDCT [127].

5.5.3 Distant Metastasis

RCC can metastasize to almost any organ (Fig. 4.16), but the lung, brain, and bone are the most common sites. The appearance of metastases, whether hypervascular, hypovascular, or cystic, typically resembles that of the primary lesion.

An evaluation of the chest is important, since the lung is the most frequent site of the distant metastasis of renal cell carcinoma. However, a retrospective study examining 120 patients with RCC concluded that plain chest radiography was sufficient for T1-stage tumors and that a chest CT is only indicated when solitary masses have been detected using plain chest radiography, when respiratory symptoms are present, and for advanced tumors [132]. A pelvic scan might not be necessary for initial staging evaluations of RCC [133, 134]. The probability of the presence of significant lesions on pelvic CT images is very low (2–3%). Very rarely, however, the renal artery divides from the common iliac artery, so a preoperative scan range that covers the pelvis might be reasonable in the CMP to clarify the arterial anatomy using CT angiography.

Bone metastases appear as large expansile lytic lesions, most commonly located in the pelvis, spine, and ribs. Bone scintigraphy may be performed in situations where bone metastasis is strongly suspected, such as the presence of bone pain, but its value as a routine examination for staging is limited [119, 120]. In a retrospective study examining 205 patients who had been pathologically diagnosed as having RCC, 34 (17%) had bone metastasis, and the sensitivity and specificity of bone scintigraphy were 94% and 86%, respectively, but the positive predictive value was as low at 57%. Also, the bone metastasis rate in patients with T1-3aN0M0 RCC without bone pain was 5% or less, leading to the conclusion that bone scintigraphy should not be recommended for such patients [119].

5.5.4 Preoperative Planning

With the introduction of multidetector CT scanners, surgical planning can now be performed using 2D multiplanar reformatted images or 3D CT images [33–35]. While 2D multiplanar reformatted images can depict pertinent and detailed surgical anatomy through the use of a continuous number of images, 3D images provide overall spatial cues that help to plan the surgical approach, to determine the resection margins, and to visualize the vascular anatomy in a single image (Fig. 5.5). 2D or 3D images reformatted from CMP images provide a detailed

depiction of the number, size, and locations of all renal arteries and veins, the major segmental arterial branches, the left adrenal vein, the gonadal veins, and any prominent lumbar veins. NP images obtained during multiphase CT can be used to determine renal position, renal tumor location, and the position of the adrenal gland, while EP images can be used to evaluate the depth of the extension and the relationship of the tumor to the pelvocalyceal system. Since the spatial resolution of MRI is not as high as that of multidetector CT, MRI is not the first choice for surgical planning, but it can be used as an alternative to CT.

5.6 Imaging After Surgery and Ablation

There are many surveillance protocols of imaging reported so far; however, there is no consensus on the imaging protocols used and the optimal interval and duration of follow-up after surgery and ablation. The use of chest X-ray and US is limited due to their low ability to detect the metastasis or recurrence. Contrast-enhanced CT is regarded as the test of choice to search for local recurrences or distant metastases after treatment [135]. MRI can be used in patients with compromised renal functions and who therefore have a higher risk of nephrotoxicity from iodinated CT contrast and also with patients who have a risk of high radiation exposure due to repeated CT scans.

Surveillance protocol of imaging depends on the risk of tumor recurrence and metastases. Risk stratification is based on integrating TNM stage, Fuhrman's grade, and Eastern Cooperative Oncology Group (ECOG) patients' performance status such as the UISS (Integrated Staging System) [136, 137]. It is also important to include clinical evaluation of renal function and cardiovascular risk factors on surveillance. We describe this section in reference mainly to the NCCN (National Comprehensive Cancer Network) guidelines [138].

5.6.1 After Partial Nephrectomy or Radical Nephrectomy

Patients who have undergone a partial nephrectomy or radical nephrectomy for stage 1 (pT1a and pT1b) tumors are recommended to obtain baseline abdominal CT (or MRI) within 3–12 months after surgery. Abdominal CT examinations should be performed with imaging during the nephrographic phase to enhance lesion conspicuity. Local recurrence after resection manifests as an enhancing mass at the resection site in the residual kidney or nephrectomy bed. If the initial postoperative is negative, abdominal CT (or MRI) may be considered annually for 3 years. Chest X-ray or chest CT is recommended to perform annually for 3 years. Since metastases to the brain or bones are usually symptomatic, routine surveillance imaging of the bones and brain is not recommended.

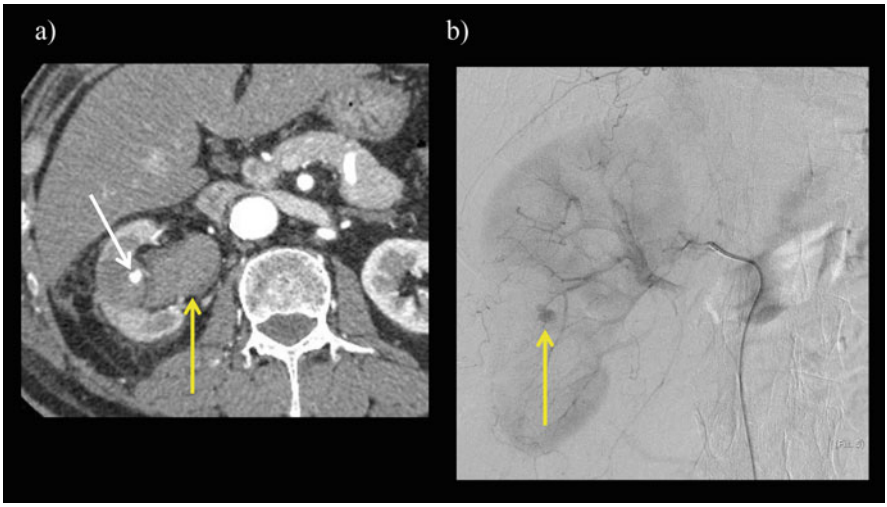

Fig. 5.20 Pseudoaneurysm after a partial nephrectomy. An enhanced CT during CMP (**a**) shows a hyperattenuating renal pelvis caused by a hematoma (*yellow arrow*) in a patient after a post-right partial nephrectomy. Small areas of contrast extravasation are visible in the renal parenchyma (*white arrow*). A renal arteriography (**b**) confirmed the presence of small rounded areas of contrast opacification (*arrow*), suggesting a diagnosis of pseudoaneurysm

Patients who have had a radical nephrectomy for stages 2 and 3 have a higher risk of both local recurrence and metastasis; therefore, an increased frequency of examinations is recommended. These patients are recommended to undergo baseline chest CT and abdominal CT (or MRI) within 3–6 months. The follow-up chest imaging (CT or chest X-ray) and abdominal CT (or MRI) are recommended to perform every 3–6 months for at least 3 years and then annually up to 5 years.

Earlier imaging is performed in patients who have clinical findings such as fever or an elevated white blood cell count, a decreasing hematocrit level, or an increased output from surgical drains. These patients undergo imaging to detect abscesses, hematomas, or urine leaks [139, 140]. Urine leaks appear as contrast-filled collections that may extend outside the renal contour or be confined within it. Hematomas are heterogeneous, soft tissue attenuation collections. Abscesses may have an enhancing wall or may contain internal gas foci. Pseudoaneurysms are seen after $<1\%$ of open partial nephrectomies [141] and $<2\%$ of laparoscopic partial nephrectomies [142] (Fig. 5.20). In the immediate postoperative period, significant operative changes occur after a partial nephrectomy, and these changes should not be confused with residual disease. These changes include perinephric fluid or scarring and a defect at the operative site.

5.6.2 *After Thermal Ablation*

RCC may be effectively treated with either radiofrequency ablation or cryoablation. Risk stratification is more difficult with these less invasive techniques as neither surgical margins nor extensive histological evaluations are available, compared with surgery, and more aggressive imaging surveillance may be needed. The American College of Radiology recommends enhanced and unenhanced abdominal CT or MRI scanning at 1, 3, 6, and 12 months after ablative therapy and at every 6–12 months after the first year, while the NCCN guidelines recommend abdominal CT or MRI at 3 and 6 months to assess treatment response followed by annual abdominal CT or MRI scans for 5 years. Early follow-up scanning is needed as the majority of incomplete treatments are detected within the first 3 months after ablation [143].

Immediately after ablation, the ablated lesion is usually larger than the pre-ablation tumor because a peripheral margin of normal renal tissue is also ablated. Perinephric stranding, which likely represents local inflammatory changes in the perinephric fat generated, could be seen [144, 145]. RFA ablated lesions showed higher attenuation than normal renal parenchyma on unenhanced CT images due to the presence of blood products in the ablation bed [144]. Within 1 week after cryoablation, false-positive tumor enhancement was observed on contrast-enhanced image in as many as 60% of patients [146] (Fig. 5.21). Although various patterns of false-positive tumor enhancement were seen, tumor enhancement areas and overall tumor size became smaller over time.

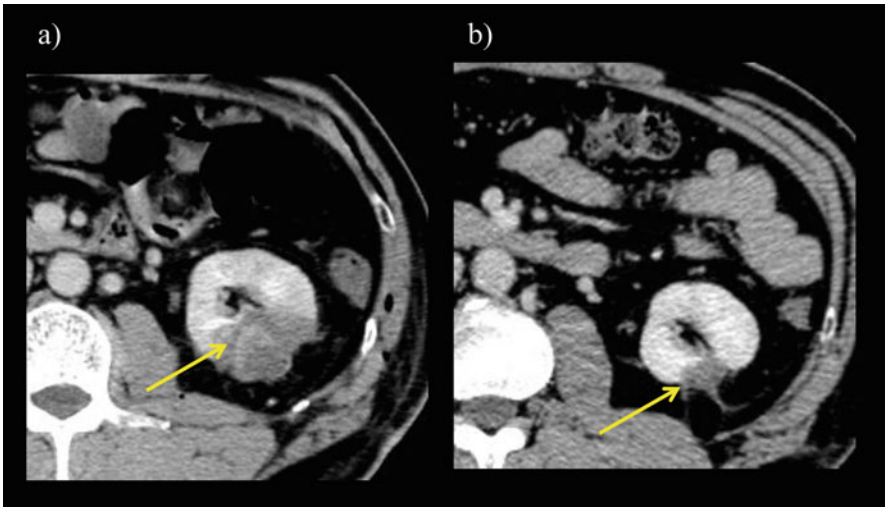

Fig. 5.21 RCC after cryoablation. False-positive tumor enhancement was observed on an enhanced CT image obtained 2 days after treatment (**a**). The tumor enhancement areas and the overall tumor size were smaller on a CT image obtained 6 months after treatment (**b**)

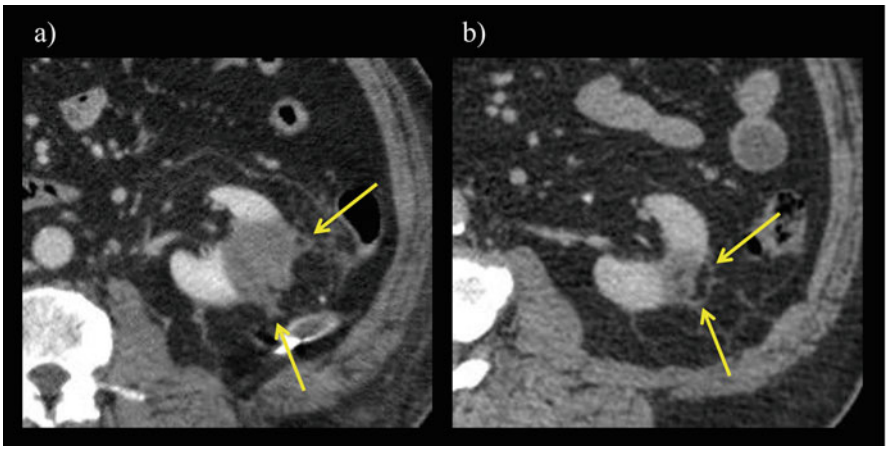

Fig. 5.22 RCC after cryoablation. An enhanced CT scan performed 2 days after cryoablation (**a**) shows perinephric stranding adjacent to the ablation site (*arrows*). An enhanced CT scan performed 6 months after cryoablation (**b**) shows a periablational halo (*arrows*)

Normal post-ablation CT or MRI findings should show no residual enhancement of the tumor with a rim of nonenhancing ablated normal renal parenchyma (Fig. 5.22). Effectively treated tumors eventually develop a "bull's eye" appearance consisting of a central dense mass surrounded by a halo of fat [143] (Fig. 5.22). These halos were persistent and are likely either fibrotic scars that occur at the periphery of the ablated lesion or the organization of perinephric stranding into a coalescent unit [144]. Incomplete ablation manifests as residual enhancing tumor [140, 141]. Enhancement of the residual nodule, especially if accompanied by enlargement, is a strong indicator of residual or recurrent active tumor [145], and repeat biopsy is recommended to these patients.

5.7 Imaging for Targeted Antiangiogenic Therapy

For many years, the Response Evaluation Criteria In Solid Tumors (RECIST) has been used to assess tumor response to immunotherapy for metastatic RCC. These criteria were established by European, American, and Canadian cancer research organizations in 2000 (RECIST 1.0) to simplify and standardize clinical trial assessment criteria, which were based on changes in the sum of the longest diameter of a patient's target tumor lesions [147]. With these criteria, a maximum of ten lesions per patient or five lesions per organ are selected for measurement. CR (complete response) is defined as the disappearance of all target lesions; PR (partial response) is defined as a reduction greater or equal to 30% relative to the pretreatment sum; PD (progressive disease) is defined as an increase greater or equal to 20% relative to the smallest sum measured during follow-up; and SD

(stable disease) is defined as the absence of a CR, PR, and PD. The RECIST was updated in 2009 (RECIST 1.1), and the maximum number of target lesions to be selected changed from ten to five per patient and from five to two per organ [148].

In 2005, the first antiangiogenic agents were approved for the treatment of metastatic RCC, and these agents were shown to be more effective than conventional immunotherapies [149]. However, defining a PR as a 30% decrease in size according to the RECIST does not seem to be optimal for antiangiogenic agents, since these antiangiogenic agents induce stabilization of the disease, rather than regression, and lead to tumor necrosis with only a modest change in size [150–152]. In fact, achieving a 30% decrease in size required several months in patients with metastatic RCC. Given these limitations, Thiam et al. proposed a modified RECIST in which PR is defined as a 10% reduction in the sum of the longest diameters to allow for a more rapid identification of patients whose diseases have responded to treatment [153]. However, a limitation of this method was that a 10% change in size may be within the margin of measurement error. Thus, several new criteria that do not rely only on tumor size have been applied in trials designed to assess the therapeutic responses to antiangiogenic agents.

van der Veld et al. applied the Choi criteria to patients with metastatic RCC who underwent antiangiogenic treatment [154]. The Choi criteria were developed in 2007 to assess gastrointestinal stromal tumors (GIST), and these criteria combine changes in tumor attenuation with tumor size to determine the tumor response [155]. A change in CT attenuation seems to be a good indicator of tumor response, since the CT attenuation of the tumor usually decreases significantly after treatment with antiangiogenic agents because of the inhibitory effect of these agents on the blood supply to the tumor. According to the Choi criteria, PR is defined as a decrease of greater or equal to 10% in a tumor size measurement or a reduction of greater or equal to 15% in the attenuation of the target lesions as measured using portal-phase (60-sec delay) contrast-enhanced CT. Although the Choi criteria had a significantly better predictive value for overall survival than the RECIST 1.1 at the first posttreatment evaluation, the use of the Choi criteria produced results similar to those achieved with RECIST 1.1 at subsequent follow-up examinations [154]. In 2010, Nathan et al. proposed a modification of the Choi criteria requiring a reduction in both the size and attenuation of the target lesions to define an objective response [156]. With these criteria, PR is therefore defined as a decrease of greater or equal to 10% in a tumor size measurement and a reduction of greater or equal to 15% in the attenuation of the target lesions as measured using arterial-phase contrast-enhanced CT. The study concluded that the modified Choi criteria provided a better median assessment of progression-free survival time than RECIST, but this study was of limited value because of its small number of subjects (20 patients).

Smith et al. proposed new criteria similar to the modified Choi criteria, evaluating changes in both tumor size and attenuation [157]. They named these criteria "the size and attenuation CT (SACT) criteria." However, the SACT criteria require a three-dimensional volumetric evaluation using proprietary software for the attenuation measurements. Therefore, applying the criteria is time-consuming, and the

reproducibility of the system at other institutions and its incorporation into routine clinical practice are limited [157]. To overcome these limitations in the SACT criteria, Smith et al. developed the new MASS (Morphology, Attenuation, Size, and Structure) criteria that eliminate the need for a three-dimensional analysis of attenuation [158]. With these criteria, PR was defined as a decrease in tumor size of more than or equal to 20% or one or more predominantly solid enhancing lesions with marked central necrosis or a marked decrease in attenuation (>40 HU) as observed using portal-phase contrast-enhanced CT. In addition, they defined specific patterns of contrast enhancement in target lesions that are indicative of progressive disease, such as a "marked central fill-in" or a "new enhancement of a previously homogeneous hypoattenuating nonenhancing mass." They examined 53 patients with metastatic RCC who were being treated with antiangiogenic agents and confirmed the importance of marked central necrosis (>50%), marked decreased attenuation (≥40 HU), and decreased size of more than 20% on the first enhanced CT after initiating the therapy [158]. Nonetheless, there are several limitations in the current use of attenuation-based criteria including the Choi, modified Choi, SAT, and MASS classifications for the assessment of metastatic RCC treated with antiangiogenic agents [159]. First, it is essential to use the same CT acquisition protocol and the injection methods for intravenous contrast agents before and after treatment to obtain reproducible data. However, comparisons between studies that use the same imaging phase depend on many other factors, such as cardiac output, kilovoltage, and the use of different scanners. Cardiac output is an important factor in patients who are being treated with antiangiogenic agents because of the cardiotoxic effects of these drugs. Second, there is a lack of agreement with regard to the most appropriate method of measuring attenuation. No consensus exists as to whether the region of interest (ROI) should cover the whole lesion (Choi and modified Choi systems) or only a part of the lesion (MASS system). Third, the injection of contrast agents may not be possible in a number of metastatic RCC patients, who have often undergone a nephrectomy, since the administration of iodinated contrast agents is contraindicated in patients with renal failure.

Other than criteria based on changes in tumor size or tumor attenuation, many criteria using perfusion CT, perfusion MRI, diffusion-weighted MRI, contrast-enhanced US, or PET findings have also been applied to the assessment of tumor response to antiangiogenic agents [160–163]. Using these criteria, nonresponders who are identified early could benefit from rapid changes in therapy, enabling costly but ineffective treatments with adverse effects to be avoided. These criteria have shown promising results but are still under investigation. At present, no widely acknowledged criteria for evaluating tumor response to antiangiogenic agents exist.

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Chapter 6
Treatment Overview

Tsunenori Kondo

Abstract Treatment strategy for patients with renal cell carcinoma (RCC) has dramatically changed during the last decades. As for the surgical treatment option, development of the laparoscopic procedure provides a great benefit for patients in terms of less invasive surgery, allowing quick recovery from the procedure. More recently, robotic surgery has been spreading and replacing the conventional laparoscopic procedure. Even more patients can be safely treated with robotic procedure. Partial nephrectomy has been reported to reduce the risk of new onset of development to chronic kidney disease and has been increasingly replacing the use of radical nephrectomy. Previously, RCC had been a contraindication for the use of tumor biopsy, but recent studies now indicate the safety of biopsy. Thus, the use of percutaneous biopsy for small renal masses is increasing, eliminating unnecessary surgery for patients with benign tumors. As for systemic therapy, introduction of targeted therapy has prolonged the survival of patients with metastatic RCC compared to that of the cytokine era. In addition, immune checkpoint inhibitors have recently introduced a major paradigm shift in sequential therapy. These treatments have been supported by a great deal of evidence. We need to heed the standard care recommended by guideline, but minor modifications are also required according to patient conditions.

Keywords Renal cell carcinoma • Surgery • Drug therapy • Algorithms • Guideline

6.1 Introduction

Treatment strategy for renal cell carcinoma (RCC) has dramatically changed during the past decades due to the development of new technologies for surgery and molecular-targeted therapy. This chapter is an overview of current treatment strategies.

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M. Oya (ed.), *Renal Cell Carcinoma*, DOI 10.1007/978-4-431-55531-5_6

6.2 Nonmetastatic Patients (Fig. 6.1)

A treatment algorithm for patients with nonmetastatic RCC is shown in Fig. 6.1 according to the stage of tumor. A detailed explanation of this algorithm is described below, stage by stage.

6.2.1 Stage 1

6.2.1.1 Surgical Therapy

Surgical therapy remains the mainstream treatment for patients with stage 1 RCC because the resection of primary tumors tends to be curative and provides the most effective oncological outcome [1–3]. Thus, surgical therapy should be considered as the primary treatment option for stage 1 disease. Radical nephrectomy was considered the gold standard of surgical treatment from the 1960s [4]. The original concept of radical nephrectomy was conceptualized by Robson et al., including early ligation of the renal arteries and veins, ipsilateral adrenalectomy, dissection of the kidney outside Gerota's fascia, and lymphadenectomy [5]. Wide resection of the kidney resulted in improved patient survival compared to simple nephrectomy, the former standard procedure.

However, these results are based on the era when most tumors were found at advanced stages due to the lack of the present radiological modalities. In current practice, however, the proportion of stage 1 disease has increased up to about 70 %,

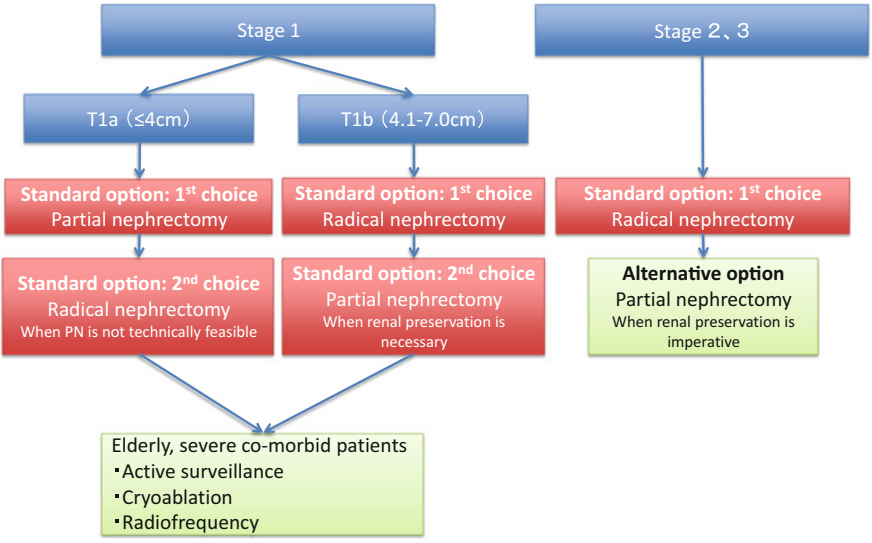

Fig. 6.1 Treatment algorithm for nonmetastatic patients with RCC

and most of it is found incidentally by radiological screening. Thus, there is controversy about the necessity of wide resection for all RCC tumors. Cumulative results show that ipsilateral adrenalectomy at the time of nephrectomy has no survival benefit; thus, prophylactic adrenalectomy is currently considered unnecessary unless abnormal radiological findings are detected in the adrenal gland [6]. The benefit of lymphadenectomy has been also examined by randomized prospective studies. Although its benefit remains undetermined for advanced stage disease, the EORTC study concludes that lymphadenectomy does not improve survival in patients with low-stage disease [7]. In addition, the disadvantage of radical nephrectomy has been emphasized due to the new onset of chronic kidney disease (CKD) and increased risk of cardiovascular disease and noncancer mortality [8–10]. Thus, the recent preference in surgical treatment for stage 1 disease trends toward partial nephrectomy.

6.2.1.1.1 Radical Nephrectomy or Partial Nephrectomy?

Partial nephrectomy was originally indicated for patients with a solitary kidney, renal dysfunction, or bilateral disease. These patients are not good candidates for radical nephrectomy [11]. But many studies have supported the equivalent oncological outcome between radical and partial nephrectomy [12]. In addition, prevention of CKD has been considered as the great advantage of partial over radical nephrectomy as mentioned above [8–10]. Progression of chronic kidney disease is reported to increase the risk of death, cardiovascular events, and hospitalization in a large community-based population [13]. Whether postoperative CKD really induces cardiovascular events or non-RCC-related death has been debated. Huang et al. reported that radical nephrectomy was significantly associated with a 1.4 times higher risk of more cardiovascular events after surgery compared to partial nephrectomy ($p < 0.05$) [10]. Population-based studies including large numbers of patients show an increased risk of noncancer mortality with radical nephrectomy as compared to partial nephrectomy despite comparable RCC-related mortality between procedures [9, 14, 15]. However, only one randomized EORTC trial comparing postoperative survival between radical and partial nephrectomy shows similar cancer-specific and overall survival [16]. In the original report, there are several points to be criticized. One is a lack of results on renal function after surgery. Thus, this trial is statistically underpowered. Functional outcome was reported thereafter, and partial nephrectomy resulted in higher postoperative renal function than that after radical nephrectomy [17]. The randomized trial did not show any benefit of partial nephrectomy over radical nephrectomy in reducing the risk of overall survival. Nevertheless, renal function was preserved at a higher level after partial nephrectomy.

There may be several other advantages in partial nephrectomy apart from the reduction of the risk of non-RCC-related death. First, about 5 % of patients develop tumors on the contralateral kidney [18]. If the functioning kidney is preserved during the first surgery, the selection of treatment for contralateral tumors can be

more flexible. Second, the proportion of benign tumors is reported to be about 20 % in Western countries [19, 20]. Performing radical nephrectomy on these patients with benign disease may constitute overtreatment. Third, preservation of renal function may allow the patients to undergo systemic therapy for other diseases with agents having renal toxicity, such as cisplatin [21].

Taking these results into consideration, the recommendation of the current guideline is partial nephrectomy for patients with stage 1 disease, especially for T1a tumors whose sizes are 4 cm or less [1, 22, 23]. Radical nephrectomy is considered only when partial nephrectomy is not technically feasible due to the anatomical complexity of the tumors.

Partial nephrectomy should be discussed as a standard care even for tumors of T1b, 4.1–7.0 cm [1]. Most studies support the preservation of renal function after partial nephrectomy [24–26]. However, renal function after partial nephrectomy for T1b tumors is slightly lower than that after nephron-sparing surgery for T1a tumors [26]. The advantage in overall survival from partial nephrectomy is even more unclear [26, 27]. Nephron-sparing surgery does have other advantages over radical nephrectomy as mentioned before. Thus, partial nephrectomy also should be considered for T1b tumors when technically feasible.

There has been controversy over which is the better procedure in terms of nephron-sparing surgery—partial nephrectomy or tumor enucleation. In partial nephrectomy, normal kidney margin is attached to the surroundings of the tumors. This concept is based on possible satellite lesions around the tumors. The incidence of satellite lesions has been reported to be 6–15 % within 1 cm from the tumor boundary [28, 29]. Recent studies, however, show no significant correlation between the thickness of normal kidney margin and local recurrence [30], and the incidence of local recurrence is likely to be very low at 1–2 % [31]. In tumor enucleation, tumors are excised without a margin of normal parenchyma [32, 33]. Tumor enucleation has some advantage in the maximum preservation of functional renal parenchyma and a low incidence of major bleeding and collecting system damage, thereby decreasing the incidence of complications such as urinoma and urinary fistula [33]. In addition, oncological outcomes are reported to be comparable between tumor enucleation and partial nephrectomy [34]. The incidence of positive surgical margin was not different between the procedures (0.2 % versus 3.4 %). Thus, either procedure can be considered acceptable.

Figure 6.2 shows the sequential change by year in the utilization of partial nephrectomy at Tokyo Women's Medical University. Partial nephrectomy has been selected for about 95 % of patients with T1a disease in recent years. Radical nephrectomy for T1a disease is considered only when the patients are over 80 years old, have CKD stage 4 or 5, and are likely to develop end-stage kidney disease soon even after nephron-sparing surgery [35]. The proportion of partial nephrectomy for T1b tumors has increased over the years and reached up to 90 % in the year 2013. Thus, the more the technique improves for partial nephrectomy, the more we can extend the indication of the nephron-sparing procedure.

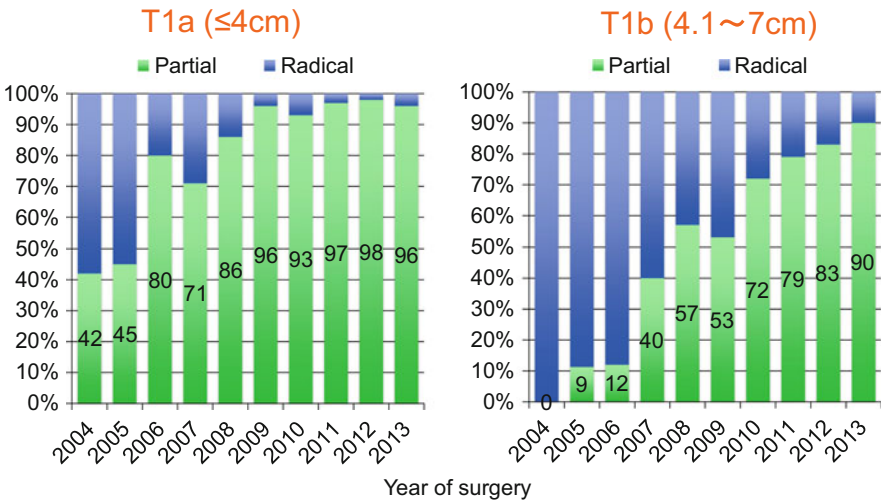

Fig. 6.2 Sequential change of the utilization of partial nephrectomy for stage I tumors at Tokyo Women's Medical University

6.2.1.1.2 Open, Laparoscopic, or Robotic Surgery?

How do we select the approach—open, laparoscopic, or robotic procedure? According to the current guidelines, the standard procedure for partial nephrectomy is open surgery since laparoscopic partial nephrectomy (LPN) is associated with a higher incidence of complications and longer ischemic time (Table 6.1) [1]. However, recent technical innovations—including early unclamping, zero ischemia, and the accumulation of experience with LPN—have resulted in better residual renal function and lowered the incidence of postoperative complications [36, 37]. Based on these results, the current guidelines allow experienced surgeons to perform LPN [22]. In addition, robot-assisted laparoscopic partial nephrectomy (RAPN) has widely spread around the world [38]. The new technology of robotic partial nephrectomy has made suturing and excising easier and more precise compared to the laparoscopic procedure. Meta-analysis showed an equivalent cancer control and safety of RAPN to LPN and the advantage of RAPN in shortening ischemic time over LPN [39]. Thus, RAPN is more feasible and likely to be indicated for more patients with stage 1 than LPN.

Laparoscopic radical nephrectomy has shown a significant benefit over open radical nephrectomy with its reduction in intraoperative bleeding, complication rate, and shorter hospital stay [40]. Laparoscopic radical nephrectomy shows equivalent long-term oncological outcome to open radical nephrectomy [41]. Thus, laparoscopic radical nephrectomy is more preferable for stage 1 disease than open radical nephrectomy (Table 6.1) [22].

Table 6.1 Recommended approach for surgical treatment by clinical stage

Clinical stage	Procedure	Approach	Comment
T1	Nephron-sparing surgery	Open	Recommended
		Laparoscopic	Optional for experienced centers only
		Robot assisted	
	Radical nephrectomy	Laparoscopic	In patients not suitable for NSS
		Open	Optional in patients not suitable for NSS
T2	Radical nephrectomy	Laparoscopic	Recommended
		Open	Optional
	Nephron-sparing surgery	Open	Alternative at experienced centers
T3, T4	Radical nephrectomy	Open	Recommended in most patients
		Laparoscopic	Feasible in selected patients

Modified from Ref. [2], Table 3

6.2.1.2 Thermal Ablation

Cryoablation and radiofrequency ablation (RFA) are two less invasive treatment options for small renal masses than surgery. Patients who are not surgical candidates can be still treated with a percutaneous approach under local anesthesia. However, there is a limitation in terms of tumor location for RFA. The most serious complication of RFA is ureteric stricture, which occurred at 3 % [42]. Thus, RFA is not recommended for central tumors located in the hilum, near the proximal ureter, or the central collecting system [22]. Cryoablation has fewer limitations for tumor location compared to RFA. However, percutaneous cryoablation is often found to be time-consuming and shows higher risk of complications about 20 %, with most involving hemorrhagic-related complications including hematoma and requirement of transfusion [43]. Local recurrence after thermal ablation therapy is seen much more frequently than after surgical therapy, 7.4 times higher after cryotherapy, and 18.2 times higher after RFA [44]. The long-term oncological outcomes of cryoablation and RFA are reported to be comparable to that of partial nephrectomy [45, 46].

One major advantage of thermal ablation is its superior preservation of renal function after the procedure. Residual renal function is reported to be higher in cryoablation than that in robotic partial nephrectomy [46, 47]. But, other researchers stated no difference between groups in long-term follow-up [48]. RFA shows better functional outcome than open partial nephrectomy [49], but this advantage decreases when compared to laparoscopic partial nephrectomy [50]. Currently, there is no definitive conclusion as to the superiority of one procedure over the other [51, 52].

Thus, patient selection is important for treatment. A recommended indication for thermal ablation is small renal masses in elderly patients, or those with severe

comorbidity, multiple tumors of hereditary background, and bilateral tumors, and patients with a solitary kidney who are at high risk of complete loss of renal function by surgery [22].

6.2.1.3 Active Surveillance

Active surveillance is one of the therapeutic options for small renal masses, since tumor growth is generally slow [53]. The mean linear growth rate of RCC tumors has been reported to be 0.1–0.3 cm per year [54, 55]. In addition, delayed intervention is not associated with increased surgical morbidity and does not compromise oncological outcome [56]. Surgical intervention may have no impact on the prolongation of overall survival of patients of 75 years or older [57]. The most problematic risk from active surveillance is the progression to metastatic disease. Recent studies show the surprisingly low risk of developing metastatic progression of small renal masses (1–2 %) [58]. Thus, the current guidelines recommend that active surveillance should be discussed if patients wish to avoid surgical treatment or are not surgical candidates due to age or severe comorbidities [1, 3, 22]. The recognition of the feasibility of active surveillance has increased the proportion of active surveillance for small renal masses up to about 20 % in a population-based study [59]. However, it should be noted that small renal masses include about 20 % high-grade tumors [60]. Thus, patient selection is important for this option.

6.2.1.4 The Role of Percutaneous Biopsy

Percutaneous biopsy for small renal masses has been increasingly performed during recent years, but its use is still uncommon. One population-based study shows that only 6 % of the patients who underwent surgery for small renal masses underwent a preoperative tumor biopsy [61]. This may reflect the current thought that tumor biopsy is not accurate enough (at 5 % false-positive and 25 % false-negative results) to warrant a change in treatment strategy [62]. However, previous studies included fine needle aspiration which is likely to be inferior in diagnostic accuracy to core biopsy [63]. Fine needle aspiration is not recommended for current practice [64].

Lately, the accuracy of core biopsy is very high. A recent literature review summarizes studies analyzing the diagnostic accuracy of percutaneous core biopsies [65]. The accuracy of differentiating malignant from benign tumors is reported to be 86 %–100 %. Sensitivity and specificity are reported to be 86 %–100 % and 100 %, respectively. Accuracy of core biopsy is 86–98 % in histological subtyping and 46–76 % in grading. The rate of accurate grading is not so high, but this may reflect the heterogeneity of RCC tumors or the interobserver variability in grading by pathologists [64]. Detecting high-grade tumors on biopsy may impact the treatment choice in which extirpative surgery is more preferable than active surveillance or thermal ablation.

Table 6.2 Indications for tumor biopsy for small renal masses

Recommendation	Condition
Strongly recommended before ablation	In the setting of ablation (before ablation)
Recommended	Active surveillance (possibility of delayed treatment)
	Without clear indication of pathological diagnosis on imaging appearance
	Suspicious of metastatic disease
Not recommended	Syndrome with monomorphic pathology
	Imaging characteristics of specific pathology
	Up-front extirpative surgery is preferred
	Watchful waiting without consideration of further active treatment

Complications of needle biopsy include tumor seeding, bleeding, arteriovenous fistula, infection, and pneumothorax [63]. The incidence of complication for needle biopsy has been reported to be about 5 % [66]. Significant complications which required active treatment or hospital admission were observed at less than 2 % in the recent series [65]. Most of these are bleeding. One of the most concerning complications is tumor seeding, but the estimated risk for this is less than 0.01 % [67]. No tumor seeding was observed in the recent series [65]. Thus, morbidity related to biopsy is low [64]. In addition, biopsy does not complicate the surgery [68].

Indication of biopsy is shown in Table 6.2 [64, 65]. In the setting of thermal ablation, tumor biopsy is strongly recommended before the procedure. This is because pathological interpretation is difficult after treatment due to possible necrosis or degeneration of the tumors. Biopsy is recommended for patients considered for active surveillance (with the possibility of delayed treatment), without clear indication of pathological diagnosis on imaging appearance, or suspicions of metastatic disease. Patients are not recommended to undergo biopsy when the following conditions are met: (1) syndrome with monomorphic pathology; (2) imaging characteristics of specific pathology; (3) conservative management is not an option, in other words, when up-front extirpative surgery is preferred; or (4) watchful waiting without consideration of further active treatment.

6.2.2 Stages 2 and 3

6.2.2.1 Surgical Therapy

Partial nephrectomy is difficult to perform in most cases for T2 tumors larger than 7 cm, although the results from high-volume centers show an equivalent cancer control between partial and radical nephrectomy for T2 tumors [69, 70]. However, these studies are retrospective, and highly selected patients underwent partial

nephrectomy. Thus, partial nephrectomy is only feasible in experienced centers for selected patients, and radical nephrectomy is indicated for most patients [22, 23]. Laparoscopic radical nephrectomy is a recommended procedure for T2 disease (Table 6.1) [22]. Open radical nephrectomy is also recommended, but it shows larger blood loss and longer hospital stay compared to laparoscopic radical nephrectomy [71, 72].

As for T3 or T4 disease, open radical nephrectomy is a standard procedure [22, 23]. Patients with tumor extension to the inferior vena cava or those with invasion to adjacent organs are definite candidates for open procedure. It has been reported that IVC thrombectomy with robotics was successfully performed in selected cases, but the number of cases reported has been still very low [73, 74]. When patients have tumor extension to the right atrium, long-term survival is possible to obtain with radical nephrectomy combined with tumor thrombectomy, which is usually performed under cardiopulmonary bypass [75]. Laparoscopic radical nephrectomy is feasible for selected patients since many reports from experienced centers show equivalent oncological outcome and superior perioperative profile to open radical nephrectomy [76]. It remains undetermined whether partial nephrectomy for T3a tumors is justified in terms of cancer control. Partial nephrectomy is unlikely to compromise the oncological outcome for pathological T2 or T3 tumors which were primarily diagnosed as clinical T1b by preoperative radiological findings [77]. Thus, partial nephrectomy is considered only for patients with a solitary kidney or with bilateral tumors of clinical stage T3 or higher.

6.2.2.2 Lymphadenectomy

One randomized prospective trial shows no therapeutic benefit from lymphadenectomy in the patients with RCC [7]. Pathological lymph node metastases were only found in 3.8 % of the clinically node-negative patients who underwent lymphadenectomy. This indicates that RCC tumors infrequently metastasize to the lymph nodes. This study, however, included only 28 % stage T3 tumors. In other words, the majority of the patients were at a lower stage. Thus, the role of lymphadenectomy in advanced disease was not clarified by this study. Blute et al. reported, on the other hand, that pathological grade 3 or higher, sarcomatoid component, tumor size at 10 cm or larger, pathological T stage at 3 or higher, and presence of tumor necrosis were the risk factors that predict lymph node metastases [78]. If patients meet two or more of these factors, the incidence of lymph node metastases exceeded 20 % [77]. Thus, lymphadenectomy may be beneficial in advanced stage, but no definitive recommendation has been drawn. Lymphadenectomy, however, should be performed to make an accurate staging if the radiological study shows lymphadenopathy [3, 22].

6.2.2.3 Ipsilateral Adrenalectomy

Ipsilateral adrenalectomy was originally advocated by Robson et al. when the modalities of radiological examination were less diagnostic than those of the

contemporary era [5]. Several studies examined the risk factors of adrenal metastases, and patients with upper pole tumors larger than 7 cm had been reported to be candidates for adrenalectomy [80]. However, recent retrospective studies show no therapeutic benefit of ipsilateral adrenalectomy as a prophylactic measure. Kutikof et al. reported that the incidence of pathological adrenal metastases was only at 4.4 % in patients with tumors 7 cm or greater [81]. Upper pole location was not a predictive factor. Weight et al. also reported that synchronous adrenal metastases were found only in 2.2 % of the patients who underwent ipsilateral adrenalectomy [6]. Both studies show that risk of metastases to the contralateral adrenal glands does not decrease even after ipsilateral adrenalectomy [6, 81]. It should be noted that most patients with adrenal metastases showed abnormal findings on their adrenal gland on preoperative radiological examination, and incidental metastases to the adrenal glands were rarely observed. Thus, the recommendation of the current guideline is not to perform adrenalectomy unless radiological examination shows a possibility of metastatic lesions in the adrenal glands [22].

6.2.2.4 Adjuvant Therapy

Immunotherapy did not prolong survival after surgery [82]. Several ongoing clinical trials are examining adjuvant-targeted therapy. Recently, the results of the ECOG-ACRIN E2085 phase 3 trial were reported. [83] Patients with a high risk of recurrence were randomly assigned to three treatment groups, including sunitinib, sorafenib, and placebo, and were treated for 54 weeks after surgery. Median disease-free survival was 5.8 years (IQR 1.6–8.2) for sunitinib (hazard ratio [HR] 1.02, 97.5 % confidence interval [CI] 0.85–1.23, $p = 0.8038$), 6.1 years (IQR 1.7—not estimable [NE]) for sorafenib (HR 0.97, 97.5 % CI 0.80–1.17, $p = 0.7184$), and 6.6 years (IQR 1.5—NE) for placebo. The conclusion of this trial is that adjuvant treatment with the VEGF receptor tyrosine kinase inhibitors sorafenib or sunitinib showed no survival benefit compared to placebo. In contrast, another randomized phase 3 trials (S-TRAC) of adjuvant sunitinib or placebo for very high risk patients shows the prologation of disease free survival in sunitinib group than that in placebo group (6.8 versus 56. years, HR:0.76; $p = 0.03$). [84] Therefore, the role of VEGF receptors tyrosine kinase inhibitors as adjuvant setting is still controversial.

6.2.2.5 Other Treatment Options

No alternative options to surgical therapy are recommended for stage 2 or 3 disease since cancer mortality is speculated to be higher than noncancer mortality in stages 2 and 3 [85]. There is no evidence to support a survival benefit from transarterial embolization or systemic therapy for nonmetastatic patients [22].

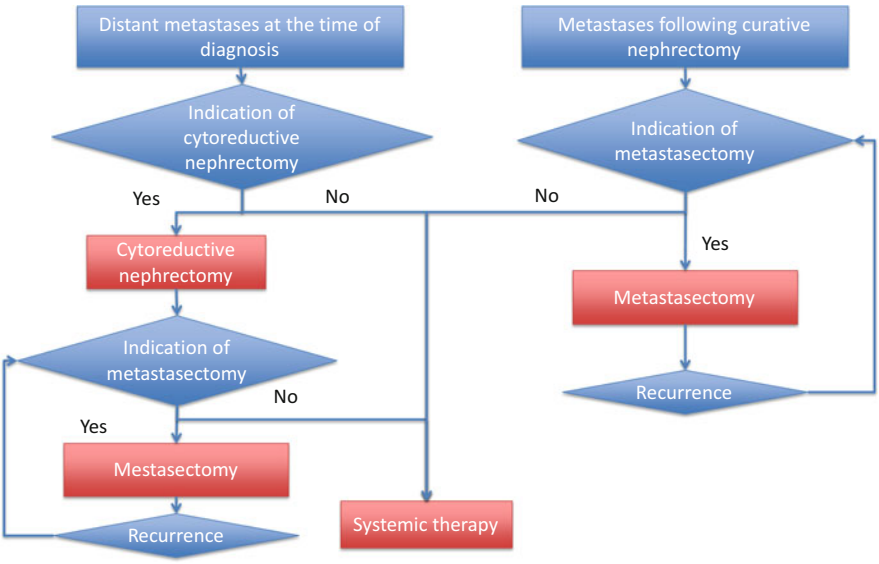

Fig. 6.3 Treatment algorithm for patients with metastatic RCC

6.3 Metastatic Patients (Stage 4) (Fig. 6.3)

The treatment algorithm for patients with metastatic RCC is shown in Fig. 6.3. The patients are divided into two groups: those who have metastatic disease at the time of diagnosis of RCC and those who develop metastases after a disease-free interval following surgery. A detailed explanation for this algorithm is described below.

6.3.1 Metastatic Disease at the Time of RCC Diagnosis

6.3.1.1 Cytoreductive Nephrectomy

The most important thing to be considered is whether the removal of primary renal tumors by cytoreductive nephrectomy (CN) would be a benefit for patients with metastatic disease. The benefit of CN was established from randomized prospective results during the cytokine era when CN followed by interferon-alpha (IFN-α) significantly prolonged patient survival compared to IFN-α treatment without CN (Table 6.3) [86–88].

The role of CN remains to be determined in the era of targeted therapy. Currently, a randomized prospective study (the CARMENA study) is in process that is comparing the results of a group receiving CN followed by sunitinib to another receiving sunitinib without CN. [89] Thus, there is as yet no determinant evidence to support the role of CN in the era of targeted therapy. It is important to

Table 6.3 Results of cytoreductive nephrectomy in the cytokine era from two prospective randomized studies

	Authors	Number of patients	50 % overall survival(months)			Objective response rate (%)			Mortality (%)
			IFNα alone	CN+IFNα	P	IFNα alone	CN+IFNα	P	
SWOG8949	Flanigan 2001	241	8.1	11.1	0.05	3.3	3.6	NS	1 (0.8 %)
EORTC30957	Mickisch 2001	85	7	17	0.03	12	19	0.38	1 (2.4 %)
Combined analysis	Flanigan 2004	331	7.8	13.6	0.002	5.7	6.9	0.60	2 (1.4 %)

IFN interferon, *CN* cytoreductive nephrectomy

Table 6.4 Predictive factors for unfavorable outcome after cytoreductive nephrectomy in the era of targeted therapy

Poor risk by risk classification
Karnofsky performance score of 70 % or less
Patients of 75 years or older
Brain metastases
Degree of reduction in tumor burden by CN less than 90 %

Refs. [90, 93]

note, however, that the retrospective study demonstrates that CN still has an additional benefit in prolonging patient survival compared to systemic therapy with targeted agents alone [90, 91]. Choueiri et al. reported that their CN group showed a longer median overall survival of 19.8 months than that of their no CN group (19.8 versus 9.4 months, $p < 0.01$) [90]. The results from the International Metastatic Renal Cell Carcinoma Database Consortium show prolongation of overall survival in patients with CN compared to those without CN (20.5 versus 9.5 months, $p < 0.001$) [91]. The results from our institution are very similar to their report. The combination with CN prolonged overall survival to 17.1 months from the 8.5 months observed in patients treated with targeted therapy alone ($p < 0.0001$). CN significantly reduced the risk of patient death after adjusting patients' risk with the criteria proposed by Heng et al. [92]. Thus, cytoreductive nephrectomy still has a role in the era of targeted therapy [22, 23].

