

# Immunohistochemical Analysis for Cytokeratin 7, KIT, and PAX2

## Value in the Differential Diagnosis of Chromophobe Cell Carcinoma

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### Abstract

*Immunohistochemical staining for cytokeratin 7 (CK7), KIT, and PAX2 expression was performed on 91 renal neoplasms, 37 conventional (clear cell) renal cell carcinomas (CRCCs), 20 papillary RCCs (PRCCs), 11 chromophobe RCCs (ChCs), and 23 oncocytomas, with available karyotypes. All ChCs, 19 PRCCs, 2 CRCCs, and 1 oncocytoma were CK7+; all ChCs, 22 oncocytomas, 2 CRCCs, and no PRCCs expressed KIT; PAX2 was positive in 31 CRCCs, 17 PRCCs, 20 oncocytomas, and 1 ChC. The predominant expression profiles were as follows: CRCC, CK7-/KIT-/PAX2+ (26/37); PRCC, CK7+/KIT-/PAX2+ (17/20); ChC, CK7+/KIT+/PAX2- (10/11); and oncocytoma, CK7-/KIT+/PAX2+ (19/23). Cytogenetic analysis showed that the sole PAX2+ ChC had a retained chromosome 10, and all ChCs with chromosome 10 loss were PAX2-. These results identify specific staining patterns of the 4 major histologic subtypes of renal neoplasms and raise the question of a relationship between chromosome 10 loss and loss of PAX2 expression in ChC.*

Chromophobe cell carcinoma (ChC), first described by Thoenes et al<sup>1</sup> in 1985, accounts for approximately 5% of malignant renal neoplasms and consists of 2 variants, classical and eosinophilic.<sup>2,3</sup> The classical variant described by Thoenes et al<sup>1</sup> is characterized by large polygonal cells with transparent, slightly reticulated cytoplasm and can be confused with conventional renal cell carcinoma (CRCC), whereas the eosinophilic variant, composed of intensely eosinophilic cells, resembles oncocytoma.<sup>4</sup> Although both variants stain positively with Hale colloidal iron, the reaction is often weak, making ChC difficult to differentiate from oncocytoma on the one hand and the more aggressive “granular” variant of CRCC on the other.

Recently, Yamazaki et al<sup>5</sup> and Petit et al<sup>6</sup> demonstrated overexpression of KIT in ChC by gene expression and immunohistochemical analysis, and Mathers et al<sup>7</sup> highlighted cytokeratin (CK) 7 staining in these tumors. ChC is also characterized by a karyotype showing loss of multiple chromosomes, especially chromosomes 1, 2, 6, 10, 13, 17, and 21. A subset of cases shows a near-triploid karyotype, with approximately 60 to 90 chromosomes per cell.<sup>8-10</sup> In the present study, we demonstrate the value of CK7 and KIT in combination with PAX2, a nuclear transcription factor that regulates the development of renal epithelia, in defining an immunohistochemical expression profile characteristic of ChC.

### Materials and Methods

#### Patients and Tumor Samples

Formalin-fixed, paraffin-embedded tissue samples from 91 renal neoplasms with available karyotypes were selected from the archives of the Department of Pathology, Columbia

University Medical Center, New York, NY. All specimens were obtained from surgical resections and represented 91 successive renal cortical neoplasms in which karyotyping demonstrated clonal abnormalities. No patient had received previous treatment. H&E-stained slides of each case were reviewed, and each case was typed according to the World Health Organization classification of renal neoplasms.<sup>11</sup> The Fuhrman histologic grade was recorded for each carcinoma. Papillary carcinomas were subclassified as type 1 and type 2, according to Delahunt and Eble,<sup>12</sup> and each ChC was classified as classical, mixed, or eosinophilic.

Cases comprised the following: 37 CRCCs (clear cell; 17 grade 2, 17 grade 3, and 3 grade 4), 20 papillary-type RCCs (PRCCs; 16 type 1 and 4 type 2; 15 grade 2 and 5 grade 3), 11 ChCs (4 eosinophilic variant, 2 classical, and 5 mixed; 3 grade 2, 7 grade 3, and 1 grade 4), and 23 oncocytomas.

