Fig. 3 – Correlation of changes in soluble plasma protein concentrations with efficacy. Plasma concentrations of potential biomarkers (mean percent change from baseline [95% confidence interval]) for (A) sVEGFR-1, 2, 3, and sKIT, and (B) VEGF. (C) Objective response rate (IRC assessment) and (D) Kaplan–Meier plots of progression-free survival (IRC assessment) by sVEGFR-2 percent change from baseline to cycle 2 day 1. IRC, independent review committee; sKIT, soluble stem cell factor receptor; sVEGFR, soluble vascular endothelial growth factor receptor and VEGF, vascular endothelial growth factor.

axitinib-induced proteinuria and efficacy, respectively. These data support further investigation of axitinib in mRCC in larger clinical studies. Axitinib is currently in phase III development in RCC.

Japan Axitinib Phase II Study Group

The following investigators and investigational sites also participated in this study: S. Nagai (National Cancer Center

Hospital East, Department of Oncology/Hematology, Chiba, Japan), T. Fujioka (Iwate Medical University School of Medicine, Department of Urology, Iwate, Japan), M. Niwakawa (Shizuoka Cancer Center, Department of Urology, Shizuoka, Japan), T. Nakamura (Kyoto Prefectural University of Medicine, Department of Urology, Kyoto, Japan), T. Shuin (Kochi University, Kochi Medical School, Department of Urology, Kochi, Japan), Y. Hasegawa (National Kyushu Cancer Center, Department of Urology, Fukuoka, Japan), N. Tsuchiya (Akita
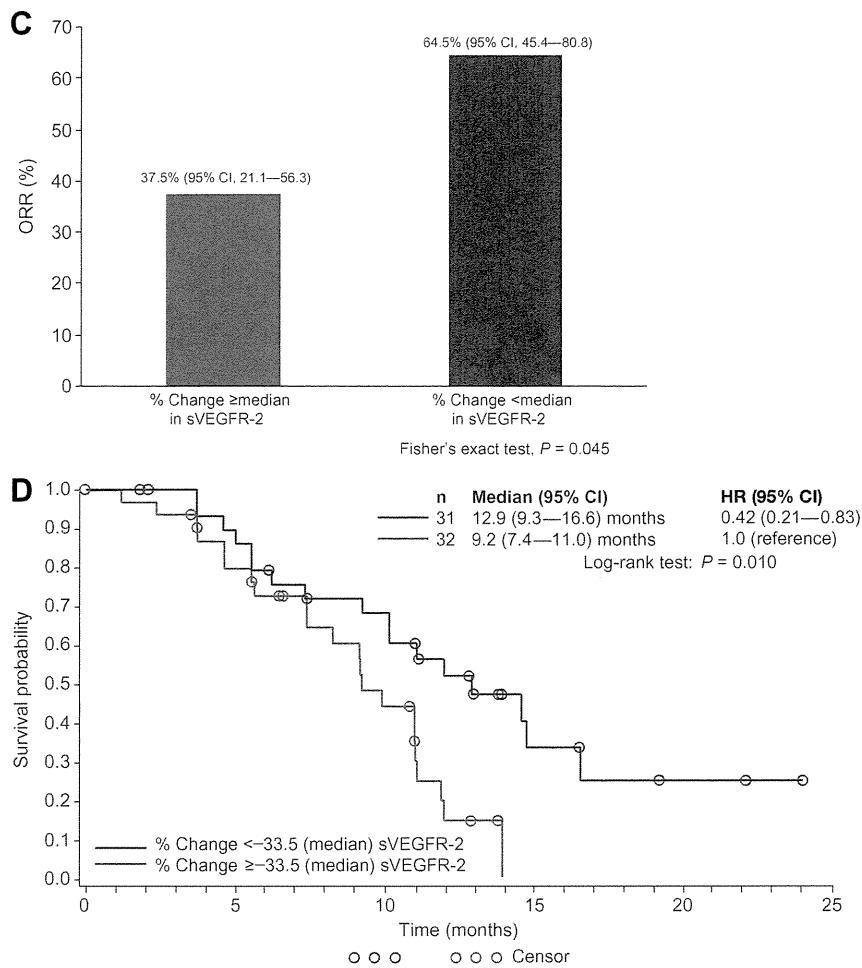
Fig 3. *(continued)*

University School of Medicine, Department of Urology, Akita, Japan), S. Takahashi (Nihon University School of Medicine Itabashi Hospital, Department of Urology, Tokyo, Japan), N. Nonomura (Osaka University Graduate School of Medicine, Department of Urology, Osaka, Japan), K. Nishiyama (Kagoshima University Graduate School of Medical and Dental Sciences, Department of Urology, Kagoshima, Japan).

Conflict of interest statement

Y. Tomita, H. Uemura, H. Kanayama, N. Shinohara and S. Ozono have received speaker honoraria from Pfizer. H. Fujimoto has nothing to disclose. H. Nakazawa, S. Naito and H. Akaza have received speaker honoraria, and consultant or advisory fees from Pfizer. K. Imai and Y. Umeyama are employees of Pfizer and own stock in Pfizer.

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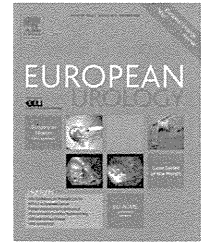
Special Edition EAU–ICUD – Review – Kidney Cancer

Current Pathology Keys of Renal Cell Carcinoma

Ferran Algaba [a,*], Hideyuki Akaza [b], Antonio López-Beltrán [c], Guido Martignoni [d], Holger Moch [e], Rodolfo Montironi [f], Victor Reuter [g]

[a] Section of Pathology, Fundació Puigvert-Universitat Autónoma de Barcelona, Barcelona, Spain; [b] Department of Urology, University of Tsukuba, Tsukuba, Japan; [c] Unit of Anatomic Pathology, Department of Surgery and Pathology, Faculty of Medicine, Cordoba University, Cordoba, Spain; [d] Department of Pathology and Diagnostics, University of Verona, Verona, Italy; [e] Institute of Surgical Pathology, Department of Pathology, University of Zurich, Zurich, Switzerland; [f] La Marche University, Ancona, Italy; [g] Pathology Department, Memorial Sloan-Kettering Cancer Centre, New York, NY, USA

Article info

Abstract

Context: Renal cell carcinoma (RCC) in adults comprises a heterogeneous group of tumours with variable clinical outcomes that range from indolent to overtly malignant. The application of molecular genetic techniques to the study of renal neoplasms has resulted in an improved classification of these entities and a better understanding of the biologic mechanisms responsible for tumour development and progression. The current 2004 World Health Organisation classification of adult renal epithelial neoplasms has expanded rapidly with new categories recently incorporated.

Objective: To review and evaluate the evidence implicating pathologic features and classification of RCC in adults as a tool to approach patients' prognosis and modulate current therapy.

Evidence acquisition: Members of Committee 3: Pathology, under the auspices of the International Consultation on Urological Diseases and the European Association of Urology (ICUD-EAU) International Consultation on Kidney Cancer, performed a systematic review using PubMed. Participating pathologists discussed pathologic categories and diagnostic features of RCC in adults.

Evidence synthesis: We reviewed and discussed articles and the personal experiences of participating uropathologists.

Conclusions: The conclusions reached by the ICUD-EAU 2010 International Consultation on Kidney Cancer emphasise the appropriate pathologic diagnosis of RCC in adults as a tool to approach patients' prognosis and modulate current therapy. Further emphasis should be placed on defining risk groups of RCC and diagnostic features of unusual tumours such as familial RCC, translocation RCC, and tubular mucinous and spindle cell carcinoma. A number of recently described entities and morphologic variants of classical categories deserves recognition because they can be important in differential diagnosis and therapy.

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* Corresponding author. Fundacio Puigvert, Department of Pathology, Calle Cartagena 340–350, Barcelona, 08025 Spain. Tel. +34 93 4169700; Fax: +34 93 4169730.
E-mail address: falgaba@fundacio-puigvert.es (F. Algaba).

1. Introduction

The introduction of the study of familial renal cell carcinoma (RCC) [1] and the new molecular therapies emerged in the 2004 classification by the World Health Organisation (WHO) [2] (Table 1) that combined morphologic and genetic characteristics and began to recognise some variations with evidence of different immunophenotype or molecular changes with clinical implications. This paper reviews the basic aspects of the different current subtypes of RCC.

Nearly each RCC type occurs in a sporadic and in a hereditary form [3]. Based on the knowledge of the different cytogenetic pathways of hereditary forms, there was an

Table 1 – World Health Organisation histologic classification of renal cell carcinoma

Clear cell renal cell carcinoma
Multilocular clear cell renal cell carcinoma
Papillary renal cell carcinoma
Chromophobe renal cell carcinoma
Carcinoma of the collecting ducts of Bellini
Renal medullary carcinoma
Xp11 translocation carcinomas
Carcinoma associated with neuroblastoma
Mucinous tubular and spindle cell carcinoma
Renal cell carcinoma, unclassified

intensive research for molecular changes associated with these chromosomal aberrations.

A surprising number of hereditary syndromes predisposes to the development of RCC. Within the last few years, seven renal cancer syndromes have been characterised. Five of the predisposing genes have been identified in the meantime: von Hippel-Lindau (*VHL*); met proto-oncogene (*MET*); fumarate hydratase (*FH*); folliculin (*FLCH*; synonym: *BHD*); and cell division cycle 73, Paf1/RNA polymerase II complex component, homolog (*CDC73*; previously known as *HRPT2*) (Table 2).