It is important to understand which patients are more likely to benefit from CN. Table 6.4 shows possible prognostic factors influencing the survival of those who undergo CN in conjunction with targeted therapy. The retrospective study shows poor risk in the risk classification system proposed by Memorial Sloan Kettering Cancer Center (MSKCC), 70 % or lower in Karnofsky performance score, the advanced age of 75 years or older, and brain metastases as significant prognostic factors [90]. The degree of reduction of tumor burden by CN is also a prognostic factor. [93] Patients show longer survival when CN removes more than 90 % of the tumor burden. These factors are not contraindications for CN, but they should be taken into consideration when deciding whether CN should be performed.

After CN, systemic therapy and/or metastasectomy should be considered. Details regarding this are described in the following section.

6.3.2 Metastatic Disease After Disease-Free Interval Following Surgery

Some patients develop metastases after prior nephrectomy followed by a disease-free interval. The treatment algorithm is shown in Fig. 6.3.

6.3.2.1 Indications for Metastasectomy

Initially, resectability of metastatic lesions (metastasectomy) should be considered. There are no randomized trials examining the survival benefit of metastasectomy. However, the many retrospective studies show longer survival in patients who received metastasectomy than those without [94–96]. Although the grade of recommendation is not high in the current guidelines because of the lack of randomized trials (grade C), metastasectomy is recommended when metastatic lesions are resectable and the patient has a good performance status [22, 23].

Prognostic factors have been examined to help predict poor prognosis after metastasectomy. Table 6.5 shows the prognostic factors and risk stratification reported by recent representative studies. Naito et al. collected data from 556 patients and report four risk factors that predict poor outcome: grade 3 at the primary tumor, brain metastases, C-reactive protein level higher than 1.0 mg/dl, and incomplete resection [95]. The overall survival of favorable risk patients who meet 0 or 1 factor is 105.6 months, whereas that of patients with 3 or more factors is only 10.3 months. Tosco et al. analyzed the results from 109 patients and report the following five predictive factors: T stage 3 or higher, Fuhrman grade 3 or higher, disease-free interval of 12 months or shorter, non-pulmonary metastases, and multiple site metastases [96]. The overall survival of patients with 0 or 1 factors is more than 108 months, but for those with four or more factors is only 13 months. Alt el al. included 887 patients in their analysis and reported risk factors for poor prognosis after metastasectomy, such as incomplete resection, ECOG-PS 1 or

Table 6.5 Risk factors for predicting poor prognosis for metastasectomy and risk stratification

	Naito 2013 Urology		Tosco 2013 Eur Urol		Alt 2011 Cancer	
Patient number	556		109		887	
Risk factors	Grade 3		T stage ≥3		Incomplete resection	
	Brain metastases		Fuhrman grade ≥3		ECOG-PS ≥ 1	
	CRP >1.0 mg/dl		Disease-free interval ≤12 months		Non-pulmonary metastases	
	Incomplete resection		Non-pulmonary metastases		Synchronous metastases to nephrectomy	
			Multiple sites			
Risk stratification		50 % OS (months)		50 % OS (months)		50 % OS (months)
	Favorable (0, 1 factors)	105.6	0, 1 factors	>108	Complete resection	59
	Intermediate (2 factors)	42.0	2	91	Incomplete resection	31
	Poor (3 or more factors)	10.3	3	38	No metastasectomy	13
			4 or more	13		

CRP C-reactive protein, *ECOG-PS* Eastern Cooperative Oncology Group, *OS* overall survival

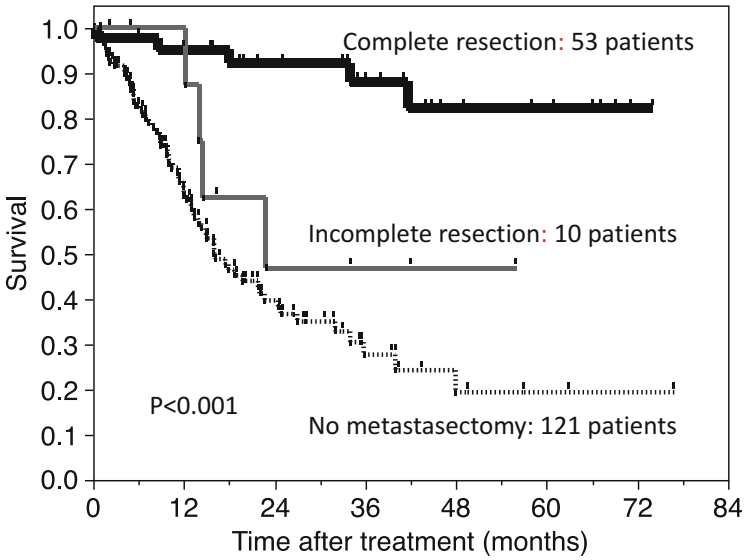

Fig. 6.4 Overall survival of patients with metastatic RCC in the targeted therapy era

higher, non-pulmonary metastases, and synchronous metastases to nephrectomy [94]. They did not perform risk stratification, but they emphasized complete resection as an important factor for selecting patients for metastasectomy. Eggener et al. reported that patients with poor risk by the MSKCC risk classification show minimum impact from metastasectomy [97]. Although there are no clear criteria for indicating metastasectomy, these factors need to be considered before performing one.

We need to keep in mind that these results are based on data from patients treated during the cytokine era. It remains to be determined whether metastasectomy is still beneficial to prolong patient survival during the targeted therapy era since there have been few reports supporting the benefit of metastasectomy. In our institute, 53 patients underwent metastasectomy after 2008 when the first agent of molecular-targeted therapy was introduced in Japan. Complete resection was performed in 43 patients and incomplete resection in ten. One hundred twenty-one patients were treated with systemic therapy alone. Figure 6.4 shows overall survival of patients with metastatic RCC after treatment for metastatic disease. Four-year overall survival of patients with complete resection is 82.2 %. This is significantly higher than that for those with incomplete resection (46.8 %) or no metastasectomy (19.4 %) ($p < 0.001$). These results are similar to those reported by Alt et al. who show incomplete resection for patients with multiple metastases provided longer survival than that of patients with no metastasectomy [94]. Thus, metastasectomy is likely to be beneficial even in the era of targeted therapy. This is also supported by results from Naito et al. who report that patients receiving metastasectomy and targeted therapy showed prolonged survival as compared to

those not receiving targeted therapy (132.2 versus 74.1 months) [95]. Collective results also support the use of metastasectomy when we treat patients with metastatic RCC. However, when patients meet the conditions related to poor prognosis after metastasectomy or when the metastatic lesions are not considered resectable, systemic therapy should be the first choice.

6.3.2.2 Systemic Therapy

Systemic therapy is mainstream for the treatment of patients with metastatic RCC. If patients are unlikely to benefit from metastasectomy, systemic therapy is initiated. Stratification of patients with the risk classification system proposed by Memorial Sloan Kettering Cancer Center (MSKCC) is necessary before starting treatment (Table 6.6). The MSKCC risk classification consists of five factors to predict poor prognosis, including time from initial RCC diagnosis to the start of systemic therapy of less than 1 year, low Karnofsky performance status (less than 80 %), hemoglobin of less than the lower limit of normal, corrected serum calcium higher than the upper limit of normal, and lactate dehydrogenase higher than 1.5 times the upper limit of normal [98]. This classification system is based on results from patients treated with interferon-alpha, but is still useful in today's era of targeted therapy. Heng et al. proposed another risk classification system for patients treated with targeted therapy [92]. This classification consists of the following six variables: time from diagnosis to treatment of less than 1 year, Karnofsky performance status less than 80 %, hemoglobin less than the lower limit of normal, corrected calcium greater than the upper limit of normal, neutrophils greater than the upper limit of normal, and platelets greater than the upper limit of normal. If patients meet no risk factor, they are designated as favorable risk, one to two factors as intermediate risk, and three or more as poor risk. Both classification systems are effective in stratifying patients.

Table 6.6 Risk classification systems for patients with mRCC

MSKCC classification	Heng classification
(1) Time from initial RCC diagnosis to start systemic therapy: less than 1 year	
(2) Low Karnofsky performance status: less than 80 %	
(3) Hemoglobin: less than the lower limit of normal	
(4) Corrected serum calcium: higher than the upper limit of normal	
(5) Lactate dehydrogenase: higher than 1.5 times the upper limit of normal	(5) Neutrophils: greater than the upper limit of normal
	(6) Platelets: greater than the upper limit of normal
The number of positive factors and classification	
0	Favorable risk
1–2	Intermediate risk
3 or more	Poor risk

Table 6.7 The currently proposed algorithm for treating patients with metastatic RCC at the year of 2016

Histology and setting	Risk group	Standard	Option
Clear cell First line	Good/intermediate risk	Sunitinib	Cytokines (including high-dose IL2)
		Bevacizumab + IFN-alpha	Sorafenib
		Pazopanib	
	Poor prognosis	Temsirolimus	Sunitinib
Clear cell Second line	Prior cytokine	Sorafenib	Sunitinib
		Pazopanib	
		Axitinib	
	Prior VEGFR-TKI	Nivolumab	Everolimus
		Cabozantinib	Sorafenib
		Axitinib	
Clear cell Third line	Post-2 TKIs	Everolimus	
		Nivolumab	
Non-clear cell histology			Temsirolimus
			Sunitinib
			Sorafenib

Another factor to be determined before starting systemic therapy is histological subtype. If prior nephrectomy has not been performed at the time of systemic therapy and if cytoreductive nephrectomy is not indicated because of poor patient condition, tumor biopsy should be considered. Histological subtype influences the choice of agents.

Table 6.7 shows the currently accepted algorithm based on evidence from clinical trials [99]. For treatment of naïve patients with clear cell histology and good/intermediate risk, standard treatment includes sunitinib, bevacizumab plus interferon-alpha, and pazopanib [100–103]. The use of cytokines, including high-dose interleukin-2 or sorafenib, is an alternative treatment option. Thus, these second options can be used when patients are unfit for the standard option. When patients are at poor risk, temsirolimus is recommended as standard care [104]. Sunitinib can be used as an alternative.

The recent development of immune checkpoint inhibitors may change the strategy for first-line treatment in the near future. Cancer cells are likely to escape from cell death through signaling pathways with immune checkpoint receptors, which are negative immune regulators that limit proliferation and activity of immune cells [105]. Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death 1 (PD-1) on immune cells are the best studied molecules. PD-1 interacts with PD-1 ligand 1 (PD-L1) and 2 (PD-L2) expressed on cancer cells [106, 107]. Nivolumab is a representative immune checkpoint inhibitor, which has been reported to restore antitumor immunity by blocking PD-1 [108, 109]. A phase 3 trial (CheckMate 025) showed prolongation of overall survival in patients with a

history of one or two previous regimens of antiangiogenic agents [110]. Currently, several immune checkpoint inhibitors are being investigated as first-line treatment in combination with another immune checkpoint inhibitor or molecular-targeted agent [111]. These clinical trials may change the treatment paradigm for first-line therapy in the near future.

After the failure of first-line treatment, a second-line therapy should be considered. For the patients with cytokine refractory, sorafenib, pazopanib, or axitinib is recommended as standard care after cytokine therapy [103, 112, 113]. Sunitinib is designated as an alternative option [114]. Before 2015, for patients who show disease progression with VEGFR-TKI, everolimus or axitinib is recommended as standard care for second-line therapy. Everolimus showed prolonged PFS compared to a placebo in patients who showed progressive disease with one or two VEGFR-TKIs (4.9 versus 1.9 months, $p < 0.01$) [115]. Axitinib prolonged PFS in patients who showed refractory to one prior regimen, as compared to sorafenib (6.7 versus 4.7 months, $p < 0.01$) [116]. In patients previously treated with sunitinib, PFS was prolonged by axitinib to 4.8 months compared to those receiving sorafenib at 3.4 months ($p = 0.01$). However, neither everolimus nor axitinib showed prolongation of overall survival compared to the control arm in each study [115, 117].

Recently, a dramatic paradigm shift occurred in sequential therapy after antiangiogenesis therapy. Two different agents have shown the ability to prolong overall survival of patients who had been previously treated with one or more VEGFR tyrosine kinase inhibitors. Nivolumab acts as an immune checkpoint inhibitor through blocking of PD-1. A randomized, open-label, phase 3 study compared nivolumab with everolimus in patients with renal cell carcinoma who had received previous treatment [110]. This phase 3 trial (CheckMate 025) showed a longer median overall survival with nivolumab compared with everolimus (25.0 versus 19.6 months). The HR for death with nivolumab versus everolimus was 0.73 (98.5 % CI, 0.57–0.93; $p = 0.002$). The objective response rate was also higher with nivolumab than with everolimus (25 % versus 5 %), although the progression-free survival was comparable. Another agent showing significant benefit in prolonging patient survival is cabozantinib, which is a tyrosine kinase inhibitor that targets vascular endothelial growth factor receptor (VEGFR) as well as MET [118]. A randomized phase 3 trial (METEOR) showed that treatment with cabozantinib increased overall survival, delayed disease progression, and improved the objective response compared with everolimus [119]. Overall survival of patients treated with cabozantinib was 21.4 months, and that with everolimus was 16.5 months. According to the current guideline, nivolumab or cabozantinib is a recommended agent for patients who are refractory to a previous VEGFR-TKI [120, 121].

Current issues in second-line therapy include (1) the lack of a randomized controlled study directly comparing axitinib and nivolumab or cabozantinib, to analyze the benefit for patient survival; (2) a lack of biomarkers that can define the best use of second-line therapy [122]; and (3) a lack of evidence to support the benefit or safety of third-line therapy after nivolumab. CheckMate 025 shows the

lack of a role for PD-L1 expression as a predictive factor in nivolumab therapy [110].

As mentioned above, third-line therapy after nivolumab has not been determined. After two previous VEGFR-TKIs, the results from the RECORD-1 trial provide stronger evidence for use of everolimus as a third-line agent [123]. According to CheckMate 025, overall survival of patients with nivolumab was similar to that with everolimus after two VEGFR-TKIs were used [110]. Thus, either nivolumab or everolimus would be recommended for patients after therapy with two VEGFR-TKIs.

Systemic therapy for patients with non-clear cell histology is the issue. Currently, there is no standard care for this category. Subgroup analysis of the phase 3 trial in poor-risk patients favored temsirolimus over interferon-alpha [104]. A phase 2 study with a large number of patients showed similar PFS for sunitinib to that for temsirolimus [124]. Thus, the 2012 ESMO guideline states that temsirolimus, sunitinib, and sorafenib are optional, but no standard care is designated [96]. In addition, the results of a subgroup analysis of patients with non-clear cell subtype were reported in the RECORD-3 trial in which sequential therapy with sunitinib followed by everolimus was compared to that with everolimus followed by sunitinib [122]. Sunitinib shows a longer PFS than everolimus (7.23 versus 5.09 months, HR, 1.54). This result also supports the use of sunitinib as first-line treatment for patients with non-clear cell histology.

6.3.2.3 Bone-Targeted Therapy

Renal cell carcinoma often metastasizes the bone. The bone is the second most common metastatic site after the lungs [126]. Bone metastases often result in skeletal-related events (SREs) such as pain, pathological fractures, spinal cord compression, and hypercalcemia, which may reduce the patient's quality of life [127]. Recent studies have clarified the mechanism of bone metastases; they progress through activation of osteoclasts which induce bone resorption [128]. RANKL (receptor activator of NFkB ligands), which are produced by osteoblasts in response to a variety of tumor-related growth factors, also stimulate osteoclasts via RANK receptors expressed on the osteoclasts [128]. Thus, blockade of these pathways is likely to play a role in inhibiting the progression of bone metastases.

Bisphosphonates induce the apoptosis of osteoclasts. Zoledronic acid, a potent bisphosphonate, has shown a significant benefit in preventing SREs shown by a phase 3 placebo-controlled randomized trial in patients with solid tumors who have bone metastases [129]. Lipton et al. also performed a subgroup analysis focusing on patients with RCC and showed that zoledronic acid significantly reduced the incidence of SREs and prolonged the time to first SRE [130]. The use of bisphosphonate in combination with sunitinib has been reported to improve the response rate, progression-free survival, and overall survival. [131] Combination therapy was unlikely to increase inadvertent interactions between two agents.

The RANKL-RANK pathway is also important in the formation of bone metastases through the activation of osteoclasts, which results in bone resorption and cancer cell migration [132]. Denosumab, a fully human monoclonal antibody that inhibits RANKL, was developed. Randomized trials demonstrated an equivalent potency of denosumab to zoledronic acid in preventing SREs in patients with bone metastases [133–135]. Combined analysis of these randomized trials showed the superior benefit of denosumab over zoledronic acid in preventing SRE in patients with bone metastases from advanced cancer [136].

Thus, the NCCN guideline recommends the use of bone-targeted agents for selected patients with bone metastases [3]. Osteonecrosis of the jaw (ONJ) and hypocalcemia are serious adverse events of these agents. The incidence of ONJ is reported to be 1.8 % for zoledronic acid and 1.8 % for denosumab [137]. Tooth extraction, oral infection, and concurrent use of antiangiogenic agents seem to be associated with the increased risk of ONJ [137]. Hypocalcemia occurred at higher incidence in denosumab than zoledronic acid (13 versus 6 %) [134]. The NCCN guideline recommends daily supplemental calcium and vitamin D [3].

6.3.2.4 Radiation Therapy

The role of radiation therapy for renal cell carcinoma is limited. Improved local control including pain relief was reported, but there was no survival benefit [22]. Stereotactic radiation therapy shows slight benefit in higher CR rate and duration of pain relief after procedure over the conventional extrabeam radiation therapy [138]. More recently, the benefit of stereotactic ablative body radiation therapy for the treatment of extracranial oligometastatic RCC has been reported, and early results show similar local control rates to metastasectomy [139]. These results suggest that, in the future, stereotactic radiosurgery may be indicated for patients with extracranial oligometastase who are not good candidates for metastasectomy. Further studies are warranted.

Brain metastases are lesions where stereotactic radiotherapy plays a role in local control. Most studies demonstrate a high rate of local control by Gamma Knife at 83–96 % [140]. The guideline from American Society for Radiation Oncology recommends radiosurgery alone, or whole-brain radiation therapy (WBRT) and radiosurgery, or WBRT for single metastasis, or a limited number of multiple metastases less than 3–4 cm [141]. It is noted, however, that addition of WBRT is associated with declined neurocognitive outcomes and quality of life [142, 143].

6.4 Conclusions

An overview of current treatment is described in this chapter. In systemic therapy, a major paradigm shift has occurred in second-line treatment, with the appearance of immune checkpoint inhibitors. The standard treatment options recommended by

guidelines are based on high-quality evidence from the results of randomized trials. Although minor modification needs to be considered according to the general condition or risk classification of each patient, we need to follow these recommendations as much as possible.

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Chapter 7
Natural History and Active Surveillance

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Abstract The last three decades have seen an increase in the detection of all stages of renal cancers due to widespread imaging. However, the largest increase is seen in cancers less than 4 cm. Surgery, either radical or partial nephrectomy, has been the mainstay of treatment of a small renal mass (SRM), defined as a usually incidentally discovered renal mass <4 cm in diameter which enhances on contrast imaging. There is morbidity associated with this standard of care. However, there has been a change in the management paradigm as a result of observations made on the natural history of SRMs. It is now known that 20–30 % are not cancer and histologically benign. This has been demonstrated with surgical series and with data from renal tumour biopsies which have been increasingly performed in the last decade. Even in SRMs proven to be renal cell carcinomas (SRM^{RCC}), the vast majority are low grade and grow slowly. Metastatic progression also appears to be a rare and late event. Initial active surveillance (AS) has therefore emerged as an attractive management option in patients with small renal cancers, especially in elderly patients or those unfit for surgery. Our chapter presents the natural history of the SRM^{RCC}, the current data with renal tumour biopsy and the experience with active surveillance including criteria, suggested protocols and triggers for treatment, and future directions in the changing landscape of renal cancer.

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M. Oya (ed.), *Renal Cell Carcinoma*, DOI 10.1007/978-4-431-55531-5_7

Keywords Small renal mass • Active surveillance • Renal tumour biopsy • Natural history

7.1 Small Renal Mass

For nearly 50 years since the time when Charles Robson from Toronto first demonstrated excellent outcomes with radical nephrectomy for early and locally advanced renal cell carcinoma (RCC), surgery has become the standard of treatment for suspected malignant neoplasms of the kidney [1]. The advent of newer imaging modalities, notably CT scans, in the 1970s and subsequent widespread uptake in the 1980s led to an increased detection of incidental renal masses [2, 3]. This was mirrored by an increase of clinical stage migration over time, with initial series reporting a diagnosis of 'incidentalomas' of about 7 % in the 1970s, going up to 25 % in the 1980s and 50–60 % in the 1990s [2, 4]. Currently, the detection rate of incidental renal cancers in contemporary series can be as high as 70 % [5].

Most of these asymptomatic renal masses are small renal cancers <3–4 cm in size. However, we now know that up to 20–30 % of tumours <4 cm thought to be renal cancer are actually benign. This rate increases with smaller tumours with 46 % of tumours less than 1 cm being benign [6].

The term 'small renal mass' (SRM) was popularised in 2000 which aptly describes the radiological entity as an enhancing solid renal mass <4 cm, rather than make an upfront cancer diagnosis which could be false in a third of cases [7]. When a SRM is histologically confirmed to be RCC, it is referred to as SRM^{RCC}. Whereas previously this term was mainly applied post-operatively, nowadays this diagnosis can be made with renal tumour biopsy (RTB) before making a treatment decision. This also lays the foundation for possible active surveillance (AS) strategies in suitable patients.

The natural history of these SRMs was not known well previously. In the 1940s, Bell postulated in his observations of renal tumours that any lesion <3 cm in size should be regarded as a benign adenoma, due to their indolent behaviour [8]. In the 1970s, Bennington rejected this theory and stated that most of these small lesions were not adenomas but merely small RCCs [9]. However, retrospective studies on SRMs since then showed that tumours less than 3 cm seldom metastasised and had a slow growth rate [10–12].

7.2 Active Surveillance

Traditionally due to the stigma associated with a diagnosis of cancer of a fear of death, clinicians and patients have sought aggressive treatments for this disease. More recently, due to advances in diagnostic and therapeutic medicine, we have

understood the natural history of various cancers and now know that many cancers are diagnosed that are not inevitably fatal. As a result, active surveillance (AS) has emerged as a management option for such cancers with an indolent behaviour in many patients. This strategy has averted overtreatment of various localised cancers, such as prostate, breast, kidney and thyroid, all of which could bear a cost to the patient as well as to global health systems [13]. Even where intervention is deemed necessary, radical treatment options have seen an evolution of organ- and function-preserving treatment options. For example, breast cancer was treated with a radical mastectomy a century ago, which was quite a disfiguring and disabling procedure associated with significant morbidity. This was subsequently modified to a less radical surgery, and nowadays, suitable patients get offered a lumpectomy. The same has occurred with radical and partial nephrectomy for RCC. However, many patients prefer the option of altogether deferring treatment until it appears necessary where intervention does not appear to alter the ultimate oncological outcome, as this is associated with a lesser morbidity than even the least invasive intervention.

Active surveillance is defined as closely monitoring the disease actively for any signs of progression with the option of delayed curative treatment before size precludes local therapy or metastatic progression occurs which in the case of RCC is usually fatal. Often AS evolves into watchful waiting where the default treatment may not necessarily be a curative treatment, as happens in an aging population with competing comorbidities and where radical treatment may be hazardous. Before offering AS as a management option to patients with RCC, clinicians have to be cognisant of the evidence and limitations, described below, and have a discussion with the patient about the pros and cons of surveillance versus initial treatment.

With the advent of more personalised medicine, the future holds promise of further developments to identify those patients in whom AS will fail due to rapid progression.

7.2.1 The Evidence for Active Surveillance for Small Renal Masses

The initial evidence for AS for SRMs comes from small series of retrospective data based on observations that unbiopsied small renal tumours were noted to have slow growth rates on serial imaging. A retrospective study by Birnbaum and colleagues of 13 SRMs of $\leq$3 cm in 11 patients followed up over a period of up to nearly 8 years showed an average growth rate of 0.5 cm/year. Seven of the 11 patients underwent surgery, and the histology showed low-grade RCCs in the majority [11].

A subsequent series, also retrospective, by Bosniak on 37 patients with 40 parenchymal renal tumours followed up for a mean of 3.25 years showed a growth rate of 0.36 cm/year with no patient developing metastases [12].

The first prospective study of the natural history of SRMs was performed by Rendon and Jewett in a small series of 13 patients who were either unfit for or declined surgery and showed that most SRMs grow at a low rate or not at all. They used tumour volume in addition to maximal axial diameter, with an average growth rate of 0.216 cm/year at a median follow-up of 3.5 years [7]. Again, no patients developed metastases in this series, although the authors concluded that those renal tumours that are destined to metastasise appear to do so early. This was followed by an update on 32 masses in 29 patients by the same group followed prospectively for a median of 2.3 years, which again showed that in the majority of patients, the average growth rate did not differ statistically from zero growth [14]. One-third of cases proceeded to surgical excision, either due to an accelerated growth rate or patient choice; however, no patient experienced progression to metastatic disease.

Several studies were performed subsequently. A study from Japan on 18 patients who were observed for 12 months and then underwent surgery also confirmed that most small RCCs are slow-growing and that the growth rate correlates with apoptosis and grade [15]. The concept of observing renal masses has also been extended to larger masses in patients who were elderly and had severe comorbidities and therefore medically unsuitable for nephrectomy in a Scottish series of 36 patients by Lamb and colleagues. The mean age was 76 years with a median tumour size of 6 cm, with an average growth rate of only 0.39 cm/year, showing that observation in such patients is a safe option. Only one case of metastatic progression was evident at a median follow-up of 2 years [16]. This notion of observing renal masses in the elderly was further supported by a series of 110 patients aged 75 or more with enhancing renal masses who were observed for a median of 2 years. None of the patients who had a median age of 81 years died from their renal mass. The mean tumour growth rate was 0.26 cm/year, with only 3.6 % of patients receiving treatment due to increasing lesion size [17]. However, there was no histological diagnosis from biopsies, unlike Lamb's series where two-thirds of patients were biopsied, with the vast majority showing RCC.

A meta-analysis of 10 studies on observed SRMs comprising 234 patients by Chawla and colleagues in 2004 showed a mean growth rate of 0.28 cm/year at a median follow-up of 2.8 years [18]. The mean lesion size was 2.6 cm. However, only 46 % of cases had a confirmed pathological diagnosis, of which 92 % was RCC [18]. The metastatic progression rate from this meta-analysis was 1 %. A subsequent meta-analysis of 6471 SRM lesions comparing nephron-sparing surgery, thermal ablation and observation showed no statistical differences in the incidence of metastatic progression regardless of whether the lesions were excised, ablated or observed [19].

As the momentum for AS for SRMs picked up after these initial observations, studies with larger patient numbers were done. A series of 172 renal tumours in 154 patients who were observed for a median of 2 years showed a low metastatic progression of 1.3 % [20]. A multicentre prospective trial of AS of 209 SRMs in 178 patients who were elderly or infirm showed local progression in 12 % with metastatic progression ion 1.1 % at a median follow-up , with an average growth rate of 0.13 cm/year, with no difference between biopsy-proven malignant and

benign lesions. Needle core biopsies in 101 lesions confirmed a malignant and benign diagnosis in 55 % and 12 %, respectively, with 33 % being non-diagnostic. About 26 % and 10 % of observed SRMs showed regression or zero growth in size, respectively [21].

A systematic review and pooled analysis of 880 patients with 963 masses again showed that a substantial proportion of SRMs remained static after initial AS. Progression to metastases occurred in only 2 %, generally as a late event. Delayed intervention in patients suitable to receive this could be offered to those that demonstrated significant linear or volumetric growth [22].

A retrospective study by Patel and colleagues from Oxford on 202 patients with SRMs managed by AS versus surgery showed no significant difference in overall or cancer-specific survival at a median follow-up of 2.8 years. The mean growth rate was 0.21 cm/year for observed masses, with 53 % of SRMs demonstrating negative or zero growth [23].

The recent study from the multi-institutional Delayed Intervention and Surveillance for Small Renal Masses (DISSRM) prospectively enrolled 497 patients with solid SRMs <4 cm in size who chose either AS or primary intervention. Whereas overall survival (OS) at 2 years was no different, patients undergoing AS had a worse 5-year OS of 75 % compared with 92 % for primary intervention. However, there was selection bias as patients who chose AS were older and had more comorbidities and worse performance status. Interestingly, the 5-year cancer-specific survival was 99 % for intervention and 100 % for AS [24].

So far, as discussed, the evidence for AS as a reasonable and sound strategy for elderly and infirm patients unsuitable for intervention has come largely from observational series, both retrospective and prospective, with a few meta-analyses or systematic reviews of the same. Some studies, also observational series, have looked at the merits of delayed intervention in those fit to receive them in patients demonstrating rapid interval growth and found initial AS as a relatively safe option. Whether AS is a safe strategy for all SRMS remains to be confirmed with evidence by way of randomised trials which is currently lacking.

The one challenge that all clinicians face is defining progression for patients on AS. Currently there are no reliable markers of progression that would help predict which SRMs will metastasise, which is relevant in patients who may benefit from curative interventions [22]. The uptake of renal tumour biopsies may help to better define the nature of SRMs where a diagnosis is made and the grade of tumour where a malignant diagnosis is made. This is discussed in detail in Sect. 7.3.

7.2.2 Prognostic Factors for Progression and Treatment Triggers

Currently, apart from the development of metastases, there is no validated confirmatory clinical or molecular marker of progression of a SRM-RCC on AS. The rate

of metastatic progression has been demonstrated to be as low as 1.1 % in the prospective Canadian trial [21] and 2 % in a pooled analysis and systematic review of 880 patients [22]. Where biopsy is diagnostic, the type and grade of RCC can help prognosticate and tailor follow-up protocols. Unlike prostate cancer, where a repeat biopsy for men on AS can help identify grade progression, this is not routinely done for SRMs. Hence, clinical stage progression on surveillance imaging coupled with growth rate is used as a surrogate for progression.

7.2.2.1 Patient Age

Age is important when selecting patients for AS or watchful waiting. The majority of patients with sporadic RCC present after the sixth decade of life. The presence of comorbidities is proportionate to increasing age and performance status and general fitness inversely proportionate. This would clearly impact on the choice of treatment. AS of SRMs is currently recommended for patients with a reduced life expectancy or in those deemed unfit for surgical treatment. In some selected cases, minimally invasive treatments such as thermal ablation by cryotherapy or radiofrequency ablation could be offered.

Several studies looking at treatment of localised renal masses in the elderly have shown similar outcomes. In one such study, 537 patients with localised renal tumours $\leq$7 cm detected at age $\geq$ 75 years were investigated whether surgical intervention improved survival compared with AS and found that at multivariate analysis, the only predictors of survival were age and comorbidity [25]. Treatment type did not alter overall survival, with the majority of patients succumbing to cardiovascular events (29 % compared to 4 % from cancer progression) [25].

In another study, 63 patients with a mean age at diagnosis of 76.6 years with renal masses were observed. For SRMs, the 5-year cancer-specific survival rate was 100 %, although histological diagnosis was only made in 18 out of the 63 cases, of which 83 % were RCC [26]. This study showed that the risk progression was higher in larger masses $>$ 4 cm. In contrast, a study from Scotland also in elderly patients showed no difference in the size of masses and disease progression. This series by Lamb and colleagues on 36 patients with a mean age of 76.1 years with renal masses with a mean size of 7.2 cm managed conservatively showed that no patient died from their cancer or from any evident progression of their disease at a median follow-up of 2 years [16].

Age has been found to be a strong predictor of mortality in population-based studies and competing risks of death, as seen in a SEER database study of 30,801 patients with localised RCC, which showed that mortality rates attributed to non-cancer deaths were highest at 11 % compared to death from other cancers (7 %) and RCC (4 %) [27]. Age was strongly predictive of mortality especially non-RCC deaths ($p < 0.001$) [27].

On the other hand, when it comes to age as a predictor of progression of SRMs, Kouba and colleagues showed in their series of 43 patients that age was the strongest predictor of tumour growth, with younger patients (60 years or younger)

showing more rapid growth rates (mean 0.90 cm/year) compared to older patients (mean 0.60 cm/year) [28]. They also showed an inverse correlation between increasing age and tumour growth rate in a meta-analysis of previously done studies [28]. As such, AS is not recommended currently as a management option of sporadic SRM^{RCCs} in the younger population. In the VHL population, where patients have multiple tumours at a young age, AS is suggested until tumours reach a size of 3 cm, based on a study done by Duffey and colleagues on 108 patients with a median age of 32 years. None of the cases developed metastases where the dominant tumours were <3 cm at a follow-up of nearly 5 years, compared to 27.4 % of cases showing metastatic progression in those with tumours larger than 3 cm [29].

7.2.2.2 Initial Tumour Size

Earlier studies of observed small renal tumours defined an axial diameter of ≤3 cm at baseline, which was consistent with the AS practice in the VHL population. However, when the term SRM was popularised in 2000, the size adopted was ≤4 cm, as there was little difference in outcomes after nephrectomy for small RCCs of 3, 4 and >5 cm tumours. Also a very small diameter left little room for growth on AS before triggering treatment [7]. A study by Frank and colleagues on size and pathology of renal tumours showed that a smaller renal tumour had a higher chance of being benign. In 2770 patients who underwent surgery for localised RCC, the overall benign rate was 12.8 %. However, it was as high as 46.3 % for tumours with a size of <1 cm, dropping to about 20 % for all tumours <4 cm, and only 6.3 % if larger than 7 cm. In SRMs confirmed to be RCC, they were more likely to be low grade with smaller tumours [6]. A study by Crispen on 172 renal tumours showed that initial size was inversely related to growth rate, with smaller tumours exhibiting significantly faster volumetric growth compared to larger tumours [20].

Increasing tumour size of incident tumours has been shown to increase the risk of biopsy-proven metastatic disease according to a study by Kunkle who predicted this to be 22 % for every 1 cm increase [30]. However, another study showed no relationship between initial tumour size and subsequent metastatic risk [31]. Population level studies that correlate tumour sizes with metastatic rate are potentially misleading as they refer to incident tumours and not increased growth rate of tumours on AS [32].

In the VHL population, a size of 3 cm is a trigger for treatment based on the increased risk of metastatic progression in tumours >3 cm in size [29]. However, in the elderly population, even larger tumours with a median size of 7.2 cm (3.5–20 cm) observed for 2 years have shown no metastatic events [16]. A series of 548 RCCs ≤4 cm found a significantly higher rate of pT3 stage and higher more grade 3 in tumours between 3.1 and 4 cm compared to tumours ≤3 cm [33]. Patel and colleagues suggested a cut-off of 3.5 cm as a trigger for intervention, based on their findings of a higher T3a rate of 25 % in SRMs >3.5 cm compared to 7.6 % for

those ≤3.5 cm. However, both these studies were retrospective and in mostly treated patients. The Canadian multicentre trial of AS on SRMs which was a prospective trial included SRMs ≤4 cm and showed that initial size was a prognosticator of worse outcome in the few cases that progressed [21].

7.2.2.3 Growth Rate

Growth rates can be measured as a change over time in either the maximal axial diameter or tumour volume. This is thought to have prognostic importance as faster-growing tumours may have a more aggressive biological behaviour. There is no universally agreed protocol for AS of SRMs. According to the Canadian multicentre trial, follow-up with serial imaging by any one modality (ultrasound, CT or MRI) is necessary, initially at 3 and 6 months, followed by 6-monthly surveillance for 3 years. If there is stability in the size of the lesion, annual surveillance is recommended thereafter [21].

Growth rates have been shown to vary between studies, but are by and large slow in most AS series. A meta-analysis of 286 SRMs in 234 patients managed by AS in 2006 showed a growth rate of 0.28 cm/year [18]. A further pooled analysis of 880 patients showed this to be 0.31 cm/year. The prospective Canadian trial of 209 SRMs in 178 patients showed a growth rate of 0.13 cm/year. Interestingly, this showed no difference in biopsy-proven malignant SRMs compared to benign SRMs at 0.14 cm/year versus 0.17 cm/year, respectively ($p = 0.8$) [21]. In this study, 63 % of SRMs grew at a mean of 0.26 cm/year, but by their progression criteria, only 12 % progressed to either reaching a size of >4 cm or doubling of initial tumour volume [21].

Most series have also reported that a number of SRMs observed over time show zero growth, and indeed some may even decrease in size. This may be explained partly by measurement error and interobserver and intraobserver variation, but these findings suggest that some RCCs may truly regress over time, something noted in other cancers as well. In the Canadian prospective trial, 26 % of SRMs actually decreased in size, while 10 % showed no growth [21]. Kunkle and colleagues showed similar rates of zero net growth between 26 and 33 % [34]. A pooled systematic review reported a zero net growth in 23 %, none of which progressed to metastases [22]. In the Oxford series by Patel of 71 patients on AS, 53 % of SRMS had zero or negative growth [23].

7.3 Role of Renal Tumour Biopsy

Renal tumour biopsies (RTBs) have been proposed to identify the histology of small renal masses (SRMs) preoperatively [35, 36]. RTBs may be performed using fine-needle aspiration (FNA) or using core biopsies. The latter are usually performed through a coaxial sheath nowadays. The accuracy of core biopsies has

been shown to be generally superior to that of FNAs [37]. Several studies have evaluated the outcome of RTBs and found a diagnostic rate that varied from 70 to over 90 % with a benign rate reported in 20–30 % of cases [35]. In the largest series presented so far ($n = 496$ biopsied masses), the group from Toronto have demonstrated a 92.9 % overall diagnostic rate [36]. Tumour size, solid consistency and a predominantly exophytic location were factors associated with a greater diagnostic yield [35, 36].

Despite their high diagnostic rates, the usefulness of RTBs has been challenged in the field partially because of questionable diagnostic accuracy. However, the rates of accurate histological subtyping have been shown, in a recent literature review, to vary between 86 and 98 %, albeit rates of accurate grading were shown to be generally lower [38]. Nevertheless, the Toronto group recently reported a 96.1 % concordance rate with surgical pathology when grades were pooled into low and high grade [36]. In addition of being a useful tool, RTBs have been shown to have low complication rates, with serious adverse events reported in less than 1 % of cases [35, 36, 38]. More importantly, needle tract seeding following RTBs has been seldom reported in the literature, and no cases have been reported since 1993 [39].

Despite their potential benefit, RTBs are still not widely applied in urologic practice because of concerns which have generally shown to be exaggerated [40]. Given their low complication rate, high rate of benign lesions among SRMs and high reliability, RTBs should be considered to aid in the management decision of SRMs.

7.4 Future Direction

Knowledge of the natural history of RCC has evolved over the last few decades and continues to do so. It has been known for decades that small renal tumours are largely indolent. Initial AS therefore remains a suitable management option in patients with SRMs, particularly in those unfit to receive primary intervention. In those in whom intervention is feasible, true AS may be offered initially with minimal harm as supported by reports of series that have offered delayed intervention. Most studies have a relatively short-term of follow-up, and more long-term and mature data is awaited.

Further research is also needed in form of randomised controlled trials whether AS is a viable option for SRM^{RCCs} in all patients, as well as in form of personalised medicine where biomarkers from renal tumour biopsies or serum markers can aid in predicting which patient will progress in order to tailor treatment recommendations. The issue of tumour heterogeneity and RTB also needs to be addressed when sampling tumour biopsies. More precise stratification by patient risk factors and tumour factors will further aid clinicians and patients in shared-decision making in the management of the small renal mass.

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Chapter 8
Surgical Treatment for Renal Cell Carcinoma

Dae Y. Kim, Jose A. Karam, and Christopher G. Wood

Abstract Systemic therapies for renal cell carcinoma have made modest improvements in patient survival but rarely offer durable cure. Thus, surgical excision of renal cell carcinoma is an integral component of oncologic management. The spectrum of renal cell carcinoma presentation from small renal masses, locally advanced disease, and in the presence of metastasis varies with the surgical armamentarium needed to treat this diverse group of patients. In general for small renal masses, a nephron-sparing approach is preferred if it can be completed safely with negative margins, and for locally advanced tumors, radical nephrectomy is preferred with excision of the affected kidney, lymph nodes, and venous thrombi if present. With metastatic disease, cytoreductive nephrectomy has been shown to prolong survival in carefully selected patients, usually with good performance status and with oligometastasis. The surgical nuances, indication, and motivation for each surgical technique will be discussed in this chapter.

Keywords Kidney cancer • Renal cell carcinoma • Nephrectomy • Partial nephrectomy • Locally advanced kidney cancer • Cytoreductive nephrectomy

8.1 Introduction

The surgical management of kidney disorders was first described by Hippocrates (B.C. 460) where in his works, he mentions "small stones like sand" cause pain and by incising into the kidney the evacuation of pus can be undertaken to relieve the kidney of the abscess and the inciting matter [1]. The first modern surgical removal of the kidney or nephrectomy is credited to Gustav Christoph Jakob Friedrich Ludwig Simon of Germany who performed the first successful procedure on Margaretha Kleb on August 1869. She had a ureteral-vaginal fistula that was unable to be closed on three previous attempts, and a nephrectomy was performed using lumbar access. She was able to leave her bed on day 28 and was discharged after

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M. Oya (ed.), *Renal Cell Carcinoma*, DOI 10.1007/978-4-431-55531-5_8

2 months [2]. Thereafter from 1870–1879, it is documented that 12 nephrectomies for "tumor" were performed with 7 mortalities. Since the first description of the radical nephrectomy, refinements in surgical technique and technological advances have evolved the treatment of the renal mass. Robson et al. in 1969 described a series of 88 patients with removal of the kidney, overlying fat, and regional lymph nodes with 5-year survival rates reported as 66 % when confined to the kidney and 42 % with lymphatic involvement [3]. With nephron-sparing surgery and the introduction of minimally invasive techniques, progressive improvements in patient mortality and morbidity have been observed, heralding the current management of the renal mass. This review will focus on the surgical management of renal cell carcinomas (RCCs) from small renal masses (SRMs) and localized disease to locally advanced and in the setting of metastasis.

The classical presentation of RCC described as flank pain and hematuria with a palpable mass is now uncommon in developed countries with a stated incidence of less than 10 % [4]. A variety of findings may signify RCC, but there is not one pathognomonic finding that defines an RCC diagnosis. Furthermore, the widespread use of cross-sectional abdominal imaging with computed tomography (CT), magnetic resonance imaging (MRI), and ultrasound (US) has propagated the detection of SRMs that is usually performed for symptoms unrelated to RCC. Currently, most SRMs are discovered incidentally [5] and account for over half of all RCC diagnosis, and while the majority are malignant, between 7 and 33 % have been reported as benign [6]. SRMs are generally defined as $\leq$4 cm, corresponding to tumor stage T1a according to the 2010 TNM staging system for kidney cancer (AJCC) [7]. The distribution of RCC stage at presentation has migrated mainly due to the increased use of cross-sectional imaging, and while the majority are discovered as localized disease, approximately 20 % are stage IV disease [8]. This mixed group of patients require careful risk-benefit counseling as the goals of treatment and complication profile are varied for active surveillance, partial nephrectomy (PN), and radical nephrectomy (RN).

Locally advanced RCCs describe a stage where the tumor may extend beyond the kidney continuously, as with venous thrombus (VT), local nodal involvement, and extension through Gerota's fascia with or without invasion of adjacent structures. As tumors progress from localized disease to locally advanced and metastatic disease, there may be an increased manifestation of clinical signs and symptoms. For locally advanced disease with VT, lower extremity edema and varicoceles may be present due to obstruction of venous return. It is estimated that 4–10 % of RCC [9] will demonstrate venous involvement in RCC, and the level of VT as a prognostic marker has been controversial [10], motivating the reclassification of the TNM staging system in 2010. The current TNM staging system divides VT level involving the renal vein as T3a, VT within the infradiaphragmatic inferior vena cava (IVC) as T3b, and VT within the supradiaphragmatic IVC or invading the IVC wall as T3c. The 5-year survival is reported as 43.2 %, 37 %, and 22 % for T3a, T3b, and T3c, respectively [11]. The surgical management of locally advanced RCC especially with VT above the diaphragm may require additional surgical

expertise including vascular, hepatobiliary, and cardiothoracic surgeons with bypass to facilitate the safe and complete removal of VT.

It is estimated that as high as one-third of patients will have RCC metastasis at initial presentation and 40 % will have RCC recurrence after treatment of their localized primary [12, 13]. The common sites of RCC metastasis are in the lung, bone, lymph nodes, liver, brain, pancreas, and thyroid [14]. These deposits may manifest symptoms such as seizures and also as pathological fractures for bony involvement. RCC may also secrete endocrine factors causing paraneoplastic syndromes such hypercalcemia and polycythemia and symptoms such as fever and cachexia. The removal of the RCC primary in the setting of metastasis is termed cytoreductive nephrectomy (CN) and has been motivated by two prospective randomized trials demonstrating a survival benefit of CN and interferon-alpha2b versus interferon-alpha2b alone [15, 16]. The selection of patients who would benefit from CN is based on prognostic risk stratification and markers in metastatic RCC (mRCC), details of which are covered in the next chapter. The discovery of metabolic pathways altered in RCC has paved the foundation for therapies that target the vascular endothelial growth factor (VEGF) and the mammalian target of rapamycin (mTOR) pathways. Although objective responses are seen with targeted therapy, complete responses are exceedingly rare, and the management of RCC in the setting of metastasis still remains a surgical disease when feasible.

8.2 Small Renal Masses

Since SRMs are a heterogenous group of benign or pathological masses, the concern for malignancy may motivate further diagnostic imaging, biopsy, and ultimately treatment. In general, SRMs are defined as $\leq$4 cm and confined to the kidney. The benefits of US include the absence of nonionizing radiation, the ability to perform in the office or outpatient setting, and the ability to differentiate between simple cysts and solid, vascular masses – a sign of malignancy. However, further anatomic detail of the tumor landscape is rather limited with US, and CT with contrast is able to characterize the internal enhancement along with details of the vascular anatomy of the kidney. MRI provides similar advantages to CT in providing SRM characterization and the adjacent landscape of structures for the contrast adverse. As many patients with RCC need serial imaging, thereby increasing the potential risk for secondary malignancies, cross-sectioning imaging in the absence of nonionizing radiation may be of benefit.

8.3 Renal Mass Biopsy

The role of renal mass biopsy (RMB) is currently being investigated, and its role in the management of SRM remains controversial. RMB is usually reserved for patients contemplating life-prolonging treatment dictated by the histopathological characterization of the biopsy via targeted therapies, local ablation, and surgical extirpation. In the setting of metastatic disease, RMB will confirm primary RCC versus a nonrenal origin of neoplasm. Patients should have adequate functional status and life expectancy, whereby the benefits of treatment outweigh the risk of RMB. Contemporary series report a low complication rate with risks of bleeding and hematoma, pneumothorax, and pseudoaneurysm formation and the potential risk of needle tract seeding with tumor [17–19]. Several technical points should be considered in RMB such as potential for a nondiagnostic sample with a reported range between 3 and 22 % [20, 21], with marked improvement in diagnostic ability in recent years. Repeat RMB may be advocated or surgical excision can be considered if the ability to obtain a diagnostic sample would be difficult secondary to the location of the mass or if repeat biopsy is considered to be a continued challenge. Another consideration is the diagnostic accuracy of the RMB with the final pathology with accuracies greater than 90 % reported in recent series [22]. In summary, RMB has a low complication rate and should be considered if the biopsy results would radically alter management of the renal mass.

