# The differential diagnosis of the chromophobe renal cell carcinoma and the renal ococytoma

**PhD Thesis**

**Ágnes Molnár, MD**

University of Pécs, Medical School, Depertment of Urology

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# 1. Introduction

The renal cancer is the 9th more frequent malignancy in male and 14th in female patients, with 1,5:1 male predominancy. The incidency is higher in the Western countries. In the era of the modern imaging techniques, a significant proportion of cancers are detected incidentally at an early stage. Symptomatic cases last for approx. 50% hematuria, 40% lumbar pain, 25% palpable mass and 30% suggestive of metastatic symptoms such as bone pain, night sweats, weakness, weight loss and hemoptoe suggest kidney cancer. Less than 10% of patients develop classical triad, palpable abdominal mass, macroscopic hematuria, and pain. The cases take approx. 30% are recognized as having paraneoplastic symptoms. In 1886 Grawitz first described the appearance of kidney tumors, deriving their origin from the lost adrenal islands, which are often seen under the kidney capsule. Based on this, it was named hypernephroma or Grawitz's tumor. In the '50s, electron microscopy found a similarity between tumor cells and the epithelium of the proximal tubule, thus uniformly labelled adenocarcinoma in American literature. The classifications published by AIFP (Armed Force Institute of Pathology) distinguished between clear cell, granular cell and mixed cell renal carcinoma and referred to their trabecular, tubular, papillary and solid forms during pathological report. In the '70s, Thoenes et al., based on electron microscopic and enzyme histochemical studies, distinguished between clear cellular, chromophilic and chromophobic renal tumors, and renal oncocytoma, which are referred to as the Mainz classification. This order is not clearly reproducible, as it is based on the phenotype of tumors, but Thoenes deserves to be the first to describe chromophobe kidney cancer as a new human kidney tumor entity. A significant proportion of tumors have a very heterogeneous appearance, which makes it difficult to accurately classify in routine histological examinations and may lead to misdiagnosis of mixed growth and cytological forms. In the late '80s, the chromosome studies started by Kovács et al in Heidelberg brought a breakthrough. Papillary renal tumor as a genetic and biological entity was isolated on the basis of the trisomy of chromosomes 7, 17 and 8, 12, 16 and 20 and the loss of chromosome Y. The most common kidney tumor, nowadays nomenclature termed

conventional kidney cancer, has reported deletion of chromosome 3 in more than 95% of tumors and confirmed that the von Hippel-Lindau gene mutation occurs only in conventional kidney cancer. In chromophobe tumors, cytogenetic studies as well as subsequent DNA studies revealed the characteristic chromosome loss as well as the monosomy of chromosomes 1, 2, 6, 10, 13, 17 and 21.It was also at this time that the characteristic chromosomal abnormalities of the kidney oncocytoma were first described. Based on this chromosome and subsequent DNA tests, a fundamentally new classification of renal tumors was born and was published as the "Heidelberg Classification of Renal Cell Tumors". In addition, the Heidelberg Group has developed a microsatellite assay for the differential diagnosis of renal tumors, which can be used to isolate the four major tumor types mentioned above in an inexpensive and reliable manner. In 1997, the US Mayo Clinic, led by UICC (Union Internationale Contre le Cancer) and AJCC (American Joint Committee on Cancer) Storkel, plagiarized the Heidelberg classification, adding a catalogue of 1-2% of all renal tumors and they have repeatedly returned to morphology-based classification. Subsequently, tumors were further subdivided according to their appearance without taking into account the genetic differences previously described. In many cases, the histological picture failed to distinguish between oncytoma and chromophobe kidney cancer, thus introducing the concept of "hybrid onocytic-chromophobe tumor" (HOCT).

Renal oncytoma accounts for 5-8% of renal tumors. Macroscopically well-defined terime in the renal cortex, most often solitary but rarely can be multifocal or bilateral. Oncytosis is most often associated with Birt-Hogg-Dubé syndrome. The histological picture is very diverse, cells typically have a highly eosinophilic cytoplasm, and they are so-called oncocytes. These cells have small, round, regular nuclei, and binuclear cells are common. In addition, solid, nested cell growth is characteristic, but rarely shows a tubular or cystic arrangement. Its immunohistochemical profile is also diverse, and no marker specific for oncocytoma has been found in the international literature. The molecular genetics of the sporadic form are

characterized by the monosomy of chromosomes 1 and 14, the balanced rearrangement of the 11q13 region, and the loss of the Y chromosome in men. In addition, in the case of Birt-Hogg-Dubé syndrome, an autosomal dominant mutation of the folliculin (FLCN) gene occurs.

