Overexpression of KIT (CD117) in Chromophobe Renal Cell Carcinoma and Renal Oncocytoma

Chin-Chen Pan, MD, Paul Chih-Hsueh Chen, MD, PhD, and Hung Chiang, MD

Key Words: KIT; CD117; Immunohistochemistry; Mutation; Renal cell carcinoma; Chromophobe renal cell carcinoma; Oncocytoma; Angiomyolipoma

DOI: 10.1309/A7M2XTMJQK0KPQER

Abstract

KIT expression has not been studied substantially in renal tumors. We analyzed the immunohistochemical expression for KIT in 256 conventional renal cell carcinomas (RCCs), 29 chromophobe RCCs, 25 papillary RCCs, 6 collecting duct RCCs, 6 unclassified RCCs, 7 renal oncocytomas, 20 urothelial carcinomas, 7 nephroblastomas, and 23 angiomyolipomas. We found that 24 chromophobe RCCs (83%) and 5 renal oncocytomas (71%) revealed membranous immunoreactivity for KIT, while none of the RCCs of other types expressed KIT immunohistochemically. Sporadic cases of urothelial carcinoma and nephroblastoma were focally positive for KIT. All angiomyolipomas were negative. Genomic DNA extracted from the chromophobe RCCs and renal oncocytomas was submitted for polymerase chain reaction and direct sequencing of the juxtamembrane (exons 9 and 11) and tyrosine kinase (exons 13 and 17) domains. No mutation was found. Our results demonstrate that KIT could be a useful immunophenotypic marker for chromophobe RCC and renal oncocytoma; therefore, it has value for the precise classification of renal cortical epithelial tumors. However, the therapeutic relevance of KIT overexpression in these tumors is uncertain owing to the lack of mutations that would lead to constitutive activation of the protein.

Chromophobe renal cell carcinoma was first described by Thoenes et al1 in 1985, and now it is accepted universally as a distinct subtype of renal cell carcinoma (RCC). Histologically, the tumor is characterized by broad trabeculae of polygonal cells with clear to granular cytoplasm, thick cell borders, and a wrinkled nuclear membrane. Although the typical cases can be distinguished easily from other renal cortical epithelial tumors by the distinctive morphologic features, potential confusion may arise in histologically borderline cases. The classic variant of chromophobe RCC might be confused with clear cell conventional RCC, while the eosinophilic variant might be confused with other renal cortical epithelial tumors with granular cytoplasm, such as predominantly granular cell conventional RCC, papillary RCC, and renal oncocytoma. Accurate classification is important not only for its correlation with the cytogenetic findings, but also for its prognostic implications. The outcome for patients with chromophobe RCC generally is better than for those with conventional RCC,2 but worse than for patients with renal oncocytoma, of which the overwhelming majority are benign and do not metastasize.3,4

Traditionally, the strong and diffusely positive reticular staining pattern of the Hale colloidal iron stain5 and the intracytoplasmic 250- to 400-nm microvesicles observed under the electron microscope6 are reported to support the diagnosis of chromophobe RCC. However, the tests needed to determine these findings are not always feasible for ordinary pathology laboratories. There also is overlapping with renal oncocytoma; thus, the interpretation requires sufficient training in identifying different patterns. For the Hale colloidal iron stain, oncocytomas can have fine dust-like positivity, and clear cell RCCs can show a coarse drop-like or bubbly pattern, both of which
might not be distinguished easily from the true reticular pattern of chromophobe RCC without experience. Ultrastructurally, renal oncocytomas can possess a few microvesicles, while the microvesicles might be rare in the eosinophilic variant of chromophobe RCCs. On the other hand, determining an immunohistochemical hallmark by using commercial antibodies workable on formalin-fixed, paraffin-embedded specimens would assist in the differential diagnosis.