8.4 Management of SRM and Localized RCC

The current management for SRM and localized RCC include (1) active surveillance, (2) partial nephrectomy, (3) radical nephrectomy, and (4) ablation. A broad spectrum of risk and benefit with variable rates of cancer control and cure rates are seen with each option. The natural history of SRMs and the role of active surveillance are covered in the previous chapter. Surgical excision is currently the recommended treatment of choice for localized RCC with partial nephrectomy when technically feasible and radical nephrectomy reserved for larger tumors that are central in location and adjacent to hilar structures, if not amenable to partial nephrectomy. Partial nephrectomy is also recommended for genetic disorders such as von Hippel-Lindau syndrome which predispose to RCC and where repeated surgical treatments are needed. Ablative techniques with various modes of energy, including cryo-, radiofrequency, and microwave ablation, are generally reserved for patients with comorbidities prohibiting or unwilling to undergo surgical removal of the tumor. These techniques are generally performed percutaneously with general and even local anesthesia, best suited for posteriorly located SRMs.

8.4.1 Partial Nephrectomy

PN is performed using open, laparoscopic, or robotic techniques. The 10-year metastasis-free estimates have been reported to be greater than 90 % for T1 tumors with both open and laparoscopic approaches [23]. On multivariable analysis, factors associated with metastasis were larger tumor size, an absolute indication for PN, and comorbidity with no significant difference noted between open and laparoscopic PN (p=0.32) [23]. The benefit of PN is the preservation of nephrons leading to a decreased risk of renal insufficiency, as renal insufficiency is associated with other secondary morbidity and mortality-causing events. In a retrospective series of 662 patients, the probability of freedom from new-onset renal insufficiency after PN was 80 % versus 35 % after RN with RN identified as an independent risk factor for new-onset renal insufficiency [24]. The renal function outcomes were recently reported for a prospective, randomized study comparing RN and nephron-sparing surgery (NSS) [25]. The estimated glomerular filtration rate (eGFR) <60 for NSS was reached in 64.7 % compared to 85.7 % in RN patients after a median follow-up of 6.7 years.

PN can be performed using a retroperitoneal or transperitoneal approach depending on the location of the tumor and surgeon preference. The technique that is traditionally described for open partial nephrectomy is a flank approach with the patient positioned in the lateral decubitus position or full flank position and an incision extending from the tip of the 11th rib providing safe and adequate exposure to the retroperitoneum. The hilum is dissected, and vascular structures are identified for clamping the artery and/or vein to decrease bleeding during tumor excision. For smaller, exophytic tumors, a clampless technique can be potentially utilized. If the collecting system is entered, absorbable sutures are used to close the collecting system, and figure-of-eight absorbable sutures are used for small vessels. The renorrhaphy is completed by closing the capsule with figure-of-eight absorbable sutures, and dependent on surgeon preference, a hemostatic agent can be applied. Other incisions used in open partial nephrectomy are subcostal, midline, thoracoabdominal, and dorsal lumbotomy approaches which are dictated by tumor location and patient body habitus.

The first laparoscopic PN is credited to Winfield et al. in 1992 in a woman presenting with a calyceal diverticulum and stone [26]. This technique was then first reported for renal tumors by Mcdougall et al. in 1993 with a wedge resection of an oncocytoma using laparoscopy [27]. Since these initial reports, minimally invasive procedures have shown varied benefit and, in general, have decreased analgesic requirements, less estimated blood loss, and shorter hospital stays while demonstrating similar cancer-specific survival [28, 29]. The context of these benefits must be weighed with the cost-effectiveness and capital investment of minimally invasive approaches along with specialized training and learning curve needed to become adept at approaching complex tumors with equal oncologic control as the open approach.

As for the surgical approach, the patient is positioned in a modified flank position with the camera port placed laterally or through the umbilicus. Working ports are placed in the subcostal area (1) and the other lateral and caudal (2) to the camera port to triangulate around the affected kidney. The assistant ports are placed in the periumbilical area, and a third assistant or laparoscopic/robotic port can be placed either lateral or medial to port 2. The operative steps are similar to the tranperitoneal approach to open PN. The large bowel is reflected medially, and the ureter/gonadal vessel can be used to assist in identifying the hilum. For right-sided tumors, the Kocher maneuver mobilizes the duodenum away from the medial kidney to expose the hilar structures. Ultrasonography is typically used to localize the tumor and mark out the margins for resection. Hilar clamping is performed using laparoscopic bulldogs, and after clamping, the tumor is excised using cold shears. For closure, interrupted figure-of-eight suturing can be used; however, techniques and tools such as Lapra-Tys and the sliding-clip renorrhaphy using hemolock clips have been developed for laparoscopic and robotic surgery to close the excised tumor bed [30, 31]. The developments of these techniques have facilitated decreasing ischemia time and blood loss. Laparoscopic and robotic PN via a retroperitoneal approach has also been described and may be suited for posteriorly located renal tumors or in patients with multiple abdominal surgeries.

8.4.2 Renal Scoring System (Nephrometry)

The variability of tumor location (anterior/posterior, upper/lower pole) and its proximity to hilar structures dictate the anatomic complexity and difficulty in performing a PN. Contemporary scoring and descriptive systems that have been developed to describe these features include mainly the following (Table 8.1): (1) RENAL nephrometry score [32], (2) PADUA classification [33], (3) C-index [34], (4) DAP system [35], and the (5) zonal NePhRO scoring system [36]. The RENAL nephrometry score uses radius of tumor, exo-/endophytic properties, nearness to collecting system/sinus, anterior/posterior location, and location to polar line to quantify a score of complexity as low, medium, and high. The PADUA classification scores tumor size, renal sinus and collecting system involvement, exophytic rate, polar location, and tumor (lateral/medial) rim location. The C-index is a centrality scoring system calculated using the Pythagorean theorem to determine tumor distance to kidney center. The DAP system integrates (D)iameter of tumor, (A)xial distance, and (P)olar distance to report nephrometry. The zonal NePhRO system uses four components, (N)earness to collecting system, (Ph)ysical location (lower, lateral, collecting system location), (R)adius of tumor, and (O) rganization (exo-/endophytic) to describe complexity of renal tumor. Although each nephrometry scoring system measures the anatomic location of kidney tumor to the complexity of excision using its own unique method, validation with clinical variables and survival characteristics is yet to be determined in large multi-institutional cohorts.

Table 8.1 Major renal nephrometry scoring methodologies

System	Variables	1 pt	2 pts	3 pts
RENAL	Radius (maximal diameter)	R ≤ 4 cm	4 < R ≤ 7 cm	R ≥ 7 cm
Low complexity: 4–6	Exo-/endophytic	≥50 %	<50 %	Endophytic
Moderate complexity: 7–9	Nearness to collecting system/sinus	N ≥ 7 mm	4 < N < 7 mm	≤4 mm
High complexity: 10–12	Anterior/posterior: a, anterior; p, posterior; x, not determined	–	–	–
h suffix if mass touches the renal artery/vein	Location relative to polar line	Entirely above or below polar line	Crosses polar line	>50 % of mass is across polar line or mass crosses the axial midline, or mass is between polar line
PADUA	Tumor size	≤4 cm	4.1–7 cm	>7 cm
Low complexity: 6–7	Renal sinus involvement	Not involved	Involved	–
Moderate complexity: 8–9	Collecting system involvement	Not involved	Involved	–
High complexity: ≥10	Exophytic rate	≥50 %	<50 %	Endophytic
	Polar location	Superior/inferior	Middle	–
	Tumor rim location	Lateral	Medial	–
DAP	Diameter of tumor	<2.4 cm	2.4–4.4 cm	>4.4 cm
	Axial distance	>1.5 cm	≤1.5 cm	Overlap
	Polar distance	>2 cm	≤2 cm	Overlap
Zonal NePhRO	Nearness to collecting system	Mass touches cortex	Mass touches medulla	Mass touches collecting system or crosses renal sinus
Low risk: 4–6	Physical location	Lower pole below collecting system	Lateral to but not touching collecting system	Upper pole or touches collecting system
Intermediate risk: 7–9	Radius of tumor (diameter)	<2.5 cm	2.5 ≤ R < 4 cm	≥4 cm
High risk: 10–12	Organization (exo-/endophytic)	>50 % exophytic	50 % endophytic 75 % exophytic	>75 % endophytic

(continued)

Table 8.1 (continued)

System	Variables	1 pt	2 pts	3 pts
C-index score:	Centrality index scoring	No point system but calculation of centrality index by the following:		
0 = mass concentric to kidney center		Cross-sectional imaging and Pythagorean theorem to calculate distance from tumor center to kidney center. Division with tumor size to obtain centrality index		
1 = periphery touching kidney center				
Larger index = increased distance to kidney center				

8.4.3 Radical Nephrectomy

Complete excision by PN is preferred for SRMs in healthy individuals. In 2009, the American Urological Association (AUA) presented guidelines for clinical T1 renal masses listing radical nephrectomy (RN) as a viable treatment option for patients where PN is not technically feasible. In a multi-institutional study (EORTC 30904) [29], patients randomized to either nephron-sparing surgery (NSS) or RN for renal tumors $\leq$5 cm showed that the 10-year overall survival (OS) was 75.7 % for NSS compared to 81.1 % for RN (p = not significant). The 10-year progression rate for NSS was 4.1 % and for RN was 3.3 % (p = 0.48). The NSS group had a slightly higher rate of complications with pleural and splenic injury, bleeding, and urine leaks. Approximately 85.7 % of patients who underwent RN had renal dysfunction with an estimated glomerular filtration rate (eGFR) <60, compared to 64.7 % of patients after NSS at a median follow-up of 6.7 years. For advanced kidney disease (defined as eGFR <30), 10 % of RN and 6.3 % of NSS patients reached this point, and about 2 % of patients in each group demonstrated extreme renal dysfunction (eGFR <15). Thus, the decreased moderate renal dysfunction seen with NSS did not demonstrate a survival benefit in this group of patients for this follow-up time period.

The removal of the whole kidney for a peripherally located/exophytic SRM theoretically seems to remove an excess amount of normal kidney parenchyma unnecessarily. The surgeon should consider patient age and comorbidities, life expectancy, and oncologic goals of treatment when considering RN versus a nephron-sparing approach. Although, EORTC 30904 did not demonstrate a survival benefit in patients with clinical T1 masses who had NSS during follow-up, the higher rates of moderate renal dysfunction in RN patients may increase progressive renal insufficiency requiring dialysis along with its associated risk factors such as cardiovascular events after longer follow-up time periods [24, 25]. From a technical

approach, RN for SRMs can be performed by laparoscopic or open surgery with similar steps as described above for PN with hilar vessel control and division. In general, RN should be reserved for masses whereby NSS is not easily possible and where the expeditious removal of the kidney facilitates recovery in patients with marginal surgical candidacy.

8.5 Locally Advanced Disease

Surgical excision of locally advanced RCC requires careful planning, patient optimization, and coordination of medical specialists and urologic surgeons. As in SRMs, the oncologic goals of locally advanced RCC are identical, to provide the greatest survival benefit with palliation of clinical symptoms, with the lowest morbidity possible. The definition of locally advanced RCC is typically defined as $\geq$T3 in the absence of distant metastasis [37]. For the surgical excision of RCC with concomitant thrombectomy in M0 (nonmetastatic) patients, the reported median survival range from 35 to 116 months with the 5-year CSS ranging between 40 and 65 % [38, 39]. For metastatic and T4 disease, the 5-year CSS is significantly lower ranging between 6.5 and 19 % [39, 40]. In comparison, the natural course or untreated RCC with VT is rather dismal with a recent Surveillance, Epidemiology, and End Results (SEER) database study reporting a median survival time of 5 months and a 1-year DSS as 29 % [41]. In this study, patients are of advanced stage and of poor performance status prohibiting primary surgical treatment. However, a subset analysis of nodal and metastasis-free (N0, M0) patients in this study demonstrate a significantly longer median survival of 14 months. Thus, the complete excision of RCC with concomitant VT removal may significantly increase survival [42].

The surgical excision of locally advanced RCC is more invasive compared to techniques developed for SRMs with surgical maneuvers performed to optimize exposure and removal. Due to the varying scope of locally advanced RCC, open, laparoscopic, and robotic techniques have been described with transabdominal and retroperitoneal approaches with different types of surgical incisions such as midline, subcostal/bilateral chevron, flank, and thoracoabdominal incisions. The midline incision allows exposure to the affected renal hilum as well as the contralateral renal vasculature and when extended to the thorax, the retrohepatic inferior vena cava, and the cardiac vasculature. Similar exposure can be obtained with bilateral chevron with an extended midline/thorax incision and with a flank incision extended to the thorax (a thoracoabdominal incision). Hepatic mobility may be facilitated by transection of the left triangular and coronary ligaments to provide exposure to the retrohepatic IVC.

The surgical steps for excision of RCC with associated venous thrombus (VT) include the isolation of the renal hilum with control of the renal artery first. The renal vein, IVC, and the contralateral renal vein are isolated and sequentially clamped cephalad and caudal to the VT. The VT can be visualized and monitored

for extraction using transesophageal echocardiography. The VT is extracted with a cavotomy en bloc with the affected renal vein. The IVC can then be reconstructed primarily, or if the diameter is less than 50 % of the original diameter, a graft can be utilized. The VT may also directly invade the IVC; in this instance, the IVC may be removed en bloc with reconstruction using vascular grafts as needed. For VT above the diaphragm and into the cardiac vasculature, cardiopulmonary (CPB) and venovenous (VVB) bypass may be used to facilitate VT removal. In general, VVB is not used for VT involving the right atrium and, due to the shorter bypass circuit, may provide shorter operative times compared to CPB.

8.6 Lymphadenectomy

The EORTC 30881 was a randomized trial examining therapeutic benefit of RN with and without lymph node dissection (LND) [43]. A total of 772 patients were selected for randomization with 383 patients in the LND group and 389 in the non-LND group. The majority (~70 %) of these patients were of lower-stage tumors ($\leq$T2). Pathological analysis of the LND dissections revealed an absence of LN metastasis in 332 patients out of 346 (96 %). Palpably enlarged LNs during surgery did not demonstrate LN metastasis as the majority (80 %) were negative and only 1 % with non-palpable nodes were positive. In the patients that did not undergo a LND, 9 % of patients had enlarged LNs. These LNs were excised for staging purposes or biopsied with 12 % demonstrating LN metastasis. In all, 96 % of the resected group did not show LN metastasis, and there were no significant differences in all survival parameters (overall, time to progression, or progression-free survival) at a median follow-up period of 12.6 years. The main criticism of this study was that the majority of patients were of low-risk disease, and benefits of a formal LND would not demonstrate much of a survival benefit.

LND may be of limited benefit for low-stage renal tumors as noted previously. On the contrary, it is hypothesized that LND may benefit higher-stage tumors and/or renal tumors with adverse pathological features. As retrospective studies have shown LN metastasis to be stage dependent ranging between 12 and 37 % for T3–4 tumors [44, 45]. At our institution, the borders of a formal LND are ipsilateral hilar LNs and para-aortic LNs from the crus of the diaphragm to the aortic bifurcation for left-sided tumors. For right-sided tumors, the interaortocaval and para-caval LNs are removed from the crus of the diaphragm to the large vessel bifurcation [46].

8.7 Cytoreductive Nephrectomy

Approximately 25 % of RCC patients will initially present with metastatic disease with treatments mainly focused on systemic therapies [47]. In 2001, two phase III randomized clinical trials reported a statistically significant survival benefit when radical nephrectomy was combined with interferon-alpha2b. In EORTC 30947 [16], 42 participants were randomly assigned to the RN before interferon-alpha2b and 43 to the interferon-alpha2b alone. The time to progression was 5 months versus 3 months (HR 0.60, 95 % CI 0.36–0.97) and median survival of 17 versus 7 months (HR 0.54, 95 % CI 0.31–0.94) with favorable survival observed when combined with RN. The Southwest Oncology Group (SWOG) randomized 121 to interferon alone versus 120 to RN plus interferon-alpha2b [15]. When combined with surgery, there was a 3-month ($P = 0.05$) improvement in median survival (11.1 vs. 8.1 months), independent of performance status and site of metastatic spread.

Although these two trials used immunotherapies, they have continued to motivate cytoreductive nephrectomy (CN) in the contemporary targeted therapy era. Targeted agents such as the tyrosine kinase inhibitors (sorafenib, sunitinib, and axitinib), mTOR inhibitors, and VEGF antibodies have been explored as agents used after CN [48–53]. These studies examined CN in subgroup analysis with the primary end point of progression-free survival to show promising trends in survival improvement. There are two ongoing randomized trials accruing to examine CN with targeted therapies. The EORTC 30073 (SURTIME trial, NCT 01099423) is a randomized phase III trial comparing immediate versus delayed (after receiving two cycles of sunitinib) nephrectomy in patients with synchronous metastatic RCC. The trial is still accruing with an expected enrollment of 458 patients. The CARMENA trial (NCT 00930033) randomizes to RN and sunitinib versus sunitinib alone with the primary end point of overall survival. The estimated accrual is 576 patients. It is expected that the results of these two trials will refine the role and timing of CN with targeted agents.

8.8 Conclusion

The spectrum of renal masses from SRMs to locally advanced and metastatic disease varies the management from active surveillance to invasive procedures including surgery. Due to the variability in biology and relative resistance to systemic therapies of RCC, surgery remains an important component of treatment. Since the first modern description of radical nephrectomy for tumor was described in the late 1800s, refinements in surgical technique have evolved to remove the kidney, perinephric fat, and regional lymph nodes for primary oncologic control. With partial nephrectomy, the removal of the whole kidney is not necessary for SRMs, and the development of laparoscopy and robotic techniques have advanced the treatment paradigm. As patients present in different stages of disease each with

their own unique clinical factors, informed counseling is paramount to meet their expectations. Furthermore, as many treatment methodologies are based on retrospective and observational studies, enrollment in clinical trials should be encouraged. As we await the conclusion of current trials with the introduction of new systemic therapies, the role of surgical excision is evolving.

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Chapter 9
Predictive and Prognostic Markers in Metastatic Renal Cell Carcinoma

José Manuel Ruiz Morales and Daniel Y.C. Heng

Abstract Predictive and prognostic factors enable clinicians to make informed decisions about therapy efficacy and patient survival. Despite advances in therapeutics, metastatic renal cell carcinoma (mRCC) remains largely incurable. Current standard targeted therapies (TT) for mRCC focus on inhibiting angiogenesis via the vascular endothelial growth factor (VEGF) or blocking the mammalian target of rapamycin (mTOR) pathway. The International mRCC Database Consortium (IMDC) criteria (Heng et al. J Clin Oncol 27(34):5794–5799, 2009), which focuses on patients who received targeted therapy, can be used to assess the prognosis of mRCC patients. Four of the MSKCC prognostic variables (anemia, hypercalcemia, Karnofsky performance status <80 %, and time from diagnosis to treatment of less than 1 year), as well as two additional factors (neutrophils and platelets greater than the upper limit of normal), were identified as key prognostic features. Patients were segregated into three risk categories: favorable (0 risk factors median overall survival (mOS) of 43.2 months), intermediate (one to two risk factors; mOS 22.5 months), and poor risk group (three or more risk factors; mOS 7.8 months). Also the IMDC criteria can be applied to different situations to evaluate prognosis in second-line treatment and in non-clear cell RCC (nccRCC) histologies. Benchmarks for survival have also been created for those treated with multiple lines of therapy to help guide clinical trial construction. Prognostic factors are better developed than predictive factors in mRCC but research continues in the field of biomarkers.

Keywords Renal cell carcinoma • Prognostic factors • Predictive factors • Biomarkers

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M. Oya (ed.), *Renal Cell Carcinoma*, DOI 10.1007/978-4-431-55531-5_9

9.1 Predictive and Prognostic Markers in Metastatic Renal Cell Carcinoma

By definition, a prognostic factor is capable of providing information on clinical outcome at the time of diagnosis, independent of therapy. In contrast, a predictive factor provides information on the likelihood of response to a given therapeutic modality [1]. Many prognostic factors have been identified and analyzed in numerous models for metastatic renal cell carcinoma (mRCC) patients. There are those inherent to the patient (performance status and symptoms), tumor burden (prior nephrectomy, bone and/or liver metastases, lactate dehydrogenase (LDH), anemia, calcium and sodium), proinflammatory markers (interleukin-6 (IL-6), erythrocyte sedimentation rate (ESR), neutrophilia, thrombocytosis, C-reactive protein), and treatment-related factors (prior therapy and radiotherapy, disease-free interval, and time from diagnosis to treatment interval). Predictive and prognostic factors ideally would enable clinicians to make informed decisions about when to initiate, stop, or change therapy for a patient or what specific therapy to choose for an individual patient [1].

9.2 Prognostic Factors at Diagnosis of mRCC

Despite advances in therapeutics, the prognosis of mRCC remains a largely incurable disease. Prognostic factors help to predetermine survival and outcome of patients. It is important for patient counseling, risk stratification, and modeling in research and clinical trials [2].

It was identified that mRCC was resistant to conventional cytotoxic agents. In the late 90's, interferon-α (IFN – α) and, later, interleukin-2 (both immunotherapy agents) were established as options for treatment after demonstrating a benefit in survival in mRCC patients, with modest objective responses between 10 and 22 % [3, 5]. There were some patients that had a longer survival and all had in common some specific clinical features. The most widely used prognostic factor model in the immunotherapy era was developed by the Memorial Sloan–Kettering Cancer Center (MSKCC), also known as the Motzer's criteria. It was based on 670 patients in clinical trials between 1975 and 1996. Pretreatment features associated with a shorter survival in the multivariable analysis were anemia, hypercalcemia, Karnofsky performance status <80 %, time from diagnosis to treatment of less than 1 year, serum LDH >1.5 times the upper limit of normal, and absence of prior nephrectomy. They categorized patients into three prognostic groups: favorable (0 risk factors, with a median overall survival (mOS) of 20 months), intermediate (1–2 risk factors, mOS 10 months), and poor risk (3 or more risk factors, mOS 4 months). It was concluded that the prognostic model is suitable for risk stratification of phase III trials using interferon-α as the comparative treatment arm and single-arm phase II trials to study progression-free survival as an end point [4].

The model was validated internally using a bootstrap resampling procedure and later validated externally by the Cleveland Clinic Group with 353 previously untreated mRCC patients enrolled onto clinical trials between 1987 and 2002. In addition, prior radiotherapy and presence of hepatic, lung, and retroperitoneal nodal metastases were found to be independent prognostic factors [2].

Other models for mRCC prognosis include the Cleveland Clinic Foundation (CCF) model [5], the French model [6], and the International Kidney Cancer Working Group (IKCWG) model [7].

Treatment of mRCC has changed dramatically since the original publication by Motzer et al [4]. Current standard targeted therapies (TT) for mRCC focus on inhibiting angiogenesis via the vascular endothelial growth factor (VEGF) pathway (sorafenib, sunitinib, pazopanib, axitinib, and bevacizumab) or the mammalian target of rapamycin (mTOR) pathway (temsirolimus and everolimus). Both have revolutionized the treatment of mRCC, doubling the mOS when compared with historical control treatment with immunotherapy. More than 80 % of patients achieve clinical benefit in the form of objective response to treatment or disease stabilization with these drugs. Seven agents are now approved or available for use in North America and Europe, and more are awaiting phase III results [8–14]. Since the initial Food and Drug Administration (FDA) approval of sorafenib in 2005 and the availability of these targeted treatments, the prognosis of mRCC required updating to help physicians in treatment decisions for mRCC and obtain contemporary numbers for overall survival in patients now rarely treated with old immunotherapy drugs [15].

Because of this new era in the treatment of mRCC, The International mRCC Database Consortium (IMDC), focused on 645 patients who received VEGF-based treatment and generated a new set of criteria that can be used to assess the prognosis of mRCC patients. These criteria have the advantage that do not require complicated mathematical models or nomograms for a risk result and are easy to remember and apply in the clinic; in fact it only needs four variables from laboratory results and two clinical factors. Four of the MSKCC variables (anemia, hypercalcemia, Karnofsky performance status <80 %, and time from diagnosis to treatment of less than 1 year), as well as two additional factors (neutrophils and platelets greater than the upper limit of normal), were identified as key prognostic features in the era of targeted treatment [16]. The accuracy of the model was tested in an external population of patients with mRCC who were treated with first-line VEGF-targeted treatment at 13 international cancer centers and who were registered in the Consortium's database but had not contributed to the initial development of the IMDC model. When patients were segregated into three risk categories, median overall survival was 43.2 months (95 % CI 31.4–50.1) in the favorable risk group (no risk factors; 157 patients), 22.5 months (18.7–25.1) in the intermediate risk group (one to two risk factors; 440 patients), and 7.8 months (6·5–9·7) in the poor risk group (three or more risk factors; 252 patients; $p<0{\cdot}0001$; concordance index 0.664, 95 % CI 0.639–0.689) [19]. At this moment, the IMDC currently includes 7400 patients from 29 institutions around the world.

Neutrophil-to-lymphocyte ratio (NLR), if elevated, is associated with worse outcomes in several malignancies, and mRCC is not the exception [17, 18]. Investigation of NLR at baseline and during therapy for mRCC has been studied also by the IMDC and recently validated. In a retrospective analysis of 1199 patients and 4350 patients from 12 prospective randomized trials (validation cohort), NLR was examined at baseline and 6 (± 2) weeks later. A landmark analysis at 8 weeks was conducted to explore the prognostic value of relative NLR change on OS, PFS, and objective response. Higher NLR at baseline was associated with shorter OS and PFS (HR per 1 unit increase in log-transformed NLR = 1.69 [95 % CI = 1.46–1.95] and 1.30 [95 % CI = 1.15–1.48], respectively). Compared with no change (decrease <25 % to increase <25 %, reference), increase NLR at week 6 by 25–50 %, and >75 % was associated with poor OS (HR = 1.55 [95 % CI = 1.10–2.18] and 2.31 [95 % CI = 1.64–3.25], respectively), poor PFS (HR = 1.46 [95 % CI = 1.04–2.03], 1.76 [95 % CI = 1.23–2.52], respectively), and reduced objective response rate (odds ratios = 0.77 [95 % CI = 0.37–1.63] and 0.24 [95 % CI = 0.08–0.72], respectively). By contrast, a decrease of 25–50 % was associated with improved outcomes. Findings were confirmed in the validation cohort [19].

With the advent of TT, the majority of patients with mRCC will be exposed to more than one line of treatment and limited data exist on outcomes for patients treated with multiple lines of therapy. There is a need for benchmark survival data from real-life patients exposed to one or more of the contemporary targeted therapies and to compare the outcomes of emerging treatment options to help shape the design of future trials (i.e., sample size calculations to create expectations of how a new drug should perform) with realistic expectations of outcomes such as progression free survival (PFS) and OS. In a recently published study, outcomes of mRCC patients from the IMDC treated with 1, 2, or 3+ lines of TT were documented [20].

mOS and PFS were calculated using different population inclusion criteria. In total, 2705 patients were treated with TT of which 57 % received only first-line TT, 27 % received two lines of TT, and 16 % received 3+ lines of TT. Overall survival of patients who received 1, 2, or 3+ lines of TT were 14.9, 21.0, and 39.2 months, respectively, from first-line TT (p < 0.0001). On multivariable analysis, 2 lines and 3+ lines of therapy were each associated with better mOS (HR 0.738 and 0.626, p<0.0001) after adjustment for the IMDC criteria (Table 9.1). This study demonstrated that patients who are able to receive more lines of TT live longer, and these results serve as a benchmark to compare the outcomes of emerging treatment options and help to shape the design of future trials with realistic expectations of outcomes such as PFS and OS [20].

Once a patient surpasses the predicted survival milestone, survival projections may lose their accuracy. At this juncture, physicians have little guidance on how to counsel patients who wish to revisit the prognosis discussion. We tend to think of prognosis only at the beginning of treatment, but in fact prognosis is a very dynamic process. The concept of conditional survival is defined as the probability of surviving an additional amount of time after the patient has already survived a specific period of time.

Table 9.1 Expected mOS and PFS for benchmark analysis from the IMDC for first-, second-, and third-line therapy for mRCC [20]

Similar trial and Inclusion criteria	Agents	Number of patients	Months (95 % IC)	Number of patients	Months (95 % IC)
Patients receiving first-line TT		**mOS from patients in IMDC**		**PFS from patients in IMDC**	
ADAPT [14] Patients with an intermediate or poor risk disease and whose diagnosis to treatment interval was less than 1 year	Autologous dendritic cell immunotherapy (AGS-003) plus standard treatment of advanced RCC	1189	14.7 (13.3–16.5)	1174	5.6 (5.3–6.1)
TIVO-1[10] Patients who previously underwent nephrectomy	Tivozanib vs. sorafenib	2117	24.8 (23.1–27.3)	2080	8.2 (7.8–8.6)
Patients who received second-line TT after at least one other VEGF-TT					
INTORSECT [21] Patients who have failed first-line Sunitinib	Temsirolimus vs. sorafenib	1157	13.0 (12.2–14.7)	1151	3.9 (3.6–4.3)
Patients who received third-line TT and were previously exposed to one VEGF inhibitor and one mTOR inhibitor					
GOLD [22] Patients with mRCC in third line after failure to one VEGF inhibitor and one mTOR inhibitor	Dovitinib vs. sorafenib	147	18.0 (11.8–24.0)	140	4.4 (3.3–5.2)

This concept can provide practical information, because it accounts for the length of survivorship and changes in hazard rates over time. It is a clinically powerful measure that can dynamically adjust prognosis as months and years pass and can aid substantially in the counseling of subsequent treatment decisions.

For this purpose, 1673 patients were analyzed from the IMDC database with a median follow-up for alive patients of 20.1 months (IQR 9.0–34.4). A patient's chance of living an additional two years (2-year conditional survival probability, conditioned on time survived regardless of whether still on the first-line targeted therapy) increases from 44 % (95 % CI 41–47) at 0 months of targeted therapy to 51 % (46–55) at 18 months since beginning targeted therapy. When stratified by the IMDC prognostic risk criteria at therapy initiation, 2-year conditional survival changed little in the favorable and intermediate groups, but in the poor-risk group, 2-year conditional survival improved from 11 % (8–15) at 0 months to 33 % (18–48) after 18 months of being on targeted therapy. It was demonstrated that conditional survival is a clinically useful prediction measure that adjusts prognosis of patients with mRCC on the basis of survival since treatment initiation

or therapy duration and it might be especially relevant to adjust prognosis for poor-risk patients [23].

9.3 Prognostic Factors in Non-clear Cell RCC

Patients with rarer histologies of mRCC are often difficult to prognosticate. The largest cohort of patients to date, with non-clear cell RCC (nccRCC) histology treated with targeted therapy (20 % of patients), was investigated in the temsirolimus phase 3 study [25]. To characterize the applicability of the IMDC prognostic model and the survival outcome of patients with nccRCC who were treated with first-line VEGF and mTOR inhibitors, the IMDC criteria have been validated in both clear cell (ccRCC) and nccRCC for mRCC. Data on 2215 patients (1963 with ccRCC and 252 with nccRCC) treated with first-line VEGF and mTOR-targeted therapies were collected from the IMDC. mOS (12.8 vs 22.3 months; $P < .0001$) and time to treatment failure (TTF) (4.2 vs 7.8 months; $P < .0001$) were worse in nccRCC patients compared with ccRCC patients. The hazard ratio for death and TTF when adjusted for the prognostic factors was 1.41 (95 % CI, 1.19–1.67; $P < .0001$) and 1.54 (95 % CI, 1.33–1.79; $P < .0001$), respectively. The IMDC prognostic model reliably discriminated the three risk groups to predict mOS and TTF in nccRCC; the median mOS of the favorable, intermediate, and poor prognosis groups was 31.4, 16.1, and 5.1 months, respectively ($P < .0001$), and the median TTF was 9.6, 4.9, and 2.1 months, respectively ($P < .0001$). Even in the targeted-therapy era, the majority of nccRCC patients still have inferior clinical outcomes compared with patients with ccRCC [26]. There is actually no other modern prognostic model that has been assessed exclusively in advanced nccRCC. Moreover, the accuracy in prognosticating mOS was slightly higher than with the MSKCC risk model.

9.4 Prognostic Factors in Second-Line Therapy

The IMDC criteria recently have been validated in second-line therapy. A total of 1021 patients who received second-line targeted therapy (TT) after progressing on 1st-line TT for mRCC at 19 centers were analyzed. For the patients who had immunotherapy (22 %) prior to their 1st TT, their second TT was examined (i.e., 3rd-line therapy). The median time on second-line TT was 3.9 months (range 0–76 +). 871 (85 %) of patients had stopped secnd-line TT by the time of analysis. Median OS since second-line TT was 12.5 months (95 % CI: 11.3–14.3 months), with 369 (36.1 %) of patients remaining alive. Five out of six predefined factors in IMDC model (anemia, thrombocytosis, neutrophilia, KPS <80 %, and <1 year from diagnosis to treatment) measured at the time of second-line TT were independent predictors of poorer OS (HR between 1.39 and 1.58, p<0.05).

Hypercalcemia was not statistically significant in multivariable analysis ($p = 0.3008$) likely due to the low incidence of hypercalcemia (9 %). The concordance index using all six prognostic factors was 0.70, and it was 0.66 compared with the three-factor-MSKCC model. When patients were divided into three risk categories using IMDC criteria, mOS was 35.8 months (95 % CI 28.3–47.8) in the favorable risk group ($n = 76$), 16.6 months (95 % CI 14.9–17.9) in the intermediate risk group ($n = 529$), and 5.4 months (95 % CI 4.7–6.8) in the poor risk group ($n = 261$) [24].

Radiologic evaluation of mRCC is also changing in TT era, and the assessment with contrast-enhanced computed tomography (CECT) is an example. About 84 standard CECT examinations with mRCC on first-line sunitinib or sorafenib therapy were retrospectively evaluated comparing morphology, attenuation, size, and structure (MASS) criteria; response evaluation criteria in solid tumors (RECIST); size and attenuation CT (SACT) criteria; and modified Choi criteria. The objective response to therapy was compared with clinical outcomes including time to progression (TTP) and disease-specific survival. A favorable response according to MASS criteria had a sensitivity of 86 % and specificity of 100 % in identifying patients with a good clinical outcome (i.e., progression-free survival of > 250 days) versus 17 % and 100 %, respectively, for RECIST partial response. The objective categories of response used by MASS criteria—favorable response, indeterminate response, and unfavorable response—differed significantly from one another with respect to TTP ($p < 0.0001$, log-rank test) and disease-specific survival ($p < 0.0001$, log-rank test). Furthermore, the use of MASS criteria for imaging response assessment showed high interobserver agreement and predicted disease outcome in patients with metastatic RCC on TT [25].

Other factors that have been identified to confer a poorer prognosis to mRCC patients are the presence of bone and/or liver metastases, compared with other metastatic sites. A retrospectively review from 2027 patients from the IMDC was conducted for this purpose. The presence of bone and liver metastases were 34 % and 19 % overall, respectively. For bone metastases (BMs), when stratified by IMDC risk groups was 27 %, 33 %, and 43 % in the favorable-, intermediate-, and poor-risk groups, respectively ($p < 0.001$). For liver metastases (LMs), the presence was higher in the poor-risk patients (23 %) compared with the favorable- or intermediate-risk groups (17 %) ($p = 0.003$). When patients were classified into four groups based on the presence of BMs and/or LMs, the hazard ratio, adjusted for IMDC risk factors, was 1.4 (95 % CI, 1.22–1.62) for BMs, 1.42 (95 % CI, 1.17–1.73) for LMs, and 1.82 (95 % CI, 1.47–2.26) for both BMs and LMs compared with other metastatic sites ($p < 0.0001$). The prediction model performance for mOS was significantly improved when BMs and LMs were added to the IMDC prognostic model (likelihood ratio test $p < 0.0001$). These two metastatic sites can be added for the risk stratification of patients with mRCC and improve the predictive accuracy of the IMDC criteria [26].

Data exists for other laboratory values, like hyponatremia and neutrophil to lymphocyte ratio (NLR) > 2.5 which are independently associated with a worse outcome in mRCC patients treated with VEGF and mTOR-targeted agents.

Hyponatremia has been associated with poor survival in many solid tumors and a recent study sought to investigate this association on treatment outcomes in mRCC patients treated with contemporary TT. Hyponatremia was found in 14.6 % of 1661 mRCC patients from the IMDC. On univariate analysis, hyponatremia was associated with shorter mOS (7.0 vs 20.9 months), shorter TTF (2.9 vs 7.4 months), and lower disease control rate (DCR) rate (54.9 % vs 78.8 %) ($p< 0.0001$ for all comparisons). In multivariate analysis, these effects remain significant (hazard ratios: 1.51 [95 % CI, 1.26–1.80] for mOS and 1.57 [95 % CI, 1.34–1.83] for TTF [30]. To evaluate the prognostic value of hematologic parameters in patients with mRCC, a retrospective review of 157 patients aimed to explore the association between NLR and platelet to lymphocyte ratio (PLR) with response to tyrosine kinase inhibitor (TKI) treatment in mRCC. On multivariable analysis NLR > 2.5 and Karnofsky Performance Status (KPS) < 90 % were associated with a lower likelihood of response. PLR did not retain association with response in multivariable analysis [31]. Unfortunately adding these factors to our current prognostic models has not led to substantially better c-indices.

9.5 Predictive Factors

Compared to prognostic factors, there are few predictive factors described in mRCC patients, aside from TT-induced hypertension and other side effects of anti-VEGF therapy (hypothyroidism and hand-foot syndrome reaction (HFSR)). Prediction is used to predetermine the efficacy of a treatment and ideally aid in treatment selection. Unfortunately, compared to other malignancies (breast, lung, colon, and melanoma) mRCC lacks effective predictive factors at this moment. We lack validated, reproducible markers to accurately predict response.

Sunitinib-associated hypertension (HTN) is associated with improved clinical outcomes. Sunitinib-induced HTN is associated with improvement in clinical outcomes (objective response rate, PFS, and mOS). It was reported that patients with mRCC and sunitinib-induced HTN defined by a maximum SBP of $\geq$140 mmHg had better outcomes than those without treatment-induced HTN (objective response rate: 54.8 % vs 8.7 %; median PFS = 12.5 months, 95 % confidence interval [CI] = 10.9–13.7 vs 2.5 months, 95 % CI = 2.3–3.8 months; and mOS =: 30.9 months, 95 % CI = 27.9–33.7 vs 7.2 months, 95 % CI = 5.6–10.7 months; $P < .001$ for all). Similar results were obtained when comparing patients with vs without sunitinib-induced HTN defined by a maximum DBP of $\geq$ 90 mmHg [32]. Comparable information has been observed with other anti-VEGF agents [27, 28].

It is suggested that sunitinib-induced hypothyroidism is also associated with improved response rates to TT. There was a statistically significant association between the occurrence of hypothyroidism during treatment and the rate of objective response: hypothyroid mRCC patients treated with targeted therapy displayed higher ORR (28.3 vs 3.3 %, respectively; $P < 0.001$) and prolonged median mOS

(not reached vs. 13.9 months, respectively; P = 0.016) than euthyroid patients. In multivariate analysis, the development of subclinical hypothyroidism was identified as an independent predictor of survival (HR 0.31; P = 0.014) [29, 30].

Attention should be paid to the potential emergence of sorafenib-induced HFSR because it has been reported it could become a predictive marker of clinical outcome in mRCC patients. In a recent retrospective review, 36 Japanese mRCC patients treated with sorafenib were analyzed. A sorafenib-induced HFSR was observed at a significantly higher rate in patients in the favorable-risk group in the MSKCC model criteria and with Eastern Cooperative Oncology Group Performance Status of one or less, prior nephrectomy, higher hemoglobin, lower lactate dehydrogenase, and lower C-reactive protein. The mean best tumor response was significantly better in the group with HFSR (16.7 %) than that in the group without it (17.9 %; P < 0.001). The median progression-free survival was significantly longer in the group with HFSR (4.6 months) compared to the group without it (1.5 months; P = 0.002). In multivariate analysis, only HFSR was shown to be a predictive factor of progression-free survival (hazard ratio 0.312, P = 0.010) [31].

All of these predictive factors (HTN, hypothyroidism, HFSR) for VEGF TT are somewhat helpful however they are not useful for treatment selection, because patients need to start the drug first and then develop toxicities for prediction. Many other described predictive factors in mRCC awaiting validation include MET [32], PDL-1 [33] and PBRM1/BAP1 [34].

Recently, it was reported that certain biomarkers could be considered as molecular entry criteria for prospective clinical studies in selecting mTOR or VEGFR inhibitors. Potential correlations between somatic mutations and treatment efficacy in RECORD-3 trial (a randomized phase 2 trial comparing first-line everolimus then sunitinib with first-line sunitinib then everolimus at progression in 471 treatment-naïve mRCC patients) [35]. Polybromo-1 (PBRM1) mutations were associated with longer PFS within everolimus (median PFS 11.1 vs 5.3 months; unadjusted p = 0.0031). Patients with PBRM1 mutations (41 % of the cohort) derived comparable PFS benefit from everolimus vs. sunitinib. Lysine demethylase 5C (KDM5C) mutations were associated with longer PFS within sunitinib (median PFS 20.6 vs 8.4 months; unadjusted p = 0.0511). However, it needs a prospective validation [36].

The benefit of cytoreductive nephrectomy (CN) for overall survival is unclear in patients with synchronous mRCC in the era of TT, and it is not well understood if CN should remain a part of the standard treatment protocol. For this purpose, retrospective data from 1658 patients with synchronous mRCC from the IMDC were used to compare 982 mRCC patients who had a CN with 676 mRCC patients who did not. All patients received targeted therapy, with most receiving first-line sunitinib (72 %). The results demonstrated that patients who had CN had better IMDC prognostic profiles versus those without (favorable, intermediate, or poor in 9 %, 63 %, and 28 % vs 1 %, 45 %, and 54 %, respectively). Fewer CN patients had non-clear cell pathology, bone metastases, and liver metastases, but CN patients had more sarcomatoid features. The median OS of patients with CN versus without CN was 20.6 versus 9.5 mo (p < 0.0001). When adjusted for IMDC criteria to

correct for imbalances, the HR of death was 0.60 (95 % confidence interval, 0.52–0.69; $p < 0.0001$). The authors concluded that CN is beneficial in synchronous mRCC patients treated with targeted therapy, even after adjusting for prognostic factors. However, patient selection is very important. Patients with estimated survival times < 12 months or four or more IMDC prognostic factors may not benefit from CN. Perhaps it is because their prognosis is so limited that they should have started on targeted therapy as soon as possible instead of delaying it with cytoreduction for which the patient will take time to recover from. This information may aid in patient selection as we await results from randomized controlled trials [43]. Although we await the results of randomized trials to truly determine the benefit of cytoreductive nephrectomy, the use of the IMDC prognostic factors may help clinicians decide which patient stands to benefit from surgery the most.

9.6 The Future of Prognostication

With at least five clinical prognostic nomograms for mRCC, we have reached the ceiling of clinical variables for prognosis. We use these models because currently they are the best available. However, the future of prognostication is closer than we think.

For example, in a recent study, more than 500 primary nephrectomy specimens were accrued from patients with histologically confirmed ccRCC and conformed to the requirements for genomic study defined by The Cancer Genome Atlas (TCGA) together with matching "normal" genomic material. Clinical and pathological features, genomic alterations, DNA methylation profiles, and RNA and proteomic signatures were evaluated. Data were divided into "discovery" (n = 193) and "validation" (n = 253) sets and platform-specific signatures were defined using Cox analyses. Kaplan–Meier analysis for each signature showed statistically significant associations with survival in the validation subset. Multivariate Cox analyses, incorporating established clinical variables, showed that the mRNA, miRNA, and protein signatures provided additional prognostic power. Top protein correlates of worse survival included reduced AMP-activated kinase (AMPK) and increased acetyl-CoA carboxylase (ACC). Poor prognosis correlated with downregulation of AMPK complex and the Krebs cycle genes and with upregulation of genes involved in the pentose phosphate pathway (glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconolactonase (PGLS), transaldolase (TALDO), transketolase (TKT)), and fatty acid synthesis (Fatty acid synthase (FASN) and ACC). Cross-platform molecular analyses indicated a correlation between worsened prognosis in patients with ccRCC and a metabolic shift involving increased dependence on the pentose phosphate shunt, decreased AMPK, decreased Krebs cycle activity, and increased glutamine transport and fatty acid production [37].

Biometric data also can provide information about the prognosis of mRCC patients. Studies have shown that skeletal muscle and adipose tissue are linked to mOS and PFS. Adipose tissue, skeletal muscle, and skeletal muscle density (SMD)

were assessed with computed tomography imaging by measuring cross-sectional areas of the tissues and mean muscle Hounsfield units (HU). A high level of mean HU indicates a high SMD and high quality of muscle. In the 149 patients studied, the median mOS was 21.4 months and was strongly associated with SMD; the median OS in patients with low SMD was approximately one-half that of patients with high SMD (14 months vs 29 months; P = .001). After adjustment for IMDC risk score and treatment, high SMD was associated with longer mOS (hazards ratio, 1.85; P = .004) and longer PFS (hazards ratio, 1.81; P = .002). Adding SMD will separate the intermediate-risk and favorable-risk groups into three groups, with different median mOS periods ranging from 8 months (95 % confidence interval [95 % CI], 6 months–12 months) for an intermediate-risk IMDC score/low SMD to 22 months (95 % CI, 14 months–27 months) for an intermediate-risk IMDC score/high SMD and a favorable-risk IMDC score/low SMD to 35 months (95 % CI, 24 months–43 months) for a favorable-risk IMDC score/high SMD. Because of this data, high muscle density appears to be independently associated with improved outcome and could be integrated into the prognostic scores thereby enhancing the management of patients with mRCC [38].

The addition of biologic markers is the next likely step for improvement of the accuracy of these models. In the near future in order to improve accuracy, the IMDC model will have to incorporate new patient-specific (biometric data) and tumor-specific markers (miRNA [39], SNPs/genomic data [40]).

9.7 Conclusion

Prognostic factors are important not only for patient counseling but also for study design and planning therapy such as in cytoreductive nephrectomy. It is a dynamic process and it needs to be constantly improved with biomarkers that are tumor and patient specific. Physicians can use IMDC criteria in the age of targeted therapy in many settings. Prognostic factors are better developed than predictive factors in mRCC, and in a near future, genomics and biomarkers will be the key to unlock further prognostic and predictive ability.

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Chapter 10
Tyrosine Kinase Inhibitors: Sorafenib, Sunitinib, Axitinib, and Pazopanib

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Abstract Over the last 10 years, the progress in our understanding of the molecular basis of RCC has led to the development of targeted therapies with significant improvement in patient outcomes. To date, targeting the vascular endothelial growth factor receptor (VEGFR) has been shown to lead to improved clinical outcomes in metastatic RCC. Currently, four VEGFR tyrosine kinase inhibitors (TKIs) have been approved by the Food and Drug Administration (FDA) for the treatment of metastatic RCC: sunitinib, sorafenib, axitinib, and pazopanib. Here, we summarize the clinical data of efficacy, safety, and tolerability of those agents in patients with metastatic RCC.