Tissue microarrays were prepared, as previously described,<sup>13</sup> selecting 3 cores of tumor and 1 core of normal tissue from each case.

### Immunohistochemical Analysis

Five- $\mu$ m sections of tissue microarrays were deparaffinized and rehydrated. Following microwave antigen retrieval in citrate, pH 6, slides were stained on a DAKO Autostainer (DAKO, Carpinteria, CA) for CK7 and KIT and a Ventana NEXes instrument (Ventana Medical Systems, Tucson, AZ) for PAX2. The antibodies used were CK7 (clone OV-TL 12/30, dilution 1:200; DakoCytomation, Carpinteria, CA), KIT (clone 104D2, dilution 1:400; DakoCytomation), and PAX2 (polyclonal, dilution 1:50; Zymed Laboratories, San

Francisco, CA). Signal was detected using the EnVision+ system (DAKO) for CK7 and KIT and the iView DAB detection system (Ventana Medical Systems) for PAX2.

Each core was evaluated for staining of tumor cells. A tumor was considered positive for a stain if greater than 10% of tumor cells showed distinct positive staining—membranous or membranous and cytoplasmic for CK7 and KIT and nuclear for PAX2.

### Cytogenetics

A portion of the tumor was obtained immediately following sectioning, and the sample was processed for cell culture. Tumor tissue was dissociated with collagenase, and cells were grown for short-term culture in complete RPMI medium supplemented with insulin–transferrin–sodium selenate. Cytogenetic analysis of 20 metaphase cells was performed using standard G-banding techniques.

### Statistical Analysis

Scoring of immunohistochemical staining highlighted 4 predominant expression profiles, ie, CK7+/KIT+/PAX2–, CK7+/KIT–/PAX2+, CK7–/KIT+/PAX2+, and CK7–/KIT–/PAX2+. A Fisher exact test was used to evaluate the distribution of each phenotypic profile in the 4 tumor categories using a 2  $\times$  4 table.

## Results

**Table 1** shows the results of immunohistochemical staining. ChCs were predominantly CK7+, KIT+, and PAX2–. Oncocytomas, on the other hand, were negative for CK7 and positive for PAX2, and they shared KIT staining with ChC. CRCCs were positive only for PAX2, whereas PRCCs were positive for CK7 and PAX2. These differences in expression were all statistically significant **Table 2** and **Image 1**.

Of the ChCs, 4 were of the eosinophilic variant and all 4 were CK7+/KIT+/PAX2–. Although the numbers are too small for a separate analysis, this indicates potential applicability of this panel as an aid to the diagnosis of this variant.

**Table 1**  
Immunostaining Results

Histologic Type	Cytokeratin 7	KIT	PAX2
Clear cell (n = 37)	2	2	31
Papillary (n = 20)	19	0	17
Chromophobe (n = 11)	11	11	1
Oncocytoma (n = 23)	1	22	20
Total (n = 91)	33	35	69