2. Evidence acquisition

A systematic review was performed using PubMed by members of Committee 3: Pathology under the auspices of

the International Consultation on Urological Diseases and the European Association of Urology International Consultation on Kidney Cancer.

3. Evidence synthesis

In this review, different subtypes of RCC are considered with special reference to their morphology, immunohistochemical features, and genetic changes.

3.1. Clear cell renal cell carcinoma

3.1.1. Macroscopy

Tumours are usually single in sporadic cases, with 4% multiplicity and 3% bilaterality. The section surface is yellow. Haemorrhagic areas are frequent. Occasionally there are scar areas, and some of them even include calcification. The cystic appearance may be due to necrosis and liquefaction (pseudocysts) or because it is formed by genuine neoplastic cysts.

There are cases with a wide cystic transformation, as well as cases with complete cystic appearance that lack a solid tumoural component. In this case a subtype was called *multilocular cystic RCC* [4] because its appearance may be similar to that of a multilocular cyst (cystic nephroma). The excellent prognosis suggests the possibility of considering it a carcinoma with low malignant potential [5].

Table 2 – Hereditary renal cell tumours

Syndrome	Chromosome	Gene	Protein	Tumour type	Extrarenal manifestations	
					Dermis	Other organs
von Hippel-Lindau	3p25	VHL	pVHL	Multiple, bilateral clear cell RCC, renal cysts	–	Hemangioblastoma of retina/CNS; phaeochromocytoma; pancreatic/renal cysts; neuroendocrine tumours; epididymal/ parametrial cysts; tumours of the inner ear
Hereditary papillary RCC	7p31	c-MET	HGF-R	Multiple, bilateral papillary RCC (type 1)	–	–
HLRC	1q42	FH	FH	Papillary RCC (non type 1)	Leiomyoma	Uterine leiomyoma/ leiomyosarcoma
Familial papillary thyroid carcinoma	1q21	?	?	Papillary RCC, oncocytomas	–	Papillary thyroid carcinoma
Hyperparathyroidism: jaw tumour (HP-JT)	1q25	HRPT2	–	Epithelial stromal mixed tumours, papillary RCC	–	Tumours of the parathyroidea; fibro-osseous jaw tumours
Birt-Hogg-Dubé	17p11	BHD	Folliculin	Multiple chromophobe RCC, oncocytic adenoma, papillary RCC	Facial fibrofolliculoma	Pulmonal cysts; spontaneous pneumothorax
Tuberous sclerosis	9q34 16p13	TSC 1 TSC 2	Hamartin tuberin	Multiple, bilateral angiomyolipomas, lymphangioleiomyomatosis; rare clear cell RCC	Angio fibroma; peau chagrin; subungual fibroma	Cardiac rhabdomyoma; adenomatous small intestine polyps; pulmonal/renal cysts; cortical tuber; subependymal giant cell astrocytomas
Constitutional transloca-tion chromosome 3	3p13-14	?	?	Multiple, bilateral clear cell RCC	–	–

RCC = renal cell carcinoma; VHL = von Hippel-Lindau; CNS = central nervous system.
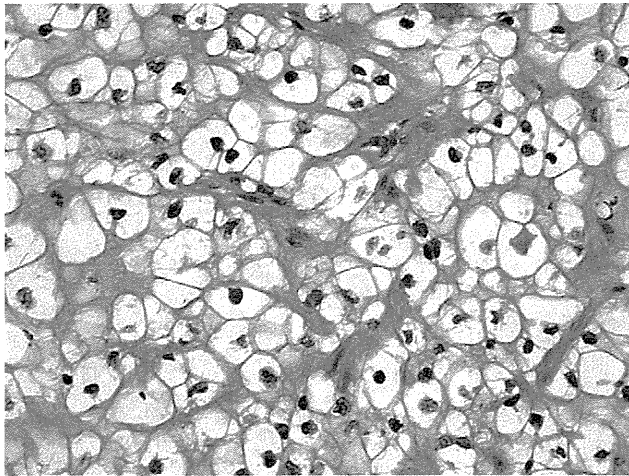
Fig. 1 – Clear cell renal cell carcinoma.

3.1.2. Microscopy

This neoplasia consists of clear cytoplasm cells; they are clear due to their high content of glycogen and lipids (Fig. 1). Cells with a higher mitochondrial content may be seen to acquire an eosinophilic or granular appearance. The predominance of this cell type is exceptional. The nuclei are rounded, and their characteristics depend on their degree of differentiation. The most frequent arrangement forms a solid pattern. Tubular and occasionally microcystic patterns can also be present. Papillary areas are very rarely observed [6]. A total of 5% of cases are of the sarcomatoid type [7].

3.1.3. Immunophenotype

The cells express low molecular weight cytokeratins (CKs; CAM 5.2 60%) more frequently [8], with vimentin in 82.6% [9] and CD10 in 94% [10] (Fig. 2). Epithelial membrane antigen (EMA) (MUC-1) appears in 85% [11] and glutathione S-transferase α in 82% [12]. c-kit and α-methylacyl-CoA racemase (AMACR) are negative [13].
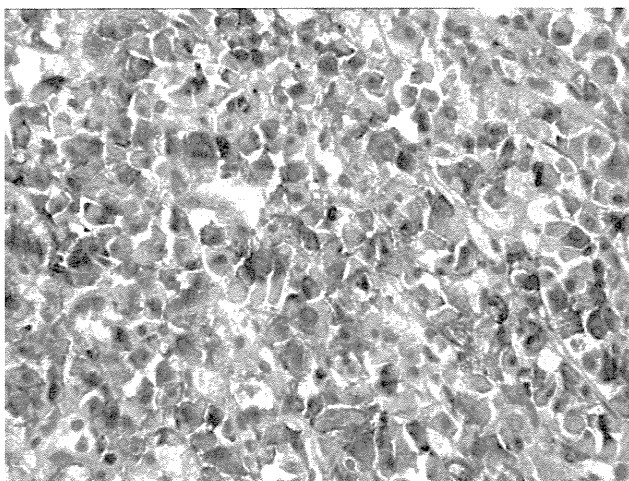
3.1.4. Genetic changes

3p deletion (LOH 3p) is the most typical genetic abnormality of this carcinoma, present in 75.8% of cases [14], which coincides with von Hippel-Lindau disease in 34–56% of sporadic carcinomas [15].

3.2. Papillary renal cell carcinoma

3.2.1. Macroscopy

Tumours with a diameter up to 5 ml are considered adenomas [2]. They are often incidental findings and occur in up to 23% of autopsy patients. The larger tumours are viewed as carcinomas, comprising 15% of all of surgically removed renal cell neoplasms. Its male-to-female ratio is 2:1 [2].

Papillary RCCs are grossly characterised by a spherical boundary and are beige to white. They can exhibit central necrosis and frequent haemorrhages. In some cases this feature can be so extensive to mimic a cyst both radiologically and grossly [16].

3.2.2. Microscopy

The epithelial neoplastic cells line a delicate fibrovascular core in which aggregates of foamy macrophages can be found. Sarcomatoid dedifferentiation is seen in approximately 5% of cases [16].

Two morphologic types of papillary RCC have been described [17]. Type 1 tumours have papillae covered by small cells with scanty cytoplasm, arranged in a single layer on the papillary basement membrane with low nuclear grade (Fig. 3). Type 2 tumours are composed of cells with higher nuclear grade, eosinophilic cytoplasm, and pseudostratified nuclei on papillary cores (Fig. 4). Type 1 tumours are more frequently multifocal. Papillary RCC entirely composed of oncocytes has been described (Fig. 5) [18]. This subset of papillary tumours shows clinicopathologic features different from type 1 and type 2 papillary RCCs and has been proposed as a third group, with the outcome intermediate between type 1 and type 2. Solid areas with morphologic features overlapping typical renal oncocytoma are often observed [19].

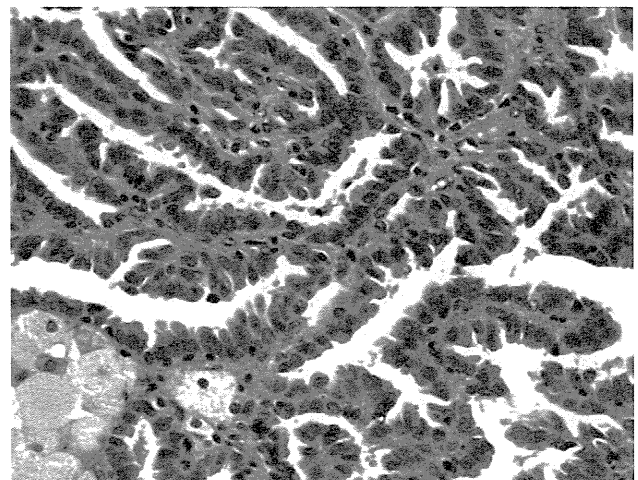Fig. 2 – Clear cell renal cell carcinoma with CD10 cytoplasmic expression.

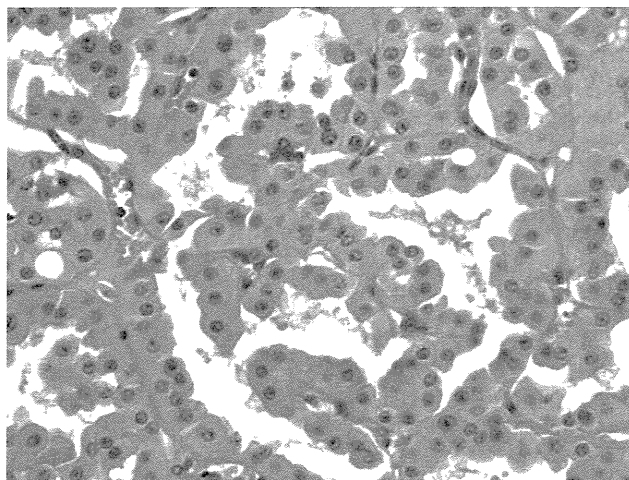Fig. 3 – Papillary renal cell carcinoma with small basophilic cells of low nuclear grade (type 1).