Keywords Kidney cancer • Tyrosine kinase inhibitors • Sorafenib • Sunitinib • Pazopanib • Axitinib

10.1 Introduction

The molecular pathogenesis of RCC was elucidated by understanding the role of von Hippel-Lindau (VHL) tumor suppressor gene in clear cell RCC [1]. VHL gene is located in chromosome 3p25, and it encodes for protein pVHL, an important protein for ubiquitin-mediated degradation of the α-regulatory subunits of the hypoxia-inducible factor (HIF) [2]. The physiologic role of HIF is to upregulate several genes that promote survival under hypoxic conditions, including the gene for vascular endothelial growth factor (VEGF) [3]. Through the activation of angiogenesis via VEGF, HIF is also involved in tumorigenesis [3]. Under normoxic conditions, HIF-α is hydroxylated, and thus it becomes amenable to recognition and ubiquitination by the pVHL-containing ubiquitin ligase complex. In contrast, under hypoxic conditions or when the VHL gene is inactivated, HIF escapes

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M. Oya (ed.), *Renal Cell Carcinoma*, DOI 10.1007/978-4-431-55531-5_10

Table 10.1 Phase III studies of sorafenib, sunitinib, axitinib, and pazopanib in metastatic RCC

Treatment	Number of patients	Line of treatment	ORR (%)	PFS (months)	OS (months)
Sorafenib vs. placebo [13, 14]	903	Second line	10 vs. 2	5.5 vs. 2.8 ($p < 0.01$)	17.8 vs. 15.2 ($p = 0.146$)
Temsirolimus vs. sorafenib [15]	512	Second line	20 vs. 20	4.3 vs. 3.9 ($p = 0.19$)	12.3 vs. 16.6 ($p = 0.01$)
Sunitinib vs. IFN-α [30, 31]	750	First line	47 vs. 12	11 vs. 5 ($p = 0.0001$)	26.4 vs. 21.8 ($p = 0.051$)
Axitinib vs. sorafenib [45]	723	Second line	23 vs. 12 ($p = 0.0001$)	8.3 vs. 5.7 ($p < 0.0001$)	20.1 vs 19.2 ($p = 0.3744$)
Axitinib vs. sorafenib [46]	288	First line	32 vs 15 ($p = 0.0006$)	10.1 vs. 6.5	Not reported
Pazopanib vs. placebo [51, 52]	435	First or second line	30 vs. 3 ($p < 0.001$)	9.2 vs. 4.2 ($p < 0.0001$)	22.9 vs. 20.5 ($p = 0.224$)
Pazopanib vs. sunitinib [53]	1110	First line	31 vs 25 ($p = 0.03$)	8.4 vs. 9.5 (HR = 1.05; 95 % CI, 0.90–1.22)	28.4 vs. 29.3 (HR = 0.91, 95 % CI, 0.76–1.08)

ubiquitination and degradation, leading to enhanced cell survival and overproduction of proangiogenic factors, such as VEGF [1].

VHL gene alterations have been found to be pivotal in the development of clear cell RCC in both VHL disease and sporadic cases. In sporadic clear cell RCC, alterations in the VHL gene through genetic or epigenetic mechanisms have been found with increased frequency [4]. As a proof of concept, overexpression of VEGF has been demonstrated in tissue samples of RCC compared to normal kidney [5, 6]. Thus, VEGF receptor became a very attractive target for the treatment of RCC (Table 10.1).

10.2 Sorafenib

Sorafenib (Nexavar™, Bayer Pharmaceuticals, West Heaven, CT, and Onyx Pharmaceuticals, Emeryville, CA) is an oral anti-VEGFR agent and the first one to be approved by the FDA in December 2005 for the treatment of metastatic renal cell carcinoma. It is a bis-aryl urea with broad anti-tyrosine kinase activity, including VEGFR-2, VEGFR-3, PDGFR-β, RAF-1, wild-type and V599E-mutated BRAF, FLT-3, c-KIT, and FGFR-1 [7].

10.2.1 Clinical Efficacy

10.2.1.1 Phase I

Four phase I studies have assessed the safety and tolerability of different dosing and treatment schedules of sorafenib in solid tumors [8–11]. The initial study [8] included 69 patients with advanced and refractory tumors that were treated with escalating doses of sorafenib, ranging from 50 to 800 mg orally once or twice daily continuously. The most common treatment-related adverse events were diarrhea, fatigue, skin toxicities, anorexia, and nausea, and most of them resolved upon treatment withdrawal. Significant dose-limiting grade 3 toxicities were diarrhea, fatigue, and hand-foot syndrome. The maximum tolerated dose from this trial was 400 mg twice daily. The only renal cell carcinoma patient that was included achieved stable disease for more than 2 years. Different treatment schedules in other phase I studies include 7 days on treatment followed by 7 days off treatment [9], 21 days on treatment followed by 7 days off treatment [10], and 28 days on treatment followed by 7 days off treatment [11]. Since the continuous treatment schedule was associated with good tolerance and stable blood concentrations of sorafenib, the recommended dose and schedule for subsequent phase II and III studies were 400 mg twice daily.

10.2.1.2 Phase II

A phase II randomized discontinuation trial evaluated the activity and tolerability of sorafenib in 202 patients with metastatic RCC [12]. Patients were allowed to enroll regardless of clear cell histology and previous treatment. All patients received sorafenib 400 mg twice daily for a total of 12 weeks. Following that initial treatment period, patients were assessed for response. Subsequently, they were either randomized to treatment with sorafenib or placebo if the change in the bidimentional size of the tumor was $\leq$25 %, or continued sorafenib if the tumor had shrunken by $\geq$25 %, or discontinued treatment if there was tumor growth of $\geq$25 %. The primary endpoint of the trial was percentage of the randomized patients that remained free from disease progression at 24 weeks of treatment, while secondary endpoints included progression free survival (PFS) for the randomized group, PFS for the entire cohort, objective response rate (ORR), and safety. After the initial 12 weeks of treatment, 69 patients (34 %) met the criteria for randomization, whereas 73 patients (36 %) had tumor shrinkage of $\geq$25 % and continued on sorafenib, and 51 patients (25 %) discontinued treatment due to either tumor growth of $\geq$25 % or other evidence of progression at or before week 12 of treatment. From the 69 eligible patients, 65 patients were randomly assigned to sorafenib ($n = 32$) or placebo ($n = 33$). Between the two arms, more patients that received sorafenib were disease free at the end of the 24 weeks compared to placebo (16/32, 50 % vs. 6/33, 18 %; $p = 0.0077$). Also the median PFS between the two

groups was in favor of sorafenib (24 vs. 6 weeks; p=0.0087). The median PFS for the entire cohort was 29 weeks. The most common treatment-related adverse events were fatigue, skin toxicities, pain, and diarrhea, with most of them being grade 1 or 2. The most common grade 3 or 4 adverse event was hypertension.

10.2.1.3 Phase III

The pivotal phase III TARGET trial examined the role of sorafenib as second-line treatment in cytokine-refractory patients [13]. Nine hundred three patients with metastatic clear cell RCC that had previously failed treatment with cytokines were randomly assigned 1:1 to receive either sorafenib 400 mg twice daily or placebo. Most patients had prior nephrectomy, and all of them had either good or intermediate-risk disease by MSKCC criteria. The primary endpoint of the study was overall survival (OS), whereas secondary endpoints included PFS and ORR. In the initial interim analysis and prior to crossover to sorafenib, the median OS was significantly prolonged for sorafenib compared to placebo (not reached vs. 14.7 months, respectively; $p = 0.02$). As a result of those favorable results, crossover to sorafenib was allowed for the patients that progressed on placebo. In the final analysis [14], there was a trend toward improved median OS for the sorafenib arm (17.8 vs. 15.2 months; $p = 0.146$); however, when post-crossover placebo survival data were censored, the difference was found to be statistically significant (17.8 vs. 14.3 months; $p = 0.029$). The median PFS was also found to be in favor of sorafenib (5.5 vs. 2.8 months; $p < 0.000001$). Common side effects were grade 1 or 2 and included diarrhea, fatigue, skin toxicities, nausea, and hypertension. The incidence of cardiac ischemia and myocardial infarction was higher in the sorafenib group compared to placebo (3 % vs. <1 %, respectively; p=0.01), including two deaths in the sorafenib group and one death in the placebo group. Finally, baseline VEGF levels were found to be a negative prognostic biomarker for PFS and OS ($p = 0.0013$ and $p = 0.0009$, respectively); however, they were not accurate predictive markers for response, since patients with either high or low baseline levels benefited from therapy with sorafenib. This trial established the first proof of principle that targeting VEGFR could lead to improved clinical outcomes in metastatic RCC patients.

The recently published INTORSECT phase III trial compared temsirolimus to sorafenib in the second-line setting in patients previously treated with sunitinib [15]. A total of 512 patients were 1:1 randomized to either intravenous temsirolimus 25 mg weekly ($n = 259$) or oral sorafenib 400 mg twice daily ($n = 253$). Both patients with clear cell and non-clear cell histologies were enrolled. The primary endpoint of PFS was not significantly different between the two groups (median PFS of 4.3 months for temsirolimus vs. 3.9 months for sorafenib; $p = 0.19$); however, the secondary endpoint of OS between the two arms favored sorafenib (median OS of 12.3 months for temsirolimus vs. 16.6 months for sorafenib; $p = 0.01$). Exploratory subgroup analyses showed favorable OS with sorafenib in patients with prior nephrectomy ($p = 0.002$), clear cell histology

($p = 0.01$), prolonged exposure to sunitinib for more than 180 days ($p = 0.02$), and intermediate MSKCC risk ($p = 0.002$). The most common side effects with temsirolimus were skin rash, fatigue, cough, anemia, and nausea, whereas the most common side effects with sorafenib were diarrhea, HFS, decreased appetite, skin rash, and fatigue. The reason for the discrepancy between PFS and OS is unclear, but these results have reinforced the practice of sequential VEGFR inhibitors in the treatment sequence for metastatic RCC patients.

Finally, the recently presented SWITCH phase III trial aimed to compare the sequential use of sorafenib and sunitinib vs. sunitinib and sorafenib in previously untreated patients [16]. Once 182 patients were assigned to the sorafenib/sunitinib arm vs. 183 patients to the sunitinib/sorafenib arm. The primary endpoint of total PFS from the time of randomization to disease progression on the second VEGFR agent was not statistically different between the two groups (12.5 months in the sorafenib/sunitinib arm vs. 14.9 months in the sunitinib/sorafenib arm; $p = 0.54$). Median OS likewise showed similar results, reaching 31.5 months in the sorafenib/sunitinib arm vs. 30.2 months in the sunitinib/sorafenib arm ($p = 0.49$). Even though this trial enrolled patient with low or intermediate MSKCC score and captured the OS after sequential treatments, the median OS is among the longest yet reported, further supporting the use of sequential VEGFR inhibitors. Unfortunately the trial was underpowered to show significant differences and thus is unlikely to impact clinical practice.

10.3 Sunitinib

Sunitinib (Sutent™, Pfizer Inc., New York, NY) is an oral, small-molecule, multitarget tyrosine kinase inhibitor, including VEGFR-1, VEGFR-2, and VEGFR-3, PDGFR-α and PDGFR-β, c-KIT, FLT-3, CSF-1R, and RET [17]. It is the most widely used agent for metastatic RCC, and it has also been approved by the FDA for the treatment of imatinib mesylate-refractory gastrointestinal stromal tumor (GIST) and locally advanced or metastatic well-differentiated pancreatic neuroendocrine tumors (pNET).

10.3.1 Clinical Efficacy

10.3.1.1 Phase I

Several phase I studies of sunitinib have been conducted in patients with both solid tumors and acute myelogenous leukemia [18–20]. The phase I trial in solid tumors [18] included 28 patients that were treated with escalating oral doses of sunitinib, ranging from 50 mg every other day to 150 mg daily for 4 weeks in a 6-week cycle. Significant dose-limiting toxicities were grade 3 fatigue and hypertension and grade

2 bullous skin toxicity, all of them reported at doses ≥75 mg daily. Based on those results, the recommended dose for subsequent studies was 50 mg daily for 4 weeks followed by a 2-week break in a 6-week cycle (schedule 4/2). At this dose level, the most common side effects were sore mouth, edema, thrombocytopenia, hair discoloration, and yellow coloration of the skin. A total of four patients with metastatic RCC were included, with three of them showing objective responses lasting from 28 to 54 weeks.

10.3.1.2 Phase II

Two consecutive phase II clinical trials examined the role of sunitinib in the treatment of metastatic RCC refractory to cytokines [21, 22]. Sixty-three and 106 patients with metastatic RCC were enrolled in those two studies, respectively. In both trials, patients were required to have an ECOG performance status of 0 to 1, and more than half of them had 0 MSKCC risk factors ($n = 34$, 54 % and $n = 61$, 58 %, respectively). The first study allowed patients with non-clear cell histologies to enroll; however, the patients in the second trial were exclusively clear cell RCC. All patients were treated with sunitinib 50 mg daily for 4 weeks followed by a 2-week break in 6-week cycles. The primary endpoint of ORR was 40 % and 34 % between the two studies, whereas the secondary endpoints of median time to progression (TTP) and median OS were 8.7 and 16.4 months in the first study and 10.7 and 23.9 months in the second study, respectively [23]. The most common side effects encountered were fatigue, diarrhea, dyspepsia, nausea, and hypertension. Based on these results, sunitinib was granted accelerated approval by the FDA for the treatment of metastatic RCC in January 2006 [24].

Even though sunitinib was approved for treatment of RCC based on those two trials, questions regarding the optimal treatment schedule remained. The EFFECT trial assessed the efficacy and safety of two different schedules of sunitinib with the aim to identify the optimal dosing and schedule [25]. Two hundred ninety-two patients with treatment-naïve advanced clear cell RCC were 1:1 randomly assigned to sunitinib 50 mg daily 4/2 ($n = 146$) or 37.5 mg daily continuously ($n = 146$). Although this trial was small and underpowered to establish differences in outcomes, the 4/2 schedule correlated with a numerically longer TTP (9.9 months in the 4/2 schedule vs. 7.1 months in the continuous treatment arm; $p = 0.09$) and no higher rates of toxicity. Steady-state plasma trough concentrations of sunitinib, its active metabolite SU12662, and total drug were higher in the 4/2 arm; however, there was no correlation between drug plasma concentrations and objective tumor response with either dosing schedule.

Based on the above results the recommended dose of sunitinib remains 50 mg daily 4/2. However, in clinical practice, maintenance of sunitinib dose at 50 mg daily 4/2 can be challenging due to treatment-related adverse events, frequently requiring dose reductions and treatment breaks. Since worsening toxicities are usually observed during weeks 3 and 4 of treatment, several retrospective studies have examined the efficacy, safety, and tolerability of alternative treatment

schedules [26–28]. Sunitinib 50 mg daily for 2 weeks followed by a 1-week break seems to be a reasonable alternative, with better tolerance and similar – if not improved – clinical efficacy. Ongoing prospective studies will provide more insight into the evolving field of optimal dosing schedule of sunitinib.

Sunitinib has also been compared to everolimus as a first-line treatment in patients with metastatic RCC. The phase II non-inferiority RECORD-3 trial [29] enrolled 471 patients with metastatic RCC (both clear and non-clear cell) that were randomized 1:1 to either everolimus followed by sunitinib upon disease progression ($n = 238$) or sunitinib followed by everolimus upon disease progression ($n = 233$). The primary endpoint of non-inferiority of everolimus was not reached, since the median PFS of first-line sunitinib was 10.7 months vs. 7.9 months with everolimus (HR 1.43, 95 % CI 1.15–1.77). Also, there was a trend toward an inferior OS in patients that received everolimus followed by sunitinib compared to sunitinib followed by everolimus (22.4 vs. 32 months; HR = 1.24, 95 % CI 0.94–1.64); however, these data are still immature and further follow-up is indicated. These data reinforce the clinical practice of frontline therapy with a VEGF inhibitor in metastatic RCC.

10.3.1.3 Phase III

A subsequent multicenter randomized phase III trial compared sunitinib to interferon-α (IFN-α), the standard of care at the time [30]. Seven hundred fifty treatment-naïve patients with metastatic clear cell RCC were assigned 1:1 to either oral sunitinib 50 mg daily for 4 weeks every 6 weeks ($n = 375$) or subcutaneous INF-α 9 million units three times weekly ($n = 375$). An ECOG performance status of 0 to 1 and absence of brain metastasis were required for study entry. Most patients had undergone prior nephrectomy (91 vs. 89 %). In addition, most patients had MSKCC favorable (38 vs. 34 %) or intermediate (56 vs. 59 %) risk disease with only a minority having poor risk disease (6 vs. 7 %). The primary endpoint of median PFS was 11 months for the sunitinib arm compared to 5 months for the IFN-α arm ($p<0.001$). In addition, sunitinib was associated with a higher ORR of 31 % vs. 6 % for IFN-α ($p<0.001$). All grades' adverse events were more common in the sunitinib arm with the most common being diarrhea, fatigue, nausea, vomiting, and hypertension in comparison to fatigue, pyrexia, nausea, chills, and myalgias in the group treated with IFN-α. However, patient-reported health-related quality of life was significantly better in the sunitinib group ($p < 0.001$).

In a follow-up report of that trial [31], median OS of the patients that received sunitinib was greater compared to IFN-α (26.4 vs. 21.8 months; p=0.51). One possible explanation for the borderline p value is the high rates of crossover to sunitinib for the patients that progressed on IFN-α. When the analysis was censored to exclude those patient, the difference in the median OS was found to be statistically significant (26.4 vs. 20.0 months; p=0.36).

10.3.1.4 Expanded-Access Trial

Since several criteria were required for participation to the initial trials with sunitinib, a subsequent expanded-access trial was undertaken in order to assess the safety and efficacy of sunitinib in patients that did not meet the prespecified criteria [32]. Patients with an ECOG performance status of $\geq$2, brain metastasis, age $\geq$65 years, and non-clear cell histology were allowed to participate. More than 4500 patients were treated with sunitinib 50 mg daily on a 4/2 schedule in a compassionate basis, with 4371 of them included in the intention-to-treat (ITT) cohort. For that cohort, the ORR was 17 %, the median PFS was 10.9 months (95 % CI 10.3–11.2), and the median OS was 18.4 months (95 % CI 17.4–19.2). This trial confirmed the activity and safety of sunitinib in an unselected patient population.

10.4 Axitinib

Axitinib (Inlyta ™, Pfizer Inc., New York, NY) is an orally bioavailable second-generation indazole derivative. It is a potent small-molecule tyrosine kinase inhibitor of VEGFR-1, VEGFR-2, and VEGFR-3 at picomolar concentrations and PDGFR-α and PDGFR-β and c-Kit inhibitor in nanomolar concentrations [33]. Thus, in therapeutic plasma concentrations, it blocks VEGF-mediated tumor vascularization by a number of signaling processes that promote the proliferation, migration, and survival of endothelial cells [34].

10.4.1 Clinical Efficacy

10.4.1.1 Phase I

The initial phase I study of axitinib [35] included 36 patients with advanced solid tumors refractory to standard therapy. Participants were treated with escalating doses of axitinib, ranging from 10 mg to 60 mg daily divided to 2 oral doses. Significant dose-limiting toxicities reported were hypertension, hemoptysis, and stomatitis with hypertension, fatigue, nausea, and diarrhea being the most common side effects observed. The recommended dose of axitinib from that trial was 5 mg twice daily in the fasted state. Six patients with metastatic RCC were included with two of them achieving a partial response (PR). Subsequent phase I studies in Japanese patients with advanced solid malignancies have shown similar pharmacokinetic and safety results and have also confirmed the effect of axitinib to soluble VEGFR-2 levels [36, 37].

10.4.1.2 Phase II

Using the recommended dose from the phase I studies, three subsequent phase II trials assessed the safety and efficacy of axitinib in patients with metastatic RCC who had failed previous therapies [38–41]. The initial single-arm, open-label phase II study allowed patients refractory to prior cytokine-based therapies to participate [38]. Patients were required to have an ECOG performance of 0 to1, measurable disease by RECIST, and no prior exposure to antiangiogenic agents. Fifty-two patients were treated with axitinib starting at 5 mg twice daily. The primary endpoint of the study was ORR, and the secondary endpoints were duration of response, TTP, OS, safety, pharmacokinetics, and patient-reported health-related quality of life. The median age of the cohort was 59 years, most patients (94 %) had prior nephrectomy, and all but one (98 %) had clear cell histology. The ORR was 44.2 % [2 complete responses (CRs) and 21 partial responses (PRs) (95 % CI, 30.5–58.7)] with a median response duration of 23.0 months (95 % CI, 20.9, not estimable; range 4.2–29.8). Twenty-two additional patients (42 %) showed stable disease (SD) for more than 8 weeks. Further, the median TTP was 15.7 months (95 % CI 8.4–23.4), and the median OS was 29.9 months (95 % CI 20.3,not estimable; range 2.4–35.8). The most commonly reported side effects were diarrhea, hypertension, fatigue, nausea, and hoarseness. A recent update of that trial [39] showed a 5-year survival rate of 20.6 % (95 % CI, 10.9 %–32.4 %). A pharmacokinetic post hoc analysis aiming to associate drug levels with efficacy was also undertaken. Patients were stratified to 4 quartiles based on their axitinib plasma concentration 1 to 2 hours post-dose on day 1 of cycle 1, with the third group exhibiting a numerically prolonged median PFS and OS. The biologic explanation was that a higher concentration of axitinib that is associated with good tolerance and no discontinuation of therapy is associated with optimal efficacy.

A subsequent phase II study evaluated the efficacy and safety of axitinib in sorafenib-refractory metastatic RCC patients [40]. Sixty-two eligible patients were enrolled in this multicenter, single-arm, open-label phase II study. All patients had disease progression while receiving sorafenib with most of them (74.2 %) having received more than one lines of treatment, including sunitinib and bevacizumab. The primary endpoint was ORR, and the secondary endpoints were safety, duration of response, PFS, OS, and patient-reported outcomes. The starting dose of axitinib was again 5 mg twice daily, and if well tolerated, a stepwise dose titration to 7 and 10 mg twice daily was undertaken. The median age of the cohort was 60 years, all patients had undergone prior nephrectomy, and the predominant histology was clear cell (95.2 %). The reported ORR was 22.6 % (95 % CI, 12.9–35.0 %) with a median duration of response of 17.5 months (95 % CI, 7.4, not estimable), a median PFS of 7.4 months (95 % CI, 6.7–11.0), and a median OS of 13.6 months (95 % CI, 8.4–18.8). Thirty-three patients (53.2 %) were able to tolerate increased doses of axitinib, whereas 11 patients (17.7 %) required dose reduction to less than 5 mg

twice daily. The most common all-causality grade 3 to 4 side effects were hand-foot syndrome, fatigue, hypertension, dyspnea, diarrhea, dehydration, and hypotension.

Another single-arm, open-label, multicenter phase II evaluated the safety and efficacy of axitinib in Japanese patients who had failed prior cytokine therapy [41]. Dose titration to 7 and 10 mg twice daily was again recommended if no hypertension developed and axitinib was well tolerated. Sixty-four eligible patients were enrolled. The reported ORR and median PFS were 50.0 % (95 % CI, 37.2–62.8) and 11.0 months (95 % CI, 9.2–12.0), respectively, confirming the favorable response to axitinib. Commonly reported side effects were again hypertension, hand-foot syndrome, diarrhea, and proteinuria.

Based on the results from the phase I study, patients receiving axitinib exhibit variable plasma drug exposure with dose-proportional pharmacokinetics suggesting higher plasma exposure with escalated doses [35]. Further, higher axitinib exposure has been associated with prolonged PFS and OS [39, 42]. Based on that rationale, a randomized, double-blind, multicenter, phase II study that examined the efficacy of dose titration of axitinib was undertaken [43]. Two hundred thirteen previously untreated metastatic RCC patients received axitinib 5 mg twice daily as the initial treatment. One hundred twelve patients who did not experience elevation in their blood pressure (>150/90 mmHg) or any grade 3 or 4 side effects were subsequently randomized to either increased dose of axitinib to 7 mg twice daily with a further increase to 10 mg twice daily or placebo, while the rest of the patients continued treatment on standard dose. Even though the ORR was higher in the axitinib titration group compared to placebo (54 vs. 34 %, $p = 0.01$), no difference in the median PFS was observed between the two groups (14.5 vs. 15.7 months; $p = 0.24$). No significant differences in the all-causality serious adverse events between the two groups were reported. These result support that dose titration of axitinib can lead to clinical benefit (improved ORR), but the precise schema for doing so requires further study, as tolerance issues in the titration group likely prevented an adequate duration of exposure to the higher dose. Smaller increments in dose and alternative parameters for titration may improve outcomes further. This is an evolving field that further studies regarding the timing and scheme of titration are indicated.

10.4.1.3 Phase III

The pivotal AXIS trial [44] compared the efficacy and safety of axitinib vs. sorafenib in 723 patients with metastatic clear cell RCC that had progressed on previous first-line treatment with sunitinib, bevacizumab/IFN-α, temsirolimus, or cytokines. The primary endpoint of PFS in the initial report was 6.7 months for the axitinib group vs. 4.7 months for the sorafenib group ($p < 0.0001$). The prolonged PFS in patients treated with axitinib was observed in patients previously treated with both sunitinib (4.8 vs. 3.4 months; $p = 0.0107$) and cytokines (12.1 vs. 6.5 months; $p < 0.0001$). Objective response rates were 19 % for axitinib vs. 9 % for sorafenib ($p = 0.0001$). In an updated report of the AXIS trial [45], no overall

survival difference was recorded between the two groups, regardless of prior treatment. Median OS was 20.1 months (95 % CI, 16.7–23.4) for patients treated with axitinib vs. 19.2 months (95 % CI, 17.5–22.3) for patients treated with sorafenib ($p = 0.3744$). Common side effects in patients treated with axitinib were diarrhea, hypertension, fatigue, anorexia, and nausea, whereas most common side effects with sorafenib were diarrhea, hand-foot syndrome, alopecia, rash, and hypertension.

Axitinib has also been directly compared to sorafenib in the first-line setting. In a recently published randomized, open-label, phase III study [46], 288 treatment-naïve metastatic renal cell carcinoma patients were treated with either axitinib or sorafenib. Despite the favorable outcomes in patients treated with axitinib as a second-line therapy, no significant difference in median PFS was observed when axitinib was compared to sorafenib as the initial treatment (median PFS of 10.1 vs. 6.5 months; stratified HR 0.77, 95 % CI 0.56–1.05; one-sided $p = 0.038$); however, this trial was small and underpowered to confirm the primary endpoint of 4.3 months of improved median PFS with axitinib. The two agents had different toxicity profiles. Diarrhea, hypertension, weight loss, and fatigue were most commonly reported with axitinib, whereas diarrhea, hand-foot syndrome, and hypertension were most commonly reported with sorafenib. Patient-reported outcomes were similar between the two groups throughout the treatment.

10.5 Pazopanib

Pazopanib (Votrient™, GlaxoSmithKline Inc., Research Triangle Park, NC) is a potent orally administered small-molecule multitargeted tyrosine kinase inhibitor. Like axitinib, it is a second-generation indazole derivative. In therapeutic levels it is highly selective for all the VEGFR subtypes (VEGFR-1, VEGFR-2, VEGFR-3), all the PDGFR subtypes (PDGFR-α and PDGFR-β), and c-kit; thus it targets both endothelial and pericyte proliferation of tumor stroma [47].

10.5.1 Clinical Efficacy

10.5.1.1 Phase I

Safety, pharmacokinetics, and clinical efficacy were evaluated in an initial phase I study [48]. Sixty-three patients with advanced-stage relapsed or refractory solid tumors were allowed to enroll. Patients were treated with escalating doses of pazopanib ranging from 50 mg three times weekly to 2000 mg daily in the dose-escalation phase ($n = 43$), followed by 300–400 mg twice daily in the dose-expansion phase ($n = 20$). Important dose-limiting toxicities included hypertension, diarrhea, hair depigmentation, and nausea. Most of those side effects were grade

1 to 2 and resolved upon treatment discontinuation, while hypertension was the most common grade 3 toxicity (25 %). Twelve patients with metastatic RCC were included in the study, with two patients achieving a PR as their best response and four patients having prolonged stable disease for more than 6 months. Clinical activity was observed at doses of $\geq$800 mg daily or 300 mg twice daily, so the recommended dose for subsequent trials was 800 mg daily.

10.5.1.2 Phase II

Following the favorable response of patients with RCC in the phase I trial, a phase II study was undertaken [49]. A total of 225 patients with metastatic clear cell RCC were treated with pazopanib 800 mg daily. More than two-thirds of the patients were treatment naïve ($n = 155$, 69 %), whereas the rest ($n = 70$, 31 %) had received one prior line of treatment with either cytokines or bevacizumab. This study was initially designed as a randomized discontinuation study that would further randomize patients with SD after 12 weeks of treatment to either continuation of pazopanib or switching to placebo. However, after an interim analysis that showed an ORR of 38 %, all patients continued in the pazopanib arm. The median age of the group was 59.8 years, the majority had undergone nephrectomy (91 %), and all patients had an ECOG performance status of 0 or 1. The primary endpoint of ORR was 35 % (95 % CI, 28–41 %), and it was independent of previous treatment. The median PFS was 52 weeks (95 % CI, 44–60 weeks), and the median duration of response was 68 weeks. Approximately 87 % ($n = 195$) of the evaluable patient had some degree of response. Subgroup analysis showed favorable PFS for patients with an ECOG performance status of 0 and a time from diagnosis to initiation of treatment of more than a year. The most commonly observed grade 3 and 4 side effects were hypertension, AST and ALT elevation, diarrhea, and fatigue.

A second multicenter, open-label, single-arm phase II study assessed the efficacy and safety of pazopanib 800 mg daily as second-line treatment [50]. Fifty-five eligible patients that had previously progressed on sunitinib ($n = 39$) or bevacizumab ($n = 16$) were enrolled. The ORR was 27 % with an additional 49 % of patients achieving SD. Pazopanib was found to be effective in patients pretreated with both sunitinib and bevacizumab. The median PFS for the entire group was 7.5 months (95 % CI, 5.4–9.4 months), and the median OS was 14.8 months (95 % CI, 12–28.8 months).

10.5.1.3 Phase III

To date, 3 phase III studies have compared pazopanib to placebo or other TKIs [51–53, 55]. The initial multicenter, double-blind phase III trial [51] included 435 patients with locally advanced and/or metastatic RCC that were randomly assigned 2:1 to treatment with pazopanib 800 mg daily ($n = 290$) or placebo ($n = 145$). Two hundred thirty-three patients (54 %) were treatment naïve, whereas

the rest 202 (46 %) had previously failed treatment with cytokines. All patients had clear cell or predominantly clear cell histology, and the majority of them had undergone nephrectomy (89 %). The patients that received pazopanib had a significantly prolonged median PFS compared to placebo (overall 9.2 vs. 4.2 months; $p < 0.0001$) that was independent of prior treatment (treatment-naïve population 11.1 vs. 2.8 months; $p < 0.0001$, cytokine-pretreated patients 7.4 vs. 4.2 months; $p < 0.001$), age, sex, MSKCC risk stratification, or ECOG performance status ($p < 0.0001$). The secondary endpoint of ORR was also significantly better for the pazopanib group (30 vs. 3 %; $p < 0.001$) with a median duration of response of 58.7 months. The most commonly reported side effects on the pazopanib arm were grade 1 and 2 and included diarrhea, hypertension, hair color changes, nausea, anorexia, and vomiting. Grade 3 or 4 adverse events were observed in 33 % and 7 % in the pazopanib group vs. 14 % and 6 % in the placebo arm, respectively. No statistically significant differences in the quality of life were recorded between the two groups.

In a recent update of the above trial [52], the median OS was not significantly different between the pazopanib and the placebo groups (22.9 vs. 20.5 months; $p = 0.224$); however, those results were probably confounded by the early and high rates of crossover to the pazopanib arm. Further, more patients in the placebo arm ended up getting a second-line VEGFR or mTOR inhibitor upon disease progression.

In an attempt to compare pazopanib to sunitinib as first-line treatment in patients with metastatic clear cell RCC, the phase III COMPARZ trial was undertaken [53]. This non-inferiority trial enrolled 1110 patients assigned in a 1:1 ratio to receive either pazopanib 800 mg daily or sunitinib 50 mg daily for 4 weeks followed by a 2-week break in 6-week cycles. The primary endpoint of median PFS was 8.4 months with pazopanib (95 % CI, 8.3–10.9) vs. 9.5 months with sunitinib (95 % CI, 8.3–11.1) and met the predefined criterion for non-inferiority (HR 1.05; 95 % CI, 0.90–1.22). The secondary endpoint of ORR was in favor of pazopanib (31 % vs. 25 %; $p = 0.03$); however, the median OS was not different between the two groups (28.4 vs. 29.3 months; $p = 0.28$). Patients that received pazopanib were more likely to develop elevation in their liver enzymes (60 vs. 43 %), whereas patients treated with sunitinib had higher incidence of fatigue (63 vs. 55 %), hand-foot syndrome (50 vs. 29 %), and thrombocytopenia (78 vs. 41 %). Overall, pazopanib was better tolerated than sunitinib in 11 out of 14 assessed health-related quality-of-life domains ($p < 0.05$).

However, several limitations in the design of the trial have not led to universal acceptance of pazopanib as the first-line agent of choice for metastatic RCC. Even though the primary endpoint of non-inferiority of pazopanib over sunitinib was met, based on the results of this trial, a conclusion that the two agents have equal efficacy cannot be reached, since the null hypothesis of an increased risk in the hazard of disease progression with pazopanib required a HR of $\geq$1.25. Further, the health-related quality of life was assessed on day 28 during cycles 1 to 9, which was the last day of the 4-week on-treatment period with sunitinib. Previously reported studies with sunitinib have shown that the severity of treatment-related side effects

accumulates during weeks 3 and 4 of treatment, and as a result patients tend to score worse in patient-reported outcomes on day 28 of treatment compared to day 42 after the 2-week break [18, 25]. In addition, the protocol was amended to include patients from another similar trial conducted in China, Taiwan, and South Korea when it became apparent that the initial study population would not yield a power of 80 %. It is unclear if this inclusion has changed the quality of final results, considering recent evidence that has shown significant differences in treatment discontinuation between Asian and non-Asian patients treated with TKIs [54].

Finally, the recently published phase III PISCES trial compared pazopanib and sunitinib in terms of patient preference, health-related quality of life (HRQoL), and safety [55]. Patients with metastatic RCC were randomly assigned 1:1 to receive sequential treatments with both agents for two 10-week periods divided by a 2-week washout break. This trial had a unique crossover design that allowed all patients to get exposed to both agents and decide which one they preferred better. HRQoL were assessed every 2 weeks during treatment, and the patients were asked about their preference at the end of the 22-week study duration, before unblinding and before informed of the final disease assessment. One hundred fourteen patients in the ITT population were treated with either pazopanib 800 mg daily for 10 weeks and then sunitinib 50 mg daily (4 weeks on, 2 weeks off, 4 weeks on) or the reverse sequence. The primary endpoint of patient preference was significantly better for pazopanib compared to sunitinib with 70 % of patients favoring pazopanib (95 % CI, 60.9–78.4 %) vs. 22 % that favored sunitinib (95 % CI, 14.7–30.6 %) vs. 8 % that had no preference ($p<0.001$). Pazopanib was preferred due to less fatigue, whereas sunitinib due to less diarrhea. The most common side effects with either drug were diarrhea, fatigue, and nausea. The crossover analyses of HRQoL also favored pazopanib in terms of less fatigue, mouth/throat pain, and hand and foot soreness.

However, several limitations apply to this trial as well. First, this was a small and underpowered trial that only included 114 patients in the ITT analysis. Further, there was no assessment of efficacy, and both pazopanib and sunitinib were considered as equally effective. Thus, the question of which is the agent of choice in the first-line setting has not been answered yet. Ultimately, patient comorbidities and different safety profiles of each VEGFR inhibitor need to be taken into consideration before making treatment decisions.

10.6 Combining TKIs with Bevacizumab or mTOR Inhibitors

Several attempts have been made to improve the outcomes of patient with metastatic RCC by combining VEGFR inhibitors with other active agents, such as bevacizumab or mTOR inhibitors (everolimus, temsirolimus) [56–61]. However, these combinations have led to higher rates of toxicity without any meaningful

clinical benefits. For that reason sequential single-agent treatment is preferred over the concurrent combination of different agents.

10.7 Conclusion

Ever since VEGFR inhibitors were included in the therapeutic armory, the treatment landscape of renal cell carcinoma has changed dramatically with significant impact in patient survival. In addition to the FDA-approved anti-VEGFR TKIs, several other agents, such as cediranib, cabozantinib, and dovitinib, are currently in different stages of development. However, despite the tremendous progress, several questions remain unanswered.

In terms of treatment preferences, several different approaches exist. To this date, both VEGFR and mTOR inhibitors have been approved for the treatment of metastatic RCC. Since a significant proportion of patients with metastatic RCC live long enough to be exposed to several lines of treatment, it is uncertain what the best sequence of agents is. Further, since metastatic RCC is an incurable disease and treatment is accompanied by variable degrees of toxicity, proper timing for initiating treatment and continuous vs. intermittent therapy remain two open questions. Several trials have examined the effect of different agents to quality of life; however, the limitations of those studies prevent us from drawing firm conclusions. Thus, treatment decisions should always take into consideration the need for treatment and patient's tolerance.

Finally, several phase II and III clinical trials with novel immune therapies, such as the anti-PD 1 agents, are underway, and the introduction of these agents is expected to revolutionize the field of kidney cancer treatment. It is unknown how these therapies will be incorporated in our current practice and how they will affect patient survival. Identification of accurate biomarkers is indicated in order to guide treatment choices and personalize therapy.

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Chapter 11
Mammalian Targets of Rapamycin Inhibitors: Temsirolimus and Everolimus

Camillo Porta, Silvia Chiellino, and Mimma Rizzo

Abstract mTOR is a key intracellular hub which integrates a number of signals coming from different sources, mainly but not exclusively related to cell metabolism; the mTOR pathway appears to be deregulated in a number of human malignancies including renal cell carcinoma.

To date, two mTOR inhibitors have been registered for the treatment of renal cell carcinoma: temsirolimus (for treatment-naïve patients with poor prognostic features) and everolimus (for the treatment of patients previously treated with one or two VEGF-targeting agents). Temsirolimus was the very first drugs which, within a randomized controlled phase III study, induced an overall survival benefit against an active comparator, while everolimus was the first drug which proved able to prolong progression-free survival in the post-tyrosine kinase inhibitor setting. Subsequent studies investigated the role of the two drugs in different settings, yielding conflicting results. Further development of these two drugs in renal cell carcinoma is expected, even though only the identification of reliable genetic or molecular biomarkers will lead to a tailored, and thus smarter, use of these drugs.

Keywords mTOR • Inhibitors • Temsirolimus • Everolimus • RCC

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M. Oya (ed.), *Renal Cell Carcinoma*, DOI 10.1007/978-4-431-55531-5_11

11.1 Introduction

The mammalian target of rapamycin (mTOR) and the phosphatidylinositol-3-kinase (PI3K)/Akt signaling pathways are heavily interconnected one with the other, thus being potentially regarded as a unique pathway [1] crucial to many aspects of cell growth and survival, in physiological as well as in pathological conditions. Furthermore, they heavily interact with many other pathways, including the hypoxia-inducible factors (HIFs) one.

mTOR is a serine/threonine kinase ubiquitously expressed in mammalian cells [2] which integrates signals initiated by nutrient intake, growth factors, and other cellular stimuli to regulate downstream signaling and protein synthesis. Through its downstream effectors, 4EBP1 and P70S6 kinase (S6 K), it is involved in the initiation of ribosomal translation of mRNA into proteins necessary for cell growth, cell cycle progression, and cell metabolism.

On the other hand, the PI3K/Akt pathway is a key regulator of survival in an intrinsically stressful environment (such as cancer is), characterized by limited nutrient and oxygen supply, as well as by low pH.

The activation of the PI3K/Akt/mTOR pathway through somatic mutations and/or gains and losses of key genes converging unto this complex, but key, intracellular hub, results in a profound disturbance of control of cell growth and survival, which ultimately leads to a competitive growth advantage, metastatic competence, angiogenesis, and therapy resistance.

Thus, this pathway has been taken into consideration as one of the most attractive targets for the development of anticancer agents [3, 4].

11.2 mTOR Structure and Functions

mTOR is a key protein evolutionarily conserved from yeast to man and is essential for life. Indeed, embryonic mutations in mTOR proved to be lethal.

In normal cells, mTOR activity is controlled by positive and negative upstream regulators [5]. Positive regulators include growth factors and their receptors, such as insulin-like growth factor-1 (IGF-1) and its cognate receptor IFGR-1, members of the human epidermal growth factor receptor (HER) family and associated ligands, and vascular endothelial growth factor receptors (VEGFRs) and their ligands, which transmit signals to mTOR through the PI3K-Akt. Negative regulators of mTOR activity include phosphatase and tensin homolog (PTEN) that inhibit signaling through the PI3K-Akt pathway and tuberous sclerosis complex (TSC) 1 (hamartin) and TSC2 (tuberin). Phosphorylation of TSC2 by Akt releases its inhibitory effect on mTOR and upregulates mTOR activity. Another negative regulator, LKB1, is in an energy-sensing pathway upstream of TSC [6].

mTOR activity is carried out by two distinct complexes: mTORC1 and mTORC2.

The mTORC1 complex is made up of mTOR, Raptor, mLST8, and PRAS40. It is extremely sensitive to rapamycin and thus represents the target of first-generation mTOR inhibitors. It also activates S6 K and inactivates 4E-BP1, leading to protein translation and cell growth [5].

The mTORC2 complex is composed of mTOR, Rictor, Sin1, and mLST8. It is less sensitive to rapamycin, but its role in normal cell function and oncogenesis has not been well characterized yet. However, it is known to activate AKT, thereby promoting cell proliferation and survival. The canonical pathway of mTOR activation depends on mitogen-driven signaling through PI3K/AKT, although alternative non-AKT-dependent activation through the Ras/MEK/ERK pathway has been recognized [7].

Altogether, mTOR activation leads to increased synthesis of multiple proteins. These include several that have been implicated in the pathogenesis of multiple tumors (including renal cell carcinoma, RCC), e.g., cyclin D1, which allows progression of cells through the cell cycle [8], and HIF, which drives the expression of pro-angiogenic growth factors, e.g., VEGF [9].

11.3 The Development of mTOR Inhibitors as Anticancer Agents

Rapamycin (sirolimus), an antifungal agent with immunosuppressive properties, was first isolated in 1975 from the soil of the island of Rapa Nui or Easter Island [10]. In the 1980s, when tested against a panel of human cancer cell lines, rapamycin showed a broad anticancer activity [11]. However, clinical development of rapamycin as an anticancer agent was hampered by unfavorable pharmacokinetic properties [12].

The relatively recent development of rapamycin analogs endowed with a more favorable pharmacokinetic profile, i.e., temsirolimus, everolimus, and ridaforolimus (a.k.a. deforolimus), opened up the present era of mTOR inhibitors as anticancer agents.

All these agents have similar structure and mechanism of action, but different pharmacokinetic properties. Indeed, all these drugs are small molecule inhibitors that function intracellularly, forming a complex with the FK506 binding protein-12 (FKBP-12) that is then recognized by mTOR. The resulting complex prevents mTOR activity, leading to inhibition of cell cycle progression, survival, and angiogenesis [1]. Notably, all these inhibitors are similar to the parental compound rapamycin in that they affect only mTORC1, and not mTORC2 [12], which thus could be used, by the cancer cell, as an escape pathway [13].

11.4 Temsirolimus

Temsirolimus (CCI-779) is a prodrug whose primary active metabolite is rapamycin. Temsirolimus is administered intravenously on a once-weekly schedule [14]. It has been approved for the treatment of patients with advanced RCC with poor prognostic features and of mantle cell lymphoma (MCL) patients.

11.4.1 Phase I Studies

Based on promising preclinical data on different tumor histotypes, phase I studies evaluating tolerability and pharmacokinetics of escalated doses of temsirolimus were performed. Raymond et al. treated 24 patients with refractory solid tumors with a 30-minute weekly infusion of temsirolimus at escalating doses (7.5–220 mg/m^2) [15]. As far as its safety profile, temsirolimus-induced adverse events proved to be manageable and reversible, grades 1–2 dermatologic reaction, mucositis, asthenia, and nausea being the commonest toxicities observed, while the major dose-limiting toxicity was thrombocytopenia. As far as temsirolimus activity, although most of the patients enrolled were extensively pretreated, two partial responses lasting around 6 months were observed in RCC and breast cancer patients. Two other patients with RCC obtained minor tumor responses lasting no more than 5 months. Notably, no dose-response relationship was observed. Pharmacokinetics analysis suggested that a fixed-dose regimen could be appropriate and the recommended doses for further testing on phase II trials were 25, 75, and 250 mg/m^2.

Using a different schedule, Hidalgo et al. yielded similar findings [14]; within a conventional phase I clinical trial, 63 patients were treated with temsirolimus at escalating doses (0.75–24 mg/m^2/day). The most common drug-related toxicities were asthenia, mucositis, nausea, and cutaneous toxicity, while the maximum tolerated dose was 15 mg/m^2/day for patients with extensive prior treatment; on the contrary, for minimally pretreated patients, the maximum acceptable dose proved to be 19 mg/m^2/day. Notably, associated immunological studies did not show any consistent trend toward immunosuppression, while temsirolimus exposure increased with dose in a less than proportional manner.

As a whole, most common adverse events were asthenia (56 %), mucositis (54 %), nausea (41 %), and dermatologic toxicity (41 %), thrombocytopenia being once again the dose-limiting toxicity. As far as signals of activity, one patient with non-small cell lung cancer had a confirmed partial response, while other three unconfirmed partial responses were observed in two RCC patients and in one soft-tissue sarcoma patient.

11.4.2 Phase II Studies

Temsirolimus clinical development proceeded with phase II studies conducted in different solid tumors, including RCC, melanoma, breast cancer, lung cancers, neuroendocrine tumors, and glioblastoma multiforme, the most promising activity being observed in RCC [16].

Indeed, in a randomized phase II study [16], 111 refractory RCC patients were exposed to 25, 75, or 250 mg of temsirolimus. Primary end point was overall response rate (ORR), but the Authors decided to include also minor responses (MR), defined as a decrease of measurable lesions between 25 and 50 % by WHO criteria. As a whole, ORR was 7 % for the entire intention-to-treat (ITT) population, but 51 % of patients yielded a clinical benefit (defined as complete responses + partial responses + minor responses + disease stabilizations). Duration of responses was about 6 months, and there were no differences in efficacy or survival between treatment groups. Most common adverse events again were rash (76 %), mucositis (70 %), asthenia (50 %), and nausea (43 %), while most frequent grades 3–4 adverse events were hyperglycemia (17 %), hypophosphatemia (13 %), anemia (9 %), and hypertriglyceridemia (6 %).