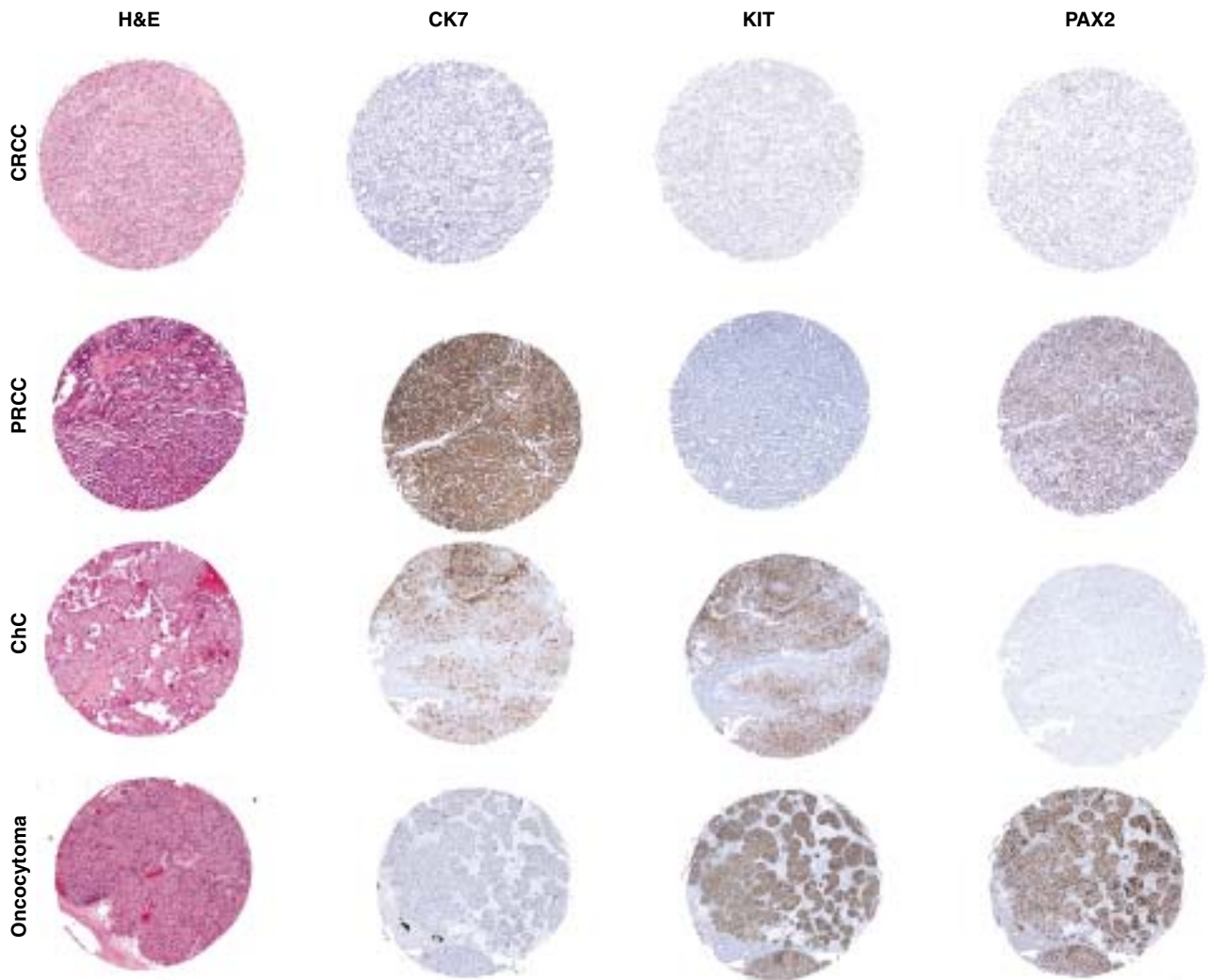
**Table 2**  
The Four Major Immunostaining Profiles of Renal Tumors\*

Staining Pattern	Chromophobe (n = 11)	Oncocytoma (n = 23)	Clear Cell (n = 37)	Papillary (n = 20)	P†
CK7+/KIT+/PAX2–	<b>10</b>	0	0	0	<.001
CK7–/KIT+/PAX2+	0	<b>19</b>	3	0	<.001
CK7–/KIT–/PAX2+	0	0	<b>26</b>	0	<.001
CK7+/KIT–/PAX2+	0	0	2	<b>17</b>	<.001

CK, cytokeratin.

\* Of 91 tumors, 77 showed one of the phenotypic profiles shown in the Table. Several cases did not fit into the predominant phenotypes: 2 cases (1 chromophobe and 1 oncocytoma) were positive for all 3 markers; 2 oncocytomas were positive for KIT and negative for CK7 and PAX2; 2 papillary carcinomas were positive for CK7 and negative for KIT and PAX2; 8 cases (6 clear cell, 1 oncocytoma, and 1 papillary carcinoma) were negative for all 3 stains. Boldface indicates the number with “characteristic” phenotype.