Recently, Yamazaki et al analyzed the gene expression profiles in 10 conventional RCCs, 2 papillary RCCs, and 3 chromophobe RCCs using high-density oligonucleotide arrays targeting on 12,000 genes. They found that the c-kit oncogene was up-regulated selectively in chromophobe RCCs. Reverse transcriptase–polymerase chain reaction (RT-PCR) and immunohistochemical stains further confirmed the presence of the c-kit transcript and overexpression of KIT protein in the chromophobe RCCs. KIT (CD117) is a transmembrane tyrosine kinase receptor protein encoded by the proto-oncogene c-kit that maps to chromosome 4 (4q11-12). KIT is expressed at high levels in several normal tissues, including hematopoietic stem cells, mast cells, melanocytes, germ cells, and the interstitial cells of Cajal. Overexpression of KIT is also observed in a spectrum of human neoplasms, chiefly gastrointestinal stromal tumor (GIST), myeloproliferative disorders, mast cell neoplasm, melanoma, and seminoma.

Because the observation of Yamazaki et al was limited by a rather small sample, we expanded the scope to encompass a substantial number of cases of renal cortical epithelial tumors. We analyzed the expression of KIT in renal cortical epithelial tumors by immunohistochemical analysis to evaluate its diagnostic usefulness as a phenotypic marker. We also attempted to detect the mutations in the c-kit gene by PCR and direct sequencing of both juxtamembrane domains (exons 9 and 11) and the tyrosine kinase domain (exons 13 and 17) in the KIT-expressing cases.

### Materials and Methods

#### Case Selection

We retrieved 322 consecutive cases of RCC and 7 cases of renal oncocytoma from the surgical pathology archives at the Taipei Veterans General Hospital, Taipei, Taiwan, for the period July 1990 to June 2000. The patients were 185 men and 144 women, with ages ranging from 18 to 85 years (mean, 63.9 years). The specimens were obtained by radical surgery. Cases with needle biopsies were excluded. The RCCs were classified based on the 1997 International Union Against Cancer/American Joint Committee on Cancer classification into 256 conventional, 29 chromophobe, 25 papillary, 6 collecting duct, and 6 unclassified type.

We also randomly selected 22 urothelial carcinomas of the renal pelvis, 7 nephroblastomas, 23 angiomyolipomas, and 10 normal renal tissue samples from the same archives and assessed them in parallel. Three cases of GIST known to express KIT were used as control samples.

#### Immunohistochemical Analysis

Immunohistochemical staining for KIT (clone: A4502; dilution 1:200; DAKO, Carpinteria, CA) was performed. The staining condition was adjusted using GIST samples as controls. The deparaffinized slides were pretreated with a 10-mmol/L concentration of citric acid buffer, pH 6.0, and heated in a microwave oven for 10 minutes. The endogenous peroxidase was quenched by using hydrogen peroxide, and the nonspecific binding was blocked by using swine serum. The bound antibodies were detected by using the DAKO EnVision system. The system is free of biotin. Immunopositivity was scored as follows: 0, undetectable; 1+, heterogeneous positivity in fewer than 50% of tumor cells; 2+, strong positivity in 50% to 80% of tumor cells; and 3+, diffuse positivity in more than 80% of tumor cells.

#### Genomic DNA Sequencing

The 29 chromophobe RCCs and 7 renal oncocytomas were evaluated for mutations in the juxtamembrane domains, exons 9 and 11, and in the tyrosine kinase domain, exons 13 and 17, by PCR and direct DNA sequencing. Under a light microscope, the tumor tissue was dissected and scraped down from the dewaxed slides cut from the formalin-fixed, paraffin-embedded blocks to ensure the greatest population of tumor cells. DNA was extracted according to the standard procedure of Proteinase K digestion, phenol-chloroform extraction, and ethanol precipitation. The intronic primers are listed in **Table 1**. PCR amplifications were performed.

#### **Table 1**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>PCR Products (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 9</td>
<td>Forward</td>
<td>5’-TCC TAG AGT AAG CCA GGG CTT-3’</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5’-GCC TAA ACA TCC CCT TAA ATT G-3’</td>
</tr>
<tr>
<td>Exon 11</td>
<td>Forward</td>
<td>5’-CCA GAG TGC TCT AAT GAC TG-3’</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5’-AGC CCC TGT TTC ATA CTG AC-3’</td>
</tr>
<tr>
<td>Exon 13</td>
<td>Forward</td>
<td>5’-GCT TGA CAT CAG TTT GCC AG-3’</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5’-AAA GGC AGC TTG GAC ACG GCT TTA-3’</td>
</tr>
<tr>
<td>Exon 17</td>
<td>Forward</td>
<td>5’-GTT TTC TTT TCT CCT CCA ACC TAA TA-3’</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5’-TTG AAA CTA AAA ATC TTT TGC AGG AC-3’</td>
</tr>
</tbody>
</table>