In another study [17], 71 RCC patients were enrolled onto a multicenter, phases I–II ascending-dose study of temsirolimus (5, 10, 15, 20, or 25 mg) administered i.v. once a week combined with interferon-α (6 or 9 MU) administered s.c. three times per week; an expanded cohort was treated at the recommended dose to obtain additional safety and efficacy information. The recommended dose which emerged from this study was 15 mg for temsirolimus and 6 MU for interferon-α, based on dose-limiting toxicities of stomatitis, fatigue, and nausea/vomiting, which were observed at higher doses of the two drugs. The most frequent grade 3 or 4 toxicities included leukopenia, hypophosphatemia, asthenia, anemia, and hypertriglyceridemia. Among patients who received the recommended dose ($n = 39$), 8 % achieved partial response and 36 % had stable disease for at least 24 weeks, with a resulting median PFS for the whole patient population of 9.1 months.

11.4.3 Pivotal Temsirolimus Phase III Trial in RCC: The ARCC Trial

Temsirolimus registration in RCC was obtained on the basis of the positive results of a randomized, controlled, phase III trial of temsirolimus, interferon-α, or a combination of the two: the ARCC (global *a*dvanced *r*enal *c*ell *c*arcinoma) trial [18]. In this study, 626 patients with previously untreated, poor-prognosis, metastatic RCC were randomized to receive 25 mg of intravenous (i.v.) temsirolimus weekly, up to 18 MU of interferon-α (however, with a starting dose of 3 MU) subcutaneous (s.c.) three times weekly, or a combination therapy with 15 mg of

temsirolimus weekly and 6 MU of interferon-α three times weekly, overall survival (OS) being the primary end point of the trial.

Patients who received temsirolimus alone had longer OS and progression-free survival (PFS) than patients who received interferon-α alone, while there was no significant difference in OS between the combination therapy group and the interferon-α group.

Indeed, the median overall survival for temsirolimus versus interferon-α alone was 10.9 and 7.3 months, with a hazard ratio (HR) of 0.73 (95 % confidence interval [CI]: 0.58 to 0.92; $p = 0.008$). The combination of interferon-α and temsirolimus failed to show benefit against interferon-α alone, with an OS of 8.4 months and an HR for death of 0.96 (95 % CI, 0.76 to 1.20; $p = 0.70$). As expected, the most common side effects associated with temsirolimus (either given as monotherapy or in combination with interferon-α) were dermatologic toxicity, peripheral edema, stomatitis, and lipid and glucose metabolism disorders; grade 3 or 4 adverse events were more often seen in the combination therapy (87 %) and interferon-α alone (78 %) groups than in the temsirolimus (67 %) group [19]. As a whole, the results of the ARCC trial are summarized in Table 11.1.

The overall survival benefit achieved in the global ARCC trial led the US Food and Drug Administration (FDA) to approve temsirolimus on May 30, 2007, as an anticancer therapy for use in the first-line setting of advanced poor-risk RCC patients [20].

However, due to difficulties in the enrollment of pure poor-risk patients (defined, according to the MSKCC criteria, as patients having three or more of the following five negative prognostic characteristics: anemia, high LDH levels, high corrected calcium levels, poor Karnofsky performance status, and an interval between diagnosis and start of treatment of less than a year), the protocol was emended, with a sixth negative prognostic feature (i.e., multiple metastatic sites) added to the

Table 11.1 Key features of the pivotal phase III trials evaluating everolimus and temsirolimus in RCC

Characteristic	Temsirolimus	Everolimus
Study population	Treatment-naive patients with poor-risk disease	Patients who had progressed on prior sunitinib and/or sorafenib
Number of patients	626	410
Randomization	Temsirolimus vs. temsirolimus/IFN-α vs. IFN-α	Everolimus/BSC vs. placebo/BSC
Primary end point	OS	PFS
Met primary end point?	Yes	Yes
ΔPFS (P-value)[a]	1.9 mos (P = NR)	3.0 mos (P, 0.001)
ΔOS (P-value)[a]	3.6 mos ($P = 0.008$)	0.39 mos ($P = 0.177$)

Abbreviation: *BSC* best supportive care

aΔPFS and ΔOS values reported for temsirolimus pertain to the comparison of temsirolimus alone to IFN-α

original MSKCC criteria to classify patients as poor risk [18]. Such a change allowed for the enrollment into the study also of patients from the intermediate MSKCC risk group.

Notably enough, a subgroup analysis of the study suggested that real poor-risk patients (i.e., those classified as such according to the original MSKCC criteria) benefited most for the treatment [19]; furthermore, patients with non-clear cell histologies seemed to achieve a preferential benefit from temsirolimus too [19].

11.4.4 Other Temsirolimus Phase III Trials in RCC: INTORSECT and INTORACT

More recently, temsirolimus was compared with the multikinase inhibitor sorafenib within the INTORSECT (Investigating Torisel as Second-Line Therapy) phase III trial, as a second-line therapy in patients with metastatic RCC after disease progression on first-line sunitinib [20]. As a whole, 512 RCC patients were randomly assigned to receive either temsirolimus, given i.v. at the dose of 25 mg once weekly ($n = 259$), or oral sorafenib at the standard dose of 400 mg twice per day, continuous dosing ($n = 253$), with stratification according to duration of prior sunitinib therapy ($\leq$ or $>$180 days), prognostic risk, histology (clear cell or non-clear cell), and nephrectomy status. No significant PFS difference between treatment arms was observed, median PFS being 4.3 and 3.9 months in the temsirolimus and sorafenib arms, respectively (stratified hazard ratio [HR]: 0.87; 95 % CI, 0.71 to 1.07; $p = .19$). Surprisingly enough, a statistically significant (and clinically relevant) OS difference in favor of sorafenib was observed: 16.6 vs. 12.3 months (stratified HR: 1.31; 95 % CI, 1.05 to 1.63; $p = .01$).

Another phase III trial was designed and conducted to prospectively determine the efficacy of a combination of temsirolimus plus bevacizumab as compared to bevacizumab plus interferon-α in previously untreated advanced RCC patients: the INTORACT (*In*vestigation of *Tor*isel and *A*vastin *C*ombination *T*herapy) study [21]. Patients were randomized to receive the combination of either temsirolimus (25 mg i.v. weekly) or interferon-α (9 MIU s.c. thrice weekly) with bevacizumab (10 mg/kg i.v. every 2 weeks), the primary end point being once again PFS. There were no significant differences in both PFS (9.1 and 9.3 months, respectively; HR: 1.1; 95 % CI, 0.9 to 1.3; $p = .8$) and overall survival (25.8 and 25.5 months, respectively; HR: 1.0; $p = .6$) with temsirolimus plus bevacizumab as compared to bevacizumab plus interferon-α, respectively. Despite differences in overall mean scores in the Functional Assessment of Cancer Therapy-Kidney Symptom Index (FKSI)-15 and FKSI-Disease-Related Symptoms subscales (in favor of the combination of temsirolimus plus bevacizumab), no differences in global health outcome measures were observed. Finally, treatment-emergent all-causality grade $\geq$ 3 adverse events were more common ($p < .001$) with temsirolimus plus bevacizumab and included mucosal inflammation, stomatitis, hypophosphatemia,

hyperglycemia, and hypercholesterolemia, whereas neutropenia was more common in the control arm (i.e., bevacizumab plus interferon-α).

As the negative results of the INTORSECT trial hampered the development of temsirolimus in second or later treatment line in RCC [22], the same happened for Temsirolimus first-line use outside the setting of poor-risk patients.

11.5 Everolimus

Everolimus (RAD001) is the other mTOR inhibitor that has been developed for the treatment of advanced RCC; it is administered orally, on a continuous daily schedule (even though a weekly schedule has been also tested, especially for combination regimens and in indications different from RCC) [23].

11.5.1 Phase I Studies

Based on preclinical data with weekly treatment schedules [24], an initial phase I trial in advanced solid tumors explored both weekly and daily dosing of the oral formulations of the drug [23]. In the first phase, patients were treated with weekly doses ranging from 5 to 30 mg. No dose-limiting toxicities (DLTs) were observed, and accompanying correlative studies assessing peripheral blood mononuclear cells (PBMCs) showed downregulation of relevant downstream moieties (i.e., p70S6 K). In the second part of the study, patients were treated with weekly doses of everolimus above 30 mg and daily doses of 5 or 10 mg. DLT was seen in one patient each at 50 mg/week (stomatitis and fatigue) and 10 mg/day (hyperglycemia). Ultimately, it was determined that doses of 70 mg weekly and 10 mg daily could be satisfactorily tolerated. Although the half-life of everolimus (∼30 h) was thought to facilitate weekly dosing of the drug, it was observed that daily dosing could produce more sustained target inhibition in preclinical models [25].

11.5.2 Phase II Study in RCC

The first published phase II trial of everolimus (dosed at 10 mg/day) for metastatic RCC, conducted by Amato et al. enrolled 41 patients with predominantly clear cell disease who had received up to one prior systemic treatment [26]. Most patients (83 %) had been previously treated, mainly in the form of immunotherapy (61 %). With 57 % of patients progression free for $\geq$6 months and median PFS of 11.2 months (95 % CI, 1.7–36.2 months), the study met the prespecified criteria for further evaluation. In all, 24 of 37 evaluable patients experienced some degree of tumor reduction. Objective responses per independent assessment were mainly

stable disease (SD), lasting for ≥3 and ≥6 months in 74 % and 58 % of patients, respectively, with an additional two patients achieving a partial response (PR). Considering all patients, median OS was 22.1 months (95 % CI, 1.4–36.4 months). Most adverse events (AEs) were of grade 1/2 severity, with no grade 4 AEs reported. The most common treatment-related grade 3 AEs were pneumonitis ($n = 7$, 18 %) and alanine aminotransferase elevation ($n = 4$, 10.3 %), followed by alkaline phosphatase elevation, hyperglycemia, and thrombocytopenia ($n = 3$ each, 8 %).

11.5.3 Pivotal Everolimus Phase III Trial in RCC: The RECORD-1 Trial

Everolimus was approved by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) for the treatment of advanced renal cell carcinoma (RCC) after failure of treatment with sunitinib and/or sorafenib, following the presentation of the results of the RECORD-1 (*Re*nal *C*ell Cancer Treatment with *O*ral *R*AD001 given *D*aily) trial.

The pivotal RECORD-1 trial was a randomized (2:1), placebo-controlled, phase III study, in which RCC patients who had failed treatment with one or two previous VEGFR tyrosine kinase inhibitors (TKIs); notably enough, the majority of patients had also failed other previous treatments [27].

A total of 416 patients were enrolled and stratified according to the number of previous treatments (sorafenib or sunitinib [1 TKI] vs. sorafenib as well as sunitinib [2 TKIs]) and prognostic risk group. Patients were then randomized in the ratio of 2 to 1 to receive everolimus (given at the standard dose of 10 mg daily, per os) plus best supportive care (BSC) or to placebo plus BSC. After the second interim analysis, the study was terminated since the prespecified efficacy end point had been met [27]. Indeed, at the final trial analysis, everolimus proved able to significantly improve PFS when compared to placebo: 4.9 months vs. 1.9 months, respectively (HR: 0.33; 95%CI, 0.25–0.43; $p < 0.001$) [28]. As a whole, the results of the RECORD-1 trial are summarized in Table 11.1.

Regarding OS, the high percentage of patients who crossed over from the placebo to the active drug precluded any chance to observe a significant difference between the two arms, although a subsequent statistical analysis, used to correct the estimate of the effect of treatment taking into account the bias generated by crossover, showed an OS 1.9 times longer in favor of everolimus [28].

Furthermore, everolimus significantly increased median PFS in each risk group regardless of whether patients had received 1 or 2 prior TKIs [29], had stopped prior therapy for intolerance [30], or of patient age [31].

11.5.4 Global Open-Label Expanded Access REACT Study

Based on the results of RECORD-1, the *R*AD001 *E*xpanded *A*ccess *C*linical *T*rial (REACT) [32] was initiated to provide everolimus in advance of regulatory approval and commercial availability to patients with metastatic RCC in whom prior VEGF TKI therapy had failed and to enable collection of safety and efficacy data in a larger and more diverse population of patients with RCC. A total of 1367 patients from 34 countries received everolimus, given at the standard dose of 10 mg, once daily. As in the RECORD-1 phase III trial, patients from all MSKCC risk categories were eligible, and the inclusion criteria were broadened to include patients with metastatic RCC of any histology, measurable or non-measurable disease, and brain metastases. Safety findings and tumor responses were consistent with those observed in RECORD-1, with no new safety issues identified. The most commonly reported serious AEs were dyspnea (5.0 %), pneumonia (4.7 %), and anemia (4.1 %), and the most commonly reported grades 3/4 AEs were anemia (13.4 %), fatigue (6.7 %), and dyspnea (6.5 %). Best overall response was stable disease and partial response in 51.6 % and 1.7 % of the treated patients, respectively. Median everolimus treatment duration was 14 weeks.

11.5.5 Multicenter, Non-interventional, Observational CHANGE Study

The efficacy and safety of everolimus in routine clinical practice in Germany was evaluated in a prospective observational study in patients with mRCC of any histology in whom one prior anti-VEGF therapy (including TKIs as well as bevacizumab) had failed [33].

Median time to disease progression (TTP), defined as the time from first everolimus intake to disease progression from any cause, was 6.6 months (95 % CI, 5.0–8.8 months) in the safety population ($n = 195$), 7.0 months (95 % CI, 5.1–9.0 months) in the efficacy population ($n = 165$), and 7.1 months (95 % CI, 5.5–9.0 months) in patients of the efficacy population who previously received only one VEGF TKI ($n = 121$). The prolonged median TTP compared with the RECORD-1 study (i.e., 4.9 months) might have been a result of the higher percentage of patients who received everolimus as a pure second-line therapy (72 % vs. 21 % for RECORD-1). The most commonly reported AEs (any grade occurring in >5 % of the safety population) associated with everolimus were dyspnea (14 %), anemia (13 %), nausea (9 %), pain (9 %), and stomatitis (8 %). Overall, more than 75 % of physicians reported a positive assessment of tolerance to everolimus and a high adherence to therapy.

11.5.6 RECORD-3: A Sequential Trial of Sunitinib Followed by Everolimus or Vice Versa

RECORD-3 is a randomized, open-label, multicenter, phase II, non-inferiority trial aimed at assessing the efficacy and safety of first-line everolimus followed by second-line sunitinib versus first-line sunitinib followed by second-line everolimus for the treatment of patients with mRCC [34].

In this trial, 471 patients with both clear (85.6 %) and non-clear cell metastatic RCC were evenly randomized between the two treatment arms. A majority of patients (86 %) presented with favorable or intermediate prognoses. Median follow-up was 22.7 months. Just 53.7 % of the patients who discontinued first-line everolimus received second-line sunitinib, and 51.6 % of patients who discontinued first-line sunitinib did receive second-line everolimus. Median PFS was 7.9 versus 10.7 months (HR = 1.43), respectively, whereas median OS was 22.4 versus 32.0 months (HR = 1.24), suggesting a trend toward improved survival in the sunitinib first arm. Common treatment-emergent adverse events for first-line everolimus vs sunitinib, respectively, were stomatitis (53 % vs. 57 %), fatigue (45 % vs. 51 %), and diarrhea (38 % vs. 57 %).

11.5.7 RECORD-4: Everolimus in a Pure Second-Line Setting

Since RECORD-1 demonstrated clinical benefit of everolimus in patients with metastatic RCC previously treated with sunitinib, sorafenib, or both (although prior treatments were also permitted), more recently the phase II RECORD-4 study prospectively assessed everolimus in a purely second-line setting, after having been exposed to sunitinib, other anti-VEGF therapy, or cytokines [35]. Overall median PFS was 7.8 months, while it was 5.7 months after sunitinib, 7.8 months after other previous anti-VEGF therapy, and 12.9 months after previous cytokines. Total median OS was 23.8 months, the same figure observed after previous sunitinib, while it was 17.2 months after other previous anti-VEGF therapy, median OS having not been reached yet after cytokines. The results of the RECORD-4 study ultimately confirmed the activity of second-line everolimus after first-line sunitinib or other anti-VEGF therapies.

11.6 The "Fall of Gods": The Checkmate-025 and Meteor Trials

After being for years one of the two standards of treatment for the second (and third) line of metastatic RCC [36], in the past 2 years, everolimus was the losing arm of two large randomized controlled, phase III trials which ultimately changed the treatment landscape in the second (and further) treatment line setting. Indeed, after the results of the nivolumab versus everolimus Checkmate-025 trial and of the cabozantinib versus everolimus METEOR trial, everolimus lost the status of standard treatment according to all the major international guidelines [37–39].

11.6.1 The Checkmate-025 Trial

Checkmate-025 was a randomized, open-label, phase III study comparing nivolumab (a fully humanized IgG4 isotype monoclonal antibody that inhibits PD-1 and thus restores anticancer immune responses) with everolimus in previously treated RCC patients [40]. A total of 821 patients with advanced clear cell RCC for which they had received previous treatment with one or two regimens of antiangiogenic therapy were randomly assigned to receive 3 mg/kg of nivolumab intravenously every 2 weeks or everolimus. The primary end point of this trial was OS, while the secondary end points included ORR and safety. The median OS was 25.0 months with nivolumab and 19.6 months with everolimus, a difference that was statistically significant and that translated into a reduction in the risk of death of 27 % in favor of nivolumab (HR for death = 0.73, 98.5 % CI = 0.57 to 0.93). However, median PFS did not significantly differ between nivolumab (4.6 months) and everolimus (4.4 months; HR = 0.88). As far as the ORR, it proved to be greater with nivolumab than with everolimus (25 % vs. 5 %).

Finally, nivolumab was also associated with quality-of-life improvement compared with everolimus [41].

11.6.2 The METEOR Trial

METEOR was a randomized, open-label, phase III trial aimed at evaluating the efficacy of cabozantinib (a multikinase inhibitor which targets all the three VEGFRs, AXL, as well as c-Met) as compared with everolimus in patients with RCC that had progressed after VEGFR-targeted therapy [42]. In this study, 658 patients were randomized to receive cabozantinib at a dose of 60 mg daily or everolimus. The primary end point was PFS, while secondary efficacy end points were OS and OR rate. Median PFS was 7.4 months with cabozantinib and 3.8 months with everolimus, representing a 42 % reduction in the risk of

progression and/or death in favor of cabozantinib (HR = 0.58; 95 % CI = 0.45 to 0.75). The OR rate was 21 % with cabozantinib and 5 % with everolimus.

As far as OS, at the final survival analysis [43], median OS was 21.4 months (95 % CI = 18.7-not estimable) with cabozantinib and 16.5 months (14.7–18.8) with everolimus, a difference that proved to be statistically significant in favor of cabozantinib (HR = 0.66 [95 % CI = 0.53–0.83]).

11.7 The Safety Profile of mTOR Inhibitors in RCC

Adverse events observed in patients treated with mTOR inhibitors are fairly constant, irrespective of each specific indication. They include cutaneous and mucosal events (i.e., stomatitis and skin rash), pulmonary dysfunction (noninfectious pneumonitis), metabolic abnormalities (elevated blood levels of glucose, cholesterol, and triglycerides), as well as immune-related events (i.e., increased incidence of infections) [44].

Metabolic and immune-related adverse events are clearly on-target effects of mTOR inhibition, while cutaneous and mucosal effects may have a less direct association with mTOR inhibition, although inhibition of mTOR-mediated growth and tissue repair and/or immune dysregulation has been proposed to be a factor in mucosal epithelia with high turnover. As far as the risk of infections is concerned, we should not forget that mTOR inhibitors were first developed as immunosuppressive agents and are still widely used as such in the transplantation setting.

As a whole, the safety profile of both mTOR inhibitors, as it comes from the two registrative studies (i.e., ARCC and RECORD-1) performed in RCC, is summarized in Table 11.2.

11.7.1 A Class-Specific Effect: Interstitial Pneumonitis

As regards mTOR inhibitors, the predominant class-effect toxicity is the occurrence of nonspecific interstitial pneumonitis. Although this is often asymptomatic or only presents with mild dyspnea and/or cough, it can be life-threatening in extent. Physiopathology of pulmonary toxicity is not fully elucidated. This event involves about 35 % of the patients receiving an mTOR inhibitor and appears after 3–-4 months of treatment [18, 28]. The clinical presentation is either a noninfectious pneumonitis that could be the result of a direct toxicity on pneumocytes, of an endothelial dysfunction, or of an immunoallergic mechanism or an infectious pneumonitis, knowing that both forms are not excluded from each other.

In the ARCC trial, temsirolimus-related interstitial pneumonitis paid limited attention as four cases (2 %) of patients of the temsirolimus arm developed this event [18]. However, a subsequent independent, blinded review in the temsirolimus group revealed all grades of drug-induced pneumonitis in 29 % of the patients

Table 11.2 Main adverse events of mTOR inhibitors (patients' percentage) [18, 28]

	Temsirolimus ($n = 208$) [18]		Everolimus ($n = 274$) [28]	
	All grades	Grades 3/4	All grades	Grades 3/4
Pulmonary				
Cough	26	1	30	0.7/0
Dyspnea	28	9	24	6.2/1.5
NIP	2	1	9.9	2.6/0
Non-pulmonary				
Stomatitis	20	1	38	4.0/0.4
Asthenia	51	11	33	2.6/0.7
Fatigue	NA	NA	31	5.5/0
Diarrhea	27	1	30	1.5/0
Rash	47	4	29	1.1/0
Nausea	37	2	26	1.5/0
Anorexia	32	3	25	1.5/0
Peripheral edema	27	2	25	0.7/0
Vomiting	19	2	20	2.2/0
Pyrexia	24	1	20	0.7/0
Mucosal inflammation	NA	NA	19	1.5/0
Headache	15	1	19	0.7/0.4
Abdominal pain	21	4	9.5	3.3/0
Laboratory investigations				
Anemia (decreased hemoglobin)	45	20	38	9.5/0.7
Hypercholesterolemia	24	1	20	3.3/0
Hypertriglyceridemia	27	3	15	1.1/0
Hyperglycemia	26	11	12	6.2/0
Raised creatinine	14	3	9.5	1.1/0
Thrombocytopenia	14	1	6.6	1.5/0

NA not available, *NIP* noninfectious pneumonitis

versus 6 % in the IFN-group ($p < 0.0001$) [45]. Most (60 %) occurred within the first 8 weeks of treatment, and only 31 % were symptomatic. Specific reccomendations for monitoring and managing temsirolimus-induced pneumonitis have been produced [46].

In the RECORD-1 trial, the incidence of all grades of noninfectious pneumonitis was 13.5 % (3.6 % grade 3, none grade 4) with a median time to occurrence of 15 weeks [28]. Clinical pneumonitis was fully reversible in 54 % of cases. This trial contained a prospective, independent monitoring of patients for pneumonitis that was reported separately [47]. On blinded review of serial images obtained with the study, baseline radiographic abnormalities were present in 17 % of all patients, in 24 % of those who went on to develop clinical pneumonitis, and in 50 % of those with subsequent grade 3 pneumonitis. New or worsening radiographic changes suggestive of pneumonitis were detected in 53.9 % of patients on everolimus, which included 38.9 % of patients without clinical suspicion for pneumonitis.

Based on these observations, the investigators issued specific management guidelines [47].

11.7.2 Other Common Toxicities

Other toxicities are common to both mTOR inhibitors with different levels of incidence: stomatitis, hyperglycemia, hypercholesterolemia, hypertriglyceridemia, hypophosphatemia, anemia, and cutaneous toxicity.

11.7.2.1 Stomatitis

The results of pivotal trials confirm what is observed in routine practices, i.e., a higher incidence of all-grade stomatitis with everolimus compared with temsirolimus (44 % versus 20 %), even if grades 3–4 stomatitis remain rare [18, 28]. Topical therapy is recommended; however, alcohol- or peroxide-containing mouthwashes should be avoided.

11.7.2.2 Hyperglycemia

The attenuating effects of the PI3K/Akt/mTOR cascade on insulin signaling have been established, and mTOR has been implicated in insulin resistance [48]. As expected, clinical trials of mTOR inhibitors highlighted an impact on glucose metabolism. The incidence of hyperglycemia is more frequent with everolimus than with temsirolimus (57 % vs. 26 %), whereas the incidence of grades 3–4 hyperglycemia is close between both mTOR inhibitors (15 % vs. 11 %, respectively) [18, 28]. Physicians should adhere to good clinical practice, which includes adequate glucose control before initiation of mTOR-directed treatment, education of patients on the symptoms of hyperglycemia, and intermittent monitoring of fasting glucose levels.

11.7.2.3 Hyperlipidemia

Effects of lipid metabolism can be explained through the roles of mTOR in cell metabolism [49]. Recent studies suggest that the TOR signaling network controls fat metabolism. In particular, mTORC1 appears to play an important role in adipogenesis as rapamycin treatment prevents adipocyte differentiation and, thus, lipid accumulation. The mechanism by which mTOR controls adipogenesis is poorly understood. In the pivotal phase III trials [18, 28], temsirolimus caused hypercholesterolemia and hypertriglyceridemia in 24 and 27 % of patients, respectively; the reported incidence was higher for everolimus as cholesterol and

triglycerides were elevated in 77 %, and 73 % of patients, respectively. As for the management of hyperglycemia, no standardized guidance has been issued. Physicians should ascertain adequate levels prior to starting treatments and monitor patients for the development of hyperlipidemia.

11.7.2.4 Hypophosphatemia

Mild hypophosphatemia has been reported in 6 % of patients using temsirolimus and 37 % (6 % of grades 3–4) for patients using everolimus in pivotal phase III trials [18, 28]. Severely low levels can impair neurologic and myocardial function and should be replenished.

11.7.2.5 Anemia

The incidence of all-grade anemia is higher with everolimus than with temsirolimus (45 % versus 92 %), whereas the incidence of grades 3–4 anemia is close between both mTOR inhibitors (13 % versus 20 %, respectively) [18, 28].

11.7.2.6 Dermatologic Toxicity

The mTOR inhibitor-associated cutaneous toxicity consists of rash, acneiform dermatitis, pruritus, ungueal toxicity, and lower limb edema [50]. Contrarily to the majority of other drug-related toxicities, the cutaneous toxicity is more frequent with temsirolimus than with everolimus (47 % vs. 29 %) [18, 28]. The management of cutaneous side effects should be based on fragrance-free moisturizer lotion and, if necessary, on topical corticosteroids.

11.7.2.7 Infections

The mTOR inhibitors were initially mainly used as an immunosuppressant in recipients of solid organ transplantation because of their ability to potently inhibit T cell function. So, these immunosuppressive properties of mTOR inhibitors may predispose RCC patients to infections with opportunistic pathogens as well as to bacterial, viral, or fungal infections. Systemic bacterial infections as pneumonia, invasive fungal infections including candidiasis, or invasive aspergillosis or viral infections such as reactivation of hepatitis B/C virus have been described with mTOR inhibitors treatment. Some of these infections have been severe (e.g., leading to respiratory failure) and occasionally fatal. Clinicians should be aware of the increased risk of infection with mTOR inhibitors and be vigilant for any symptoms and clinical signs of infection. Preexisting infections should therefore be treated appropriately before starting treatment with mTOR inhibitors. If a severe

infection occurs during mTOR inhibitors' administration, the treatment should be discontinued temporarily or permanently.

11.8 Combination Therapy of the Two mTOR Inhibitors with Other Targeted Agents

Concurrent treatment of RCC with mTOR inhibitors and other targeted agents has been studied in hopes of enhancing antitumor effects by parallel inhibition of multiple oncogenic signaling pathways. Unfortunately, this has been limited by treatment-associated toxicity.

Several trials have evaluated the safety of combining an mTOR inhibitor (temsirolimus or everolimus) with a VEGF TKI (sunitinib or sorafenib) which has typically required attenuated dosing schedules [51–54]. In a phase I trial of sunitinib plus temsirolimus, DLTs were seen in two of three patients at the starting dose of temsirolimus 15 mg weekly and sunitinib 25 mg daily (1 grade 3 acneiform rash, 1 grade 3 cellulitis). Because of efficacy concerns at lower doses, the study was terminated early [51]. The combination of sunitinib and everolimus proved to be toxic in a separate phase I trial [52], and investigators switched to a weekly schedule of everolimus, as two of two patients suffered DLT even at attenuated doses of sunitinib 37.5 mg and everolimus 5 mg. Even so, chronic treatment was only tolerable at the lowest weekly dosing schedule of everolimus 20 mg weekly, with sunitinib 37.5 mg daily (4 weeks on, 2 weeks off). DLTs included mucositis, vomiting, and leukopenia. Five patients (25 %) achieved PR, three of these had non-clear cell RCC [52]. In a phase I trial of sorafenib plus temsirolimus in advanced solid tumors, investigators reported nine DLT in 23 patients treated up to a level of temsirolimus 25 mg weekly and sorafenib 400 mg twice daily. Toxicities were predominantly mucocutaneous, but also included thrombocytopenia and loss in renal function [53]. Sorafenib was better tolerated when combined with everolimus, as per preliminary reports of another dose-finding study [54]. Still, two out of four patients in the second cohort suffered DLT (grade 4 uricemia and grade 3 elevation in lipase with concurrent pancreatitis, respectively) with everolimus 5 mg daily plus sorafenib 400 mg twice daily. Three of ten evaluable patients achieved PR, two had SD, and five showed evidence of progression.

Better tolerance was also seen for combinations of mTORs with bevacizumab. A phase I/II trial of temsirolimus and bevacizumab [55] established safety at standard doses (temsirolimus 25 mg IV weekly, bevacizumab 10 mg/kg IV every 2 weeks) with one DLT in six patients (grade 3 mucositis). The tolerability at full doses prompted phase II (TORAVA and BEST) and phase III (INTORACT, described above) trials [21]. The TORAVA trial (bevacizumab plus temsirolimus vs. single-agent sunitinib vs. bevacizumab plus IFN-α in the first-line setting) revealed higher no improvement in efficacy for the combination [56]. In the four-arm phase II BEST trial (bevacizumab/temsirolimus vs. bevacizumab/sorafenib vs. sorafenib/

temsirolimus vs. sorafenib alone) temsirolimus combined with bevacizumab or sorafenib offered no improvement in efficacy, but did add toxicity [57].

The combination of everolimus and bevacizumab is tolerated at full doses as demonstrated in a phase I trial that reported DLT and grades 1–2 toxicities [58]. A subsequent phase II study conducted in targeted therapies-naïve as well as targeted therapies-pretreated advanced RCC patients [59] yielded an ORR of 30 % and 23 % in the targeted therapies-untreated and pretreated groups, respectively. Median PFS and OS were reported at 9.1 and 21.3 months for untreated patients and 7.1 and 14.5 months for the pretreated group, respectively. The RECORD-2 phase II trial [60] has compared bevacizumab + everolimus to bevacizumab + interferon-α in untreated clear cell RCC. There was no significant difference between the everolimus and interferon-α groups in objective response rates (27 % vs. 28 %) or median progression-free survival based on central review (9.3 months vs. 10 months; HR = 0.91; $P = .485$).

11.9 Conclusions and Future Perspectives

The two mTOR inhibitors temsirolimus and everolimus have contributed to expand our armamentarium against kidney cancer offering patients suffering from this neoplasm an alternative to VEGF-targeting agents.

To date, according to the results of both the ARCC and the RECORD-3 trials, we know that in the first line, mTOR inhibitors should not be given, unless in the case of patients with poor-risk features, as defined by the ARCC study criteria.

As far as the second line, nivolumab and cabozantinib are presently the new standards of treatment, and the still unresolved issue of using another VEGFR TKI (e.g., axitinib) or the mTOR inhibitor everolimus after a first-line VEGFR TKI [35] retains importance only in those countries where nivolumab and cabozantinib are not yet available (or reimbursed). As a consequence of the Checkmate-025 and METEOR trials, everolimus has thus been practically pushed in the third or even fourth line, although data of activity in these setting are scarce and retrospective.

Novel molecular and genetic insights in the pathogenesis of RCC could probably help us to tailor the treatment of our patients in the future and possibly give new life to everolimus (and mTOR inhibitors as a whole).

Indeed, we can now subdivide RCC in two distinct entities: a disease of chromosome 3p and a metabolic disease, the latter being characterized by mutations in the kinase and FAT domain of the mTOR gene [61]; patients harboring these mutations are characterized by a profound metabolic disturbance and could theoretically derive benefit from an inhibition of mTOR, as already suggested by two different groups [62, 63]. Only prospective studies will address with fascinating hypothesis, hopefully leading to a more tailored and personalized use of mTOR inhibitors in advanced RCC.

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Chapter 12
Immunotherapy for Renal Cell Cancer (RCC)

Shigehisa Kitano, Ayumu Ito, and Youngji Kim

Abstract It is estimated that approximately 30–40 % of patients are diagnosed in the advanced stage and require systemic therapy. However, clinical development of cytotoxic chemotherapies for RCC has failed for many years. Historically, investigators have focused on the immunogenicity of RCC. Because many types of tumor-infiltrating lymphocytes (TILs) were frequently observed in tissue sections, in rare cases spontaneous tumor regression was experienced. Based on these observations, clinical development of immunotherapies has been attempted. This chapter introduces the history of clinical development of the conventional immunotherapies, including cytokine- and vaccine-based approaches, and then, does the novel immunotherapies, 'immune checkpoint inhibitors' as an emerging option for advanced RCC.

Keywords Immunotherapy • Cancer vaccines • Cytokine therapy • Immune checkpoint inhibitor • Anti-PD-1 antibody

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M. Oya (ed.), *Renal Cell Carcinoma*, DOI 10.1007/978-4-431-55531-5_12

12.1 Introduction

Renal cell carcinoma (RCC) accounts for 80–90 % of primary renal neoplasms. While the incidence rate of RCC varies globally among regions, it is increasing in most countries [1]. Approximately 30–40 % of patients are diagnosed in the advanced stage [2], and the majority requires systemic therapy. Historically, the prognosis of RCC patients who received cytotoxic chemotherapy was dismal [3]. For many years, investigators have focused on the immunogenicity of RCC, as many types of tumor-infiltrating lymphocytes (TILs) were frequently observed in tissue sections, and in rare cases spontaneous tumor regression was experienced [4, 5]. Previously (starting in the 1980s), immunomodulatory therapy, using cytokines such as interferon alpha (IFNα) and/or interleukin-2 (IL-2), was one of the standard-of-care treatments and demonstrated some efficacy, though toxicities were significant, achieving a median progression-free survival (PFS) of 3–5 months and a median overall survival (OS) of 1.5 years (summarized in Tables 12.1 and 12.2) [6–8]. In more recent years, molecular targeted agents, directed against VEGF (vascular endothelial growth factor) or mTOR (mammalian target of rapamycin) pathways [9–12], have largely replaced cytokine-based therapy and brought about substantial changes in the treatment strategies for advanced RCC [13]. Although molecular targeted therapy with tyrosine kinase inhibitors (TKIs) and mTOR inhibitors has provided a longer median OS of 2.5 years, only a small percentage of patients experience a long-term complete response (CR) [14]. Cabozantinib, a multikinase inhibitor of VEGF receptors, AXL, RET, and MET, is one of the promising agents being investigated [15, 16]. In this context, developing a novel therapeutic approach providing a major breakthrough remains critically important. In this chapter, we will briefly review the conventional immunotherapies, including cytokine- and vaccine-based approaches and then discuss the clinical development and future challenges of novel immunotherapies, focusing on "immune checkpoint blockade" as an emerging option for managing advanced RCC. All publications were obtained from the PubMed database, and the ongoing clinical trials were identified by searching the official website designated as follows: www.clinicaltrials.gov.

Table 12.1 Immunotherapy (IFN-α, IFN-γ, and IL-2) for metastatic renal cell carcinoma

Agent	n	Phase	References
Proleukin (IL-2)	255	Phase II	Fyfe G. et al. [19]
IFN-α	350	Meta-analysis	Medical Research Council Renal Cancer Collaborators [22]
(vs medroxyprogesterone acetate)			
IFN-α+ VLB	160	RCT	Pyrhonen S et al.[23]
(vs VLB alone)			
IFN-γ	197	RCT	Gleave et al. [24]
Placebo			

Table 12.2 Therapeutic RCC vaccines

Agent	n	Phase	References
IMA901	28	Phase I	Walter S et al. [27]
	68	Phase II	
	Ongoing	Phase III	NCT01265901
Nephrectomy + Reniale®	379	Phase III	Jocham D. et al. [28]
Nephrectomy			
MVA-5 T4	733	Phase III	Amato RJ. et al. [30]
Placebo			
Vitespen (formerly Oncophage®)	818	Phase III	Wood C. et al.[31]
TG-4010	37	Phase II	Oubard S. et al.[32]
AGS-003	21	Phase II	Amin A. et al. [34]
AGS-003	Ongoing	Phase III	NCT01826877
DC-vaccine	148	Meta-analysis	Draube A. et al. [35]
DC-CIK	Ongoing	Phase III	NCT00862303

12.2 Cytokine Therapy (IFN-α, IFN-γ, and IL-2) for Metastatic RCC

For metastatic RCC, cytokine therapies with a focus on interferon (IFN-α) and interleukin (IL-2) have been studied (Table 12.1). However, based on elucidation of the mechanisms underlying RCC onset and progression, many molecular targeted drugs have been developed. Currently, molecular targeted drugs are used as the first-line treatment for metastatic RCC.

Bolus high-dose intravenous recombinant human IL-2 treatment, particularly in patients with metastatic RCC, was reported in 1985 and 1987 [17, 18]. Based on the results obtained, cytokine therapy for oncological use was developed. For IL-2, no randomized controlled trial (RCT) has yet been performed, but the response rate in the phase II trial was 14 % and the CR rate 5 % [19]. Patients who obtained CR showed long-term survival, raising the possibility of a potentially curative treatment. As a result of this clinical trial, IL-2 was approved by the US Food and Drug Administration (FDA) for the treatment of metastatic RCC in the 1990s. In addition, the objective response rate (ORR) to cytokines was reportedly 12.9 % and the CR rate was 3.6 % in a meta-analysis [20]. Furthermore, IL-2 therapy was found to be effective, achieving long-term clinical responses [21].

There are also two reports of homogeneous RCT examining IFN-α for metastatic RCC [22, 23]. One was from the Medical Research Council Renal Cancer Collaborators [22]. Their 350 cases in the target population were divided into two groups, one treated with IFN-α, the other with medroxyprogesterone. The proximity effect, as indicated by nonprogressive survival, showed IFN-α to be effective, and the OS findings were consistent with this observation. Another RCT was reported by Pyrhonen [23]. The 160 cases in the target population were divided into two groups, one receiving a combination of vinblastine (VBL) and IFN-α and the other VBL

only. The combination of IFN-α and VBL was more effective than VBL alone in terms of the proximity effect, nonprogressive survival, and OS. Based on these results, the efficacy of IFN-α monotherapy for advanced RCC has been confirmed.

On the other hand, with respect to IFN-γ therapy for metastatic RCC, Gleave reported different RCT findings [24]. The target population of 197 patients was divided into two groups, one given IFN-γ and the other a placebo. However, IFN-γ had no impact on either the proximity effect or nonprogressive survival.

Currently, IFN-α therapy appears to be inferior, in terms of both the response rate and nonprogressive survival, as compared to some of the molecular targeted drugs [7, 8]. Therefore, molecular targeted therapy has recently been standardized. Once the responsiveness of patients to IFN-α and IL-2 therapy has been determined, it will become necessary to review the future role of cytokine monotherapy regimens for RCC.

12.3 Therapeutic RCC Vaccines

It has been suggested that cancer vaccines are safe as well as showing efficacy against some carcinomas [25, 26]. IMA901, Reniale®, TroVax® (MVA-5 T4), Vitespen (formerly Oncophage®), TG4010, AGS-003, and the dendritic cells (DC) vaccines are now recognized as therapeutic RCC vaccines (Table 12.2).

IMA901

IMA901 is composed of multiple tumor-associated peptides (TUMAPs). In a phase I study [27], the IMA901vaccine induced T-cell immune responses. In addition, the regulatory T cell number needed to suppress the immune response was low. Moreover, in a randomized phase II trial, a single dose of cyclophosphamide prior to IMA901 vaccine administration reduced the number of regulatory T cells (Treg) and confirmed that immune responses to multiple TUMAPs were associated with longer OS. Currently, a randomized phase III trial is being conducted to determine the clinical benefits of treatment with IMA901. Three hundred and forty patients were randomized to a sunitinib alone regimen or a combination of first-line sunitinib and IMA901.

Reniale® (Autologous RCC-Tumor Lysate Cell-Based Vaccine)

Reniale® is an autologous RCC-tumor lysate cell-based vaccine. In a phase III trial, 379 metastatic RCC patients who had undergone nephrectomy in an adjuvant setting were given Reniale® [28]. PFS rates at 5 years and 70 months were 77·4 % and 72 % in the vaccine group and 67·8 % and 59·3 % in the control group, respectively. Adjuvant treatment with Reniale® after radical nephrectomy thus appeared to be beneficial.

TroVax® (MVA-5 T4)

TroVax® (MVA-5 T4) is a therapeutic vaccine targeting the tumor antigen 5 T4 expressed in human cancer cells [29]. In a randomized, placebo-controlled phase III trial, MVA-5 T4/placebo in combination with either sunitinib, IL-2, or IFN-α as first-line metastatic RCC therapy produced no significant increase in OS

[30]. However, patients treated with MVA-5 T4 plus IL-2 with a good prognosis showed a significantly better OS than comparable patients receiving the placebo plus IL-2 (HR, 0.54; 95 % CI, 0.30–0.98; P = 0.046). These findings indicated that there might be subsets of patients who would benefit from MVA-5 T4.

Vitespen (Formerly Oncophage®)

Vitespen (formerly Oncophage®) is composed of an autologous tumor-derived heat shock protein (glycoprotein 96) peptide complex (HSPPC-96) [31]. In a phase III trial, there was no difference in recurrence-free survival between the vitespen group and those who received no treatment after nephrectomy for RCC.

TG4010

TG4010 is a therapeutic vaccine targeting the vaccinia virus expressing MUC1, which is overexpressed in RCC and is related to RCC progression [32]. No objective clinical response (clinical response, safety, time to treatment failure, OS, and immune response) was observed in a phase II study evaluating TG4010 efficacy and tolerability, alone or in combination with IFN-α2a and IL-2, for metastatic RCC. Stable disease for more than 6 months was reported in 5 of 27 evaluable patients (18 %) receiving TG4010 alone and 6 of 20 patients (30 %) given TG4010 plus cytokines. MUC1-specific $CD8^+$ T-cell responses were associated with OS [33].

AGS-003

AGS-003 is an autologous immunotherapy employing a DC-based vaccine in which mature DCs are electroporated with amplified tumor RNA plus synthetic CD40-ligand (CD40L) RNA. In a phase II study, AGS-003 was evaluated in combination with sunitinib in intermediate and poor-risk, treatment-naïve patients with metastatic RCC eligible for nephrectomy [34]. Median PFS was 11.2 months (95 % CI: 6.0, 19.4) and median OS was 30.2 months (95 % CI: 9.4, 57.1) for all patients. AGS-003 showed no major toxicity other than grade 1 local reactions. In addition, the magnitude of the increase in the absolute number of cytotoxic (CD8 (+), CD28 (+), CD45RA (−) effector/memory) T cells (CTLs) correlated with OS. Currently, a phase III trial is underway to examine the combination of AGS-003 plus sunitinib versus sunitinib alone, in newly diagnosed unfavorable-risk metastatic RCC patients.

DC Vaccines

Dendritic cells (DC) are potent antigen-presenting cells that play a role in the induction of antigen-specific T-cell responses. In a meta-analysis of 12 trials, objective response rates averaged 12.7 % and clinical benefit was seen of 48 % of RCC cases [35]. However, DC subtypes and antigen types differed among these trials.

12.4 Immune Checkpoint Blockade Therapy for RCC

Recent clinical advances in cancer immunotherapy have been remarkable. This growing class of therapeutic agents is designed to modulate and activate a patient's physiological immune activities to fight cancer and includes a wide variety of

approaches [36]. "Immune checkpoint blockade" is the leading strategy and has been actively investigated in clinical trials during this decade [37–39]. It is based on blocking monoclonal antibodies (Abs), "immune checkpoint inhibitors," which antagonize the "immune checkpoint molecules" expressed on immune cells, or their ligands on immune cells and/or certain types of tumor cells. The immune checkpoint molecules, by interacting with their ligands, send inhibitory intracellular signals to suppress immune cell activities. This inhibitory axis ordinarily attenuates excessive immune responses and follows normal tissue injury; in other words it maintains self-tolerance [40, 41]. The knockout mouse model with impairment of this pathway develops an autoimmune-like syndrome, which is potentially lethal, and prominent T-cell infiltrations into multiple organs. One of the mechanisms by which cancer cells evade antitumor immune attack is hijacking this immunosuppressive pathway, by either directly utilizing the immune checkpoint molecules or indirectly recruiting regulatory immune cell subsets [42]. Based on our understanding of these mechanisms, immune checkpoint blockade, which is often compared to "releasing the brakes on immune cells," aims to redirect and boost antitumor immune activities. Due to this mechanism of action, unlike anti-angiogenic therapy or other antitumor Abs such as anti-CD20 rituximab and anti-HER2 trastuzumab, target molecules on the tumor cell surface do not need to be identified (Fig. 12.1). Based on the encouraging data obtained from animal models, a number of early clinical trials for patients with refractory advanced malignancies have been launched.

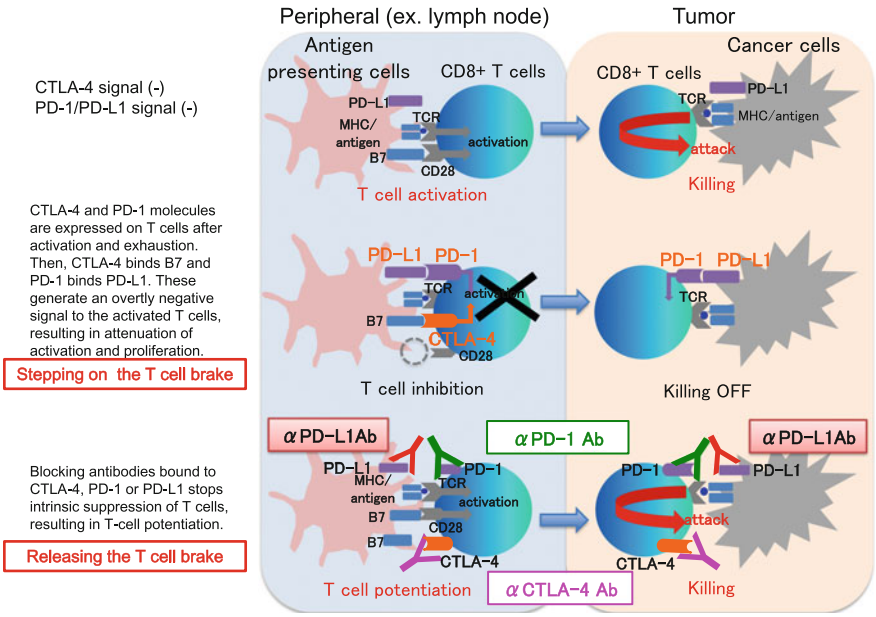

Fig. 12.1 Mechanisms of action of immune checkpoint inhibitors

Several agents have been rapidly advanced into phase III trials, and some have even obtained regulatory approval based on their safety profiles and durable antitumor efficacies. Ipilimumab (BMS, Princeton, NJ), which is directed against the CTL antigen-4 (CTLA-4), is a first-in-class drug that was approved for the treatment of advanced melanoma by the US FDA in 2011 [43, 44]. The long-term safety and efficacy profiles of ipilimumab for melanoma provide a platform for the clinical development of this class of agents targeting several other immune checkpoint molecules [45, 46]. At present, intensive investigations of these agents, as monotherapy or with combination therapy, in a variety of tumors are underway [47–49]. In the following sections, we will focus on the clinical development of immune checkpoint inhibitors for RCC patients (summarized in Table 12.3).