The chromophobe renal cancer accounts for 5-7% of all renal tumors. Macroscopically well defined, and usually large. Microscopically they typically have sparingly stained reticular cytoplasm but they are most often mixed with cells with mild eosinophilic cytoplasm. Their nuclei have an irregular appearance, surrounded by a perinuclear halo. The broad cell groups are usually separated by a thin septum, but also show tubular and papillary growth. 2-8% of them can undergo sarcomatic transformation. Cytokeratin 7 (KRT7) has been shown to be a specific marker for chromophobic renal tumors in immunohistological studies. Its molecular pathology is characterized by the monosomy of chromosomes 1, 2, 6, 10, 13, 17 and 21.

In everyday practice, the diagnosis of chromophobe kidney cancer and oncocytoma is based on haematoxylin and eosin stained sections, which is clear in typical histological cases. However, diagnosis of atypical sections is difficult even for a trained pathologist. In such cases, the next step would be to perform an immunohistological examination, which, however, was difficult in the absence of a suitable marker. Methods, which have diagnostic value and based on the lesions of chromosomes and DNA are rarely used.

## 2. Objectives

- Histological analysis of 77 chromophobe kidney cancers and 42 kidney oncocytomas, which were clearly confirmed with genetic method with methods available in a general laboratory
- Investigation of the origin of chromophobe kidney cancer and renal oncocytoma: developing from principal cell, α- and β-intercalated cells
- identification of immunohistochemical markers for differential diagnosis, that clearly differentiates between oncocytoma and chromophobe kidney cancer.

# 3. Materials and methods

Previously such kidney tumors were investigated in Heidelberg, that was clearly diagnosed by DNA testing. Fresh tumor and normal kidney tissue samples used for RNA-based studies were collected from patients operated at the Urology Clinic of Ruprecht-Karls University in Heidelberg in 1995-1996.Fresh tissue from a homogenous tumour sample was freeze-dried in liquid nitrogen and stored at -80 ° C until further processing. The rest was fixed in 4% formaldehyde and then submitted for histological examination. The use of tissue samples was approved by the Ethics Committee of Heidelberg University. The study material was prepared and the data evaluated in the Molecular Oncology Laboratory under the leadership of Prof. Gyula Kovács at Ruprecht-Karls University in Heidelberg. Hybridization was performed by the Molecular Oncology Laboratory at the European Molecular Biology Laboratory (EMBL) in Heidelberg. The biomarkers examined in the dissertation were selected based on the evaluation of Affymetrix data. Immunohistochemistry was performed on chromophobe renal tumors and an oncocytomas, which was sent to the Heidelberg Laboratory of Molecular Oncology and were operated in the Departement of Urology of University of Pécs between January 2000 and December 2014. To assess the specificity of the markers, 220 conventional and 121 papillary renal tumors as well as healthy fetal and adult kidney tissues were included in the work. In all cases, the histological diagnosis was reviewed according to the Heidelberg Classification. Tissue specimens were collected and processed with the permission of the Ethics Committee of the University of Pécs (Ethics Approval Number: 5343/2014).

## 3.1. DNA extraction, array-CGH and data analysis

Array CGH assays were performed in collaboration with Dr. Maria Yusenko (Münster, Germany).

Tumor items were marked on haematoxylin and eosin stained sections, and after removal, the fragments were deparaffinized with xylene, followed by rehydration and drying. DNA was isolated using Dneasy Blood and Tissue Kit (# 69504, Qiagen), dissolved in water and assayed for quality using the NanoDrop®ND-100 spectrophotometer. Only DNAs with an A260 / A280 ratio above 1: 8 were used. The DNA was then labelled with the ULS-Cy5 and ULS-Cy3 Universal Linkage System and hybridized to the 4x44K HG-CGH array (Amadid 014950, Agilent Technologies Deutschland GmbH, Böblingen, Germany). Labelled tumor and normal DNA were combined and hybridized at 65 ° C for 40 hours in a rotary chamber. After washing and drying, the array was read immediately with the Agilent DNA Microarray Scanner. The data were further evaluated using Agilent CGH Analytics Software where the increase and decrease in DNA copy number is log2 ratio> 0.25 and log2 ratio <-0. 5 values.