Fig. 4 – Papillary renal cell carcinoma with eosinophilic cells of high nuclear grade (type 2).
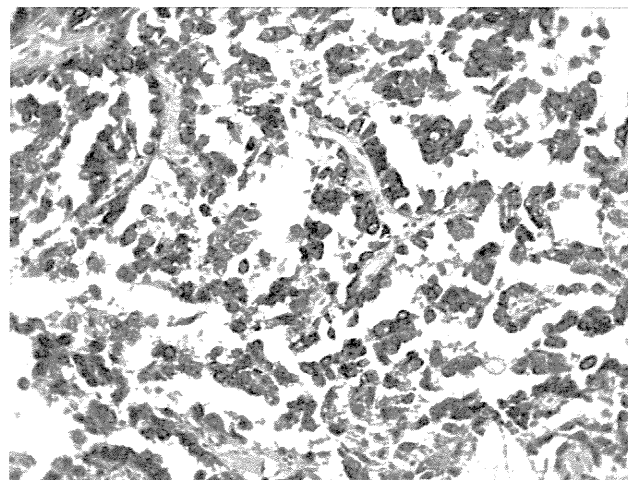

Fig. 6 – Papillary renal cell carcinoma with α-methylacyl-CoA racemase expression.

Clear cell changes can be present in papillary RCC in 20–90% of the total neoplastic area. Spindle cell areas in papillary RCC generally signify sarcomatoid change and are high grade. It has been reported that low-grade spindle cell foci, closely mimicking mucinous tubular spindle cell carcinoma, can occur [20].

3.2.3. Immunophenotype

The reaction for CK7 was strong or moderate in 78% of type 1 tumours, and reaction was null in 80% of type 2 tumours [17]. The oncocytic papillary RCCs with clear cell changes exhibited strong, diffuse, and granular positivity for AMACR (Fig. 6). Tumoural cells demonstrated variable positivity for CKs, AE1/AE3, CK8-18, CK7, CK19, and EMA. There was diffuse positivity for vimentin, and some cases were positive for parvalbumin [18].

3.2.4. Genetic changes

All papillary RCCs are characterised by trisomy of chromosomes 3q, 7, 8, 12, 16, 17, and 20 and loss of the Y
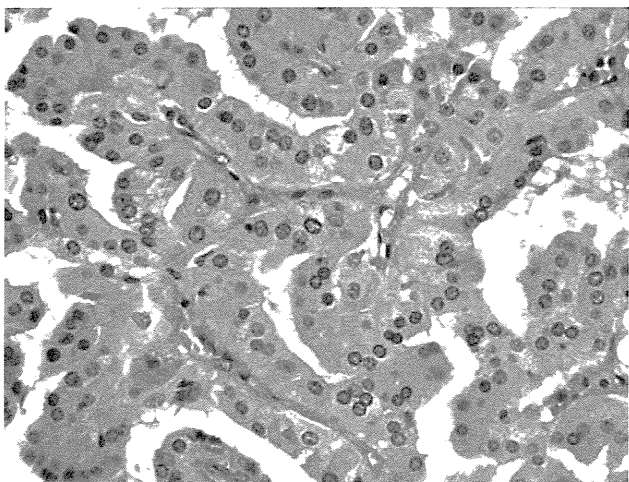

Fig. 5 – Papillary renal cell carcinoma with eosinophilic cells of low grade with oncocytic aspect.

chromosome [18,20]; these most consistent genetic abnormalities are present in both solitary and multifocal papillary RCCs, and they occur early in the evolution of this neoplasm. Some authors have suggested genetic differences between types; type 1 papillary RCC cases seem to have a significantly higher frequency of allelic imbalance on 17q than type 2 cases, and type 2 cases have a higher frequency of allelic imbalance on 9p than type 1 cases [21,22]. The c-Met proto-oncogene mutation on chromosome 7 characterises hereditary and a subset of sporadic papillary RCCs. Patients with hereditary leiomyomatosis and RCC syndrome are at risk for cutaneous and uterine leiomyomas and solitary papillary RCC type 2. The FH gene, which causes this autosomal dominant syndrome, encodes fumarate hydratase, a Krebs cycle enzyme.

3.3. Chromophobe renal cell carcinoma

3.3.1. Macroscopy

Chromophobe RCC accounts for approximately 5% of surgically removed renal epithelial tumours. It consists of one or more solid tumour nodules with a slightly lobulated surface. The cut surface appears homogeneously orange, turning beige or sandy after formalin fixation.

3.3.2. Microscopy

The basic chromophobe cell type is characterised by large polygonal cells with a transparent slightly reticulated cytoplasm for numerous sometimes invaginated vesicles, 150–300 nm in diameter resembling those of the intercalated cells type B of the cortical collecting duct, with prominent cell membrane leading to a plant cell–like appearance (Fig. 7). An eosinophilic variant does exist [23]. Sarcomatoid transformation does occur [24]. A diffuse cytoplasmic staining reaction with Hale's iron colloid stain is characteristic. Chromophobe cells usually show condensed and hyperchromatic and sometimes binucleated nuclei. In general, the growth pattern is solid/compact, sometimes cribriform, associated with focal calcifications
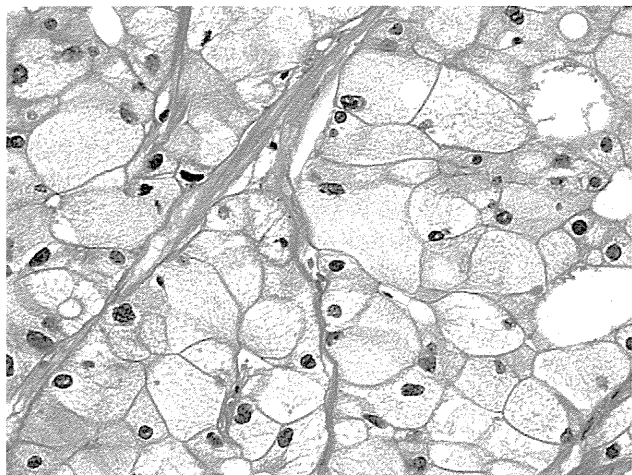
Fig. 7 – Chromophobe renal cell carcinoma.

and broad fibrotic septae. The so-called hybrid tumours share histopathologic characteristics of chromophobe carcinoma and oncocytoma because both cell types are intermingled.

3.3.3. Immunophenotype
Pan-CK, EMA, and parvalbumin are positive; vimentin and CD10 are negative.

3.3.4. Genetic changes and differential diagnosis with oncocytoma
This tumour is cytogenetically characterised by a massive loss of chromosomes 1, Y, 2, 10, 6, 21, 13, and 17 [25]. At the molecular level, the association between loss of chromosome 17 and mutation of the tumour protein p53 (TP53) tumour suppressor gene is referred in 27% of cases. The Birt-Hogg-Dubé syndrome is an autosomal dominant disease characterised by trichofolliculomas, trichodiscomas, and lung cysts. The BHD gene is localised on the short arm of chromosome 17.

Chromophobe RCCs, especially the eosinophilic variant, are frequently difficult to distinguish from renal oncocytomas on histologic sections stained with haematoxylin and eosin. Hale's colloidal iron stain shows a diffuse and strong reticular pattern in almost 100% of chromophobe RCC. Genetic alterations of oncocytoma have not yet been well characterised. However, renal oncocytomas have been reported to bear either rearrangements or translocations involving chromosome 11q13 [26] or partial or complete losses of chromosomes 1, 14, and/or a sex chromosome (Y or X). Several groups of investigators have reported that chromosome 3p loss is not detectable in oncocytoma. Because of the frequent association between oncocytomas and chromosome 1p alterations, the loss of a tumour suppressor gene residing on chromosome 1p has been proposed as the earliest genetic event associated with the development of renal oncocytoma. Oncocytomas have also been shown to exhibit microsatellite instabilities. Alterations in mitochondrial DNA have also been implicated in the development of oncocytomas. It is noteworthy that there are no apparent overlapping genetic alterations shared by

eosinophilic chromophobe RCC and oncocytoma, despite their morphologic similarities. It has been postulated that eosinophilic chromophobe RCC originates from renal oncocytoma and represents the malignant form of this tumour.

Renal oncocytosis is characterised by the presence of multiple tumours with oncocytic features, often associated with small clusters of tubule-like structures with oncocytic change.

3.4. Collecting duct carcinoma, renal medullary carcinoma

3.4.1. Macroscopy
Renal collecting duct (or Bellini's duct) RCCs represent from 0.4% to 1.8% [27] of all RCCs in Western countries. Several studies have shown a male predominance and a tendency for this disease to occur more frequently in relatively younger adults.

Renal medullary carcinoma (RMC) was first seen and is still detected almost exclusively in individuals with sickle cell trait or anaemia. This tumour shares many histologic features with collecting duct carcinoma (CDC), and some consider it a subtype of CDC or at least a closely related tumour, although the relationship between these two entities still remains controversial [28].

CDCs are derived from the medulla, but many are infiltrative; extension into the cortex is common. Typically CDCs are white to grey and have a firm consistency on sectioning.