12.4.1 Anti-CTLA-4 Ab

CTLA-4 (also known as CD152) is a member of the CD28 family of co-inhibitory receptors expressed on the surfaces of $Foxp3^+$ Treg cells and activated by T cells [40, 50]. CTLA-4 binds its ligands, B7.1 (CD80) and B7.2 (CD86), expressed on antigen-presenting cells. The inhibitory effect on T-cell activities is derived mainly from two mechanisms. One is the competition between CTLA-4 and costimulatory receptor CD28, for binding shared ligands (B7.1 and B7.2). As CTLA-4 has stronger binding affinity than CD28, it reduces CD28-derived T-cell stimulation. The other is a direct intracellular signal that CTLA-4 sends to attenuate the T-cell receptor (TCR)-mediated signaling pathway [51]. It has also been suggested that the anti-CTLA-4 Ab ipilimumab, which has a humanized IgG1-type Fc domain with the highest affinity for Fcγ receptors among the known human IgG isotypes, might have an exceptional mechanism of action [52]. In a mouse model, it was shown that intra-tumoral Tregs with higher CTLA-4 expression relative to other T-cell subsets were preferentially depleted by antibody-dependent cellular cytotoxicity (ADCC) [53]. On the basis of encouraging preclinical data demonstrating this antitumor effect, CTLA-4-blocking antibodies have been extensively evaluated in melanoma and other histological tumor types.

Ipilimumab is a fully humanized IgG1 monoclonal Ab that blocks CTLA-4. A phase II trial compared two cohorts of RCC patients receiving either continuous 3 mg/kg or one dose of 3 mg/kg followed by 1 mg/kg of ipilimumab (both every 3 weeks) [54]. In the former (higher-dosing) cohort, treated with the approved and commonly used regimen, 5 of 40 patients including IL-2 refractory cases showed a partial response (PR), three of whom experienced a durable (> 1 year) response. Grade 3–4 immune-related adverse events (irAEs) were seen in 33 % of all patients.

Table 12.3 Immune checkpoint blockade as a monotherapy for RCC

Target	Agent	Patient setting	Development status	Trial ID	Phase	Trial status	N (N planned, if recruiting)	Regimen	Primary end point	Response	Survival	AEs	Reference
CTLA-4	Ipilimumab	≧ 2nd line		NCT00057889	II	Completed	61	(3 mg/kg every 3 weeks) vs. (3 mg/kg 1d followed by 1 mg/kg every 3 weeks)	Safety	ORR (in 40 pts. receiving 3 mg/kg): 12.5 %	NA	Gr. 3–4 irAEs: 33 %	[54]
PD-1	Nivolumab	≧ 2nd line	Approved	NCT00441337 (MDX1106–01)	I	Completed	1	3 mg/kg, three doses	Safety	PR after three doses CR after 4 years from the last dose	NA	Hypothyroidism	[64, 65]
				NCT00730639 (MDX1106–03)	Ib	Ongoing (not recruiting)	34	1 vs. 3 mg/kg, every 2 weeks	Safety	ORR: 29 %, median duration of 12.9 mo	Median PFS: 7.3 mo median OS: 22.4 mo	Gr. 3–4 trAEs: 18 %	[66, 67]
				NCT01358721	I	Ongoing (not recruiting)	80	0.3 vs. 2 vs. 10 mg/kg, every 3 weeks	Immunomodulatory activity	NA	NA	NA	https://www.clinicaltrials.gov
				NCT01354431	II	Ongoing (not recruiting)	168	0.3 vs. 2.0 vs. 10.0 mg/kg, every 3 weeks	PFS	ORR (0.3 vs. 2.0 vs. 10.0): 20 % vs. 22 % vs. 20 %	Median PFS: 2.7 vs. 4.0 vs. 4.2 mo median OS: 18.2 vs. 25.5 vs. 24.7 mo	Gr. 3–4 trAEs: 11 %	[68]
				NCT01668784 (CheckMate-025)	III	Ongoing (not recruiting)	820	(Nivo: 3 mg/kg every 2 weeks) vs. (eve: 10 mg daily)	OS	ORR (nivo vs. eve): 25 % vs. 5 %	Median PFS: 4.6 vs. 4.4 mo median OS: 25.0 vs. 19.6 mo	Gr. 3–4 irAEs: 19 % vs. 37 %	[69]
	Pembrolizumab	As neoadjuvant		NCT02212730	I	Ongoing (recruiting)	36	(pemb + surgical resection) vs. (surgical resection only) Pemb: 200 mg, every 3 weeks, up to three cycles	Safety	NA	NA	NA	https://www.clinicaltrials.gov/
PD-L1	Atezolizumab (MPDL3280A)	≧ 2nd line		NCT01375842	I	Ongoing (recruiting)	604	3 vs. 10 vs. 15 vs. 20 mg/kg, every 3 weeks	Safety	ORR: 15 %	Median PFS: 5.6 mo median OS: 28.9 mo	Gr. 3 trAEs: 17 %, Gr. 3 irAEs: 4 % No Gr. 4–5 AE occurred	[75]
	BMS-936559	≧ 2nd line		NCT00729664 (MDX1105–01)	I	Completed	17	10.0 mg/kg, every 2 weeks	Safety	ORR: 12 %, SD (≧24 weeks): 41 %	PFS (@24 weeks): 53 %	NA	[76]

12.4.2 Anti-PD-1 and Anti-PD-L1 Ab

Programmed cell death protein-1 (PD-1, also known as CD279) is a member of the CD28 family of co-inhibitory receptors, which are inducibly expressed on the T, B, and NK cells [40, 55]. The ligands of PD-1 are PD-L1 (B7-H1) and PD-L2 (B7-H2), expressed on antigen-presenting cells [56]. The difference between PD-1 and CTLA-4 is that CTLA-4 functions in the priming phase of T-cell activation, PD-1 in the effector phase. PD-1 has a cytoplasmic domain that sends inhibitory signals to the TCR-derived signaling pathway [51]. It has been shown that PD-L1 is expressed in some tumor types including RCC [57–59] to evade antitumor immune surveillance and eradication [60]. PD-1 and/or PD-L1 expression in tumor tissues from RCC cases is associated with tumor aggressiveness and a poor prognosis [61, 62]. Preclinical and clinical studies have shown that blocking the PD-1-PD-L1/2 pathway leads to reactivating antitumor immune responses and tumor regression [63].

Nivolumab is a fully human IgG4 monoclonal antibody against PD-1 [63]. A first-in-human, dose-escalating phase I clinical trial evaluated nivolumab in 39 patients with advanced metastatic solid tumors, including one RCC patient who had VEGF inhibitor refractory disease [64]. Nivolumab was generally well tolerated, and the maximum tolerated dose with a single administration was not defined. The RCC patient showed a durable PR after three doses of 10 mg/kg of nivolumab and then a CR after 3 years without any antitumor therapy [65]. A subsequent phase I trial evaluated nivolumab in a larger cohort of patients, including 34 RCC patients with advanced disease [66]. The long-term follow-up data from the RCC cohort was recently published [67]. The patients, 71 % of whom had already been treated with 2–5 regimens, received either 1 mg/kg (N=18) or 10 mg/kg (N=16) of nivolumab every 2 weeks in an 8 week cycle up to 96 weeks. In all patients, the ORR was 29 %, with a median duration of 12.9 months. The median PFS was 7.3 months (95 % CI: 3.6–10.9), and the median OS was 22.4 months (95 % CI: 12.5, not estimable). Grade 3–4 adverse events (AEs) occurred in approximately 18 % of patients. Based on the encouraging results of these early studies, a randomized, dose-ranging phase II trial was conducted [68]. The 168 RCC patients received either 0.3 (N = 60), 2.0 (N = 54), or 10.0 (N = 54) mg/kg of nivolumab administered once every 3 weeks. There were 118 patients (70 %) who had received at least two prior systemic therapies. The primary end point was PFS. The ORRs were 20 %, 22 %, and 20 % in the 0.3-, 2.0-, and 10.0-mg/kg cohorts, respectively. There was no dose-response relationship for PFS: the median PFS rates were 2.7 (80 % CI: 1.9–3.0), 4.0 (80 % CI: 2.8–4.2), and 4.2 (80 % CI: 2.8–5.5) months, respectively (P = 0.9). The median OS rates were 18.2 (80 % CI: 16.2–24.0), 25.5 (80 % CI: 19.8–28.8), and 24.7 (80 % CI: 15.3–-26.0) months, respectively. Compared with the previous phase I trial, this study showed lower ORR and PFS but similar OS. A phase III RCT (CheckMate 025 trial) compared nivolumab with the mTOR inhibitor everolimus, which is a standard salvage therapy, in previously treated RCC patients [69]. In July 2015, this

study was stopped ahead of schedule after the independent data monitoring committee found significant survival superiority in those receiving nivolumab as compared to everolimus. In total, 820 patients had been randomly assigned at a 1:1 ratio to nivolumab (3 mg/kg every 2 weeks; N = 410) or everolimus (10 mg daily; N = 411). The primary end point was OS. The ORR was 25 % in the nivolumab vs. 5 % in the everolimus cohort (odds ratio 5.98; 95 % CI: 3.68–9.72; P < 0.001). The median PFS was 4.6 (95%CI: 3.7–5.4) vs. 4.4 (95%CI: 3.7–5.5) months (HR: 0.88; 95 % CI: 0.75–1.03; P = 0.11), and the median OS was 25.0 (95%CI: 21.8-NE) vs. 19.6 months (95%CI: 17.6–23.1) (HR 0.73; 98.5 % CI: 0.57–0.93; P = 0.002), respectively. Grade 3–4 irAEs occurred in 19 % of the patients receiving nivolumab, an incidence significantly lower than that in the everolimus cohort (37 %).

The Checkmate 025 trial validated earlier trials showing OS prolongation with nivolumab as salvage therapy. The lack of consistent PFS extension might be attributable to variable patterns of responses to immune checkpoint blockade, with some of the treated patients initially showing progression on computed tomographic scans due to transient inflammation giving the appearance of tumor enlargement. This "pseudo-progression" highlights the necessity of establishing specific response criteria for cancer immunotherapy [70, 71]. Another clinically important point underscored by this study was the lack of an association between PD-L1 expression in the tumor and the response to nivolumab. Pretreatment biomarkers predicting therapy responsiveness and/or toxicity are urgently needed to select patients who would actually benefit from this class of therapy. As to this point, which we will discuss separately (see section below), nivolumab was approved by the US FDA for patients with advanced RCC refractory to anti-angiogenic therapy, in November 2015.

Atezolizumab (MPDL3280A) is a humanized IgG-1κ monoclonal Ab to PD-L1 [72]. It has a genetically modified Fc domain that is involved in impairing the ADCC-dependent cellular cytotoxicity directed to PD-L1-expressing cells. Following studies in other types of tumors [73, 74], atezolizumab was recently evaluated in a phase I dose-escalation study in 70 patients with previously treated advanced RCC [75]. The doses ranged from 3 to 20 mg/kg (administered every 3 weeks). Atezolizumab was well tolerated and no maximum tolerated dose was defined. Grade 3 treatment-related AEs and irAEs were seen in 17 % and 4 % of patients, respectively, but no grade 4–5 AEs occurred. The ORR was 15 % in 62 evaluable patients. There was a trend toward a higher response rate in patients with PD-L1 expression in TILs. Importantly, an antitumor response was seen in patients with known poor prognostic features, including high Fuhrman grade 4 and/or sarcomatoid features (22 %), and poor Memorial Sloan-Kettering Cancer Center (MSKCC) prognostic risk (25 %).

Several other agents are presently being investigated ([76], summarized in Table 12.3). Recent clinical developments of antibody therapies for use with costimulatory/inhibitory molecules on T cells (immunomodulators) are shown in Fig. 12.2.

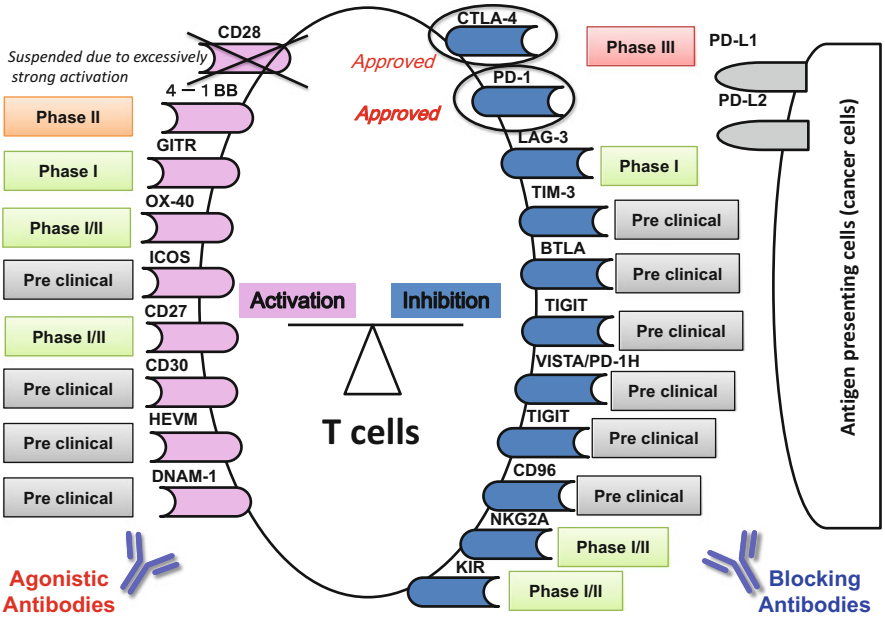

Fig. 12.2 Recent clinical developments of antibody therapies for costimulatory/inhibitory molecules on T cells (immunomodulators)

12.4.3 Combination Therapy

Although immune checkpoint inhibitors have opened up a new era in the treatment of advanced RCC, and there are many promising agents under investigation, the accumulating data on the leading drugs suggest that only a limited number (approximately 20 %) of patients can expect long-term survival with monotherapy [45]. The future challenges include exploring combination therapies that can improve antitumor efficacy without increasing toxicity [47–49]. There are numerous possible novel regimens being evaluated in ongoing clinical trials, seeking the optimal pairing, dosing, and administration sequence ([77], summarized in Table 12.4].

Among these, one of the promising approaches to treating RCC is immune checkpoint blockade with anti-angiogenic therapy. A synergistic antitumor effect is expected based on the TKIs also immunologically affecting the tumor microenvironment [78–84]. An ongoing phase II study (NCT01984242, IMmotion150 study), which recently completed participant enrollment, compared atezolizumab, atezolizumab plus bevacizumab, and sunitinib (control, serving as one of the standard first-line therapies) in patients with untreated advanced RCC. This combination (atezolizumab + bevacizumab) was moved forward to a randomized phase

Table 12.4 Immune checkpoint blockade in combination therapy for RCC

Target	Agent	Patient setting	Trial ID	Phase	Trial status	N (N planned, if recruiting)	Regimen	Primary end point	Response	Survival	AEs	Refs.
CTLA-4	Tremelimumab	≧ Second line	NCT00372853	I	Completed	28	Trem + suni trem: 6.0 vs. 10.0 vs. 15.0 mg/kg, every 12 weeks Suni: 37.5 mg/kg daily vs. 50 mg/kg, daily for 4 weeks, every 6 weeks	Safety	PR: 43 %	NA	Rapid onset renal failure was common as DLT	[77]
PD-1	Nivolumab	≧ Second line	NCT01968109	I/IIa	Ongoing (recruiting)	540	BMS986016 (anti-LAG-3 monoclonal Ab) ± nivo	Safety/ efficacy	NA	NA	NA	https://www.clinicaltrials.gov
	Pembrolizumab (MK-3475)	≧ Second line	NCT02178722	I/II	Ongoing (recruiting)	374	Pemb + epacadostat (INCB024360: IDO inhibitor)	Safety/ efficacy	NA	NA	NA	https://www.clinicaltrials.gov
		First line	NCT02014636	I/II	Ongoing (recruiting)	228	Pemb + pazo Pemb: 1–10 mg/kg Pazo: 400–800 mg/day	Safety/ efficacy	NA	NA	NA	https://www.clinicaltrials.gov
		First line	NCT02133742	I	Ongoing (recruiting)	60	Pemb + axit Pemb: 2 mg/kg, every 3 weeks, to find the MTD Axit: starting dose of 5 mg and 3 mg BID	Safety	NA	NA	NA	https://www.clinicaltrials.gov
		≧ Second line	NCT02179918	I	Ongoing (not recruiting)	45	Pemb + PF-05082566 (4-1BB agonist) Pemb: 2 mg/kg, every 3 weeks PF-05082566: starting dose of 0.45 mg/kg, every 3 weeks, dose escalation	Safety	NA	NA	NA	https://www.clinicaltrials.gov
	Pidilizumab (CT-011)	—	NCT01441765	II	Ongoing (not recruiting)	44	Pidi ± DC/RCC fusion cell vaccine Pidi: 3 mg/kg, every 6 weeks, for 4 cycles	Safety/ Efficacy	NA	NA	NA	https://www.clinicaltrials.gov

PD-L1	Atezolizumab (MPDL3280A)	First line	NCT01984242 IMmotion150)	II	Ongoing (not recruiting)	305	(Atez + bevacizumab) vs. atez vs. suni Atez: 1200 mg, every 3 weeks, 6-week cycle Bevacizumab: 15 mg/kg, every 3 weeks, 6-week cycle Suni: 50 mg/day, for 4 weeks, 6-week cycle	PFS	NA	NA	NA	https://www.clinicaltrials.gov
			NCT02420821 (IMmotion151)	III	Ongoing (recruiting)	830	(Atez + bevacizumab) vs. suni Atez: 1200 mg, every 3 weeks, 6-week cycle Bevacizumab: 15 mg/kg, every 3 weeks, 6-week cycle Suni: 50 mg/day, for 4 weeks, 6-week cycle	OS, PFS	NA	NA	NA	https://www.clinicaltrials.gov
		—	NCT01988896	I	Ongoing (recruiting)	90	Atez + cobimetinib (MEK inhibitor)	Safety	NA	NA	NA	https://www.clinicaltrials.gov
PD-1 & CTLA-4	Nivolumab and ipilimumab	—	NCT01472081 (CheckMate-016)	I	Ongoing (not recruiting)	175	(Nivo + suni) vs. (nivo + pazo) vs. (nivo + ipi) Nivo + ipi: (1+3) vs. (3+1) vs. (3+3) (mg/kg, every 3 weeks, for 4 doses) Suni: 50 mg/day, for 4 weeks, 6-week cycle Pazo: 800 mg/day	Safety	ORR: 18 % (N3 + I1), 20 % (N1 + I3)	Median PFS: 30.3 weeks (N3+I1), 36.0 weeks (N1+I3), PFS (@24 weeks): 53 % (N3 + I1), 64 % (N1 + I3), OS: NA	trAE: 88 %, Gr. 3–4 trAE: 34 % (N3 + I1) and 64 % (N1 + I3)	[85]
		First line	NCT02231749 checkMate-214)	III	Ongoing (recruiting)	1070	(Nivo + ipi) vs. (nivo + suni) Nivo: 3 mg/kg, every 2 weeks Ipi: 1 mg/kg, every 3 weeks, for 4 doses Suni: 50 mg/day, for 4 weeks, 6-week cycle	OS, PFS	NA	NA	NA	https://www.clinicaltrials.gov

(continued)

Table 12.4 (continued)

Target	Agent	Patient setting	Trial ID	Phase	Trial status	N (N planned, if recruiting)	Regimen	Primary end point	Response	Survival	AEs	Refs.
		As neoadjuvant	NCT02210117	II	Ongoing (recruiting)	60	Nivo vs. (nivo + ipi) vs. (nivo + beva) Nivo: 3 mg/kg, every 2 weeks, for 6 weeks Ipi: 1 mg/kg, every 3 weeks, for 6 weeks Beva: 2 mg/kg, every 2 weeks, for 6 weeks	Safety/ efficacy	NA	NA	NA	https://www.clinicaltrials.gov
	Pembrolizumab and ipilimumab	≧ Second line	NCT02089685 (MK-3475-029/KEY-NOTE-29)	I/II	Ongoing (not recruiting)	343	Pemb vs. (pemb + ipi) vs. (pemb + IFN2b)	Safety/ efficacy	NA	NA	NA	https://www.clinicaltrials.gov
	MEDI4736 and tremelimumab	≧ Second line	NCT01975831	I	Ongoing (recruiting)	105	MEDI4736 + trem	Safety	NA	NA	NA	https://www.clinicaltrials.gov
PD-L1 and CTLA-4	Atezolizumab and ipilimumab	—	NCT02174172	I	Ongoing (recruiting)	200	(Atez + ipi) vs. (atez + IFN2b)	Safety	NA	NA	NA	https://www.clinicaltrials.gov
PD-1 and PD-L1	MEDI0680 and MEDI4736	—	NCT02118337	I	Ongoing (recruiting)	196	MEDI4736 + MEDI0680 (AMP-514)	Safety	NA	NA	NA	https://www.clinicaltrials.gov

*Abbreviations: *ipi* ipilimumab, *trem* tremelimumab, *nivo* nivolumab, *pemb* pembrolizumab, *pidi* pidilizumab, *atez* atezolizumab, *suni* sunitinib, *pazo* pazopanib, *beva* bevacizumab, *IFN* interferon, *axit* axitinib, *OS* overall survival, *PFS* progression-free survival, *trAE* treatment-related adverse event, *irAE* immune-related adverse event, *DLT* dose-limiting toxicity, *MTD* maximum tolerated dose, *NA* not available

III trial (NCT2420821, IMmotion151 study). This study is currently recruiting participants (estimated enrollment number = 830).

Another approach is combining two immune checkpoint inhibitors. Combination therapy with nivolumab (at 1 mg/kg every 3 weeks for four doses and then nivolumab alone at 3 mg/kg every 2 weeks) and ipilimumab (at 3 mg/kg every 3 weeks for four doses) was approved for the treatment of patients with advanced melanoma by the US FDA. As to RCC, the updated results of a phase I study (NCT01472081, CheckMate-016 study) were recently reported [85]. In this study, the patients were randomized to receive either nivolumab 3 mg/kg plus ipilimumab 1 mg/kg (N3 + I1), nivolumab 1 mg/kg plus ipilimumab 3 mg/kg (N1 + I3), or nivolumab 3 mg/kg plus ipilimumab 3 mg/kg (N3 + I3) every 3 weeks for four doses, and then nivolumab alone at 3 mg/kg every 2 weeks until either progression or intolerable toxicity was documented. The latter cohort (N3 + I3) was abandoned due to early toxicity in 6 patients, and the former two cohorts (N3 + I1 and N1 + I3) were expanded to include 47 more patients overall. In both expanded cohorts, grade 3–4 treatment-related AEs occurred in 34 % of the N3 + I1 cohort, and 64 % of the N1 + I3 cohort. The ORR was approximately 40 % in both cohorts, with a median duration of 67.7 weeks (4.1–91.1) in the N3 + I1 cohort, and 81.1 weeks (6.1–81.1) in the N1 + I3 cohort. Currently, a phase III trial (NCT02231749, CheckMate-214 study) is underway to evaluate this combination in patients with untreated advanced RCC. In this study, the patients are being randomized to receive either nivolumab plus ipilimumab (the same regimen as that given to the N3 + I1 cohort in the previous phase II study) or sunitinib. The primary end points are PFS and OS. This study is currently recruiting participants (estimated enrollment number = 1070).

12.5 Biomarkers Predicting Efficacy and Toxicities

As immunotherapies are effective in only a limited number of patients, biomarker development is a very important issue. Immune checkpoint inhibitors have shown promising safety and efficacy, to date, though only a small proportion of patients treated with monotherapy have achieved long-term survival, with severe irAEs occurring on occasion. Biomarkers predicting clinical benefit support appropriate selection of individualized treatments for patients and maximize clinical benefits. Thus, there is an urgent need to identify "baseline (pretreatment)" biomarkers predicting responses or toxicities.

In general, biomarkers are defined as belonging to two functional categories, prognostic and predictive.

A prognostic biomarker can define the effects of patient or tumor characteristics on the patient's outcome. This includes patients at high risk for disease relapse who may thus derive benefit from earlier treatments. A predictive biomarker, on the other hand, defines the effects of treatment, including tumor response and improvements in OS and DFS.

In RCC, very limited data from biomarker studies have been reported. Some of these biomarker studies focused mainly on advanced melanoma, though some results would be applicable to RCC.

1. Analysis of cancer cells

Immunohistochemical (IHC) PD-L1 expression in a tumor specimen is among the potential markers for PD-1-PD-L1- directed therapies. In a phase I study of nivolumab, though the data obtained were preliminary, an objective response was obtained only in patients who showed IHC-PD-L1 expression in pretreatment tumor specimens [86]. In advanced melanoma, non-small cell lung cancer, RCC, colorectal cancer, and prostate cancer patients treated with anti-PD-1 antibody (nivolumab), IHC-PD-L1 expression in pretreatment tumor specimens was related to infiltration of immune cells, and PD-L1 and PD-L2 expressions were most **clearly** related to clinical benefit [87]. Pretreatment tumor specimen analysis showed more infiltration of $CD8^+$ T cells with TCR clonality, while immune cells expressing PD-1 and PD-L1 within the tumor or peri-tumoral areas were observed in advanced melanoma patients in whom an anti-PD-1 antibody (pembrolizumab) had been clinically effective [88]. A large study of anti-PD-L1 antibody (MPDL3280A/atezolizumab) against several solid tumors showed clinical benefit to be associated with a higher rates of PD-L1 expression on tumor-infiltrating immune cells, T-helper type 1 gene expression, and CTLA-4 expression, as well as the absence of fractalkine (CX3CL1), in pretreatment tumor samples [73]. MPDL3280A was suggested to be more effective in patients in whom the pre-existing immune response was suppressed by PD-L1 expression and then re-stimulated upon the introduction of anti-PD-L1 antibody. These observations may support the strategy of selecting PD-L1- positive patients for therapy. Furthermore, technical evaluation employing PD-L1 immunostaining is still needed. Also, the value of IHC-PD-L1 staining as a predictive biomarker for combination therapy with nivolumab plus ipilimumab has yet to be validated [89]. As PD-L1 expression on tumor cells is inducible and is susceptible to influences mediated by the tumor microenvironment, the applicability and significance of PD-L1 expression as a baseline biomarker must be interpreted with caution. Further prospective evaluations are thus needed.

Recent genetic analyses using whole exome sequencing have shown the significance of somatic mutational load as predictive biomarker of clinical benefit in advanced melanoma patients treated with CTLA-4 blockade [90] and non-small cell lung cancer with PD-1 blockade [91]. The neopeptide signature associated with achieving a clinical response was identified, and the predicted mutant peptides were verified to activate patient T cells in vitro [92].

2. Analysis of immune cells

Several biomarkers for examining T-cell proliferation or activation and other forms of antigen-specific immunity have been assessed in the context of investigating immune checkpoint inhibitors.

In patients with advanced cancer, immune suppressive cells such as Tregs, monocyte myeloid-derived suppressor cells (m-MDSCs), tumor-associated

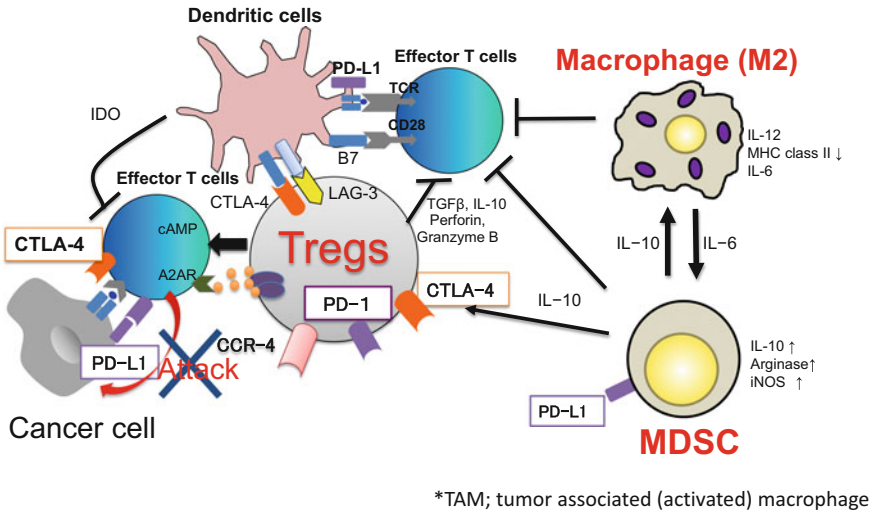

Fig. 12.3 Inhibition of antitumor effect by immune suppressor cells

(activated) macrophages (TAM; M2), and so on increase in number and thereby inhibit effector T cells attacking cancer cells (Fig. 12.3). Another potential biomarker is thus pretreatment levels of m-MDSCs. A recent retrospective study suggested higher pretreatment quantities of lineage^{-}CD14^{+}HLA-DR$^{low/-}$ m-MDSCs to be associated with inferior OS in patients with metastatic melanoma treated with ipilimumab [93, 94]. The results of a prospective analysis are awaited.

3. Others

Gene profiling analysis clarifies immune status as well as that of tumor cells in cancer patients [95]. Activation of WNT/β-catenin signaling in malignant melanoma cells is reportedly related to T-lymphocyte infiltration of tumors [96]. These reports suggest that gene analysis in the near future will unveil the interactions between cancer cells and host immune systems.

Using serum/plasma and urine samples which are relatively easy to collect, measurements of circulating tumor cells and secretions derived from cancer cells have been attempted. Elevated VEGF in peripheral blood prior to treatment was related to a poor prognosis in advanced melanoma patients treated with anti-CTLA-4 antibody (ipilimumab) [97]. The significance of various proteins, amino acids, circulating DNA, and micro-RNA derived from cancer cells in peripheral blood as potential biomarkers have yet to be clarified and the results of further research are eagerly anticipated.

Detecting biomarkers relevant to immunotherapy is challenging. This is largely because the "therapeutic" target may be different from the "immune" target, for example, an immune checkpoint molecule on T cells. These possibilities merit further study.

Previously reported biomarkers for immunotherapy are shown in Fig. 12.4.

Tumor (microenvironment)

- Immunohistochemistry (IHC)
  - PD-L1 expression on tumor cells
  - PD-L1 expression on immune cells
  - PD-L2 expression on tumor cells
  - PD-1 expression on T cells
  - Infiltration of CD8+ cells
  - infiltration of CD4+ cells (Th1)
  - Regulatory T cells
  - MDSC（myeloid derived suppressor cells)
  - Tumor associated macrophages (M2)
  - The balance of effector cells / immune suppressor cells (CD8+/FoxP3^{+}, CD8^{+}/CD204^{+} etc.)

- Gene profiling（expression/mutation/amplification）
  - Number of somatic mutations (non synonymous mutations)
  - Micro-satellite instability
  - Amplification of WNT/β-catenine signaling
  - Activation of IFN-γ signaling

Peripheral blood

- Number of Lymphocytes
- Number of Regulatory T cells
- Number of MDSCs（myeloid derived suppressor cells)
- Concentration of VEGF
- Concentrations of cytokines（IL-6、IL-8、IL-10、TGF-β, etc.）

Fig. 12.4 Examples of biomarkers for immunotherapy

12.6 Conclusion

In response to the successful clinical development of an anti-PD-1 antibody (nivolumab), various immune checkpoint inhibitors are now being developed. In the near future, the role of immunotherapy in the clinical management of RCC is anticipated to become increasingly important.

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Chapter 13
Treatment for Non-clear Cell Renal Cell Carcinoma

Makoto Sumitomo

Abstract Non-clear cell (NCC) renal cell carcinoma (RCC) is less common and accounts for 25 % of all patients with metastatic RCC. Metastatic NCCRCC and clear cell (CC) RCC both respond infrequently to cytotoxic and cytokine therapy; however, compared with CCRCC patients, NCCRCC patients have a poorer prognosis. Tyrosine kinase inhibitors (TKIs) such as sorafenib and sunitinib have significant activity in metastatic NCCRCC, but the efficacy of each agent seems to vary between different NCCRCC forms. Preliminary clinical data for temsirolimus, one of the mammalian targets of rapamycin (mTOR) inhibitors, appear to be promising, but future phase III trials should include patients with RCCs with NCC histology as well as CCRCC, with appropriate stratification to take the balance between each treatment arm. Many ongoing phase II trials should provide interesting preliminary insights into the antitumor efficacy of particular agents in these tumors. These approaches will lead us to improvements in the management of NCCRCC so as we have already achieved with the more common CCRCC.

Keywords Non-clear cell renal cell carcinoma • Molecular targeted therapy • Metastasis • Tyrosine kinase inhibitors • mTOR inhibitors

13.1 Introduction

Recent genetic and clinical studies have shown that renal cell carcinoma (RCC) is not one disease but comprises several different types of cancer that occur in the same organ. Each can have a distinct histological type and a different clinical course, caused by alteration of different genes and respond differently to systemic therapy. RCC is generally divided into two major groups: clear cell renal cell carcinoma (CCRCC) and non-clear cell RCC (NCCRCC) [1]. CCRCC is the most common histopathological subtype of kidney tumors (70–75 %), and

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M. Oya (ed.), *Renal Cell Carcinoma*, DOI 10.1007/978-4-431-55531-5_13

consequently, clinical trials for advanced-stage kidney cancer have focused on patients with this RCC subtype. In contrast, patients who had less common RCC tumors with NCC histology, e.g., papillary, chromophobe, sarcomatoid variant, and collecting duct tumors, have often been ignored [2]. Therefore, it is not surprising that immunotherapy and/or chemotherapy which has successfully been used for some patients with CCRCC does not appear to have any significant activity in other RCC subtypes [3]. Novel targeted therapies are currently under investigation in the treatment of NCCRCC.

In the last decade, targeted therapies using tyrosine kinase inhibitors (TKIs) that blocks angiogenic activity mediated by the vascular endothelial growth factor (VEGF) signaling pathway or using the mammalian target of rapamycin (mTOR) inhibitors have shown significant effects on the clinical outcome of patients with metastatic RCC. However, because of the relatively high prevalence of CCRCC, clinical trials of targeted agents have typically focused on this population of patients while frequently excluding those with NCC histology. The optimal treatment of patients with RCC with NCC histology, including the role of targeted therapy, remains uncertain and is under investigation.

In this review we discuss current clinical trials that include patients with metastatic NCCRCC; the results of these early studies are summarized and translated into therapeutic options and recommendations (Table 13.1).

13.2 Targeted Therapies for Metastatic Papillary RCC (PRCC)

Compared with CCRCC, metastatic PRCC is rare, but once the disease is systemic, the prognosis is just as bad or worse. Immuno(chemo)therapy has been inactive in papillary RCC [3], and therefore, until recently, there was no rational therapeutic option for this tumor subtype. PRCC has been further divided into type 1 and type 2 subtypes; it can occur sporadically or as part of a hereditary syndrome. Comparative genomic microarray analyses showed two highly distinct molecular PRCC subclasses well associated with morphologic subclasses. The first class, with excellent survival, corresponded to three histologic subtypes: type 1, low-grade type 2, and mixed type 1/low-grade type 2 tumors. The second class, with poor survival, corresponded to high-grade type 2 tumors [4].

13.2.1 The Efficacy of Sorafenib to PRCC

Ratain et al. [5] firstly reported the efficacy of sorafenib in patients with metastatic PRCC in 2006. In their phase II clinical trial, they used sorafenib to treat 152 and 15 patients with metastatic CCRCC and PRCC, respectively. The antitumor effect

Table 13.1 Summary of data from published clinical trials for the treatment of metastatic NCCRCC

Author	Study design	Setting	Population	Treatment line	Patients, *n*	Patients with NCCRCC, *n*	NCCRCC histologies	Targeted agent
Ratain et al. [5]	2	Metastatic	RCC	2nd	152	15	PRCC	Sorafenib
Stadler et al. [6]	EAT	Metastatic	RCC	≥2nd	2504	202	Mixed	Sorafenib
Beck et al. [7]	EAT	Metastatic	RCC	≥2nd	1159	118	Mixed (PRCC)	Sorafenib
Plantade et al. [8]	2	Metastatic	RCC	≥2nd		28	PRCC	Sorafenib
Gore et al. [10]	EAT	Metastatic	RCC	≥2nd	4371	588	Mixed	Sunitinib
Choueiri et al. [11]	2	Metastatic	RCC	≥2nd		53	41 papillary and 12 chromophobe	Sorafenib or sunitinib
Molina et al. [12]	2	Metastatic	RCC	≥2nd		23	Mixed 8 with PRCC	Sunitinib
Lee et al. [13]	2	Metastatic	RCC	≥2nd		31	Mixed (PRCC, ChRCC, Xp11.2 translocation)	Sunitinib
Hudes et al. [14]	3	Metastatic	RCC	1st	526	20 %	Mixed	Temsirolimus
Dutcher et al. [16]	3	Metastatic	RCC	1st	209	37	Mixed	Temsirolimus
Blank et al. [18]	EAT	Metastatic	RCC	≥2nd	1367	75	Mixed	Everolimus
Choueiri et al. [22]	2	Metastatic	RCC	≥2nd		74	PRCC	Foretinib
Gordon et al. [25]	2	Metastatic	RCC	≥2nd		45	PRCC	Erlotinib

(continued)

Table 13.1 (continued)

Author	Study design	Setting	Population	Treatment line	Patients, *n*	Patients with NCCRCC, *n*	NCCRCC histologies	Targeted agent
Oudard et al. [28]	2	Metastatic	RCC	≥2nd		23	CDRCC	Gemcitabine and platinum
Golshayan et al. [37]	Retrospective	Metastatic	Sarcomatoid	≥2nd	43	–	RCC with SD	Sunitinib, sorafenib, bevacizumab
Haas et al. [39]	2	Metastatic	Sarcomatoid	1st	39	–	RCC with SD	Doxorubicin-gemcitabine

in PRCC was similar to that of the CCRCC population. Two patients with PRCC showed partial response (PR), and an additional three had tumor shrinkage of 25–49 %, suggesting that sorafenib could have significant efficacy in patients with metastatic PRCC.

The 202 patients with NCCRCC enrolled in the US Advanced RCC Sorafenib (US-ARCCS) expanded access trial (EAT) included 107 patients with PRCC [6]. The rate of clinical benefit (complete response [CR] + PR + stable disease [SD] for at least 8 weeks) was 84 % in patients with PRCC similar to that in the entire population. The median progression-free survival (PFS) in the overall population was 24 weeks (95 % CI: 22–25 weeks) and was the same when patients with NCCRCC were excluded. The median PFS in patients with NCCRCC was 21 weeks; the median overall survival (OS) in this cohort was 40 weeks compared with 50 weeks (95 % CI: 46–52 weeks) in the overall population. Reported adverse events (AEs) did not differ from those observed in patients with CCRCC. The authors concluded that sorafenib was well tolerated and had significant antitumor activity in patients with metastatic PRCC. Beck et al. [7] presented similar results from the European (EU)-ARCCS trial, an open-label, non-comparative phase III study. That study included 15 PRCC histologies. Of these patients, one with PRCC achieved a PR with sorafenib (objective response rate [ORR] 3.4 %), compared with an ORR of 9.3 % in patients with CCRCC. Two patients with papillary histology exhibited tumor shrinkage.

On the contrary, within a recent French study [8], there were no objective responses in 28 patients with advanced PRCC treated with sorafenib. However, the median PFS and overall survival was 5.7 and 19.6 months, respectively.

13.2.2 The Efficacy of Sunitinib to PRCC

Within the US-sunitinib EAT, 276 evaluable patients with NCCRCC received sunitinib (4 weeks on, 2 weeks off), in most of whom previous cytokine-based therapy had failed [9]. Even though the results were less notable than for CCRCC, sunitinib also showed significant activity against NCCRCC. Overall response and disease control rates for NCCRCC compared with all RCC subtypes were 5.4 % vs 9.3 % and 47.0 % vs 52.4 %, respectively. Unfortunately, the authors did not discriminate between different NCCRCC subtypes. In 2009, Gore et al. updated the results of the US-sunitinib EAT including 588/4371 patients with NCCRCC, comprising 13 % of the overall study population [10]. In this study, the overall median PFS was 10.9 months (95 % CI: 10.3–11.2 months), and the median OS was 18.4 months (95 % CI: 17.4–19.2 months); the corresponding survival times in the subgroup of patients with NCCRCC were 7.8 months (95 % CI: 6.8–8.3 months) and 13.4 months (95 % CI: 10.7–14.9 months), respectively [10]. Although the sunitinib benefit in NCC histologies appeared lower than in the overall population, the median OS compares favorably with historical data [3].

Sunitinib efficacy was also observed in a retrospective analysis of 53 patients with PRCC treated with sunitinib ($n = 20$) or sorafenib (n = 33) at five cancer centers in France and the USA [11]. Among the sunitinib-treated patients, 2 of 13 patients with PRCC achieved a PR (15 %), and median PFS in this histological subtype was 11.9 months. None of the 28 patients with PRCC treated with sorafenib achieved an objective response. The median PFS in this cohort was 5.1 months, significantly less than the 11.9 months achieved by sunitinib treatment in patients with PRCC ($P < 0.001$).

Data from several prospective phase II studies of sunitinib in advanced NCCRCC have been presented or published. For the most part, there was a low response rate to sunitinib (ORR 0–7 %), although the majority of patients showed SD [12]. However, a recently published phase II study of 31 patients with NCCRCC (papillary, $n = 22$; chromophobe, $n = 3$; unclassified, $n = 5$; and Xp11.2 translocation, $n = 1$) reported an overall ORR of 36 % (95 % CI: 19 % to 52 %) and median PFS of 6.4 months (95 % CI: 4.2–8.6 months). The median OS had not been reached, but the 1-year survival rate was 65 % [13].

13.2.3 The Efficacy of mTOR Inhibitors to PRCC (NCCRCC)

In 2007, a phase III trial compared the efficacy and safety of temsirolimus along with temsirolimus in combination with interferon α (IFNα) or IFNα alone for the first-line treatment for poor-risk RCC [14]. This phase III study is of particular interest when considering the treatment of NCCRCC, as it is the only phase III RCC trial to date with NCC histology representation; of the 626 patients enrolled, 20 % had RCC of NCC histology. Dutcher et al. [15, 16] presented a subgroup analysis of the temsirolimus vs IFNα trial, comparing the activity of temsirolimus and IFNα in metastatic NCCRCC and the efficacy of temsirolimus in CCRCC and NCCRCC. That study included only treatment-naive patients with an intermediate and poor risk, and 76 % of those with NCCRCC had PRCC. In this population, the median OS was 11.6 months with temsirolimus and 4.3 months with IFNα (HR 0.49; 95 % CI: 0.29–0.85 months); median PFS, based on independent assessment, was 7.0 months with temsirolimus and 1.8 months with IFNα (HR 0.38; 95 % CI: 0.23–0.62 months). These outcomes are at least comparable with those for patients with clear cell RCC. The impact of temsirolimus on health-related quality of life also showed a trend for superiority over IFNα in RCC of NCC histology [17]. Taken together, these analyses strongly suggest that temsirolimus provides clinical benefit for the first-line treatment of NCCRCC.

Data on the use of the mTOR inhibitor everolimus in NCCRCC are limited, although a subgroup analysis of patients with NCCRCC enrolled in the RAD1001 Expanded Access Clinical Trial in RCC (REACT) was presented at the ASCO 2012 Genitourinary Cancers Symposium [18]. REACT enrolled RCC patients of any

histology who were intolerant to, or had progressed on, VEGFR inhibitors; of 1367 patients enrolled, 75 patients (5.5 %) had NCCRCC. Median treatment duration was similar in the NCC subgroup and in the overall REACT population (12.14 weeks versus 14.0 weeks, respectively), as was the ORR (1.3 % versus 1.7 %) and rate of stable disease (49.3 % versus 51.6 %), suggesting that everolimus shows similar results in NCCRCC compared with CCRCC.

13.2.4 Other Targeted Therapies in PRCC

One causative gene responsible for hereditary papillary RCC has been identified on chromosome 7q31 and encodes the receptor tyrosine kinase MET proto-oncogene [19]; this proto-oncogene is also dysregulated/duplicated in a significant proportion of sporadic type 1 cases [20], but the exact role of c-MET in the development of PRCC is yet unknown. Foretinib is an oral, multikinase inhibitor targeting VEGFR-2, MET, and other receptors. Preliminary data from a phase I trial of advanced PRCC [21] suggested that the agent may have activity in this setting. In a recently reported multicenter phase II study of patients with sporadic and hereditary PRCC (N = 74), foretinib was associated with an ORR of 13.5 % (while tumor shrinkage was reported in 50 out of 68 patients), a disease stabilization rate (ORR + stable disease) of 88 %, median PFS of 9.6 months, and 1-year OS of 70 % (median OS not reached) [22]. Toxic effects were manageable and typical of anti-VEGF therapy.

EGFR pathway has also been implicated in the development of metastatic RCC [23, 24]. Gordon et al. [25] evaluated the efficacy of erlotinib, an oral epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor in 45 patients with advanced PRCC. The overall RR was 11 % (five of 45 patients; 95 % CI, 3 % to 24 %), and the disease control rate was 64 % (i.e., five partial response and 24 stable disease). The median overall survival time was 27 months (95 % CI, 13 to 36 months). Probability of freedom from treatment failure at 6 months was 29 % (95 % CI, 17 % to 42 %). There was one grade 5 AE of pneumonitis, one grade 4 thrombosis, and nine other grade 3 AEs. The design of future trials of the EGFR axis in PCC should be based on preclinical or molecular data that define appropriate patient subgroups, new drug combinations, or potentially more active alternative schedules. Clinical phase II studies are ongoing to determine the efficacy of the combination of bevacizumab and erlotinib as a treatment for patients with metastatic NCCRCC (NCT01399918) or papillary RCC (NCT01130519) in the USA (Table 13.2). These studies might provide new insights on sequential or combinational therapy for NCCRCC such as PRCC.