## 3.2. Tissue microarray (TMA)

In the study, paraffin blocks containing fetal and adult healthy kidney tissue, chromophobe kidney cancer and renal oncocytoma, and papillary and conventional kidney cancer were used to make tissue microarray (TMA). During the review of the hematoxylin-eosin painted tumor blocks, we highlighted the sampling location. Subsequently, 0.6 mm diameter tissue rolls were removed from the paraffin-embedded tissue block according to the designated area using a Manual Tissue Arrayer (MTA1, Beecher Instruments, Inc., Sun Prairie, USA).The resulting tissue rolls were embedded in a common paraffin block with the aid of the MTA, allowing simultaneous examination of 150 different samples on a single section.

## 3.3. Immunohistochemistry

The investigations were performed in the Laboratory of the Department of Urology, University of Pécs. All antibodies were tested for optimal dilution, pH of digestion buffer, and

incubation with the primary antibody. Paraffin was removed from the TMA, fetal and adult kidney 4 μm thick sections with xylene and rehydrated in a series of descending ethanol. Subsequently, antigen digestion was achieved by boiling in either 10 mM sodium citrate buffer (pH 6.0) or Tris-EDTA (TE) buffer (pH 9.0) performed on a 2100-Retriever (Pick-CellLaboratories, Amsterdam, The Netherlands).Endogenous peroxidase activity and non-specific binding sites were blocked in 0.3% hydrogen peroxide containing 1% normal horse serum for 10 minutes at room temperature. The sections were then incubated overnight in a humid chamber at 4 ° C.

The following antibodies were used:

- rabbit polyclonal anti-*AQP2*antitbody (PA5-38004, ThermoFisher, Budapest, Hungary) in 1:500 dilution,

-  rabbit polyclonal anti-*FOXI1* antibody (PA5-30031, ThermoFisher) in 1:200 dilution,

- rabbit polyclonal anti-*SLC4A1* antibody (HPA015584, SigmaAldrich Budapest, Hungary) in 1:200 dilution,

- mouse polyclonal anti-*SLC26A4*antitbody (LotNo. 12065, Abnova, Taipei, Taiwan) in 1:100 dilution,

- rabbit polyclonal anti-CD82 antibody (HPA 028900, SigmaAldrich) in 1:100 dilution.

- rabbit polyclonal anti-AQP6 antibody (PA5 53296, Thermo Fisher) in 1:100 dilution

- mouse monoclonal anti-KRT7 antibody (ab9021, Abcam, Cambridge, UK) in 1:300 dilution

Rabbit and mouse specific antibody bound to Horse-radish-peroxydase (HRP) was used as a secondary antibody and the plates were incubated for 30 minutes. AEC or DAB was used as the chromogenic substrate, nuclear staining was performed with Mayer's Haematoxylin (Lillie'smodification), and the plates were either covered with Glycergel or Pertex. Evaluation was performed with a Leica Laborlux S microscope and photographs were taken with a ProgRes C14 camera mounted on a Leitz DMRBE microscope.

# 4. Results

## 4.1. Chromosome aberrations

The most frequent chromosome loss in 77 chromophobe kidney tumor we investigated were on chromosome 1 (95%), 2 (94%), 10 (90%) and 17 (90%). In addition, chromosome 6 (86%), 13 (82%) and 21 (66%) were common, specific lesions. Monosomy of 9, 3, 8 and chromosome were present in 12-36% of the chromophobe kidney cancers. The following differences were seen in the 42 kidney oncocytomas we examined. Monosomy of chromosome 1 occurred in 23% of cases, while chromosome 14 occurred in 9% of cases. In addition, some random chromosomal abnormalities were observed.

## 4.2. The distribution of characteristic chromosome aberrations in chromophobe kidney cancer

The number of monosomies varied between 3 and 12, resulting in a chromosome number of 34 to 43. In extreme cases, this means an almost haploid chromosome number. Most frequently chromosome 6 and 7 loss was observed. Tumors with eosinophilic cytoplasm have monosomy of chromosomes 3, 4, 6, 9 and 11, which is similar to those seen with classical chromophobic forms.