bp, base pairs; PCR, polymerase chain reaction.
using FastStart Taq DNA polymerase (Roche Molecular Biochemicals, Mannheim, Germany). The annealing temperature was 56°C. The amplification products were electrophoresed on 2% agarose gels, purified from the gel, and sequenced using a fluorescent automatic sequencer (Prism 377 DNA sequencer, Applied Biosystems, Foster City, CA).

**Results**

Of 29 chromophobe RCCs **Image 1**, 24 (83%) revealed membranous immunoreactivity for KIT, as did 5 of 7 renal oncocytomas **Image 2**. In most of the positive cases, the staining was strong. None of the conventional, papillary, collecting duct, or unclassified RCCs in this series immunohistochemically expressed KIT. The infiltrating mast cells could serve as an internal control. The results are given in **Table 2**.

In 4 nonneoplastic renal tissue samples, the renal tubules disclosed a focally weak to moderate cytoplasmic immunoreactivity. In the remaining 6 normal renal tissue samples, no immunoreactivity was observed **Image 3**. No membranous immunoreactivity was observed in any of the normal renal tissue samples. Occasional cytoplasmic reactivity also was observed in the adjacent normal renal tissue in the KIT-positive tumor cases. The cytoplasmic reactivity was unrelated to the membranous positivity in the tumors.

Urothelial carcinomas and nephroblastomas infrequently showed focal membranous reactivity. All angiomyolipomas were negative **Image 4**. No cytoplasmic positivity was observed.

We found no mutations in exons 9, 11, 13, and 17 in the chromophobe RCCs and renal oncocytomas.

**Discussion**

Our more extensive series of a total of 329 cases confirms the findings of Yamazaki et al⁷ that KIT is overexpressed in chromophobe RCC but is absent in other types. Accordingly, we consider KIT to be a useful adjunct for discriminating chromophobe RCC from other subtypes of RCC. The KIT immunostain is technically easier to use than the Hale colloidal iron stain and electron microscopy. The distinctive membranous pattern in the positive cases is rather straightforward to interpret in comparison with previously
proposed immunohistochemical markers for chromophobe RCC. For example, the immunohistochemical stain for anti-mitochondrial antibody 113-1 is subject to differing staining patterns (diffuse or peripheral accentuation, coarse or fine granular) among different subtypes. The diagnostic usefulness of cytokeratins 7 and 20 for chromophobe RCC also has been questioned for its low sensitivity and specificity.

For practicing pathologists who are unfamiliar with the Hale colloidal iron stain, electron microscopy, and cytogenetic examination and are willing to perform additional studies to support the histologic impression of chromophobe RCC, we recommend the immunohistochemical stain for KIT as a substitute method. In our series, 3 KIT-positive chromophobe RCCs were signed out as conventional RCC by the pathologists who initially viewed the cases. After careful review, we reclassified the 3 cases as chromophobe RCC. Our experience shows the potential usefulness of the KIT immunostain in precise classification.

In addition to chromophobe RCC, renal oncocytoma is another type of renal cortical epithelial tumor that overexpresses KIT. To our knowledge, this observation has not been described before. Therefore, KIT cannot be used to differentiate between chromophobe RCC and renal oncocytoma. The shared overexpression of KIT in these 2 kinds of tumor raises the possibility that they might be closely related and form a spectrum. Concurrent chromophobe RCC and renal