3.4.2. Microscopy
CDC is characterised by a tubulopapillary architecture that consists of an admixture of dilated tubules and papillary structures typically lined by a single layer of cuboidal cells, often creating a cobblestone appearance and associated with a desmoplastic stromal reaction (Fig. 8). However, it has been reported that the most common histologic appearance of RMC is cribriforming glands surrounding by a desmoplastic reaction. Both CDC and RMC are considered to be somewhat similar to poorly differentiated urothelial carcinoma.
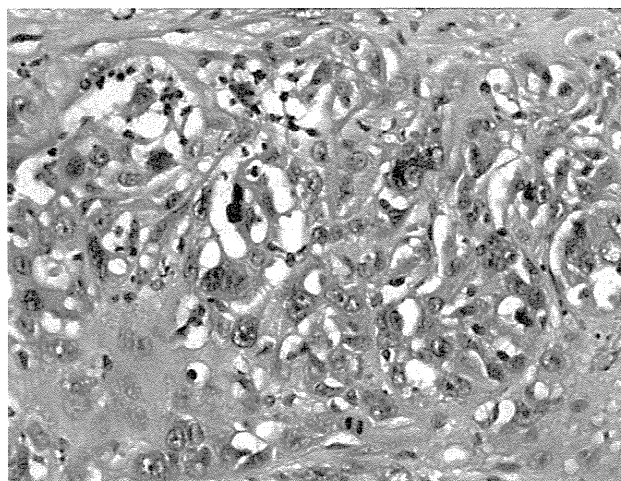

Fig. 8 – Collecting duct (Bellini) renal cell carcinoma.

3.4.3. Immunophenotype

The characteristic feature is coexpression of low and high molecular weight CKs and a positive reaction to *Ulex europaeus* [27]. There is a variable expression of Leu M1 and EMA, whereas markers of proximal renal tubules are almost always negative. Some studies reported that RMC strongly expresses keratin 19 and topoisomerase 2-α [29].

3.4.4. Genetic changes

The most constant change would be 1q32 deletion [30]. The molecular pathogenesis of CDC is not known. Although loss of chromosome 3p including the *VHL* gene was not common in CDC, some papers have described that activation of vascular endothelial growth factor (VEGF) signalling, which is analogous to the clear cell RCC hypoxia pathway, may be related to RMC carcinogenesis [28].

3.5. Mucinous tubular and spindle cell carcinoma

3.5.1. Macroscopy

This entity was included for the first time in the current WHO classification [2]. There is a female predominance, and the mean age is 53 yr at diagnosis [2]. It presents as a circumscribed asymptomatic mass on ultrasound examination.

3.5.2. Microscopy

Mucinous tubular and spindle cell is a low-grade carcinoma composed of tightly packed tubules separated by pale mucinous stroma and a spindle cell component [2] (Fig. 9) with mucinous material. It seems to derive from the distal nephron, but some authors believe it could be a variant of papillary RCC with proximal tubule origin [20,26]. Rare cases showing true well-documented sarcomatoid change were reported in 2009 [31].

3.5.3. Immunophenotype

There is an overlap with papillary RCC, and some authors believe it is a variant of papillary RCC with spindle cell differentiation [26]. Expression of AMACR (89%), CK7 (82%), vimentin (80%), EMA (78%), 34βE12 (45%), and E-cadherin
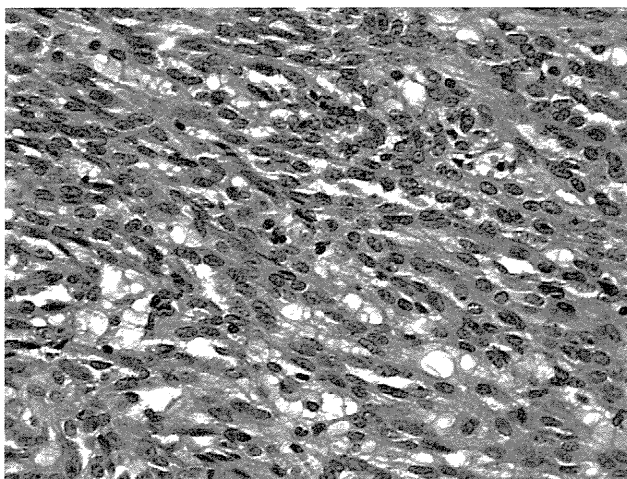

Fig. 9 – Mucinous tubular and spindle cell renal cell carcinoma.

(28%), and negativity for CK14, CD10, villin, RCC antigen, c-kit, and *U europaeus* agglutinin are characteristic [2,20].

3.5.4. Genetic changes

There is discordance in the studies. Some authors reported losses involving chromosomes 1, 4, 6, 8, 13, 14, and 15 and gains of chromosomes 2, 5, 7, 9, 10, 11, 12, 16, 17, 19, 20, 22, and X. Others found no lack of the gains of chromosomes 7, 17, and Y [32].

3.6. Renal carcinomas associated with Xp11.2 translocations/TFE3 gene fusions

These carcinomas are defined by several different translocations involving chromosome Xp11.2, all resulting in gene fusions involving the transcription factor binding to IGHM enhancer 3 (*TFE3*) gene. The first reported translocation was the t(X;1)(p11.2;q21), which results in fusion of the papillary renal cell carcinoma (*PRCC*) and *TFE3* genes. Another chromosome translocation is the t(X;17)(p11.2;q25), which results in fusion of the alveolar soft part sarcoma chromosome region, candidate 1 (*ASPSCR1*, also known as *ASPL*) and *TFE3* genes. Of note, the identical *ASPL-TFE3* gene fusion is also characteristic of alveolar soft part sarcoma (ASPS), where it was originally identified. Other reported translocations include t(X;1)(p11.2;p34), resulting in fusion of the *PSF* and *TFE3* genes, and an inv(X)(p11;q12), resulting in fusion of the non-POU domain containing, octamer-binding (*NONO* [$p54^{nrb}$]) and *TFE3* genes. These carcinomas predominantly affect children and young adults, although rare adult cases have been reported. The *ASPL-TFE3* carcinomas characteristically present at an advanced stage; almost all cases have been associated with lymph node metastases at diagnosis, even with small primaries [33–35]. Very little is known about the clinical behaviour of these carcinomas. Although the *ASPL-TFE3* renal carcinomas usually present at an advanced stage, their clinical course thus far appears to be indolent. Several *PRCC-TFE3* renal carcinomas have recurred late, up to 20–30 yr after the initial diagnosis.

3.6.1. Macroscopy

These carcinomas resemble clear cell RCCs on gross examination. They are most commonly tan or yellow and often necrotic and haemorrhagic.

3.6.2. Microscopy

The morphologic appearance of carcinomas associated with specific chromosome translocation breakpoints differs. The *ASPL-TFE3* renal carcinomas are characterised by papillary growth cover by cells with voluminous clear to eosinophilic cytoplasm, discrete cell borders, vesicular chromatin, and prominent nucleoli. Psammoma bodies are constant and sometimes extensive, often arising within characteristic hyaline nodules (Fig. 10). The *PRCC-TFE3* renal carcinomas generally feature less abundant cytoplasm, fewer psammoma bodies, fewer hyaline nodules, and a more nested, compact architecture. Too few *PSF-TFE3* and *NONO-TFE3* renal tumours have so far been studied to comment on their distinctive histopathologic features, if any.
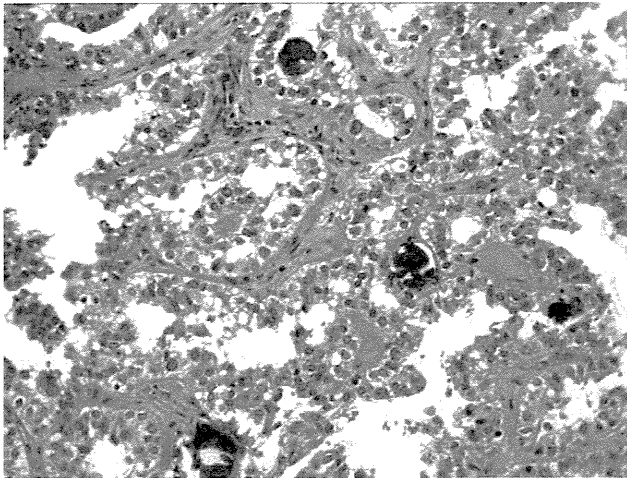
Fig. 10 – Translocation renal cell carcinoma.

These are the major differential diagnoses:

- Papillary RCC: Translocation RCCs sometimes often show well-developed papillary structures. However, they lack the cytogenetic abnormalities typical of classic papillary RCC: trisomy of 7 and 17, and loss of chromosome Y.
- Clear cell RCC: Some translocation RCCs exhibit the presence of clear cells forming nested architecture, and they are difficult to distinguish from classic clear cell RCC; however, translocation RCC lacks the chromosome 3p deletion typical of clear cell RCC.

3.6.3. Immunophenotype

Only about 50% of RCCs with Xp11.2-associated translocations express focally epithelial markers. Vimentin is also often focal, which contrasts with conventional RCCs. S-100 protein, desmin, and HMB45 are consistently negative. The tumours are consistently labelled for the RCC marker antigen and CD10, similar to conventional RCCs. The most distinctive immunohistochemical feature of these tumours is nuclear immunoreactivity for TFE3 protein, which is a common feature in all Xp11.2-associated carcinomas and ASPS.