## 4.3. Histological diversity of chromophobic kidney cancer

Examining the histological sections of 77 chromophobe kidneys that were unequivocally genetically determined, we were able to distinguish four types with different cytogenetic appearance. These are large, reticular cytoplasmic cells, fine reticular cells with microvesicles, pale eosinophil-staining plasma cells, cells with perinuclear halo and double nucleus, and medium-sized eosinophil cytoplasm, which most often appear in mixed form. We found only

four tumors that consisted exclusively of cells with pure reticular cytoplasm and only seven tumors composed exclusively of eosinophils. Metastasis was observed in 6 cases, 3 of which were eosinophilic tumors. In one case, histological examination of liver metastasis confirmed the dominance of eosinophils. Another eosinophilic cell tumor has confirmed multiple bone metastases, previously reported as metastatic renal oncocytoma. The third tumor also showed sarcomatic transformation. In the literature, subtypes of growth were also examined in our 77 cases. In 5 tumors, microcystic and papillary forms were identified in each case in combination with solid growth. At seven tumors, Psammoma body with pigmented areas was detected. In one case, an unusual comedo-like lumen filled with dead cells was observed, the wall of which was clearly covered by cells that proved to be chromophobe kidney cancer.

## 4.4 Histological diversity of renal oncocytoma

Sections of 42 renal oncocytomas confirmed by genetic examination were also examined for histological diversity, with special regard to the eosinophilic variation of the chromophobe kidney tumor. In 7 cases, strong nuclear polymorphism was found with many binuclear cells. In four tumors, tubular-nested arrangement of eosinophils was confirmed by perinuclear thinning characteristic of chromophobe tumor.

## 4.5. Immunohistochemistry of the oncocytoma and chromophobe renal cell carcinoma

The renal tubule is made up of three cell types with specific functions, such as principal, α-intercalated and β-intercalated cells. Previous immunohistological studies have concluded that chromophobe kidney cancer originates from β-intercalated cells and renal oncocytoma originates from α-intercalated cells. Based on experimental in vitro studies and knockout mice, the differentiation of IC is controlled by the FOXI1 transcription factor. To answer this question, we examined the expression of three cell types immunohistological markers, namely

AQP2, SLC4A1, SLC26A4 and FOXI1 in TMA containing chromophobe kidney cancer and kidney oncocytoma. In addition, two genes strongly expressed in oncocytoma (AQP6) and chromophobic kidney cancer (CD82) were selected from the preliminary Affymetrix gene expression map. Finally, we examined the expression of KRT7, which is uncertain in the literature, in TMAs containing 83 kidney oncocytomas, 90 chromophobe kidneys and 220 conventional and 121 papillary kidney cancers.

### 4.5.1. Aquaporin 2 (AQP2)

The vast majority of the cells in the outlet duct are principal cells on which AQP2 is expressed. Accordingly, we found strong AQP2 expression on the surface of adult renal cortical and medullary channels. Neither oncocytoma nor chromophobe renal tumor showed a positive response, as well as conventional and papillary renal carcinomas.

### 4.5.2. SLC4A1

In nephrological studies, expression of SLC4A1 was associated with α-intercalated cells expressed on the basolateral surface of cells. According to the literature it can be found in the cells of the cortical ducts and in a smaller number in the cells of the medullary ducts. We found that SLC4A1 showed a positive response in the majority of the cells in the connective channels, but no tumor specific expression was found. 11% of the chromophobe kidney tumors and 60% of the oncocytomas responded positively. In addition, no conventional or papillary renal tumors showed positive staining.

### 4.5.3. SLC26A4

According to the literature, SLC26A4 is expressed on the surface of β-intercalated cells. They appear in the fetal kidney from the 14th week of pregnancy, and in the adult kidney in the cortical β-intercalated cells. No SLC26A4 expression was observed in any of the 83 kidney oncocytomas. The β-intercalated cell marker SLC26A4 was positive in only one chromophobe kidney cancer. SLC26A4 immunohistochemistry showed no positive response in conventional and papillary renal tumors.

### 4.5.4. FOXI1

Immunohistochemical examination of FOXI1 showed positive staining in the nuclei of cortical outlet and connective channels. Of the 83 oncocytomas tested, 80 were positive (96%). Of the 90 chromophobe kidney tumors, only 3 showed positive nuclear staining, while none of the 220 conventional and 121 papillary renal tumors stained.