Table 13.2 Summary of ongoing and planned trials in NCCRCC

Drug	Phase	Status	Characteristics	ClinicalTrials.gov identifier
Sunitinib	2	Ongoing, not recruiting	Single arm, all NCCRCC	NCT00465179
Sunitinib	2	Unknown	Single arm, all NCCRCC	NCT01219751
Sunitinib	2	Unknown	Single arm, all NCCRCC	NCT01034878
Axitinib	2	Recruiting	Single arm; after progression to temsirolimus, all NCCRCC	NCT01798446
Pazopanib	2	Not recruiting	Single arm, all NCCRCC	NCT01538238
Everolimus vs sunitinib	2	Recruiting	Randomized trial, all NCCRCC	NCT01185366
Everolimus vs sunitinib	2	Recruiting	Randomized trial, all NCCRCC	NCT01108445
Temsirolimus vs sunitinib	2	Completed, results not available	Randomized trial, all NCCRCC	NCT00979966
Everolimus (RAD001)	2	Recruiting	Single arm, papillary NCCRCC	NCT00688753 RAPTOR
Everolimus (RAD001)	2	Ongoing	Single arm, any type of NCCRCC	NCT00830895
Everolimus and bevacizumab	2	Recruiting	Single arm, all NCCRCC	NCT01399918
Everolimus and bevacizumab	2	Recruiting	Single arm; hereditary leiomyomatosis and renal cell carcinoma (HLRCC) or sporadic papillary RCC	NCT01130519
Bevacizumab + gemcitabine + capecitabine	2	Ongoing	Sarcomatoid RCC	NCT00496587
Sunitinib versus sunitinib + gemcitabine	2	Ongoing	Sarcomatoid RCC	NCT01164228

13.3 Targeted Therapies for Metastatic Chromophobe RCC (ChRCC)

This variant is rare and clinical data are limited; the US-ARCCS trial of 2502 patients included 18 with advanced ChRCC [26]. With 17 patients the disease control rate was high (95 %) and included one confirmed and three unconfirmed PRs. Choueiri et al. [11] used sorafenib and sunitinib to treat five and seven patients with metastatic ChRCC, respectively. Within this small study, the median PFS and OS were only 9.3 and 14.2 months. Two of five and one of seven patients who received sorafenib and sunitinib, respectively, achieved objective responses.

In the USA, clinical phase II studies (NCT01185366, NCT01108445) are ongoing to determine the efficacy of the combination of sunitinib and erlotinib as a treatment for patients with metastatic NCCRCC including ChRCC (Table 13.2).

One recent study reported abnormal c-kit expression in ChRCC and the usefulness of imatinib for the treatment of ChRCC [27], although it is a very small population study.

13.4 Targeted Therapies for Metastatic Collecting Duct RCC (CDRCC)

In US-ARCCS EAT, ten with metastatic CDRCC were evaluable for response, but three of the ten patients treated with sorafenib had an SD [6]. Since the histology of collecting duct carcinoma is similar to that of urothelial carcinoma, the standard chemotherapy regimen defined by a gemcitabine and platinum salts combination was prospectively investigated in patients with metastatic collecting duct carcinoma [28]. There were one CR and five PRs for an ORR of 26 % (95 % CI 8 to 44). Median PFS and OS were 7.1 (95 % CI 3 to 11.3) and 10.5 months (95 % CI 3.8 to 17.1), respectively. Toxicity was mainly hematological with grade 3–4 neutropenia and thrombocytopenia in 52 % and 43 % of patients, respectively. The severity of granulocytopenia and the number of metastatic sites were associated with OS on univariate and multivariate analyses.

13.5 Treatment of Xp11.2 Translocation RCC

Recent case reports suggest that Xp11.2 translocation RCC may be successfully treated by targeted therapies using sunitinib, sorafenib, or temsirolimus [29–31]. In addition, a retrospective review of 15 adult patients with metastatic Xp11.2 RCC suggests that TKI-based targeted therapy may provide some clinical benefit in these patients [32]. In this case series, three patients had PR, seven patients had SD, and five patients developed progression disease (PD). The median PFS was 7.1 months and the OS was 14.3 months [32]. In another case series of 21 patients with metastatic translocation RCC, PFS time in the first-line setting was greater with sunitinib than with cytokine therapy (8.2 months vs 2 months; $p = 0.003$); mTOR inhibitors, sorafenib, and sunitinib all showed disease control in second and subsequent lines of therapy [33].

13.6 Targeted Therapies for Metastatic RCC with Sarcomatoid Differentiation

Sarcomatoid differentiation of RCC occurs in all histological subtypes of RCC, with an incidence of 1–23 % of all RCCs. It is uniformly associated with a poor prognosis, with a median survival of 2–9 months [34, 35]. Surgical resection alone does not seem to affect the clinical course significantly, because these tumors are usually metastatic or locally advanced at the time of diagnosis. Given its uncommon nature and the failure of traditional approaches such as immunotherapy, currently the efficacy of the novel targeted drugs is being investigated in RCC with sarcomatoid differentiation.

Within the EU-ARCCS trial [36], 46 RCC patients with sarcomatoid differentiation received standard-dose sorafenib (400 mg twice daily). At 67 % and 4.3 months, respectively, the disease control rate and median PFS were considerable but significantly lower and shorter than in patients with conventional RCC (75 % and 7.5 months). A report of a retrospective series of patients ($n = 43$), treated with TKIs or bevacizumab, noted an association between the outcome and amount of sarcomatoid change in the primary tumor; those patients with limited sarcomatoid component (<20 %) appeared to have a better outcome with anti-VEGF therapy [37], suggesting the limitation of the conventional TKI-based molecular targeted therapy for patients sarcomatoid RCC.

On the other hand, active chemotherapy appears to be promising for the treatment of RCC with sarcomatoid differentiation than without, with some reports of long-term responders to doxorubicin plus gemcitabine [38] and a median OS of 8.8 months in a phase II study of this regimen [39].

13.7 Ongoing Clinical Trials

Table 13.2 lists details of ongoing (partially completed) and planned trials of targeted therapies in NCCRCC. NCT00465179 is ongoing to evaluate the efficacy and safety of sunitinib for patients with metastatic NCCRCC. NCT01798446 is currently recruiting participants to evaluate the efficacy of axitinib for patients with metastatic NCCRCC especially with temsirolimus resistance. There are also a number of phase II studies focusing on NCCRCC currently ongoing to compare temsirolimus with sunitinib (NCT00979966) and everolimus with sunitinib (NCT01108445 by Duke University and NCT01185366 by MD Anderson Cancer center). In addition, the single-arm RAPTOR trial (RAD001 in Advanced Papillary Tumor Program in Europe, NCT00688753) evaluated the efficacy and safety of everolimus as first-line therapy for patients with metastatic PRCC. This study showed that everolimus was well tolerated by and provided clinical benefit to patients with both type 1 and type 2 PRCCs, although both PFS and OS were longer in type 1. These results may provide additional evidence on adequate first-line therapy among VEGF TKIs and mTOR inhibitors.

13.8 Current Clinical Guidelines and Future Directions

While prospective randomized data are not currently available, several systemic therapies are recommended for the first-line treatment of stage IV, relapsed, or recurrent NCCRCC based on data from phase III trial subgroup analyses, EATs, and small retrospective studies. Both the ESMO Clinical Practice Guidelines [40] and National Comprehensive Cancer Network (NCCN) guidelines [41] recommend enrolment in an appropriately designed clinical trial as the preferred treatment option. The NCCN then recommends temsirolimus (category 1 for poor-risk patients, category 2A for other risk groups) or sorafenib (category 2A) or sunitinib (category 2A). Pazopanib, erlotinib, or axitinib are alternative options (category 3). Chemotherapy with gemcitabine + doxorubicin or gemcitabine + capecitabine is also given a category 3 rating for clear cell or non-clear cell RCC with predominantly sarcomatoid features [41]. The ESMO Guidelines recommend temsirolimus, sunitinib, or sorafenib, all with level IIIB evidence, for the treatment of metastatic non-clear cell disease [40].

Evidence-based treatment recommendations regarding systemic therapy for patients with metastatic NCCRCC are thus limited. Nonetheless, the available data suggest that targeted agents currently approved for RCC are active to some degree in NCC histologies. Similar sets of common functional capabilities may exist in these tumor subtypes characterizable by the sum of molecular features occurring in RCC, irrespective of any histologic, clinical, or single molecular parameters. It is very important, however, for us to recognize that NCCRCCs are phenotypically and genotypically clearly different from CCRCC. The role of targeted therapies in NCCRCC needs to be developed in two ways. First, further molecular research into the similarities and differences between RCC subtypes should be promoted. Second, more clinical trials specifically designed to evaluate current targeted agents in NCCRCC are needed. A number of phase II trials are now ongoing or planned for patients with NCCRCC, and these should provide interesting preliminary insights into the antitumor efficacy of particular agents in these tumors. With the advent of novel therapeutic options, specific controlled multicenter trials are urgently needed to define their exact value and efficacy for treating the historically resistant NCCRCC forms. These approaches will lead us to improvements in the management of NCCRCC so as we have already achieved with the more common CCRCC.

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Chapter 14
Forthcoming Drugs for Metastatic Renal Cell Carcinoma Therapy

Keiichi Ito

Abstract The development of tyrosine kinase inhibitors (TKIs) and inhibitors of mammalian target of rapamycin (mTOR) have led to great progress in the treatment of advanced renal cell carcinoma (RCC). However, in most cases, RCC cells eventually develop drug resistance or drug treatments are stopped because of their adverse events (AEs).

TKIs that are selective for targeted molecules have potent antitumor effects and produce few side effects are ideal. Although TKIs that inhibit VEGFR signaling have been widely used, treatment strategies that inhibit other pro-angiogenic signaling pathways are also attractive. Furthermore, the PI3K/Akt/mTOR pathway can be inhibited by the widely used inhibitors of mTORC1. Because these agents only inhibit the mTORC1/S6K pathway, and compensatory pathways upregulate HIF, new drugs that inhibit mTOR2 and/or PI3K and/or Akt activity have been developed. In addition to these inhibitors, immune checkpoint inhibitors have shown strong efficacy and reduced toxicity. We expect the number of drugs that are useful for treating RCC will further increase. If we can use drugs that have potent antitumor activity and little toxicity, the prognoses of RCC patients would be further improved.

In this chapter, we introduce forthcoming TKIs and PI3K/Akt/mTOR pathway inhibitors, which are potential treatments for RCC.

Keywords Renal cell carcinoma • Tyrosine kinase inhibitor • PI3K/Akt/mTOR pathway inhibitor • PI3K • mTORC2

14.1 Introduction

Because molecular mechanisms are involved in pathogenesis and progression of renal cell carcinoma (RCC), attempts to inhibit major signaling pathways, involving proliferation and angiogenesis, have been tried as treatments for advanced

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M. Oya (ed.), *Renal Cell Carcinoma*, DOI 10.1007/978-4-431-55531-5_14

RCC. RCC, particularly clear cell RCC, is usually highly vascularized. Therefore, the main strategy for treating advanced RCC was to inhibit pro-angiogenic pathways, primarily the vascular endothelial factor (VEGF)/VEGF receptor (VEGFR) pathway. Another strategy for advanced RCC was to inhibit the PI3K/Akt/mammalian target of rapamycin (mTOR) pathway associated with proliferation and angiogenesis. Tyrosine kinase inhibitors (TKIs), such as sunitinib, sorafenib, axitinib, and pazopanib, and mTOR inhibitors, such as temsirolimus and everolimus, have been used to treat advanced RCC with clinical efficacy [1–6]. In fact, some patients with metastatic RCC (mRCC) and poor prognostic factors, such as liver metastasis in cytokine era, benefited from these molecular targeted therapies (MTTs).

Although MTTs are an effective treatment for mRCC, their clinical response usually has a short duration with monotherapy. The median progression-free survival (PFS) was less than 12 months in most clinical trials using monotherapy as a molecular targeted agent. Furthermore, MTTs can cause various adverse events (AEs). In some cases, patients have stopped MTTs because of AEs. Therefore, agents that have high antitumor activity and few side effects should be developed. In addition to new TKIs and PI3K/Akt/mTOR pathway inhibitors, new immunotherapies such as anti-PD-1 antibodies, anti-PD-L1 antibodies, and anti-CTLA-4 antibodies have also been developed [7] and are expected to have clinical efficacy. Patients treated with these novel immunotherapies occasionally show long-term responses and reportedly show fewer side effects than TKIs or mTOR inhibitors.

If agents with high antitumor activity and limited toxicity are developed, we could appropriately manage AEs and maintain the patient's performance status. Using these novel drugs, the prognoses of patients with advanced RCC can be further prolonged. In this chapter, we present new and novel therapies (TKIs and PI3K/Akt/mTOR pathway inhibitors) for advanced RCC.

14.2 Receptor TKIs (Summarized in Table 14.1)

RCC is one of the most highly vascularized tumors among the solid tumors. In RCC, particularly clear cell RCC, pro-angiogenic signals such as the VEGF/VEGFR pathway are upregulated. VEGF-targeted therapies, including sunitinib, sorafenib, and bevacizumab, also have been developed [1, 2, 8], and the prognoses of patients with advanced RCC treated with these therapies have improved. Although VEGF-targeted therapies have shown significant efficacy as an RCC therapy, these drugs can cause various AEs. VEGF-targeted TKIs usually inhibit other receptor tyrosine kinases (RTKs), such as platelet-derived growth factor receptor (PDGFR) and c-kit. Because the first-generation RTK inhibitors had a low affinity for target molecules, a significant amount of the drug was required to adequately inhibit the targeted RTKs. The nonspecific effects of TKIs also can lead to various "off-target effects." To decrease the off-target effects of TKIs, VEGFR-TKIs that have a high affinity for VEGFR have been developed.

Table 14.1 Summary of clinical activities of novel RTK inhibitors for the treatment of renal cell carcinoma (RCC)

Agent	Molecular targets	Phase (comparator)	Patient population	Clinical activity in RCC	References
Tivozanib (AV-951)	VEGFR-1, 2, 3	Phase III (TIVO-1) (vs. sorafenib)	Clear cell RCC	PFS (11.9 vs. 9.1 mo)	[13]
			More than one prior systemic therapy (does not include targeted therapy)	OS (28.8 vs. 29.3 mo)	
				ORR (33 vs. 23 %)	
Cediranib (AZD2171)	VEGFRs, c-kit, PDGFRβ, FGFR1	Phase II (vs. placebo)	Metastatic or recurrent clear cell RCC	PFS (12.1 vs. 2.8 mo)	[16]
				ORR (34 vs. 0 %)	
Dovitinib (TKI258)	FGFR1, 2, 3, VEGFR-1, 2, 3	Phase II (single arm)	Advanced RCC treated with at least a VEGFR inhibitor and a mTOR inhibitor	PFS (3.7 mo)	[20]
				OS (11.8 mo)	
Regorafenib (BAY73–4506)	VEGFR-1, 2, 3, PDGFRβ, FGFR1, Tie2	Phase II (single arm)	Previously untreated advanced RCC (Clear cell RCC)	PFS (11.0 mo)	[25]
				ORR (39.6 %)	
Cabozantinib	MET, VEGFR-2	Phase II (single arm)	Advanced RCC, at least one or two lines of prior systemic therapy	PFS (14.7 mo)	[29]
	RET, c-kit, AXL, FLT3			OS (not yet reached)	
				ORR (28 %)	
Tivantinib (ARQ 197)	MET	Phase I (single arm)	Advanced RCC	Clinical benefit (60 %)	[33]
Foretinib (XL880)	MET, VEGFRs, RON, AXL, Tie2	Phase II (single arm)	Advanced RCC	PFS (9.3 mo)	[34]
			No more than one prior systemic therapy	OS (not yet reached)	
				ORR (13.5 %)	

Other strategies for developing new RTK inhibitors are inhibiting other pathways, except for the VEGF pathway, which relates to the RCC biology, or inhibiting pathways that are activated when RCCs develop resistance to VEGFR-targeted therapy.

In addition, inhibition of the VEGF pathway is reportedly less effective in non-clear cell RCC, including papillary RCC, than in clear cell RCC [9]. Therefore, different strategies are needed to treat non-clear cell RCC. For instance, MET

inhibitors such as tivantinib and foretinib were developed because papillary RCC is frequently associated with upregulation of the c-MET pathway [10].

14.2.1 Tivozanib (AV-951)

TKIs which have a high affinity for the target molecule may decrease their off-target effects. Tivozanib is a quinoline-urea derivative TKI, a potent VEGFR TKI with high activity against VEGFR-2, and strongly inhibits VEGFR-1 and VEGFR-3 [11, 12]. Tivozanib blocks the VEGFR-1, VEGFR-2, and VEGFR-3 tyrosine kinases at picomolar concentrations [11]. In the Phase II randomized discontinuation trial, 272 patients with mRCC received open-label tivozanib 1.5 mg/day (3 weeks on followed by 1 week off) for 16 weeks [13]. Most of the primary renal tumors revealed a clear cell histology (83 %). In the overall study population, the ORR was 24 % and the median PFS was 11.7 months with tivozanib treatment. After 16 weeks of tivozanib treatment, the 118 patients with a stable disease (SD) were randomly assigned to a placebo or tivozanib group. The median PFS was 10.3 months in the tivozanib group and 3.3 months in the placebo group. The common AEs were hypertension, dysphonia, diarrhea, and asthenia. Among these, hypertension was the most common (all grade; 45 %). TIVO-1 trial was an open-label, randomized Phase III trial for patients with clear cell RCC and no more than one prior systemic therapy that could not include VEGF- or mTOR-targeted therapies [14]. A total of 517 patients were randomly assigned to either a tivozanib (1.5 mg/day for 3-week-on 1-week-off schedule) or sorafenib (400 mg twice daily) group. In the overall population, PFS was significantly longer in patients receiving tivozanib (11.9 months) than in those receiving sorafenib (9.1 months; hazard ratio = 0.797; 95 % CI, 0.639–0.993; p = 0.042). The overall response rate (ORR) favored tivozanib, which was 33 % for tivozanib and 23 % for sorafenib. The final overall survival (OS) was not significantly different between sorafenib and tivozanib (median, 29.3 vs. 28.8 months, $p = 0.105$), and the tendency for a longer OS was observed in the sorafenib group. A greater proportion of patients in the sorafenib group received the next-line targeted RCC treatment (63 %), whereas 13 % of the patients in the tivozanib group received the next-line treatment. Of the patients who received sorafenib, 61 % (156 patients) switched to tivozanib. With regard to AEs, hypertension (44 % vs. 34 %) was more common with tivozanib and dysphonia with sorafenib (21 % vs. 5 %). Reactions involving the skin of the hand or foot (54 % vs. 14 %) and diarrhea (33 % vs. 23 %) were more common with sorafenib than with tivozanib.

14.2.2 Cediranib (AZD2171)

Cediranib is an oral VEGFR TKI that has an affinity for VEGFRs, c-kit, PDGFRβ, FGFR-1, and several other kinases [15]. Cediranib inhibits VEGFR-2 tyrosine kinase activity at a subnanomolar concentration and VEGFR-1 and VEGFR-3 tyrosine kinase activity at a nanomolar concentration [16]. A Phase II, randomized, double-blind trial was performed that compared the efficacy of cediranib with a placebo in patients with metastatic or recurrent clear cell RCC [17]. The patients had not previously received a VEGFR inhibitor. Patients in the placebo group could cross over to open-label cediranib at 12 weeks or earlier if their disease had progressed. The cediranib group included 53 patients, whereas the placebo group included 18 patients. After 12 weeks of therapy, a significant difference was observed in the mean percent change in tumor size from baseline (primary end point) between the cediranib and placebo groups (20 % reduction vs. 20 % increase). PR was achieved in 34 % of patients treated with cediranib and 47 % of the patients experienced SD. Of the 18 responders, 11 (61 %) had responses lasting for more than 1 year. Cediranib treatment significantly prolonged PFS compared with the placebo (median PFS 12.1 vs. 2.8 months, $p = 0.017$). The most common AEs in patients treated with cediranib were diarrhea, hypertension, fatigue, and dysphonia. Cediranib monotherapy demonstrated clinical activity in patients with advanced RCC with an acceptable AE profile.

14.2.3 Dovitinib (TKI258)

Tumor escape from anti-VEGF therapy may include various signaling pathways, including the fibroblast growth factor (FGF) signaling pathway. FGF signaling is one of several pathways responsible for tumor resistance after inhibition of the VEGF pathway [18]. Dovitinib is a potent inhibitor of FGFRs (1, 2, and 3) and 3 VEGF receptors [19]. In mouse xenograft models (Caki-1 cells), dovitinib treatment showed an 83 % reduction in tumor volume, whereas sunitinib and sorafenib treatments showed a 66 % and 16 % reduction, respectively [20]. The Phase I trial for dovitinib was performed for mRCC pretreated with VEGFR inhibitor, mTOR inhibitors, cytokine therapy, or a combination of these treatments [20]. A maximum tolerated dose was determined as 500 mg dovitinib (5-day-on 2-day-off schedule). The most common AEs were nausea, vomiting, diarrhea, and asthenia. In the Phase II trials, 67 patients with advanced RCC were treated with dovitinib (500 mg/day) [21]. The patients were previously treated with at least a VEGFR inhibitor and an mTOR inhibitor. Patients with RCC who were previously treated with mTOR and VEGFR inhibitors showed high baseline FGF levels. The elevated baseline FGF levels after VEGF inhibition and the cross talk between FGF and VEGFR signaling led us to consider that a strategy that simultaneously targeted both signaling pathways is possibly useful. The ORR and disease control rate

8 weeks after the treatment were 1.8 % and 52.7 %, respectively. The median PFS and OS were 3.7 and 11.8 months, respectively. Dovitinib treatment resulted in an increased circulating FGF23 level, indicating inhibition of FGFR1. In addition, there were significant decreases in soluble VEGFR-2, and modest increases in VEGF and placental growth factor. The most frequent AEs for all grades were nausea, diarrhea, vomiting, decreased appetite, and fatigue. The GOLD trial (**ClinicalTrials.gov., NCT01223027**) is an ongoing Phase III trial that compares dovitinib with sorafenib in patients with advanced RCC after failure of one VEGF inhibitor and one mTOR inhibitor.

14.2.4 Regorafenib (BAY73–4506)

Regorafenib is a potent inhibitor of several angiogenic RTKs, including VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-β, FGFR-1, and Tie2. In addition, regorafenib inhibits various oncogenic RTKs (c-KIT and RET) and intracellular signaling kinases such as RAF [22]. A unique characteristic of regorafenib is that it inhibits Tie2. The angiopoietins are an important pro-angiogenic growth factor family that signals through the Tie2 receptor [23, 24]. Tie2 is not found in normal vasculature but found only in tumor vascular endothelial cells [25].

A Phase II open-label, nonrandomized study was conducted with 49 patients with advanced RCC [26]. Patients with previously untreated metastatic or unresectable clear cell RCC received regorafenib (160 mg per day, 3 week-on 1-week-off schedule). The tumor response was assessable for 48 patients and 19 patients (39.6 %) showed an objective response (PRs in all patients). Ten of the 19 partial responders maintained a response for more than 12 months and 5 had a response for more than 18 months. Tumor shrinkage occurred in 39 patients (81 %) with 25 patients (52 %) showing a shrinkage of at least 30 %. A clinical benefit (CR, PR, or SD) was noted in 39 patients (81 %; median PFS was 11.0 months). Patients who showed greater increases in plasma concentration of CK18M30 were more likely to show a tumor shrinkage of at least 40 %. Baseline concentrations of soluble Tie1 and TIMP2 tended to be higher in the group that achieved at least 40 % maximum tumor shrinkage. AEs occurred in 48 patients (98 %) and serious AEs in 17 (35 %). Grade 3 AEs were common with regorafenib treatment, which most frequently were skin reactions of the hand and foot, diarrhea, renal failure, fatigue, and hypertension. Two patients had grade 4 treatment-related AEs and 4 patients died during study treatment or within 30 days of their last dose.

14.2.5 Cabozantinib

MET/hepatocyte growth factor (HGF) is a signal involved in angiogenesis and malignant behavior [27]. It appears to be an important resistance pathway for

VEGF-targeted therapy because MET levels increase after exposure to anti-angiogenic therapy [28]. Cabozantinib was identified as a dual inhibitor of VEGFR-2 and MET and shows additional activity against RET, KIT, AXL, and FLT3 [29].

Twenty-five patients with advanced RCC were enrolled in a Phase II trial for cabozantinib [30]. All patients had at least one line of prior therapy with systemic agents, 50 % had three or more lines of prior therapy, and 88 % of the patients had prior anti-VEGF therapies. Fourteen patients (56 %) were previously treated with sunitinib. The cabozantinib dose of 140 mg per day was administered orally that was previously determined as the MTD. Twenty-one of 25 patients were in the intermediate-risk group according to the Heng criteria [31], and 3 were in the poor-risk group. Of the 25 evaluated patients, 7 (28 %) achieved PR, 13 had SD (52 %), and 1 (4 %) showed disease progression. The median PFS was 14.7 months, but the median OS could not be calculated with a median follow up of 14.7 months (range: 11.2–21.8 months). Four patients had bone metastases, and 3 of these 4 patients showed a response. Pain was significantly and durably palliated in 2 patients reporting bone pain. The most common AEs were fatigue, diarrhea, hypophosphatemia, and hyponatremia.

Cabozantinib appeared to be effective in treating advanced RCC. Two Phase III trials have been conducted for patients with advanced RCC. One of the trials compares cabozantinib to sunitinib in previously untreated patients with advanced RCC (**ClinicalTrials.gov., NCT01835158**). The other (the METEOR trial) compares cabozantinib (60 mg/day) to everolimus in patients with RCC who previously received treatment with at least one VEGF-TKI (**ClinicalTrials.gov., NCT01865747**).

14.2.6 Tivantinib (ARQ 197)

Papillary RCC is the second most common subtype of RCC. Patients with hereditary papillary RCC have mutations in c-MET, which is also present in sporadic papillary RCC [32]. Therefore, MET-target therapy appears to be an attractive strategy for this disease. Tivantinib is a selective and non-ATP competitive inhibitor of c-MET that has shown a higher selectivity for c-MET compared with other kinase inhibitors [33]. In a Phase I trial including 10 patients with RCC, 6 patients (60 %) achieved SD, with 4 of these 6 patients (2 clear cell RCC, 2 unspecified renal cancer) maintaining treatment for more than 24 weeks [34]. The dose-limiting toxicities for tivantinib were neutropenia, thrombocytopenia, vomiting, and dehydration. Clinical trials for tivantinib in papillary RCC are currently being conducted (**ClinicalTrials.gov., NCT01688973**).

14.2.7 Foretinib (XL880)

Foretinib is an oral multikinase inhibitor that targets c-MET, RON, AXL, Tie-2, and VEGFRs. Foretinib potently inhibits c-MET and VEGFRs with a subnanomolar concentration [35]. In a Phase II study of foretinib that examined 74 patients with papillary RCC, the ORR was 13.5 %, and the median PFS was 9.3 months. The presence of a germ line *MET* mutation predicted the response to foretinib. Five of 10 patients (50 %) with the mutation responded to foretinib. In contrast, 10 of 57 patients (17.5 %) without the mutation responded. The most common AEs were fatigue, hypertension, gastrointestinal toxicities, and nonfatal pulmonary emboli. Foretinib demonstrated efficacy in patients with advanced papillary RCC. Therefore, patients with papillary RCC-associated germ line *MET* mutations are expected to respond to foretinib with relatively high rate.

14.3 PI3K/Akt/mTOR Pathway Inhibitors

Although cytokine therapies including interferon and interleukin-2 have shown limited efficacy, mTORC1-targeted therapies such as temsirolimus and everolimus have shown an improved PFS and OS in patients with advanced RCC. In a Phase III trial, temsirolimus demonstrated a better PFS and OS when compared to IFN-α (PFS: 5.5 vs. 3.1 months; OS: 10.9 vs. 7.3 months) [5]**.** Following a Phase III trial that demonstrated everolimus had an improved PFS compared with placebo (4.9 vs. 1.9 months) in patients with mRCC who had progressed with VEGF-TKI therapies, everolimus was approved as a treatment for advanced RCC [6]. Many patients with mRCC are first controlled with mTORC1 inhibitors; however, most of these patients who responded to mTORC1 inhibitors eventually demonstrate relapse. Resistance to mTORC1 inhibitors is considered to occur via pathways that can compensate or serve as alternatives for mTORC1 signaling, and this may lead to HIF accumulation [36]. Possible mechanisms (Fig. 14.1) for acquired mTORC1 inhibitor resistance are dysfunctional negative feedback loops that function normally during mTORC1/S6K pathway activity [37, 38]. One feedback loop prevents mTOR complex 2 (mTORC2) activation. Therefore, mTORC1/S6K inhibition leads to mTORC2-dependent AKT activation and HIF upregulation [37–41]. Another possible mechanism for mTORC1 inhibitor resistance is an inactive negative feedback loop that normally prevents activation of insulin receptor substrate 1 (IRS1)/PI3K/Akt signaling. Considering S6K typically suppresses IRS1 activation through the negative feedback loop, IRS1 activation occurs with mTORC1/S6K pathway inhibition. IRS1 activation subsequently activates HIF via the IRS1/PI3K/Akt pathway [37, 41–44]. Furthermore, HIF-2α is considered to have a greater contribution to RCC development and progression as compared with HIF-1α [46–48]. Recent studies demonstrated that HIF-2α translation requires mTORC2 activity [50], which indicates mTORC2 inhibition is a potentially viable

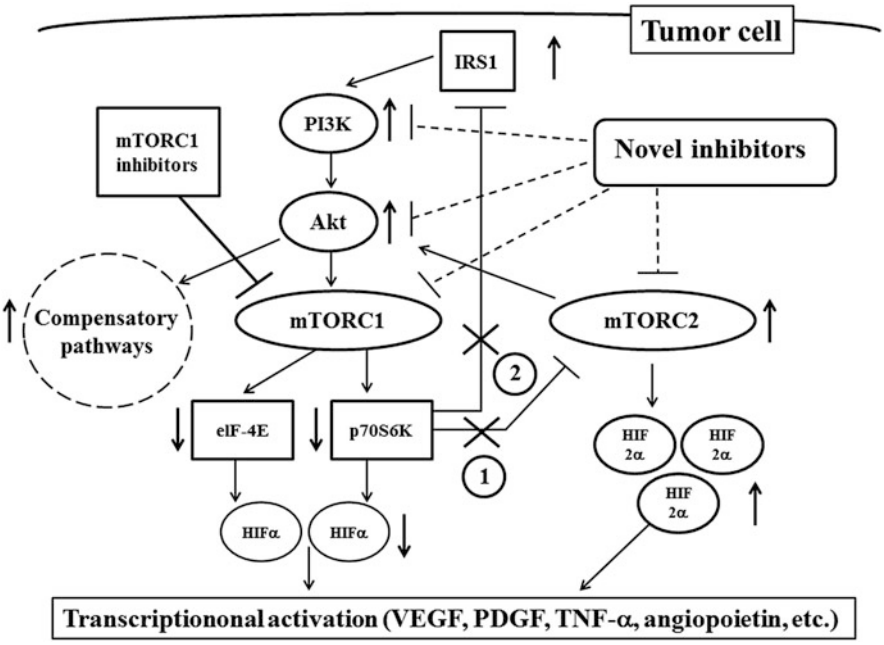

Fig. 14.1 Possible mechanisms for resistance of RCC cells to mTORC1 inhibitors. When mTORC1/S6 K pathway is active, one feedback loop prevents mTORC2 and Akt activation. Therefore, mTORC1/S6 K inhibition leads to mTORC2-dependent HIF upregulation and AKT activation (*circle 1*). Another feedback loop prevents activation of IRS1/PI3K/Akt signaling (*circle 2*) when mTORC1/S6 K pathway is active. mTORC1/S6 K inhibition leads to IRS1 activation and HIF upregulation through the IRS1/PI3K/Akt pathway. Novel inhibitors of PI3K/Akt/mTOR pathway which inhibit PI3K and/or Aki and/or mTORC2 activity have been developed

treatment for RCC. The proposed mechanisms for mTORC1 therapy resistance have led to the development of agents targeting PI3K, Akt, and mTORC2.

14.3.1 NVP-BEZ235

NVP-BEZ235 is a novel dual PI3K/mTORC1/2 inhibitor. BEZ235 inhibits class 1 PI3K activity by binding to its ATP-binding domain [49]. BEZ235 also binds directly to the mTOR ATP-binding domain and inhibits both TORC1 and TORC2. BEZ235 showed strong inhibition of cell proliferation; suppression of Akt, Mnk-1, eIF4E, and 4EBP-1 phosphorylation; and suppression of cyclin D1 and HIF-2α levels in RCC cell lines [50]. Furthermore, BEZ235 inhibited tumor growth in RCC tumor xenografts (from 786-O) with inhibitory effects resulting from its antiproliferative and pro-apoptotic activity [50]. In another experiment, RCC cell lines (786–0 and Caki-1) were treated with BEZ235 alone, sorafenib alone, and these two agents combined. The antitumor efficacy (reduced proliferation and

increased apoptosis) of BEZ235 in combination with sorafenib was superior to NVP-BEZ235 or sorafenib alone [51]. In a multicenter, open-label Phase I trial in 59 patients with advanced solid tumors, including breast cancer and RCC, BEZ235 was well tolerated and demonstrated preliminary efficacy [52]. The common AEs were nausea, vomiting, diarrhea, fatigue, asthenia, anemia, and anorexia. A Phase I/II trial for BEZ235 was completed in patients with advanced RCC, the results of which will be revealed in the near future (**ClinicalTrials.gov., NCT01453595**). A Phase I trial for BEZ235 combined with everolimus is being tested in patients with advanced solid tumors including mRCC (**ClinicalTrials.gov., NCT01482156**).

14.3.2 GDC-0980

GDC-0980 is a selective and potent inhibitor of class I PI3K and mTORs (TORC1/2) [53]. In preclinical studies, GDC-0980 has shown activity against several cancer cell lines (RCC cell line was not included) and xenograft models [53, 54]. A Phase I study was conducted for advanced solid tumors that enrolled 33 patients. GDC-0980 treatment showed tumor shrinkage in 3 patients with mesothelioma (29–64 % decrease), a patient with a gastrointestinal stromal tumor (58 % decrease) who remained on study for more than 6 months, and a patient with adrenal cell carcinoma who remained on study for more than 1 year [55]. The most common AEs observed in more than 10 % of patients were fatigue, diarrhea, decreased appetite, nausea, rash, mucositis, hyperglycemia, vomiting, and constipation. A Phase II trial for GDC-0980 in comparison with everolimus in patients with mRCC who have progressed after a VEGF-targeted therapy is currently underway (**ClinicalTrials.gov., NCT01442090**).

14.3.3 Perifosine (KRX-0401)

Akt is a possible therapeutic target for RCC. Perifosine is a synthetic substituted heterocyclic alkylphosphocholine that can inhibit Akt activity [56]. Perifosine interfered with phosphoinositide metabolism, inhibition of protein kinase C, and inhibition of protein kinase B/Akt phosphorylation [57]. Perifosine targets the pleckstrin homology domain of Akt, preventing its translocation to the plasma membrane and subsequent activation by PDK1 and mTORC2 complexes. Perifosine has also been shown to block cell cycle progression by inducing p21 WAF1 [58, 59]. Two independent Phase II trials (Perifosine 228 and 231) have been conducted. In one Phase II trial (Perifosine 228 trial), 24 patients with advanced RCC received oral perifosine (100 mg daily) [60]. One patient achieved a PR (ORR = 4 %) and 11 patients (46 %) had a SD. The median PFS was 14.2 weeks. Another Phase II trial (Perifosine 231) includes two groups of patients that received perifosine (100 mg daily). Group A included 32 patients who had not previously

received mTOR inhibitor, whereas Group B included 18 patients who had received one prior mTOR inhibitor. In Group A, 4 patients experienced a confirmed objective PR (ORR = 13 %), and 9 patients experienced SD. The median PFS for Group A was 14.1 weeks. In Group B, 1 patient experienced a confirmed objective PR (ORR = 6 %) with a duration of 23 months and 7 patients experienced SD. The median PFS for Group B was 14 weeks. The overall median PFS for both Group A and Group B was 14 weeks (95 % CI, 12.9–20.7 weeks). The ORR for the Perifosine 231 trial was 10 %. Overall, perifosine was well tolerated. The most common AEs included nausea, diarrhea, musculoskeletal pain, and fatigue.

14.3.4 BKM120

BKM120 is a pyrimidine-derived pan-PI3K inhibitor with specific activity against class I PI3Ks that has no inhibitory activity against the class III PI3K or mTOR. Moreover, BKM120 inhibits PI3Ks at nanomolar concentrations [61]. In a Phase I trial, 35 patients with advanced solid tumors, including RCC, were treated with BKM120 [62]. The treatment was well tolerated with a MTD of 100 mg/day. The most frequent AEs were anorexia, rash, diarrhea, hyperglycemia, nausea, fatigue, pruritus, and mucositis. One patient demonstrated a confirmed PR (triple negative breast cancer); 7 patients (20 %, 2 patients each with breast cancer and colorectal cancer, 1 patient with prostate cancer, angiosarcoma, and lung adenocarcinoma) were on the study for $\geq$8 months. Another current Phase I trial that will examine the combination of BKM120 and bevacizumab is currently recruiting patients with advanced RCC (**ClinicalTrials.gov., NCT01283048**).

14.3.5 AZD8055

AZD8055 is an mTORC1/2 and Akt inhibitor that shows strong antitumor activity against the VHL null clear cell RCC cell lines (UOK139 and UOK140) **[63]**. AZD8055 downregulates 4EBP1, a critical component of cap-translation that is regulated by mTORC1. In contrast, rapamycin cannot downregulate 4EBP1. Preliminary data from a Phase I study in 49 patients with advanced solid tumors suggested that AZD8055 was well tolerated (64). The maximal tolerated dose was 90 mg, but dose-limiting toxicities of grade 3 transaminases were reported.

14.3.6 INK128

INK128 is an mTORC1/2 inhibitor of downstream substrates for mTORC1 (S6 and 4EBP1), phosphorylation of Akt, and induced G1 cell cycle arrest [65]. INK128

showed antitumor effects in a mouse tumor model through a direct inhibitory effect on cell proliferation; these effects could be enhanced when combined with sorafenib or bevacizumab because of their inhibitory effects on tumor angiogenesis. INK128 is currently being tested in a Phase I study in patients with advanced solid malignancies as a single agent (**ClinicalTrials.gov., NCT01058707**). As a monotherapy, INK128 was well tolerated in 52 patients who had pathologically confirmed advanced solid tumors, and preliminary antitumor activity was observed in some patients with RCC and lung cancer [66]. The most common AEs included nausea, hyperglycemia, mucosal inflammation, rash, asthenia, vomiting, and diarrhea. All reported AEs were reversible.

14.3.7 SF1126

The PI3K/mTORC1/2 inhibitor SF1126 significantly suppressed signaling pathways downstream of PI3K (Akt, ERK) and abolished hypoxia-induced stabilization of HIF-2α in a RCC cell line (786-O) [67]. SF1126 inhibited tumor growth by more than 90 % in RCC xenograft models and demonstrated to potent anti-angiogenic activity [67]. Furthermore, in 786–0 RCC cells, a combination of SF1126 with rapamycin showed greater antiproliferative activity and apoptosis as compared with either agent alone, and the combination enhanced the antitumor activities compared with each monotherapy alone in RCC xenograft models [68]. SF1126 is currently being tested in a Phase I trial in patients with advanced or metastatic solid tumors (39 patients) and B-cell malignancy [69]. There were 19 of 33 patients (58 %) with a solid tumor experienced SD. SD was the best response with a mean duration of 21 weeks; 8 patients had SD for at least 16 weeks. Moreover, a patient with clear cell RCC had SD for 84 weeks. The majority of AEs were grade 1 or 2. The most common AEs were nausea, fatigue, vomiting, diarrhea, pyrexia, chills, anorexia, anemia, pruritus, and headache.

14.3.8 WYE-132

WYE-132 is an mTORC1/2 inhibitor. WYE-132 inhibited cell proliferation and cell cycle progression and induced cell apoptosis in tumor cell lines, including RCC cell lines. WYE-132 completely inhibited tumor growth and induced tumor regression at higher doses in a mouse tumor model (A498 RCC cells). The combination of WYE-132 with bevacizumab enhanced the tumor regression caused by WYE-132 alone or bevacizumab alone [70].

14.3.9 OSI-027

A second dual mTORC1/2 inhibitor, OSI-027, is currently being tested in a Phase I trial in patients with advanced solid tumors or lymphoma (**ClinicalTrials.gov., NCT00698243**). OSI-027 was well tolerated in 34 patients with the doses tested till date, and 8 patients (26 % of the treated patients) have achieved SD lasting at least 12 weeks [71]. In the 8 patients with SD, 1 patient had RCC. MTD has not yet been determined, and a dose escalation of OSI-027 is ongoing. AEs included nausea, vomiting, pneumonia, fatigue, diarrhea, anorexia, elevated creatinine, and a reversible increase in QTc.

14.3.10 XL765

The PI3K/mTORC1/2 inhibitor XL765 is currently being investigated in two Phase I trials in patients with solid tumors. One of these was conducted as monotherapy (**ClinicalTrials.gov., NCT00485719**). Another trial was conducted in combination with erlotinib (**ClinicalTrials.gov., NCT00777699**). As a single agent [72], XL765 was generally well tolerated in 34 patients; 5 patients achieved SD lasting more than 3 months, including 1 patient with RCC. The most commonly related AEs were elevated liver enzymes, nausea, and diarrhea.

14.3.11 GSK2126458

A PI3K/mTORC1/2 inhibitor, GSK2126458, is currently being tested in Phase I trials as a single agent in relapsed or refractory advanced solid tumors (**ClinicalTrials.gov., NCT00972686**). Also, another Phase I trial is being conducted in combination with an MEK inhibitor in advanced solid tumors (**ClinicalTrials.gov., NCT01248858**). In the single-agent study, 2 of 78 patients have achieved a PR to date, including 1 patient with RCC [73]. The most commonly reported drug-related AEs were fatigue, nausea, and diarrhea.

14.4 Conclusion

Novel drugs for advanced RCC were discussed in this chapter. Recent progress in RCC therapy, particularly molecular targeted therapy, has led to remarkable improvement in the prognoses for patients with RCC. Attractive drugs are now increasing in availability. However, we need to reconsider which drugs should be selected for the individual patient in order to prolong patient survival with a

maintained general condition. Although most drugs for RCC therapy have been developed for the treatment of clear cell carcinoma, we should develop effective therapies for patients with advanced non-clear cell RCC. Further progress will be needed for the treatment of advanced RCC.

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Chapter 15
Refractory Mechanisms

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Abstract Vascular endothelial growth factor (VEGF)-targeted agents have brought significant progress in pharmacotherapy of advanced renal cell carcinoma (RCC). Some RCCs, however, appear to have innate resistance to treatment. Moreover, majority of patients who primarily respond to VEGF-targeted therapy eventually develop disease progression after prolonged treatment. The mechanism of resistance is supposed to be so multifactorial that it cannot be explained by single molecular events. Despite various factors including cytokines, bone marrow-derived cells, microenvironment, and genetic or epigenetic abnormality could be involved, tumor hypoxia seems to play one of crucial roles in most of the processes. The multiple mechanism in developing drug resistance might just represent the diversity of cellular response to hypoxia and nutrient deprivation, induced by the disruption of the blood vessels. In this chapter we comprehensively review current efforts aiming to clarify these process and to develop pharmacological strategies leading to overcome the drug resistance in RCC.

Keyword Renal cell carcinoma • Antiangiogenic therapy • Mechanisms of drug resistance • Acquired resistance • Intrinsic resistance

15.1 Introduction

Several clinical studies have demonstrated that antiangiogenic therapies have a significant therapeutic benefit in terms of overall survival and disease control in patients with metastatic RCC [1–5]. The agents designed to blocking the angiogenic pathway include tyrosine kinase inhibitor (TKI), such as sorafenib, sunitinib, axitinib, and pazopanib, and a humanized anti-VEGF monoclonal antibody, bevacizumab. Despite these VEGF-targeted agents have brought significant progress in RCC pharmacotherapy, some RCCs appear to have innate resistance to treatment [6]. Clinical data of 4564 patients from 52 countries, treated with

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M. Oya (ed.), *Renal Cell Carcinoma*, DOI 10.1007/978-4-431-55531-5_15

sunitinib for advanced or metastatic RCC, showed that 24 % of the patients were primarily refractory to the TKI, having progressive disease as best response or stable disease less than 3 months [7]. Through the histological examination on RCC specimens from patient receiving neoadjuvant TKI therapy, Tsuzuki et al. found TKI-induced vasculopathy of tumor vessels, which is characterized by decreased density of endothelial cells, within most of necrotic or degenerative area [8]. Interestingly, they also demonstrated that those tumors always accompanied TKI-insensitive areas which did not show vasculopathy, suggesting that TKI has different efficacies within same tumor. Intratumor heterogeneity could be one of the mechanisms explaining the limitation of this one-size-fits-all approach to systemic therapy for patients with kidney cancer [9].

Majority of patients who primarily respond to VEGF-targeted treatment eventually develop disease progression after prolonged treatment. This so-called acquired resistance has made it difficult to achieve complete and durable responses in patients with RCC and then eventually leads to death. Imaging studies have shown evidence that antiangiogenic therapy affects tumor perfusion in RCC [10, 11]. In patients with treatment resistance, those studies showed increasing rebound vascularization identified by contrast CT. These suggest that the mechanism that induced re-vascularization could be involved in the process of establishing acquired resistance.

In this chapter, we review the basic research data that inform the current view of the mechanism of resistance in antiangiogenic treatment. Those could provide insight into overcoming the clinical problem facing oncologists who care for patients with advanced RCC.

15.2 Mechanism of Resistance in Antiangiogenic Therapy

Resistance to anticancer drug therapy is defined as evidence of progressive disease measured by RECIST criteria despite continued treatment. Two general modes of tumor resistance have been proposed: intrinsic resistance which has no therapeutic benefit despite the administration of antiangiogenic drug and an adapted-acquired resistance, which induced progressive disease following a period of tumor control.

15.2.1 Intrinsic Resistance

Current evidence in literature have shown that under anti-VEGF therapy, particular clones can be selected that leads to development of more aggressive and metastatic tumor [12–14]. For example, mice bearing p53−/− human colorectal cancer cells have shown less response to anti-VEGF antibody therapy comparing to mice bearing p53+/+ tumors. Meanwhile, the selection of hypoxia-resistant p53−/− clones has been observed if the tumors from mixtures of p53+/+ and p53−/−

clones are treated by VEGFR-2 inhibition [13]. However, it is hard to regard this scenario as likely in RCC, because p53 mutation is not a frequent event in RCC. Although mutation in a gene encoding a key tyrosine kinase receptor is a common cause of drug resistance development in other types of cancers [15, 16], it is not likely that this mechanism also could explain the drug resistance of anti-VEGF therapy. Because several evidences have demonstrated that resistance to VEGFR antagonists is reversible [17, 18], moreover, the target of VEGFR antagonists is endothelial cells, which are usually supposed to be less prone to mutation than genetically unstable tumor cells. However, several studies have been clarifying the genetic difference between tumor endothelial cells and their normal counterpart [19–21]. Bussolati et al. [20] obtained tumor-derived endothelial cells from renal cell carcinoma and observed that endothelial cells originated from tumor upregulated several proangiogenic growth factors and antiapoptotic survival pathway. Indeed, it has been demonstrated that tumor endothelial cells are cytogenetically abnormal and could have tumor-related mutation [22–24]. Possible mechanisms that result in tumor endothelial cell genetic mutation are not understood yet, but the involvement of tumor microenvironment producing cytokines leading to genetic instability and transdifferentiation of tumor cells into endothelial cells have been suggested [24]. Overall, several evidences have been suggesting the scenario that genetic mutation in tumor endothelial cells could be involved in the mechanism of resistance to antiangiogenic therapy; however, further comprehensive and meticulous analysis is warranted to prove this hypothesis.