Table 2
Expression of KIT in Renal Cell Carcinoma and Renal Oncocytoma

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. Positive/Total No. (%)</th>
<th>Score*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Renal cell carcinoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conventional</td>
<td>0/256 (0)</td>
<td>256</td>
</tr>
<tr>
<td>Chromophobe</td>
<td>24/29 (83)</td>
<td>5</td>
</tr>
<tr>
<td>Papillary</td>
<td>0/25 (0)</td>
<td>25</td>
</tr>
<tr>
<td>Collecting duct</td>
<td>0/6 (0)</td>
<td>6</td>
</tr>
<tr>
<td>Unclassified</td>
<td>0/6 (0)</td>
<td>6</td>
</tr>
<tr>
<td>Renal oncocytoma</td>
<td>5/7 (71)</td>
<td>2</td>
</tr>
<tr>
<td>Urothelial carcinoma</td>
<td>4/20 (20)</td>
<td>16</td>
</tr>
<tr>
<td>Nephroblastoma</td>
<td>2/7 (29)</td>
<td>5</td>
</tr>
<tr>
<td>Angiomyolipoma</td>
<td>0/23 (0)</td>
<td>23</td>
</tr>
</tbody>
</table>

* The scoring system was as follows: 0, undetectable; 1+, heterogeneous positivity in fewer than 50% of tumor cells; 2+, strong positivity in 50% to 80% of tumor cells; and 3+, diffuse positivity in more than 80% of tumor cells.
oncocytoma and the existence of “hybrid” tumors exhibiting both histologic features have been reported. Previous studies also demonstrated some overlapping histochemical or ultrastructural features. These findings support the hypothesis that chromophobe RCC and renal oncocytoma might have a common precursor but differentiate in different directions. Nevertheless, distinction between the 2 tumors has clinical importance because of their differing biologic behavior. Ancillary methods to separate these 2 categories such as histochemical analysis, immunohistochemical analysis, electron microscopic examination, and genetic studies have been surveyed vigorously, but so far, histologic examination has remained the “gold standard.”

The underlying mechanism for the overexpression of KIT in chromophobe RCC and renal oncocytoma is unknown. We believe the overexpression is an acquired phenomenon during the oncogenesis of these tumors. Mutations or altered methylation in promoter regions or genomic amplification of the KIT locus possibly contribute to the upregulation of KIT transcription. The method of the present study cannot determine the functional status of KIT protein. The common KIT genomic mutations that lead to the constitutive activation were not present in the chromophobe RCCs and renal oncocytomas in this series. Further phosphorylation studies are required.

KIT cannot be taken as a prognostic indicator of aggressiveness, because renal oncocytoma is a benign tumor, and chromophobe RCC, in general, has a better prognosis than other types of RCC. Without elucidating these points, we currently do not propose that patients with chromophobe RCC would benefit from tyrosine inhibitor therapy (imatinib mesylate).19

We observed that normal renal tubules occasionally showed weak cytoplasmic reactivity for KIT. Yamazaki et al also noted a similar phenomenon. However, the staining was inconsistent, so we are not certain about its significance. A recent study showed that the cytoplasmic staining for KIT probably results from a faulty staining technique, and it varies greatly with different antibody sources and dilution and with the antigen retrieval method.20 Since there is no clear genetic evidence to support the KIT staining in these cases, and the previous RT-PCR data are from a small number of cases, the present observation also might be a result of nonspecific staining. It is judicious to regard only membranous reactivity as a genuine expression of KIT. Because the renal tubular cells are rich in mitochondria, the cytoplasmic staining might be also due to nonspecific adsorption or cross-reactivity for some unknown mitochondrial proteins.

Another finding in the present study worth noting is that we failed to identify any immunoreactivity for KIT in the 23 renal angiomylipomas we examined. In contrast, Makhlof et al reported that all 21 angiomylipomas (15 hepatic, 6 renal) in their series revealed cytoplasmic reactivity for this protein. The discrepancy is likely to be caused by the different clones of the antibody. Makhlof et al applied a Santa Cruz antibody (clone C-19, Santa Cruz Biotechnology, Santa Cruz, CA), which has been shown to be prone to produce such cytoplasmic staining compared with the DAKO antibody (clone A4502) that we used. Whether angiomylipoma is a true KIT-expressing tumor requires more studies, such as with Western blot for the KIT protein or RT-PCR or in situ hybridization for the c-kit transcript. The disparate immunohistochemical findings underscore the necessity for pathology laboratories to establish optimized immunohistochemical procedures to achieve reliable and reproducible results for each antibody, especially for an indicator with therapeutic consideration.22

References