### 4.5.5. Aquaporin 6 (AQP6)

AQP6 is expressed in the intercalated cells of the renal cortical and medullary outlet ducts. The proportion of positive cells in cortical tubules was higher than in distal tubules. 92% of kidney oncocytomas were positive, compared with only 6% of chromophobe kidney tumors. In the former case it was observed in 80-90% of the cells, while in the latter cases it applied only to a part of the cells and showed extremely weak staining. In case of both conventional and papillary tumors, no positive reaction was seen.

### 4.5.6. CD82

In the kidney, cells in the connective ducts, as well as in the cortical and medullary collecting ducts, show a positive reaction with CD82. In chromophobe kidney tumors, the cell membrane was strongly positively stained, with cytoplasmic staining followed by cellular cytological diversity, i.e., weak or stronger staining according to reticular or eosinophilic cytoplasm. 70 of the tumors we investigated showed positive immunoreaction No cells with positive staining were found in any kidney oncocytoma, as in conventional and papillary renal tumors.

### 4.5.7. Citokeratin 7 (KRT7)

Immunohistochemical analysis of KRT7 showed a diffuse positive reactor in 84 chromophobe neoplasms, which was independent of the cytological variation of the tumors and their growth form. Different intensities were observed within the same biopsy, but 91% of the tumor cells were positive. Diffuse staining was also found in the case of chromophobe renal tumor previously diagnosed as metastatic oncocytoma. In the "oncocytoma-like" part, the cytoplasm of tumor cells contained granular cytokeratin, whereas in chromophobr tumor-like areas, the cell membrane was highly positive. The basis for differential diagnosis is that at least 90% of the cells of chromophobe kidney tumors of different morphologies show a positive immune response to KRT7. By this staining, only the small cellular tubulopapillary portions of the oncocytoma are positive.

Among the antibodies tested, CD82, KRT7, FOXI1 and AQP6 were found to be useful diagnostic markers in everyday practice. Neither KRT7 nor CD82 produces positive results in renal onocytoma, so their specificity is extremely high, so in doubt cases the positivity of these markers is always in favour of the diagnosis of chromophobe kidney cancer. Both FOXI1 and AQP6 were found to be positive in more than 90% of the renal oncocymomas, but unfortunately these two markers also produced positive results in 3% and 6% of the chromophobe cancers.

# 5. Discussion

The chromosomal aberrations evaluated in this study clearly demonstrate that renal oncocytoma and chromophobe kidney cancer correspond to two independent tumor entities, regardless of cell morphology and growth diversity. Kidney oncocytoma may also have a different histological picture than textbook data and may be confused with the so-called eosinophilic form of chromophobe kidney cancer. Given the biological behaviour of the two tumors, differential diagnosis plays a crucial role in the clinical judgment of tumors. In such cases, genetic testing can be decisive. Most pathologists have returned to morphology-based classification without taking into account the genetic differences behind the "reclassified" histological types based on changing cytology. As a result, several kidney oncocytomas were included in the group of so-called eosinophil chromophobe tumors, which distorted the results of genetic tests. A work group, using the next generation sequencing (NGS) technique, compiled a tumor cohort of 66 chromophobe renal cancers, including 13 eosinophilic chromophobe cancers. Characteristic chromosome loss has been found in "classic" chromophobe kidney cancer, but in 7 of the 13 eosinophilic tumors, no genomic divergence characteristic of chromophobe kidney cancer occurred. This data indicates that the diagnostic pathologist made a misdiagnosis in 7 of the 66 tumors because these "eosinophilic" chromophobe kidney cancers were actually kidney oncocytomas. In a series of 33 chromophobe kidney cancers reported in another international collaboration, no specific chromosomal aberrations were found in one "classic" and three eosinophilic chromophobe cancers. Here too, there can be no doubt that the pathological diagnosis was incorrect. In addition, the two analysis groups concluded that eosinophilic chromophobe kidney cancer is characterized by extremely low chromosome loss and thus the classification of "classical" and "eosinophilic" chromophobe kidney cancer is warranted. In our study, we found no difference in the number of monosomies between "classic" and eosinophilic chromophobe tumors. Based on this, we do not consider it necessary to subclassify by cytological appearance. Of the 77 chromophobe kidney tumors we examined, 7 showed only eosinophilic cytology, and in each