3.7. Renal cell carcinoma unclassified

In surgical series, unclassified RCCs represent 4–7% of renal tumours [2], and at presentation most are of high grade and stage at diagnosis with poor survival [2,36,37]. Limited reported data suggest it is an aggressive form of RCC, mainly because most cases are at an advanced stage at presentation [2,36,37]. Compared with the clear cell variety, unclassified disease was associated with larger tumours ($p = 0.005$), increased risk of adrenal gland involvement (25% of cases; $p = 0.0001$), direct invasion to adjacent organs (42%, $p = 0.00001$), bone (52%; $p = 0.022$), and regional (52%; $p = 0.0042$) and nonregional lymph node (41%; $p = 0.03$) metastases [36]. Unclassified histology was a significant indicator for poor prognosis on multivariate

analysis ($p < 0.0001$). Median survival in patients with unclassified RCC was 4.3 mo [36].

According to the current WHO classification of kidney cancer [2], the features to define unclassified RCC include (1) composites of recognised types, (2) pure sarcomatoid morphology without recognisable epithelial elements, (3) mucin production, (4) rare mixtures of epithelial and stromal elements, and (5) unrecognisable cell types.

3.8. Non-2004 World Health Organisation renal cell carcinoma subtypes considered

3.8.1. Tubulocystic carcinoma
3.8.1.1. Macroscopy. These tumours are usually a solitary unencapsulated tumour with a white or grey spongy cut surface. They can vary in size, although most of them are pT1 tumours. Multifocal cases have been reported, and associations with papillary neoplasms have been provided [38,39].

3.8.1.2. Microscopy. Tumours are composed of packed tubules and cysts separated by bland fibrous stroma. The lining cells are cuboidal to columnar; hobnail cells are commonly seen. The cells have an abundant eosinophilic or amphophilic cytoplasm, and the nuclei are large and have prominent nucleoli (Fig. 11). Occasional cells with low-grade nuclear changes may be seen [38,39].

3.8.1.3. Immunophenotype. A wide range of marker positivity with CKs (CK8, CK18, and CK19) is consistently positive. CD10 and AMARC are positive in >90% of tumours. CK7 is variable expressed, although that pattern may be weak and focal. Staining for kidney-specific cadherin and Pax-2 may also be seen. The 34BE12 is nearly always negative [38,39].

3.8.1.4. Genetic changes. It has a molecular signature consisting of gains of chromosomes 7 and 17 [38,39].

3.8.2. Thyroid-like (follicular) renal carcinoma
3.8.2.1. Macroscopy. The seven tumours reported were tan and of various sizes. Follow-up data were available for all
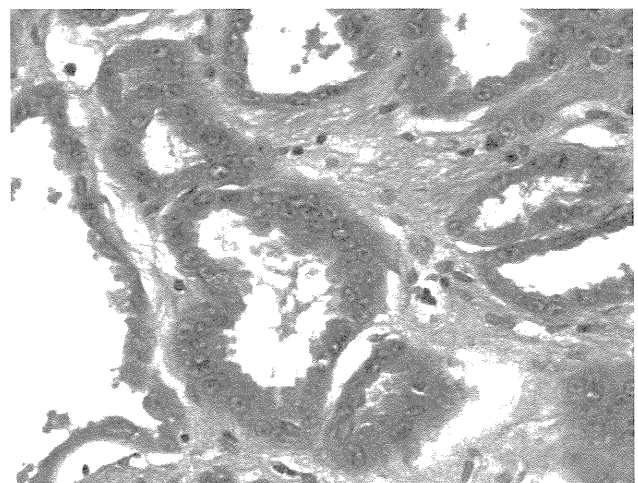

Fig. 11 – Tubulocystic renal cell carcinoma.

seven cases, and all patients remained tumour free after 6–84 mo [40].

3.8.2.2. Microscopy. The tumours are composed of cells showing low-grade pleomorphism with amphophilic to eosinophilic cytoplasm. The cells aggregate into micro- and macrofollicles. Colloid-like proteinaceous fluid may be revealed. Pseudo-inclusions and nuclear grooves may be present.

The main differential diagnosis for these tumours is metastases from either a primary thyroid follicular carcinoma or a thyroid carcinoma arising in a teratoma [40].

3.8.2.3. Immunophenotype. Variable expression of CK7 and CD10 have been reported. Six of the cases were negative for RCC, WT1, vimentin, Ksp-cadherin, Pax 2, AMACR, CD56, CD57, and TTF1 [40].

3.8.2.4. Genetic changes. One case showed gains of chromosome 8q24, 12, and 16, and loss of 1p36.3 and 9q21.33,103, whereas gene expression profiling showed widespread underexpression or overexpression, particularly involving chromosomes 1, 2, 3, 5, 6, 10, 11, 16, and 17 [41].

3.9. Acquired cystic disease–associated renal cell carcinoma and clear cell papillary renal cell carcinoma

3.9.1. Macroscopy
Studies indicate an increased prevalence of carcinoma in patients with end-stage renal disease. Most of the cases present more than one tumour in a single kidney. A wide spectrum of renal neoplasia was noted [42,43].

They are usually well circumscribed and large with dystrophic calcification. Clear cell papillary RCC frequently contains a prominent pseudocapsule and cystic feature.

3.9.2. Microscopy
A variety of architectural patterns with solid, acinar, cystic, and papillary patterns are present. Irregular lumina may give the tumour a cribriform appearance. In most of the reported cases, the tumour appeared to arise in a cyst. The tumour cells contain eosinophilic cytoplasm with a rounded nucleus and large nucleolus. Oxalate crystals are present in most tumours and also calcium aggregates [44].

Clear cell papillary RCCs show a pronounced cystic component in 50% of cases; solid, tubular, and microcystic areas are also present. The tumour cells show a clear cytoplasm and a low-grade nuclear pleomorphism with nuclei situated toward the surface of the papillary tufts [42,43].

3.9.3. Immunophenotype
Acquired cystic disease RCCs are positive for vinculin and AMACR. A proportion of cases show variable focal staining for CK7 and parvalbumin, discordant positive immunoexpression for CK AE1/AE3 and CD10, and variable expression for vimentin CAM 5.2 [45].

Clear cell papillary RCCs show positive staining for CK7 and are negative for AMACR and parvalbumin.

3.9.4. Genetic changes
In a kidney showing acquired cystic disease, genetic analysis showed gains of chromosomes 7 and 17, and multiple gains of numerous chromosomes including chromosomes1, 2, 6, 10, 3, 7, 17, and Y.

Clear cell papillary RCC did not show either 3p or trisomies of chromosomes 7 and 17 [45].

3.10. Leiomyomatous renal cell carcinomas

3.10.1. Macroscopy
Grossly the tumours measured 1.8–14 cm (mean: 4.6 cm) and were variously described as tan, brown, yellow, or white with the frequent presence of a thick investing capsule. Of cases for which details are available, four were pT1a and one was pT1b at diagnosis.

3.10.2. Microscopy
The tumours are composed of nests, cords, and sheets of epithelial cells frequently forming solid areas, tubules, or papillary structures. There is slight nuclear pleomorphism with abundant clear cytoplasm. The stroma is composed of mature smooth muscle that is often more pronounced at the periphery and in some cases appears to extend into adjacent renal tissue or into perirenal fat.

The differential diagnosis for these tumours is clear cell RCC, angiomyolipoma, and sarcomatoid RCC [46,47].

3.10.3. Immunophenotype
The epithelial components of the tumour are positive for AE1/AE and CAM 5.2, CD-10, S-100 protein (focal), EMA, and vimentin. There was variable expression of 34bE12, whereas smooth muscle actin and HMB45 were negative. The stroma component was positive for smooth muscle actin, caldesmon, desmin, and vimentin, and negative for HMB45, CD117, CKs, EMA, ER, and PR.

3.10.4. Genetic changes
Genetic studies are contradictory. In three cases, fluorescent in situ hybridisation showed loss of VHL and FHIT, with loss of chromosome 3 in one case and 3p in another. In a separate study there was no evidence of 3p deletion in the three cases examined.

3.11. Molecular basis of renal cancer treatment

The *VHL* tumour suppressor gene is epigenetically silenced or mutated in most cases of sporadic clear cell RCC. The decreased pVHL expression leads to a stabilisation of the hypoxia induced factor (HIF)-α and consequently to the transcription of HIF-α target genes, many of which are involved in tumour-promoting processes such as proliferation, angiogenesis, and cell motility [48]. Targeting the transcription factor HIF directly is difficult, but a variety of agents have been identified that downregulate HIF-α levels indirectly, for example, inhibitors targeting the mammalian target of rapamycin [49,50]. Another approach is to target HIF-α regulated genes directly. HIF-α responsive genes of major importance in

tumour biology are VEGF, platelet-derived growth factor (PDGF), tumour necrosis factor (TGF)-α, epidermal growth factor receptor (EGFR), and carbonic anhydrase IX (CAIX). VEGF is overexpressed in RCC and has prognostic properties. PDGF correlates to higher Fuhrman grades and appears to be a prognostic marker in RCC. TGF-α is overexpressed in RCC and induced by hypoxia. TGF-β is overexpressed in various malignant tumours including RCC. EGFR (ErbB-1) is over-expressed in most RCCs [51]. CAIX is overexpressed in >90% of clear cell RCC but is usually not found in normal tubulus epithelium. Other target genes not associated with HIF-α are kinase tyrosine (KIT), cyclo-oxygenase (COX)-2, and matrix metalloproteinases (MMPs). KIT is a type 3 tyrosine kinase receptor that is overexpressed in chromophobe RCC and oncocytoma but rarely in clear cell RCC [52]. COX-2 is upregulated in many malignant tumours including RCC. COX-2 is possibly a predictive marker for therapy response to COX inhibitors in RCC. MMPs are associated with a poor prognosis in RCC. Eichelberg et al. recently published a summary of relevant biomarkers in RCC [53].