15.2.2 Acquired Resistance

In orthotopic tumor models of glioblastoma in which VEGF or HIF1α was genetically or therapeutically blocked [25–27], hypoxia in the tumor microenvironment accompanied with initial tumor shrinkage could eventually lead or facilitate increased invasiveness along host vessels or recurrent tumor growth. Increased invasiveness and metastasis in mice also have been observed in pancreatic neuroendocrine carcinoma, exhibiting marked hypoxia following inhibition of VEGFR and PDGFR signaling by sunitinib [28]. Tumor-independent (host-mediated) pathways of resistance to angiogenesis inhibition have also been demonstrated to facilitate metastasis [29]. Although several evidences have not been definitive yet, there are six distinct different mechanisms involved in acquired resistance to antiangiogenic therapy. Those mechanisms are (1) mediated by the production of pro-angiogenic factors, (2) recruitment of pericyte coverage, (3) recruitment of bone marrow-derived cells, (4) epigenetic change to adapt environment, (5) lysosomal sequestration, and (6) increasing invasiveness and metastasis through epithelial to mesenchymal transition (EMT).

15.2.2.1 Upregulation of Pro-angiogenic Pathway

HGF

Ligand-dependent c-MET activation through paracrine HGF induces proliferation, survival, and invasiveness of solid tumor through a series of signaling pathways including PIK3/Akt, STAT, and Ras/Mek. It also regulates tumor angiogenesis by inducing endothelial cell migration and tubular formation. The significant role of the HGF/c-met pathway in the mechanism of resistance to anti-VEGFR therapy has been indicated by several authors [30, 31]. Shojaei et al. [32] demonstrated that HGF levels in the sunitinib-resistant xenograft tumor such as mouse lymphoma (EL4) and Lewis lung carcinoma (LLC) are much higher when compared to that of sunitinib-sensitive tumor such as B16F1 mouse melanoma and TIB6 mouse myeloma cells, especially after treated by sunitinib. Combination of sunitinib and selective c-MET inhibitor had an additive effect of inhibiting the growth of sunitinib-resistant xenograft. Moreover, treatment efficacy of sunitinib for B16F1 or TIB6 cells was significantly decreased after the exposure of tumor cells to HGF. This approach clearly confirms the significance of c-MET activation in the mechanism of developing anti-VEGFR drug resistance in xenograft of several malignancies. Recently, cabozantinib, which is a novel tyrosine kinases inhibitor of c-MET and VEGFR2, has been highlighted for targeting both axes, expected to offer significant benefit comparing to targeting each individual pathway [33]. In phase I trial for RCC, the patients, most of whom had VEGF pathway inhibiting therapy, have shown a favorable safety profile and antitumor activity [34].

FGF

Casanova et al. provided the first report of fibroblast growth factor (FGF) involvement in the resistance to anti-VEGFR therapy by using RIP-Tag2 model, which is a genetically engineered mouse model producing pancreatic islet carcinoma [35]. In their study, although VEGFR2 blockade with an antibody initially achieved an impaired tumor growth and angiogenesis, significant regrowth of the treated tumor occurred during 4 weeks of treatment. Those tumors which grew during anti-VEGFR2 therapy showed a more invasive and malignant phenotype. Quantitative mRNA expression analysis in relapsing tumors revealed a significant upregulation of a set of proangiogenic factors, such as FGFs, ephrins, and angiopoietin, when compared with untreated tumors. In the condition of hypoxia, upregulation of some of these proangiogenic families was also observed in the cultured cell line, derived from RIP-Tag2 tumors. Interestingly, blocking of FGF by using a soluble form of the FGF receptor achieved a remarkable decrease in tumor volume and angiogenesis during the regrowth phase, especially when they are combined with anti-VEGFR2 treatment. Those indicated that increased production of FGFs could be involved in alternative pro-angiogenic pathway and partly explained the

mechanism of development of resistance to VEGFR blockade. Subsequent related studies also have provided the effect of FGF blockade in the face of VEGF inhibition. Welti et al. reported that although sunitinib alone could provide a significant inhibition of endothelial tube formation in the presence of VEGF [36], FGF2 could suppress this antiangiogenic activity of sunitinib. Addition of FGF2 to cultures incubated with VEGF and sunitinib increased the endothelial proliferation and de novo tubule formation. Furthermore, Bello et al. demonstrated by using a human RCC xenograft model that renal tumors that had developed resistance to sunitinib still had sensitivity to a dual inhibitor of VEGFR and FGFR tyrosine kinase, E-3810 [37], suggesting the clinical utility of FGF antagonist in advanced RCC. However, the recent clinical trial using dovitinib, a multi-tyrosine kinase inhibitor of FGFR, VEGFR, and PDGFR, could not prove the superiority of FGFR-targeting therapy in the treatment of anti-VEGFR-refractory RCC patients [38].

IL-8

Many of the proangiogenic factors upregulated under the hypoxic microenvironment of tumor contain hypoxia-response element (HRE) on their gene promoter [39]. This suggests that the HIF pathway partly regulates the compensatory gene expression in the tumors which has responded to antiangiogenic treatment, leading to the development of intratumoral hypoxia. However, Mizukami et al. revealed that even in the absence of functional HIF-1 protein, IL-8 could be induced and maintain vascularity of colon cancer xenograft. They also confirmed that neutralizing antibody to IL-8 achieves the regression of xenograft tumor lacking HIF-1 [40]. Wyscozynski et al. showed that blockade of both MAPK and PI3K-AKT pathways was necessary to inhibit the hypoxia-induced IL-8 expression in rhabdomyosarcoma cells, suggesting that nuclear factor (NF)-KB and activating protein (AP)-1 have an essential role in regulating its gene expression [41].

The potential role of IL-8 on mediating resistance to sunitinib treatment in RCC was demonstrated by Huang et al. [42]. They established a sunitinib-resistant human RCC xenograft model by administrating minimal dosage of drug with an intermittent dosing schedule. The levels of various secreted human cytokines derived from the xenograft tumor were screened. They found that IL-8 level of plasma sample from mice bearing sunitinib-resistant tumor was higher than that from mice with sunitinib-sensitive tumor. To demonstrate the functional significance of IL-8 in development of sunitinib resistance, neutralizing antibody to IL-8 was used in xenograft models. The combination treatment with IL-8 neutralizing antibody and sunitinib had a significant effect in decreasing the size and microvascular density of sunitinib-refractory tumor, even though the neutralizing antibody alone did not reduce the growth of the tumor. They conclude that inhibition of IL-8 function could resensitize the resistance tumors to sunitinib treatment, which result in decreasing RCC growth.

PlGF

Although placental growth factor (PlGF) is a member of the VEGF family, it has a different characteristic from that of VEGF-A isoforms, which bind to VEGFR-1 and VEGFR-2. There is much evidence that VEGFR-2 is the major mediator of VEGF signaling pathway in endothelial cells [43], but PlGF exclusively binds to VEGFR-1, which is expressed by tumor cells, endothelial cells, bone marrow-derived proangiogenic and proinflammatory cells, and stromal cells [44]. The functional properties of VEGFR-1 are still under debate, because they seem to be different depending on the cell type. Although VEGFR-1 has ten times higher affinity to VEGF than VEGFR-2, it undergoes weak tyrosine phosphorylation in response to VEGF [43]. Therefore, VEGFR-1 is considered rather as a decoy receptor, which is able to negatively regulate the activation of VEGFR-2 by VEGF. Indeed, PlGF-deficient mice are healthy and fertile under physiological condition. However, in pathological conditions such as wound healing and inflammation, defect in pathological angiogenesis becomes obvious when a PlGF-deficient mouse was analyzed [45, 46]. The therapeutic potential of PlGF blocking in tumor growth was initially demonstrated by Luttun et al. [47]. Later on, Fisher et al. [48] demonstrated in a xenograft model of melanoma and colonic and pancreatic cancer that blockade of PlGF with a monoclonal antibody enhanced the antitumor activity of anti-VEGFR-2 therapy by inhibiting tumor angiogenesis and intratumoral macrophage recruitment. As for RCC, Rini et al. have revealed that serum levels of PlGF are increased in patients treated with anti-VEGFR therapy [49]; however, the treatment efficacy of PlGF blocking strategy in anti-VEGF treatment-resistance RCC is still under research [50].

Angiopoietin

In addition to the VEGF-VEGFR system, Tie receptors, and their ligands, angiopoietin has been regarded as second tyrosine kinase receptor signaling system which is specific to vascular endothelial cells [51]. This signaling is supposed to have an essential role in maturation and stabilization of the blood vessels. In RIP-Tag2 model, upregulation of angiopoietin mRNA was demonstrated in the tumor relapsing after initial anti-VEGFR2 treatment [35]. The association between evasive tumor resistance and anti-VEGF therapy and adaptive upregulation of Ang2 was revealed in the same model recently by Rigamonti et al. [52]. Therefore, it is not surprising that several recent evidences have shown that angiopoietin production decreases the effect of VEGFR-targeted therapies in other types of cancer. For example, Hashizume et al. demonstrated that the combination of Ang2 inhibitor with anti-VEGF antibody could limit the tumor growth and vasculature expansion more effectively than either of those agents alone in a colon cancer xenograft model [53]. The efficacy of human anti-Ang2 monoclonal antibody was confirmed in several other types of xenograft tumor, including RCC [54]. However,

as for RCC, there is only limited evidence which suggests the therapeutic benefit of the combination therapy targeting Ang2 along with VEGF inhibition [55].

15.2.2.2 Recruitment of Pericyte Coverage

Pericytes are perivascular support cells, which are not only providing a scaffold but communicate with endothelial cells by direct physical contact and reciprocal paracrine signaling. Tumor pericytes appear to be responsible for maintaining the integrity and functionality of the blood vessels [56], even though they are more loosely attached to tumor vessels and less abundant in the tumor tissue, when compared to healthy tissue [57]. Pericytes may play a role as a local source of VEGF for adjacent endothelial cells, and several studies have indicated that pericytes protect the vascular network against anti-VEGF treatment. For example, Helfrich et al. [58] demonstrated that tumor vessels in human melanoma metastasis, which grew during bevacizumab therapy, were featured by increase in the diameter of blood vessels and normalization of the tumor vascular bed covered by mature pericytes, compared with those from patients without anti-VEGF treatment. The blood vessels of treatment-resistant tumor had also an enhanced expression of desmin and α-SMA, which are immature and mature markers of pericytes, respectively, suggesting that mural cell differentiation and stabilization of the vascular wall could contribute to the therapeutic effect of antiangiogenic therapy. Indeed, several approaches have done targeting tumor pericytes to overcome the resistance to VEGF pathway inhibition. Tyrosine kinase inhibitor of PDGF receptor has been considered as a relatively selective pericyte-targeting drug, which could disrupt pericyte support [59]. Pietras et al. revealed that PDGF inhibition could increase sensitization of endothelial cells to antiangiogenic chemotherapy, resulting in regression of tumor vasculature in a xenograft model of pancreatic islet cancer [60]. However, inhibition of PDGF might be a double-edged sword, since the decreased coverage by pericytes could lead to vascular destabilization and subsequent escape of tumor cells into the blood vessel, resulting in hematogenous metastasis [61]. To date, clinical trial for renal cell carcinoma treated by the inhibition of VEGF and PDGF pathway showed no treatment benefit compared to single anti-VEGF therapy, despite the fact that the combined regimen exhibited increased toxicity [62].

15.2.2.3 Recruitment of Bone Marrow-Derived Cells

Hypoxia induced by tumor vessel regression through anti-VEGF treatment recruits not only other proangiogenic factors but also bone marrow-derived cells, which facilitate vascular remodeling and tumor growth. Those cells are tumor-associated macrophages [63], immature TIE2+ monocytes [64], VEGFR1+ hemangiocytes [65], and CD11b+ myeloid-derived suppressor cell [66, 67], all of which expressing CXCR4 receptor. Ceradini et al. showed marked increase in SDF-1 mRNA and

protein expression in ischemic tissue which was proportional to the reduction of oxygen tension in the tissue [68]. SDF-1 expression was induced by HIF-1, then CXCR-4 positive circulating cells were recruited to the site of ischemic tissue. Du et al. [69] had shown in mouse model of glioblastoma that HIF1 arufa induces recruitment of bone marrow-derived CD45+ myeloid cells, through the upregulation of VEGF and SDF-1 in tumor cell. Those monocytic cells comprised of heterogeneous population, such as CD11b+ monocyte cells, F4/80+ macrophages, TIE2-expressing monocytes, and VEGFR1 hemagiocytes. Shaked et al. illustrated that antiangiogenic treatment leads to acute reactive mobilization of circulating endothelial progenitors to damaged vessels, which could contribute to the rapid regrowth of xenograft tumor [70]. As for RCC, loss of VHL function has been shown to overexpress both CXCR4 and SDF-1 through the constitutive activation of HIF-1 protein [71]. Pan et al. indicated that the expression of CXCR4 was associated with metastatic behavior in RCC xenograft mice and neutralization of SDF-1 could reduce metastasis in this model [72]. Recently, increased tumor infiltration of CD11b+ myeloid cells, comparing to untreated control, was demonstrated in RCC xenograft treated by sunitinib [73]. Those preliminary evidences suggest CD11b+ myeloid might play a substantial role in acquired resistance to antiangiogenic treatment in RCC.

15.2.2.4 Epigenetic Change to Adapt Environment

Recent studies have focused on the epigenetic change of gene expression in cancer under hypoxic environment [74]. Mechanism of epigenetic regulation involves DNA methylation, histone modification, and nucleosome remodeling, all of which control gene expression without alternation of DNA sequences. High-throughput genetic studies of RCC have identified the several mutated genes whose functions are implicated in epigenetic modification, such as PBRM1, UTX, SETD2, and JARID1C [75–77]. However, mutation patterns of those genes were not concordant between primary and metastatic region, and mutation of SETD2 histone methyltransferase and JARID1C histone demethylase genes tend to be identified in metastatic sites [78]. Histones are regulators of chromosomal activity by altering electrostatic charge by changing chromatic structure or by providing protein recognition sites through specific modification. Histone modifications included methylation, acetylation, and ubiquitination. In active gene, promoters are mainly marked by methylated H3 at lysine 4 (H3K4me3), and transcribed regions are enriched for H3K36me3 and H3K79me2. Histone H3K9 methylation and H3K27 tri-methylation usually associate with gene repression [79]. Dysregulation of histone-modifying enzymes in RCC leads to global change in those histone modifications [80–82], which may contribute to aberrant gene expression.

Screening of genes regulated by hypoxia has identified promoters of several histone demethylase (JMJD1A, JMJD2B, and JARID1B) as direct binding target of HIFs [83–85].

Krieg et al. [86] demonstrated that the subset of protumorigenic genes, such as adrenomodullin (ADM) and growth and differentiation factor 15 (GDF15), induced by hypoxia, are regulated by JMJD1A-dependent histone modification. In their study, loss of JMJD1A did not have an effect on growth of cancer cells in vitro but reduced the rate of xenograft tumor growth. Those suggest that induction of JMJD1A in the cells exposed to a hypoxic environment facilitates both hypoxic and oncogenic gene expressions and then enhances tumor growth.

Recent study has elucidated that JMJD1A inhibition reduces vascular formation and macrophage infiltration into xenograft tumor tissue [87]. Moreover, JMJD1A inhibition increases the effect of anti-VEGF therapy possibly through the reduction in the expression of tumor-derived FGF2, HGF, and Ang2. These data indicate that targeting epigenetic modifier, such as JMJD1A, could be a novel strategy to overcome the resistance to antiangiogenic therapy.

15.2.2.5 Lysosomal Sequestration

Gotink et al. [88] have revealed that prolonged exposure of RCC cells to sunitinib in vitro resulted in transient resistance to the drug whose mechanism could be explained by not decreased but increased drug concentration within the cell. Their imaging analysis revealed that sunitinib was predominantly colocalized with lysosomal staining. Those subcellular localization of sunitinib was disturbed by co-incubation with bafilomycin A1, which abolished the acidification of lysosomes. Sunitinib has chemical features of a hydrophobic, weak base; therefore, it is supposed to be easily sequestered in acidic lysosomes. Although sequestering sunitinib from a cytoplasmic compartment was associated with decreasing growth inhibitory activity, phosphorylation levels of ERK and Akt were similar between parental and sunitinib-resistant cells. Those suggest that increased lysosomal sequestration could be a novel possible mechanism of resistance to sunitinib.

15.2.2.6 EMT

Epithelial to mesenchymal transition (EMT) is a recent recognized phenomenon. During EMT, epithelial cells lose cell-cell adhesion and begin to show the migratory and invasive phenotype. Molecular features of EMT are characterized by loss of epithelial markers such as E-cadherin or by upregulation of mesenchymal markers such as fibronectin and vimentin. In advanced RCC, parts of the tumor commonly show a sarcomatoid pattern, and transition of epithelial clear cells to sarcomatoid ones is considered as EMT in RCC [89]. Several EMT regulators play a critical role as E-cadherin repressor. Among them, Snail appears to be an important player in development of sarcomatoid RCC progression [90].

Several evidences have supported the idea that EMT has associated with the mechanism of drug resistance including gefitinib and paclitaxel [91, 92]. To explore the relation of EMT with a resistance to antiangiogenic treatment, Hammers et al.

[17] obtained skin metastatic lesion from a patient with advanced clear cell RCC, which initially had responded to sunitinib treatment but eventually developed resistance. The minced metastatic tumor tissue was implanted subcutaneously in a nude mouse, and then sunitinib treatment was performed on the fourth and fifth generation of primary xenograft. Surprisingly, the tumor regained sensitivity to the same sunitinib treatment, coupled with the reduction in microvascular density. Moreover, although the primary skin metastatic tissue showed pure sarcomatoid feature without clear cell pattern, conventional clear cell histology was restored during the development of xenograft tumor. At the same time, mesenchymal markers, such as vimentin and HIF-1, were upregulated in the spindle-shaped cells of sarcomatoid structure. These suggest that an EMT-like phenotype in a patient with clear cell RCC associated with acquired resistance to antiangiogenic treatment.

Several studies have suggested the link between the EMT and the expression of cancer stem cell (CSC) phenotype [93, 94]. CD44 is one of putative CSC surface markers in several types of cancer. Breast cancer cells with CD44 expression are resistant to chemotherapy [95]. In fact, chemotherapy itself could lead to increase the number of cells with CD44 high expression, which suggests that drug-induced CSC phenotype may play a crucial role in the mechanism of acquired drug resistance [96]. Mikami et al. demonstrated that TNF-α stimulation on ccRCC cells enhanced not only EMT marker but upregulated the expression of CD44. [97] Interestingly, hypoxic condition also could induce both CD44 and TNF-α expression. They also demonstrated that the expression of TNF-α and CD44 was predominant in high-grade ccRCC, and those had an inverse correlation with progression-free survival of the patient. Moreover, CD44 was highly expressed in metastatic ccRCC specimens which had been resected from patients receiving sunitinib treatment. Those data indicated that CD44 induced by TNF-α under specific microenvironment, such as intratumoral hypoxia, could be involved in the process of acquired antiangiogenic drug resistance in ccRCC.

15.3 Conclusion

The mechanisms of nonresponsiveness to VEGF-targeted therapies, we have outlined above, clearly show that the resistance to antiangiogenic treatment is so multifactorial that it cannot be explained by single molecular mechanism. Despite various factors including cytokines, bone marrow-derived cells, microenvironment, and genetic or epigenetic abnormality could be involved, tumor hypoxia seems to play a crucial role in most of the processes. In other words, the multiple mechanism in developing drug resistance just represents the diversity of cellular response to hypoxia and nutrient deprivation, induced by the disruption of the blood vessels. Interestingly, there may be a delicate balance between destruction of vasculature and induction of intratumoral hypoxia, because tumor vascular regression possibly leads to transient vessel normalization by reopening of previously collapsed

vessels, which could facilitate tumor growth and metastasis [98, 99]. Furthermore, deprivation of oxygen and nutrient supply not only decreases tumor growth but selects or increases the malignant phenotype of cancer cells. Those complexities have undermined the possibility that the inhibition of just one additional proangiogenic cytokine would consistently solve the problem of resistance. Targeting various stress-induced pathways activated by hypoxia seems to be an attractive strategy to overcome the drug resistance in RCC.

The problem of permanent intrinsic resistance to molecular-targeted therapy is usually supposed to be resolved through the comprehensive analysis of individual genetic patterns. However, different from other types of solid tumor, such as breast and lung cancer, biomarkers, which could easily predict treatment outcome at the onset, have not been identified in RCC yet. The presence of complex genomic abnormality within a different region of a tumor, and a different metastatic site, could be the reason for the difficulty in RCC [9]. Tissue collection protocols based on multi-region tumor analyses to identify relatively common genetic change should be served in clinical trials to find more accurate predictors of disease biology and treatment outcome.

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Chapter 16
Optimization of Therapy by Pharmacokinetic–Pharmacodynamic Analyses

Chiyo K. Imamura

Abstract In cytotoxic anticancer agents, efficacy depends on tumor heterogeneity and is not evaluated immediately after administration, and toxicities are severe and life threatening such as neutropenia and thrombocytopenia. Therefore because toxicity is often more readily measured than efficacy, there are more reported pharmacodynamic (*PD*) studies defining relationships between pharmacokinetic (*PK*) parameters and the toxicity. However, retrospective studies have shown that molecular targeted agent systemic exposure correlates with treatment response (efficacy and toxicity) in various cancers including renal cell carcinoma (RCC). The evidence of the relationship between *PK* and *PD* for imatinib currently exists in the treatment of leukemia and gastrointestinal stromal tumor (GIST). It is important to evaluate the relationship between *PK* and *PD* prospectively in clinical trials rather than extrapolating from retrospective analyses. Based on these findings, therapeutic levels should be defined for molecular targeted agents in the treatment of RCC such as that that already occurs for antiepileptic, immunosuppressive, and antibiotic agents. Optimization of systemic exposure by dose modification to eliminate individual variability can increase the probability of efficacy, decrease the probability of toxicity, or both in each RCC patient treated with molecular targeted agents.

Keywords Pharmacokinetics • Pharmacodynamics • Tyrosine kinase inhibitors (TKIs) • Mammalian targets of rapamycin (mTOR) inhibitors • Renal cell carcinoma (RCC)

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M. Oya (ed.), *Renal Cell Carcinoma*, DOI 10.1007/978-4-431-55531-5_16

16.1 Introduction

Recent evidence indicates that systemic exposure reflected in area under the concentration-time curve (AUC) and trough blood concentration (C_{trough}) of molecular targeting anticancer agents including tyrosine kinase inhibitors (TKIs) and mammalian targets of rapamycin (mTOR) inhibitors correlates with response (efficacy and/or toxicity) for the treatment of renal cell carcinoma (RCC). Therefore the clinical outcome is affected not only by genetic heterogeneity of drug targets but also by the pharmacokinetic (*PK*) variability influenced sometimes by pharmacogenetic background of the patient (e.g., polymorphisms of transporters and metabolic enzymes). This chapter deals with the factors that cause wide interindividual variability in *PK* and the relationship between *PK* and pharmacodynamics (*PD*) in targeted therapy for RCC. The current evidence is summarized in Table 16.1.

16.2 Tyrosine Kinase Inhibitors

16.2.1 Sorafenib

16.2.1.1 Factors Influencing PK

Sorafenib is metabolized by cytochrome P450 (CYP) 3A4 and UDP-glucuronosyltransferase (UGT) 1A9. It was reported that the genetic polymorphisms of UGT1A9 influence on the PK of sorafenib [1]. A population pharmacokinetic (PPK) analysis of sorafenib in 111 patients with solid tumor from five phase I and II clinical trials demonstrated that baseline bodyweight was a statically significant covariate for distribution volume, accumulating for 4 % of interindividual variability. In other PPK analyses, no clinically important PK covariates were identified in evaluating the possible effects of genetic polymorphisms of CYP3A4/5 and UGT 1A9 [2].

16.2.1.2 PK/PD Relationship

Early clinical trials showed that higher C_{trough} in sorafenib-treated patients were moderately predictive of prolonged progression-free survival (PFS). A weak relationship between C_{trough} and skin toxicity, as well as hand-foot skin reactions and hypertension, was also observed [3]. In a preliminary study in 58 patients with advanced or metastatic solid tumors treated with sorafenib, increased cumulated sorafenib exposure (AUC_{cum}) between day 0 and day 30 was independently associated with any grade $\geq$3 toxicity ($P = 0.037$). Additionally, the threshold AUC_{cum} value of 3161 mg·h/L was associated with the highest risk to develop any grade $\geq$3 toxicity ($P = 0.018$) [1].

Table 16.1 Factors influencing PK and PK/PD relationships of molecular targeted agents for RCC

Drug	Factor influencing PK	PK/PD relationship	
		PK parameter	PD (efficacy, toxicity)
Tyrosine kinase inhibitors			
Sorafenib		C_{trough}	PFS
			Skin toxicity
			Hand-foot reaction
			Hypertension [3]
		AUC_{cum}	Any grade $\geq$ 3 toxicity [1]
Sunitinib	Body size	AUC_{ss}	TTP
	Polymorphisms of ABCG2		OS
			SD
			Fatigue [6]
		C_{trough}	dBP [6]
		AUC_{cum}	ANC [6]
Axitinib		AUC_{ss}	PFS
			OS
			dBP [15]
Pazopanib	Fat meals	$C_{trough,\ ss}$	PFS
			Tumor shrinkage
			BP
			Hand-foot syndrome [18]
Mammalian targets of rapamycin inhibitors			
Everolimus		$C_{trough,\ ss}$	Tumor size reduction
			Risk of PFS events
			Grade $\geq$ 3 pulmonary
			Grade $\geq$ 3 stomatitis
			Grade $\geq$ 3 metabolic events [22]
Temsirolimus	Body surface area	AUC_{sum}	Severity for thrombocytopenia, pruritus, hyperlipidemia [25]
	Hematocrit		

C_{trough} trough concentration, $C_{trough,\ ss}$ trough concentration at steady state, AUC_{cum} cumulative AUC
AUC_{ss} AUC at steady state, AUC_{sum} sum of temsirolimus and sirolimus AUCs

16.2.2 Sunitinib

16.2.2.1 Factors Influencing PK

Body size affects volume of distribution but not clearance for sunitinib in patients with RCC [4]. CYP3A4 metabolizes sunitinib to its active *N*-desethyl metabolites, SU12662, and subsequently into SU14335 and other inactive metabolites. Sunitinib is a substrate of the efflux transporters ATP-binding cassette transporter P-glycoprotein and the breast cancer-resistant protein encoded by the ABCB1 and ABCG2 genes, respectively. Genetic polymorphism of ABCG2 was identified as a

significant covariate for the prediction of oral clearance (*CL/F*) of sunitinib by PPK analysis [5].

16.2.2.2 PK/PD Relationship

In a PK and PD meta-analysis, tentative relationships were identified between the following:

1. Steady-state AUC (AUC_{ss}) of total drug (sunitinib + its active metabolite SU12662) and time to tumor progression (TTP); overall survival (*OS*), with AUC significantly associated with longer TTP and *OS* in patients with mRCC; and incidence, but not severity, of fatigue
2. Steady-state AUC of sunitinib and response probability, with AUC significantly associated with objective response and stable disease (*SD*) in patients with mRCC (Fig. 16.1)
3. Dose and tumor size reductions
4. Total drug C_{trough} and diastolic blood pressure (dBP), with a typical patient on sunitinib 50 mg QD (the recommended dose) predicted to experience a maximum dBP increase of 8 mmHg
5. AUC_{cum} of total drug and absolute neutrophil count (ANC), with ANC reductions occurring predominantly after one treatment cycle (Fig. 16.2) [6]

The frequency of the *ABCG2* 421 AA genotype, which developed severe toxicities due to high exposure of sunitinib and SU12662 [5], is higher in Asian populations (Japanese, 7 % [7]; Korean, 8 % [8]; and Chinese, 12 % [9]) than in non-Asian populations (Caucasian, 1.7 % and African, 0.2 % [9]). Thus, this racial difference in the frequency of the *ABCG2* 421 AA genotype could explain the

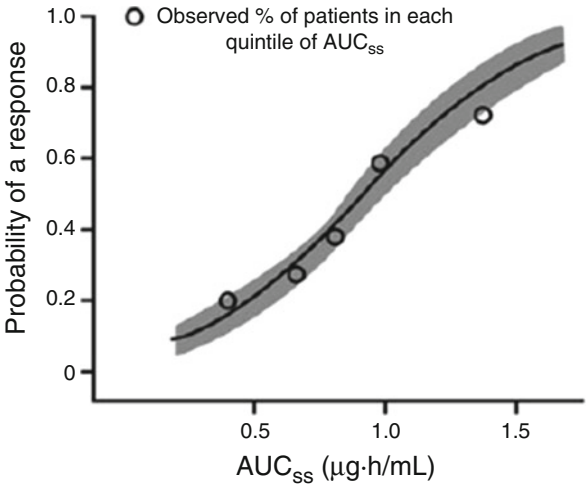

Fig. 16.1 Probability of partial or complete response (by RECIST criteria) versus average daily AUCss to sunitinib in mRCC. *Lines* represent model prediction and shaded area represents 95 % confidence interval

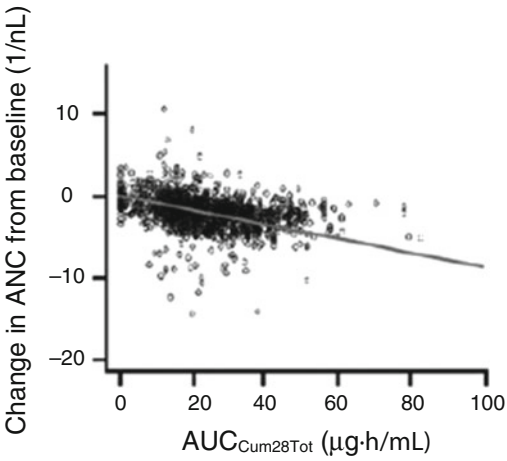

Fig. 16.2 Measured and population-directed (*straight line*) changes in absolute neutrophil count (ANC) by sunitinib exposure in mRCC

higher frequency of grade 3 and 4 sunitinib-related toxicities in Asians [10, 11] than in non-Asians [12].

16.2.3 Axitinib

16.2.3.1 Factors Influencing PK

Axitinib is metabolized primarily by CYP 3A4/5 and to a lesser extent (<10 % each) by CYP1A2, CYP2C19, and UGT1A1. Axitinib is also a substrate for the drug transporters P-glycoprotein and OATP1B1 encoded by the ABCB1 and SLCO1B1 genes, respectively. Meta-analysis was performed using $AUC_{0\text{-}\infty}$ data measured in 315 healthy subjects from 11 clinical pharmacology trials to examine potential influences of genetic polymorphisms in drug-metabolizing enzymes or transporters on axitinib pharmacokinetics. The results demonstrated no statistically significant associations between the specific genetic polymorphisms analyzed and axitinib plasma exposures and that none of them contributed >5 % to the overall pharmacokinetic variability of axitinib [13]. The lack of the pharmacogenetic effect of CYP2C19 and UGT1A1 on axitinib pharmacokinetic variability was also supported by the PPK analysis of axitinib in 337 healthy subjects from 10 phase I studies [14].

16.2.3.2 PK/PD Relationship

In a PPK analysis of 383 healthy volunteers, 181 patients with mRCC and 26 patients with other solid tumors in 17 trials, the median(range) for AUC at the end of 4 weeks (AUC_{ss}) was 375 ng·h/mL (32.8–1728). The relationship between

axitinib plasma exposure and the probability of response (i.e., 1.5-fold increase in the probability of achieving a partial response for every 100 ng·h/mL increase in AUC_{ss}) in 168 metastatic RCC patients was demonstrated ($P < 0.0001$). Patients were stratified by having an AUC_{ss} greater or equal to axitinib total daily therapeutic exposure of 300 ng·h/mL (high AUC_{ss}) or <300 ng·h/mL (low AUC_{ss}). Median PFS in the high-AUC_{ss} group was significantly longer than median PFS in the low-AUC_{ss} group (13.8 months vs. 7.4 months, respectively; hazard ratio [HR] 0.558; $P = 0.003$). Similarly median *OS* of 37.4 months in the high-AUC_{ss} group was considerably longer than the 15.8 months in the low-AUC_{ss} group (HR 0.489; $P < 0.001$) shown in Table 16.2. These results indicated significant associations between AUC_{ss} and clinical responses for axitinib. When used as a continuous variable, the result was more significant than using a cut-off value of 300 ng·h/mL. For PFS and *OS*, the HR was 0.871 ($P = 0.001$) and 0.810 ($P < 0.001$) for every 100 ng·h/mL increase in AUC_{ss}, respectively (Table 16.2). In toxicity, a weak correlation between exposure and dBP (r^2 value<0.10) was shown [15].

16.2.4 Pazopanib

16.2.4.1 Factors Influencing PK

Administration of pazopanib with both low- and high-fat meals increased maximum observed plasma concentration (C_{max}) and AUC by approximately twofold as compared with the corresponding values when administered to patients in the fasted condition [16].

16.2.4.2 PK/PD Relationship

The steady-state concentration of pazopanib determined from preclinical activity showed a strong correlation with antitumor activity in a phase I clinical trial [17]. In 225 patients with locally advanced or metastatic RCC from phase II trial, the mean and median C_{trough} on week 4 were 28.8 and 28.1 μg/mL, respectively (Fig. 16.3). Dose reductions were allowed in this study. In all, 184 of the 205 patients with week 4 C_{trough} data received pazopanib 800 mg once daily for at least 2 weeks; the mean week 4 C_{trough} value in those patients was 29.3 μg/mL. For patients who experienced dose reductions or dose interruptions within 2 weeks of collection of the week 4 plasma sample, the mean week 4 C_{trough} value was 24.8 μg/mL. The C_{trough} on week 4 threshold of >20.5 μg/mL was associated with improved efficacy (PFS, $P < 0.004$; tumor shrinkage, $P < 0.001$), but there was no appreciable benefit in absolute PFS or tumor shrinkage from C_{trough} on week 4 >20.5 μg/mL. The median PFS for patients with C_{trough} on week 4 ≤20.5 μg/mL was 19.6 weeks. In contrast, the median PFS for patients with a C_{trough} on week 4 >20.5 μg/mL was 52.0 weeks

Table 16.2 Univariate cox proportional hazards analysis of progression-free and overall survival

Covariates	mPFS, months	HR(95 % CI)	P[a]	mOS, months	HR(95 % CI)	P[a]
Age						
Continuous	–	1.006(0.986,1.026)	.593	–	0.992(0.972,1.01)	.480
Gender						
Male	13.0	1	–	27.7	1	–
Female	7.63	1.63(1.09,2.46)	.018	19.6	1.26(0.799,1.97)	.324
Prior therapy						
Cytokine refractory	13.0	1	–	30.0	1	–
Sorafenib refractory	7.63	1.55(1.02,2.34)	.038	15.8	2.15(1.39,3.34)	<.001
ECOG PS						
0	13.7	1	–	41.6	1	–
1	7.13	2.17(1.46,3.23)	<.001	10.7	3.63(2.40,5.48)	<.001
Hemoglobin(g/dL)						
≤13 for male, ≤11.5 for female	7.69	1	–	15.9	1	–
>13 for male, >11.5 for female	14.6	0.537(0.364,0.792)	.001	43.3	0.282(0.179,0.443)	<.001
Corrected serum calcium, mg/dL						
<10	11.1	1	–	27.7	1	–
≥10	8.38	1.21(0.688,2.13)	.507	16.4	1.74(1.03,2.96)	.038
AUC, ng·h/mL						
Continuous[b]	–	0.871(0.801,0.947)	.001	–	0.810(0.733,0.897)	<.001
<300	7.4	1	–	15.8	1	–
≥300	13.8	0.558(0.379,0.823)	.003	37.4	0.489(0.324,0.738)	<.001
dBP, mm Hg						
Continuous[c]	–	0.604(0.487,0.750)	<.001	–	0.652(0.524,0.811)	<.001
<90	7.86	1	–	18.5	1	–
≥90	14.6	0.590(0.402,0.866)	.006	29.5	0.622(0.411,0.942)	.024

AUC area under the plasma concentration-time curve, *CI* confidence interval, *dBP* diastolic blood pressure, *ECOG PS* Eastern Cooperative Oncology Group performance status, *HR* hazard ratio, *mOS* median overall survival, *mPFS* median progression-free survival

[a]Based on log-rank (score) test at significance level of $P = 0.1$ for inclusion in multivariate model

[b]Hazard ratio per 100 ng·h/mL increase of AUC

[c]Hazard ratio per 10 mm Hg increase of dBP

(Fig. 16.4). For patients with solid tumors enrolled in the phase I study, strong correlation between increased blood pressure and C_{trough} on week 4 was observed ($r^2 = 0.91$) (Fig. 16.5). And the association of C_{trough} on week 4 with certain adverse reactions, particularly hand-foot syndrome, was continuous over the entire C_{trough} on

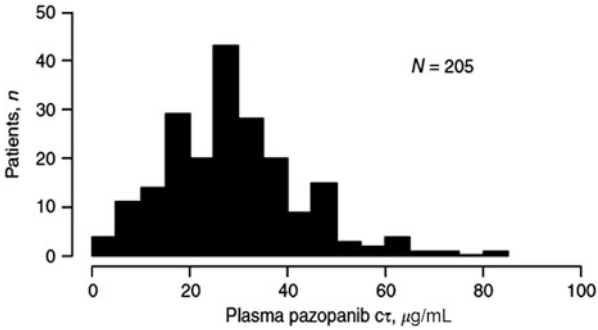

Fig. 16.3 Distribution of predose steady-state plasma pazopanib concentrations (C_{trough})

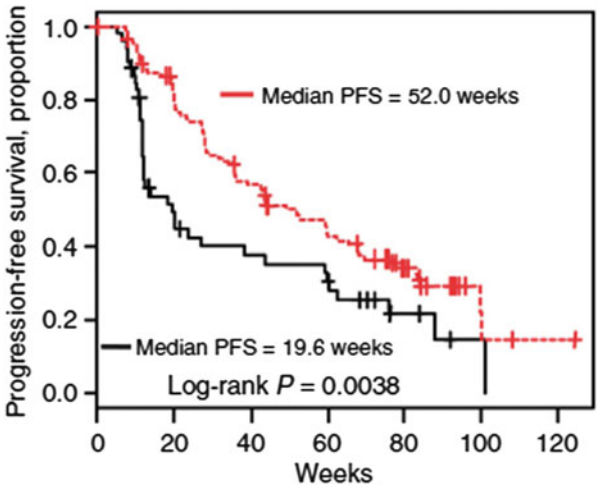

Fig. 16.4 The Kaplan–Meier curves in patients with C_{trough} on week 4 of pazopanib >20.5 μg/mL (*red*) and ≤20.5 μg/mL (*black*)

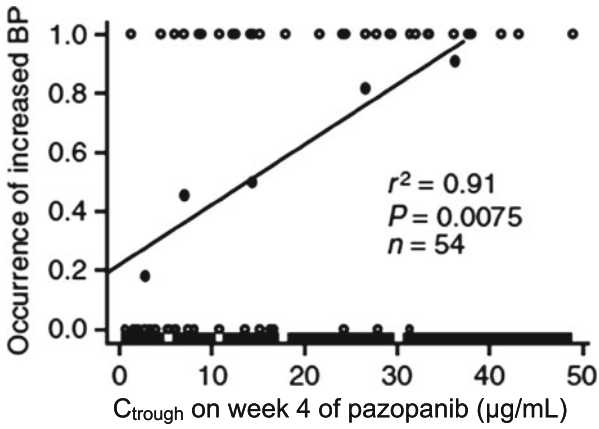

Fig. 16.5 C_{trough} on week 4 of pazopanib and blood pressure in patients with solid tumors. *Open circles* represent individual observations (0. no significant increase; 1. significant increase), and *closed circles* represent the proportion of patients with a significant increase in blood pressure within each C_{trough} on week 4 quintile range. The *thick line* represents C_{trough} on week 4 quintile range

week 4 range for patients with mRCC in phase II study. Thus, the threshold concentration for efficacy overlaps with concentrations at which toxicity occurs, although some toxicities increase over the entire C_{trough} on week 4 range [18].

16.3 Mammalian Targets of Rapamycin Inhibitors

16.3.1 Everolimus

16.3.1.1 Factors Influencing PK

Everolimus is a substrate for CYP3A4 and P-glycoprotein. It was reported that the polymorphisms in genes coding for CYP3A5 and ABCB1 had no clinically relevant effect on everolimus pharmacokinetics in renal and cardiac transplant patients administered with everolimus 2.44 ± 0.75 twice daily and 1.4 ± 0.5 mg/day by PPK analysis, respectively [19, 20].

16.3.1.2 PK/PD Relationship

$C_{trough,\ ss}$ of everolimus was correlated with inhibition of tumor downstream proteins of mTOR in patient with solid tumor in phase I study. A trend was also observed in the relationship between $C_{trough,\ ss}$ and tumor peIF-4G inhibition and significant correlation with inhibition of tumor p4E-BP1 [21].

Efficacy and safety were evaluable for 945 and 938 patients in patients with solid tumors administered with everolimus 10 mg/day from five phase II and III studies, respectively. A twofold increase in everolimus $C_{trough,\ ss}$ significantly increased the likelihood of tumor size reduction by approximately 40 % in patients with solid tumors (odds ratio 1.40, 95 % confidence interval (CI) 1.23–1.60). Conversely, although there was a trend for a reduced risk of PFS events with increased everolimus exposure (risk ratio [RR] 0.90, 95 % CI 0.69–1.18), the relationship was not statistically significant. The lack of statistical significance may be partly explained by the much greater effect of the underlying cancer on PFS: median PFS ranged from 1.74 months in patients with NSCLC and everolimus $C_{trough,ss} < 10$ ng/mL to 25.17 months in patients with carcinoid tumors and everolimus $C_{trough,\ ss}$ 10–30 ng/mL (Table 16.3). Additionally, PFS is a combined end point and sensitive to censoring, whereas tumor size reduction is not. A twofold increase in C_{trough} of everolimus was also associated with increased risk of grade ≥ 3 pulmonary (RR 1.93, 95 % CI 1.12–3.34), stomatitis (RR 1.49, 95 % CI 1.05–2.10), and metabolic (RR 1.30, 95 % CI 1.02–1.65) events (Fig. 16.6) [22]. In the other report, a total of 113 first 3 months C_{trough} were analyzed in 42 mRCC patients who have failed prior anti-angiogenic therapies. The median C_{trough} was 14.1 μg/L (range 2.6–91.5). Fourteen patients (67 %) versus 8 (38 %) patients with median C_{trough} above or below 14.1 μg/L were free from progression at 6 months ($P = 0.06$).

Table 16.3 Progression-free survival by everolimus trough concentration and patient population (full analysis set)

Patient population	C_{min}, ng/mL	Total patients, *N*	Patients with event, *n*	Patients censored, *n*	Median PFS (95 % CI), mo
Carcinoid	<10	69	31	38	16.82 (13.77–27.27)
	10–30	62	20	42	25.17 (13.86–NA)
	>30	5	1	4	NA (2.60–NA)
NSCLC	<10	14	8	6	1.74 (0.76–1.87)
	10–30	42	39	3	2.86 (1.87–3.65)
	>30	20	20	0	1.68 (0.99–2.69)
RCC	<10	26	10	16	4.47 (1.87–NA)
	10–30	112	60	52	5.52 (3.88–8.44)
	>30	40	21	19	5.36 (3.65–7.62)
pNET	<10	131	50	81	13.80 (11.01–18.79)
	10–30	114	43	71	22.70 (14.00–22.70)
	>30	6	2	4	NA (1.87–NA)

CI confidence interval, C_{min} minimum everolimus concentration in whole blood, *NA* not achieved, NSCLC non-small cell lung cancer, *PFS* progression-free survival, *pNET* pancreatic neuroendocrine tumor, *RCC* renal cell carcinoma

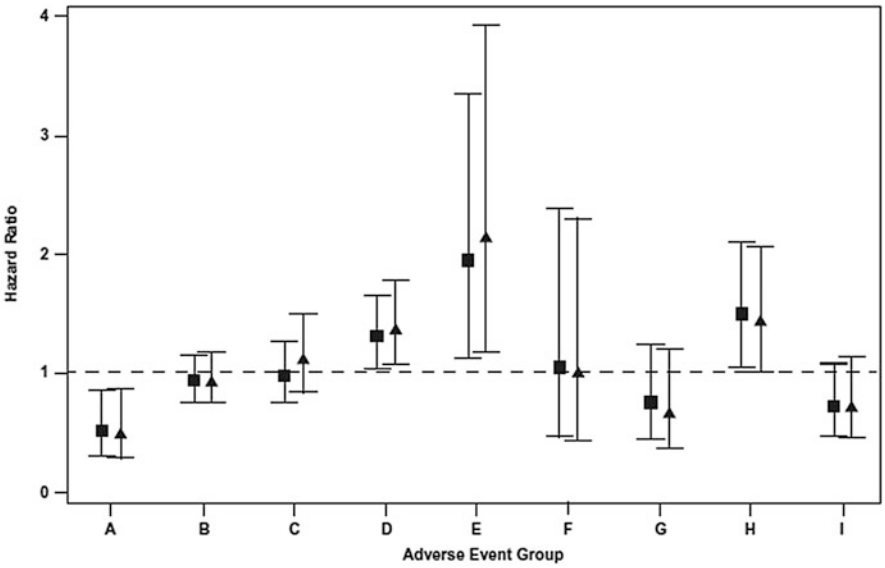

Fig. 16.6 Relationship between twofold increase in everolimus C_{trough} and time to the first selected grade 3 or 4 adverse events by grouping. Selected adverse event groupings: *A* = bleeding events; *B* = hematopoiesis decreased/cytopenias; *C* = infections/infestations; *D* = metabolic events; *E* = pulmonary events; *F* = rash and similar events; *G* = renal events; *H* = stomatitis/oral mucositis/ulcers; *I* = thromboembolic events. Results for grade 3 or 4 hepatic events are not presented; the results were not interpretable because of the low number of events. *Squares* = time averaged C_{trough}; *triangles* = instant C_{trough}

Median PFS was 13.3 versus 3.9 months (HR 0.66 95 % CI 0.33–1.31; $P = 0.23$), and the median *OS* was 26.2 versus 9.9 months (HR 0.62 95 % CI 0.28–1.37; $P = 0.24$), for patients above or below the median value of C_{trough}, respectively [23]. For adverse reactions, a trend was observed between time to oral ulcers and their duration and dose escalation [24].

16.3.2 Temsirolimus

16.3.2.1 Factors Influencing PK

In the PPK analysis of temsirolimus and its active metabolite, sirolimus was performed in 235 and 305 observations from 50 patients with advanced RCC treated with temsirolimus. Body surface area and hematocrit were shown as significant pharmacokinetic covariates for temsirolimus and sirolimus, respectively [25].

16.3.2.2 PK/PD Relationship

AUC_{sum} calculated by the sum of temsirolimus and sirolimus AUCs correlated with adverse reaction severity for thrombocytopenia ($P = 0.007$), pruritus ($P = 0.011$), and hyperlipidemia ($P = 0.040$) [25].

16.4 Conclusion

It is important to evaluate the relationship between PK and PD prospectively in clinical trials rather than extrapolating from retrospective analyses. Based on these findings, therapeutic levels should be defined for molecular targeted agents in the treatment of RCC such as that that already occurs for antiepileptic, immunosuppressive, and antibiotic agents. Optimization of systemic exposure by dose modification to eliminate individual variability can increase the probability of efficacy, decrease the probability of toxicity, or both in each RCC patient treated by molecular targeted agents.

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