case we found a lack of specific chromosomes, which is in significant contradiction with the two studies mentioned above. The conclusion of the two studies mentioned above is that the eosinophilic tumors that do not show the differences characteristic of chromophobe kidney cancer are extremely good prognostic, with 100% of patients remaining tumor-free during follow-up. Of the 7 eosinophilic chromophobe tumors we have genetically verified, 3 have metastasized at the time of surgery, which contradicts the supposed good prognosis of eosinophilic chromophobe kidney cancer. These data further support our suspicion that kidney oncocytoma diagnosed with chromophobe kidney cancer has been included in the study material. The 42 kidney oncocytomas, despite having several tumors with unusual appearance, were diagnosed by renal oncocytoma based on the histological picture. Taking into account our own data as well as literature data, chromosome 1, 2, 6, 10, 13, 17, and 21 are undoubtedly characteristic of chromophobe kidney cancer, regardless of cytological variations. The absence of these chromosome aberrations, the loss of chromosome 1 or 14, and the possible random chromosomal aberration are all clearly in favour of the diagnosis of renal oncocytoma. The cells of the outlet and collecting ducts contain cells with different functions with their characteristic markers: principal cells (AQP2), α-intercalated cells (SLC4A1), β-intercalated cells (SLC16A4). The expression of SLC4A1 and SLC26A4 and thus the differentiation of α- and β intercalated cells is regulated by the FOXI1 transcription factor. The α-intercalated cell marker SLC4A1 was positive in 60% of kidney oncocytomas, but also stained in 11% of chromophobe kidney cancers. The β-intercalated cell marker SLC26A4 gave a positive reaction in only one chromophobe kidney cancer, thus ruling out the possibility that the chromophobe kidney cancer would start from the β-intercalated cells. The transcription factor FOXI1 gave a positive response in 97% of the kidney oncocytomas and thus showed a relationship between the kidney oncocytoma and FOXI1. This is also surprising because FOXI1 controls the differentiation of α-intercalated and β-intercalated cells and their characteristic markers SLC4A1 and SLC26A4 were not associated with FOXI1 positivity. According to our studies, KRT7 is a specific marker for chromophobe kidney cancer. In our experience, chromophobe tumors show diffuse positivity, whereas only a few tubular formulas

show positive staining in kidney oncocytoma. CD82 showed a positive response in 78% of the chromophobe cancers, but no oncocytoma was positive. This identified another immunohistological marker that may be useful in routine work. AQP6 was positive in 92% of renal oncocytomas, but unfortunately was also expressed in 6% of chromophobe kidney cancers. In that case only slight staining was observed and not in all cells but in the intensity of positivity overlapping that observed in renal oncocytomas. Thus, it cannot be used to clearly distinguish between the two tumors.

After reviewing the literature and based on my own research, I have concluded that KRT7 and CD82 can be used reliably to characterize chromophobe kidney cancer, provided that positive cases can be clearly identified as chromophobe kidney cancer. FOXI1 and AQP6 were present in 97% and 92% of oncocytomas, due to the overlapping positivity, especially AQP6, they are of informative diagnostic value only.

| Antibody | Oncocytoma (83) | Chromophobe (90) |
|---|---|---|
| CD82 | 0 % | 78 % |
| KRT7 | 0% | 93 % |
| FOXI1 | 97% | 3% |
| AQP6 | 92 % | 6 % |

# 6. Conclusion

- Chromophobe kidney cancer and kidney oncocytoma are biological and genetic entities whose clear distinction is of clinical importance.

- There is no biological or genetic basis for distinguishing between "classical" and "eosinophilic" chromophobe kidney cancer. This variant of cytology, just like the form of growth, can be described in the histological findings, but should not be included in the diagnosis.

- There is no HOCT, which is equivalent to either renal oncocytoma or chromophobe kidney cancer.

- The origin of the renal oncocytoma and the chromophobe kidney cancer could not be found, and in both tumors a positive reaction is observed for α and β intercalated cells.

- FOXI1 gene expression is a reliable marker for the diagnosis of kidney oncocytoma

- The CD82 and KRT7 genes are a marker for chromophobe kidney cancer

- In conclusion, the histological diagnosis of most renal oncocytomas and chromophobe kidney cancers is not a problem for an experienced uropathologist. In cases where the diagnosis is unclear, the immunohistological panel we have developed, or where appropriate methods are available at pathological institutes, we recommend genetic testing.

# Acknowledgments