4.   Conclusions

The WHO's 2004 classification makes a clear distinction between certain tumour subtypes having a better progno-sis and others that do not. The differences regarding prognosis of the most usual forms is statistically signifi-cant. In spite of this, some cell subtypes do seem to be related to different carcinogenesis, and their response to future therapies may be different too. This is why we must consider widely the morphology and the genetics of the different RCC subtypes.

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Combined Immunotherapy with Low-dose IL-2 Plus IFN-α for Metastatic Renal Cell Carcinoma: Survival Benefit for Selected Patients with Lung Metastasis and Serum Sodium Level

Hideyuki Akaza[1,2,*], Taiji Tsukamoto[3], Tomoaki Fujioka[4], Yoshihiko Tomita[5], Tadaichi Kitamura[6], Seiichiro Ozono[7], Tsuneharu Miki[8], Seiji Naito[9], Hitoshi Zembutsu[10] and Yusuke Nakamura[10]

[1]Department of Urology and Andrology, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Ibaraki, [2]Department of Strategic Investigation on Comprehensive Cancer Network, Research Center for Advanced Science and Technology (RCAST), The University of Tokyo, Tokyo, [3]Department of Urologic Surgery and Andrology, Sapporo Medical University School of Medicine, Sapporo, [4]Department of Urology, Iwate Medical, University School of Medicine, Morioka, Iwate, [5]Department of Urology, Faculty of Medicine, Yamagata University, Yamagata, [6]Department of Urology, Faculty of Medicine, The University of Tokyo, Tokyo, [7]Department of Urology, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, [8]Department of Urology, Kyoto Prefectural University of Medicine, Kyoto, [9]Department of Urology, Graduate School of Medical Sciences, Kyushu University, Fukuoka and [10]The Laboratory of Molecular Medicine and Laboratory of Genome Technology of Human Genome Center, Institute of Medical Science, The University of Tokyo, Japan

*For reprints and all correspondence: Hideyuki Akaza, Department of Strategic Investigation on Comprehensive Cancer Network, Research Center for Advanced Science and Technology (RCAST), The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8904, Japan. E-mail: akazah@med.rcast.u-tokyo.ac.jp

Objective: To clarify the survival benefit of immunotherapy for renal cell carcinoma patients with lung metastasis using low-dose interleukin-2 plus interferon-α, we examined survival outcomes and factors associated with prognosis.

Methods: This was a multicenter prospective study. Nephrectomized renal cell carcinoma patients with lung metastasis were treated with interleukin-2 (0.7×10^6 unit, 5 days a week) and interferon-α (6×10^6 IU, 3 days a week) for the first 8 weeks, and then with both interleukin-2 and interferon-α, 2 or 3 days a week for 16 additional weeks.

Results: Median follow-up period for 42 patients was 28.3 months (range: 4.2–43.8). Two-year overall survival rate was 82% and the probability of 3 year survival rate was 71%. Median progression-free survival was 10.4 months. While no difference was found in survival among patients assessed as complete response, partial response and no change, survival of patients assessed as NC or better was significantly better than those assessed as progressive disease ($P < 0.0001$). Furthermore, multivariate analyses identified pre-treatment serum sodium ($P = 0.004$) as an independent prognostic factor. The sodium level was also statistically associated with tumor response ($p = 0.035$). Patients with normal sodium level survived significantly longer ($P = 0.0005$) than those with low sodium level showing median survival of 12.2 months.

Conclusions: Combination immunotherapy with low-dose interleukin-2 plus interferon-α showed survival benefit for patients with lung metastasis whose tumor responded as no change or better. This combination immunotherapy could be beneficial for patients selected by metastatic organ and their pre-treatment serum sodium level.

Key words: renal cell carcinoma – interleukin-2 – interferon-α – lung metastasis – sodium

INTRODUCTION

The prognosis for patients with advanced renal cell carcinoma (RCC) is poor. It is reported that the median survival for patients with advanced RCC is 10 months and 5-year survival rate is <15% (1). RCC is highly resistant to conventional cytotoxic chemotherapy, while RCC evokes an immune response, which occasionally results in spontaneous remission (2,3). Such observations provide the rationale for developing immunotherapeutic approaches to treatment and have led us to a clinical investigation of immunostimulatory cytokines, such as interleukin-2 (IL-2) and interferon-α (IFN-α). Positive response rates of 10–20% are reported with these cytokines and some patients achieve a complete and long-lasting remission (4–6).

Among the effective immunotherapy options, administering a high-dose bolus i.v. IL-2, IFN-α and low/intermediate dose of IL-2 plus IFN-α have shown some evidence of anti-tumor activity, but no impact on overall survival (7). A number of uncontrolled studies, however, have shown that low doses of IL-2 plus IFN-α are associated with less toxicity and capable of inducing partial and complete remission with a comparable effect on median survival (8–10). Naito et al. (11) have recently reported a large retrospective study of 1463 Japanese patients that cytokine-based therapy, including IL-2 and IFN-α, improved the prognosis of advanced RCC patients.

Many studies have suggested that the great benefits of the cytokines can be achieved when applied to appropriately selected patients (12,13). Improvements in patient selection will be necessary to ensure that patients who might attain durable remission with IL-2 will not miss this opportunity. The important issue is how these individuals can be selected more accurately. A prognostic model by the Memorial Sloan Kettering Cancer Center (MSKCC) (14) is the most extensively used guide for optimal treatment. In terms of histological characteristics, it has been reported that patients with RCC of clear cell histology respond well to cytokine therapy (15). Although many efforts have been undertaken to clarify clinical or molecular factors associated with response to cytokines, the potential remains largely untapped.

Recently, novel molecular-targeted agents have been developed for the treatment of metastatic RCC (16). These include tyrosine kinase inhibitors, such as sorafenib and sunitinib as well as mammalian target of rapamycin inhibitors. These agents have been designed to target tumor-related angiogenesis and signal transduction. Although we now have an increasing number of effective new agents for patients, extensive experience has shown that they rarely induce durable regressions of metastatic RCC (17,18).

Our previous pilot study has shown that combination treatment with low-dose IL-2 (0.7 × 10⁶unit/person) plus IFN-α is effective for metastatic RCC patients, especially those with metastasis limited to lung (19). In addition, the combination therapy was tolerated well and no additional adverse event was observed in comparison with the monotherapy using either low-dose IL-2 or IFN-α. Thus, in order to confirm the efficacy of the treatment and to explore genetic markers that may be useful in patient selection, we have tried a new prospective and multicenter trial of the combination therapy on patients who had radical nephrectomy, lung metastasis and no previous systemic therapy. The efficacy for tumor responses has already been described in our recent report (20); briefly, the efficacy for patients with metastasis limited to lung has been reproduced with similar response rate of 35.5% and the disease control rate of 80.6%. A separate paper reports that expression levels of HLA-DQA1 and HLA-DQB1 are candidate markers for predicting the tumor response to this combination therapy using oligoDNA microarray analysis after enrichment of the cancer cells with laser microbeam microdissection technology (21).

In this paper, we report survival outcomes of this study and examined factors associated with the prognosis of patients receiving the combination therapy with low-dose IL-2 plus IFN-α. We show that the combination therapy produced superior survival outcomes with a 2-year overall survival rate of 82%. Furthermore, better survival was shown to be significantly associated with tumor responses including NC (no change) and with normal baseline serum sodium level, indicating that the combination immunotherapy will be beneficial to patients selected by their pre-treatment serum sodium in addition to their metastatic organ limited mainly to lung.

PATIENTS AND METHODS

PATIENTS AND TREATMENT

Study design and patient inclusion criteria have been previously described (20). Briefly, this was a prospective, multicenter and open-label trial for Japanese patients with metastatic RCC, who had received radical nephrectomy, measurable lung metastasis, the possibility of providing blood and specimens from primary tumors to determine genetic markers, and who had received no previous systemic treatment. Patients were enrolled from September 2006 to April 2008. The study was approved by the institutional review board at each center.

Administration of IL-2 (Imunace, Shionogi, Osaka, Japan) and IFN-α (Sumiferon, Dainippon Sumitomo, Osaka, Japan) was commenced simultaneously and continued for 8 weeks at following doses: IL-2 administrated by intravenous infusion at 0.7 × 10⁶unit/person per day, 5 days a week and IFN-α subcutaneously or intramuscularly at dose 6 × 10⁶ IU, 3 days a week. From week 9 to week 24, IL-2 and IFN-α were administered 2 or 3 days a week to patients showing evidence of objective response or NC. When this 24-week treatment was completed, progressive disease was detected, or this regimen could not be continued because of severe side effects, subsequent therapy was determined on each case by each center. The patients who were assessed as PD could continue to receive treatment with IL-2 and/or IFN-α

(continuous cytokine therapy) when centers determined it to be beneficial to them, because continuation of cytokine treatment despite progression of disease was reported to add a survival benefit to patients (11) and alternative agents (molecular target drugs) other than cytokines had not been approved in Japan by April 2008. Before their official approval, however, target drugs became available for clinical trials during the present study and were given to some patients who experienced relapse.

OUTCOME VARIABLES

The efficacy of tumor response has reported in our recent paper (20). Tumor response was assessed by up to 24 weeks plus an additional 4-week follow-up after commencement of the treatment according to the criteria of the Japanese Urological Association (JUA) (22) which is similar to the WHO criteria (23). We used JUA criteria instead of RECIST in order to compare the efficacy with our previous pilot study (19). Response evaluation was reviewed by external independent radiologists following investigators' assessment and further confirmed by central assessment. Progression-free survival (PFS) was defined as the time from the date of registration to disease progression or death, whichever occurred first. Overall survival was defined as the time from registration until death from any cause. Baseline serum sodium was determined in each center and low sodium level was determined based on the criterion of each center.

STATISTICAL ANALYSIS

For time-to-event endpoints, medians and 95% confidence intervals (CI) were estimated using the Kaplan–Meier method and the differences were assessed using log rank test. Uni- and multivariate survival analyses were based on the Cox proportional hazards regression model. Univariate parameters with $P < 0.05$ were used in the multivariate analyses using the backward selection.

RESULTS

PATIENT CHARACTERISTICS

From September 2006 to April 2008, a total of 44 Japanese patients were enrolled in this study and treated with low-dose IL-2 plus IFN-α therapy as a first-line setting. One patient was excluded due to violation of inclusion criteria and one discontinued treatment in the first week by withdrawal of consent. The baseline characteristics of 42 patients, which have been previously described in part (20), are shown in Table 1. All patients had undergone radical nephrectomy and had lung metastasis. Thirty-one patients (73.8%) had metastasis limited to lung. Others (11 patients) had multiple organ metastases, including lymph node, bone, liver, pancreas, adrenal gland and/or cardiac membranes in addition to lung. The number of measurable metastatic

Table 1. Patient characteristics

	n	%
Gender		
Male	32	76.2
Female	10	23.8
Age		
Less than 65	28	66.7
65 or greater	14	33.3
ECOG PS		
0	33	78.6
1	9	21.4
Nephrectomy		
Yes	42	100
No	0	0
Pathological T stage		
pT1	9	21.4
pT2	9	21.4
pT3	23	54.8
pT4	1	2.4
Histology		
Clear cell	38	90.5
Papillary	1	2.4
Mixed	3	7.1
Metastatic organ		
Lung	42	100
Lymph node	7	16.7
Bone	5	11.9
Others	7	16.7
Number of metastatic organ		
Single (lung only)	31	73.8
Multiple	11	26.2
Number of metastatic lesion		
1	3	7.1
2	9	21.4
3–5	16	38.1
6–10	12	28.6
17–26	2	4.8
MSKCC risk group		
Favorable	1	2.4
Intermediate	29	69
Poor	12	28.6

ECOG, Eastern Cooperative Oncology Group; Others, included liver, pancreas and cardiac membrane; MSKCC, Memorial Sloan Kettering Cancer Center.

lesions in each patient varied from 1 to 26 with a median number of 4. Among patients with only lung metastasis, the number of lesions varied from 1 to 16 with a median
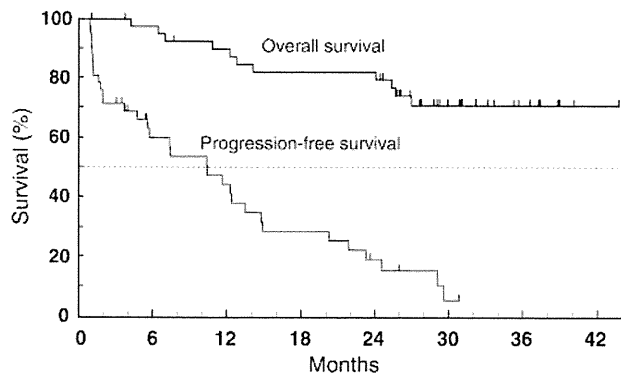
Figure 1. Kaplan–Meier estimates for progression-free survival (PFS) and overall survival (OS) for patients receiving first-line IL-2 plus IFN-α. Median PFS was 10.4 months. OS has not been reached to median during observation period (median: 28.3 months, range: 4.2–43.8).

Figure 2. Cause-specific survival and tumor response of patients treated with IL-2 plus IFN-α. The tumor response was assessed by up to 24 weeks plus additional 4-week follow-up after the first dose (20). There was no difference between survival of patients assessed as complete response (CR)/partial response (PR) or no change (NC), while for those assessed as PD it was significantly different ($P < 0.0001$). For the PD subpopulation, the median survival time was 13.2 months, while the survival for CR/PR or NC has not been reached to median during observation period (median: 28.3 months, range: 4.2–43.8).

number of 3. Thirty-eight (90.5%) of 42 patients had pure clear cell carcinoma, 1 papillary and others (3 patients) had mixed cell type with clear cell carcinoma. Based on MSKCC prognostic criteria (14), patients were categorized mostly in the intermediate (69.0%) and poor (28.6%) risk groups with only one patient categorized as favorable group (2.4%). To utilize the primary tumor specimens for marker analysis, the present study had mainly enrolled patients (92.9%: 39/42) who had metastasis at nephrectomy, which is one of the risk factors in the MSKCC criteria.

OVERALL SURVIVAL AND PFS

Median follow-up period for 42 patients was 28.3 months (range: 4.2–43.8). The overall survival had not reached the median by June 2010. In the first 12 months and the next 12 months after the registration, 3 and 4 deaths had occurred, during these respective periods. The 1- and 2-year overall survival rates were 89.9% (95% CI: 75.4–96.1) and 82.0% (66–91%), respectively. Figure 1 shows the overall survival curve estimated by the Kaplan–Meier method. The probability of 3-year survival rate was estimated to be 70.9% (54–83%). The patients ($n = 7$) who died in 2 years had either multiple organ metastases ($n = 4$) or poor risks ($n = 5$) by MSKCC criteria (14), although 7 of 12 poor risk patients have survived for over 2 years (data not shown).

The median PFS was 10.4 months (5.6–14.8) (Fig. 1). While one of the two patients assessed as complete response (CR) has relapsed after a follow-up period of 13 months but surviving over 32.2 months, another patient remained with no evidence of disease for over 25 months by continued therapy with IL-2 plus IFN-α. One patient with papillary type RCC (type not classified) in the lung, who had responded to the combination therapy (assessed as PR), was progression free for 10 months and survived for over 29 months.

Survival was compared between patient groups with only lung metastasis ($n = 31$) and with extrapulmonary organs ($n = 11$). The difference was not statistically significant, but patients with only lung metastasis tended to survive longer
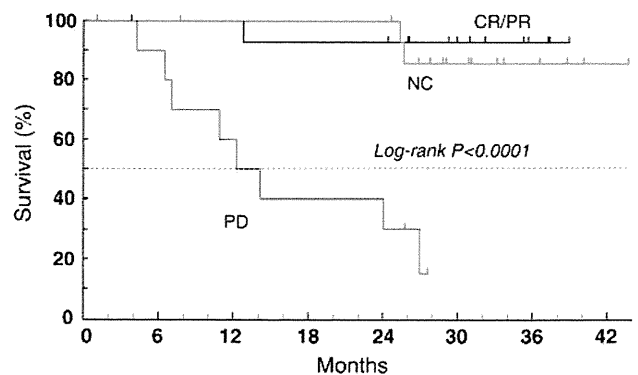
than those with extrapulmonary metastasis (log-rank $P = 0.0745$, data not shown). The 2-year survival rates of patients with only lung metastasis and with extrapulmonary metastases were 89.7% (71.3–96.5) and 61.4% (26.6–83.5), respectively.

RELATIONSHIP BETWEEN TUMOR RESPONSE AND CAUSE-SPECIFIC SURVIVAL

In our subgroup analysis, a strong correlation was found between diagnosis of tumor response (20) (the response assessed by 24 weeks after the first dose) and cause-specific survival (Fig. 2). In the patient group achieving CR or PR ($n = 15$), only one death occurred in 24 months with a 2-year survival rate of 92.9% (59.1–99.0) and no death occurred in patients assessed as NC ($n = 16$) in 24 months. Thus, the 2-year survival rate was 96.6% (77.9–99.5) for patients achieving objective response or NC. A patient diagnosed as PR who had died after 12 months had baseline characteristics, including multiple organ metastases (lung plus mediastinal lymph node), 16 lung metastatic lesions and poor risk factors (<1 year from initial visit to metastasis, >10 mg/dl high corrected calcium and low hemoglobin) by MSKCC prognostic criteria.

In contrast, 6 deaths had occurred in patients diagnosed as PD ($n = 11$) in 24 months with a 2-year survival rate of 40.0% (12.3–67.0). The median survival time was 13.2 months (7.0–27.0) for the PD subpopulation. All of the 6 patients have been assessed as PD by 8 weeks from the first dose with a median of 4 weeks.

PROGNOSTIC AND PREDICTIVE FACTORS

To identify clinical factors predicting prognosis in patients who received the combined IL-2 plus IFN-α therapy,

Table 2. Univariate and multivariate analyses of baseline parameters for overall survival of patients receiving IL-2 plus IFN-α

Risk factors	Categories	Univariate analyses			Multivariate analyses		
		Hazard ratio	95% CI	*P*	Hazard ratio	95% CI	*P*
Sodium	Low vs. N[a]	6.48	1.94–21.6	0.002	16.1	2.45–105	0.004**
Lymphocyte	Low vs. N[a]	7.91	2.04–30.8	0.003	14.7	2.25–96.6	0.005**
Corrected Ca	>10 mg/dl	5.51	1.56–19.4	0.008	13.2	1.83–94.2	0.010**
Albumin	Low vs. N[a]	4.72	1.02–21.8	0.047	1.94	0.28–13.4	0.500
CRP	>0.3 mg/dl	5.35	1.15–24.9	0.032	1.04	0.14–7.57	0.966

[a]N, normal.
**$P < 0.05$ on multivariate analysis.

Table 3. Correlation between pre-treatment serum sodium and tumor response to IL-2 plus IFN-α

	Sodium level, *n* (%)	
	Normal	Low
n	34	8
Tumor response		
CR/PR	15 (44.1)	0 (0)
NC	13 (38.2)	3 (37.5)
PD	6 (17.6)	5 (62.5)
Clinical benefit		
CR/PR/NC	28 (82.4)	3 (37.5)
p-value*		
CR/PR vs. NC/PD	0.035	
CR/PR/NC vs. PD	0.020	

The tumor response was assessed by up to 24 weeks plus additional 4-week follow-up after the first dose (20). Response evaluation was reviewed by external independent radiologists following investigators' assessment, and further confirmed by central assessment.
*p-value: Fisher's precision test.

univariate and multivariate analyses using the Cox proportional hazard regression model were performed on baseline parameters, including pathological, blood and urinary tests. Survival was significantly associated with corrected calcium, CRP, serum albumin, sodium and lymphocyte count on univariate analyses (Table 2). Multivariate analyses showed that baseline serum sodium ($P = 0.004$), lymphocyte count ($P = 0.005$) and corrected calcium ($P = 0.010$) were independent risk factors for shorter survival, although a small number of patients in the present study seemed to exclude some potential factors. Serum sodium level was also found to be associated with tumor response to this therapy (Table 3; responder (CR/PR) vs. non-responder: $P = 0.035$). Furthermore, more strong correlation ($P = 0.020$) was found between patients with clinical benefit (CR/PR/NC) and without benefit (PD). Using the Kaplan–Meier estimate and log-rank test, serum sodium
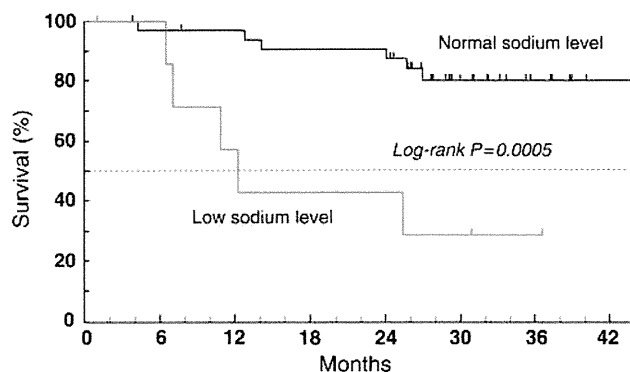

Figure 3. Survival and baseline serum sodium level of patients treated with IL-2 plus IFN-α. Survival was significantly different between patients with normal and low sodium levels ($P = 0.0005$). The median survival time of patients with low sodium level was 12.2 months, while the survival for patients with normal sodium level has not been reached to median during observation period (median: 28.3 months, range: 4.2–43.8).

levels were also shown to be statistically significant predictor of survival time ($P = 0.0005$, Fig. 3). The 2-year survival rates for patients with normal sodium and low level of sodium were 90.7% (73.9–96.9) and 42.9% (9.8–73.4), respectively. The median survival time of patients with low sodium level was 12.2 months.

In MSKCC risk factors (14), corrected calcium was shown to be the only factor associated with survival on multivariate analyses. Prognostic groups by MSKCC criteria were also found to have a correlation with survival. Because only one patient was categorized in a favorable group, survival for intermediate ($n = 29$) plus favorable group was compared with that of the poor group ($n = 12$), and the difference was statistically significant ($P = 0.036$, data not shown). The 2-year survival rates for the favorable/intermediate and poor groups were 92.9% (74.3–98.2) and 58.3% (27.0–80.1), respectively. The median survival of the poor group was 25.4 months.

DISCUSSION

Our previous pilot study has shown that combination therapy with low-dose IL-2 plus IFN-α is effective for metastatic

RCC patients, particularly those with metastasis limited to lung (19). The present trial has confirmed the efficacy of tumor response for patients with lung metastasis (20) and the present study further showed that this regimen provides a good survival benefit. The treatment was well tolerated and no additional adverse events occurred to those observed with monotherapy using either low-dose IL-2 or IFN-α (20). The overall survival did not reach the median in the median follow-up of 28.3 months (range: 4.2–43.8). The median PFS was 10.4 months with 1- and 2-year survival rate of 89.9 and 82.2%, and the probability of 3-year survival rate of 70.9%. While the data from the USA showed that the 1- and 3-year survival rates were 54 and 19%, respectively, in 463 metastatic RCC patients who received IFN-α (14), a large retrospective study on Japanese patients (11), 82% of whom had received cytokine therapy, including IFN-α and/or IL-2, showed 64.2 and 35.2% of 1- and 3-year survival rates, respectively. The 1- and 3-year survival rates of the present study are similar to or even better than those (86 and 46%, respectively) of favorable risk subpopulation in a randomized trial of IFN-α with/without IL-2 and fluorouracil (24).

It is noted that patients enrolled in this study were categorized mostly in intermediate (69.0%) and poor (28.6%) risk groups with only one patient categorized as favorable in the MSKCC prognostic model. To utilize the primary tumor specimens for gene marker analysis, the present study had mainly enrolled patients who had metastasis at nephrectomy, which is one of the risk factors in the MSKCC criteria. Despite the small proportion of favorable patients, on the whole, the survival outcomes were superior.

One reason for the better outcomes in the present study can be attributed to our patient selection by the criteria that included prior radical nephrectomy, ECOG performance status of 0–1 and limited metastasis mainly to lung. Upfront nephrectomy has been shown to enhance survival time for immunotherapy of metastatic RCC patients (25). In fact, nephrectomy improved the median survival period from 10.3 to 14.3 months in patients with only lung metastasis (26). In addition, racial differences may affect the survival of metastatic RCC patients as reported in one study (27).

The baseline serum sodium was found to have a significant positive correlation with tumor response and survival. Most recently, Jeppesen et al. (28) have reported that the level of baseline serum sodium is one of the prognostic and predictive factors in metastatic RCC patients who have been treated with IL-2-based therapy with/without IFN-α. In their work, low serum sodium has been shown to be a prognostic factor for short survival and a predictive factor for a lack of response to the immunotherapy. In the present study, the responders were found only in patients with normal sodium levels. The survival was significantly longer in patients with normal sodium than those with low sodium ($P = 0.0005$). Thus, our observations in the present study were consistent both with prognostic and predictive values of the serum sodium. These results imply that the tumor response and

survival can be further improved by patient selection with baseline serum sodium levels in addition to the pathological criteria, including limited metastasis to lung.

Furthermore, the present study showed that tumor responses were closely associated with survival. The survival of patients assessed as NC was not different from those as CR or PR, while survival for patients assessed as PD was significantly shorter than those assessed as the objective response or NC ($P < 0.0001$). Since similar observations have been shown in our previous pilot study of IL-2 plus IFN-α combination therapy (19), our two independent prospective trials demonstrated that patients showing objective responses or NC can anticipate a survival benefit from this combination therapy. This finding is in agreement with previous reports on IL-2-based immunotherapy (29,30). In the present study, patients who died within 2 years had been diagnosed as PD by 8 weeks from the first dose. Thus, it might be possible to consider that the patients who are assessed as not PD in the first 2 months could continue the combination therapy and could benefit from the treatment.

It is of interest to mention that IFN-α has recently been shown to play a role in the dynamic balance between activated regulatory and effector T cells (31,32). Pace et al. (31) have reported that IFN-α inhibits IL-2-induced regulatory T cell (Treg) proliferation and function through antigen-presenting cell activation. IL-2 plays important roles in tumor immunity by enhancing dendritic cell function, and T cell and NK cell effector activities, while IL-2 also delivers essential signals for the activation of Treg, which suppresses the functions of effector T cells in their homeostasis (33). Therefore, the combination of IL-2 with IFN-α may enhance antitumor activity through suppression of Treg with the aid of IFN-α as suggested by Tatsugami et al. (34).

Administration of targeted agents has become a routine practice for treatment of patients with metastatic RCC. However, none of the novel targeted agents seem to be curative. Furthermore, both randomized and expanded-access trials on sunitinib and sorafenib have shown that PFS and overall survival of both agents have been reported not to be significantly different between treatment-naïve and cytokine-refractory patients (17,18,35–38), indicating that the agents are as effective for patients who are refractory to cytokines. From above, it is thought to be possible to improve the survival benefit for metastatic RCC patients, if the combination therapy with IL-2 plus IFN-α is chosen as the first-line treatment, seeing it has better outcomes, even to the extent that complete remission can be expected. In the case of a patient who is refractory to this treatment, an alternative treatment with targeted agents can commence without delay and provide additional benefits.

A more accurate patient selection would ensure that the benefits they receive from the treatment are maximized. Our separate paper reports that expression levels of HLA-DQA1 and HLA-DQB1, the genes known to form heterodimers in antigen presentation process, are candidate markers for predicting the tumor response to the combination therapy with

IL-2 plus IFN-α (21). Exclusion of patients with tumors lacking either expression of these two genes is likely to improve the response rate to IL-2 plus IFN-α from 36 to 67%, indicating that a pretreatment genetic test would provide useful information in narrowing down the patients in order to improve the efficacy of this treatment and reduce unnecessary medical costs. Thus, by extending the patient selection criteria to metastatic organs, baseline sodium levels and a genetic test, the efficacy of the treatment can improve further.

Although the present study is a non-randomized prospective study, including a relatively small number of patients with a short follow-up period, the results showed that the combination therapy with low-dose IL-2 plus IFN-α provides survival benefits for selected patients who had limited metastases mainly to lung. Furthermore, the present study suggests that if patients are selected by their baseline serum sodium levels, combined immunotherapy would be a great benefit for them.

Conflict of interest statement

None declared

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