† Fisher exact test for 2  $\times$  4 table.



**Image 1** Predominant staining patterns of conventional (clear cell) renal cell carcinoma (CRCC), papillary RCC (PRCC), chromophobe RCC (ChC), and oncocytoma (original magnification  $\times 4$ ).

Review of karyotypes of the 11 ChCs **Table 3** showed that 9 tumors had multiple chromosome losses (6 with chromosome 10 losses). Four tumors (cases 2, 4, 6, and 8 in Table 3) exhibited a near-triploid karyotype with 55 to 74 chromosomes; 5 (cases 4, 5, 9, 10, and 11) were hypodiploid; 1 harbored a chromosomal translocation,  $t(1;9)(q31;q22)$ ; and 1 case showed trisomy 7 in a minority of cells, most likely reflecting a normal variant.<sup>14</sup> **Image 2** shows a representative hypodiploid karyotype.

## Discussion

Our results of immunohistochemical staining with CK7 and KIT are similar to those of most previously reported studies. Petit et al<sup>6</sup> reported immunohistochemical expression of KIT in 88% of ChCs, and Yamazaki et al<sup>5</sup> showed that KIT

expression was up-regulated in ChC by using high-density nucleotide arrays. Wang and Mills<sup>15</sup> found KIT expression in 100% of eosinophilic ChCs and oncocytomas, whereas Pan et al<sup>16</sup> detected KIT immunoreactivity in 83% of ChCs and 71% of oncocytomas, with neither study detecting KIT staining in CRCC. Other investigators<sup>17</sup> reported KIT expression in only 4 (57%) of 7 cases of ChC, but this difference could be a consequence of small samples. CK7 seems to be valuable in the differentiation of ChCs from other tumors, especially oncocytomas, which are generally CK7-.<sup>7,18</sup> However, immunostaining with a single antibody in individual cases can be notoriously unreliable. When addressing a differential diagnostic question in a difficult case, it is useful to have a panel of markers, some relatively specific for one condition and others for the alternative condition(s). The demonstration of PAX2 staining in oncocytomas and not in ChCs provides such a marker. Furthermore, the differences in KIT immunostaining provide

**Table 3**  
**Karyotypes of 11 Chromophobe Cell Carcinomas**

Case No.	Final Karyotype	PAX2
1	46,XY,t(1;9)(q31;q22)[8]/46,XY[8]	-
2	70-73,<3N>,XXY,-1,-2,+5,-6,-9,+13,+mar1.2,+dmin,+acex4[cp5]/46,XY[17]	-
3	47,XX,+7[4]/46,XX[14]	-
4	55-74,XXXX,<3N>,-1,+2,-3,add(3)(q29),-5,-6,+7,t(8;?)(p23;?),der(9)hsr(9)(p23),+12,t(12;13)(p13;q11),add(14)(q32),+16+18,mar1-4[cp13]/46,XX[7]	+
5	38,X,-Y,-1,-2,t(3;8)(p21.3;p12),-5,-6+8,-10,-13,-17,-21[17]/76,<4N>XX,-Y,-Y,idem[3]	-
6	68,<3N>,XXY,-1,-2,+4,+7,-10,+12,-13,+15,-17,-21,+22[20]	-
7	38,XY,-1,-2,-5,-6,-10,-13,-17,-21[5]/46,XY[15]	-
8	69-180,<3N>,XXX,-1,-2,+4,+5,-6,+7,+9,-10,-11,+12,-13,+14,+15,-16,-17,+18,+19,+20,-21,+22,+der(?)r(?)x2,+mar1-6[cp14]	-
9	38,XX,-1,-2,-6,-8,-10,-13,-17,-21[12]/76,<4N>,XXXX,idemx2[3]/38,XX,-1,-2,-6,-8,-10,der(12)add(12)(p13),-13,-17,-21[2]/76,<4N>,XXXX,idemx2[3]	-
10	37,X,-X,-1,-2,-6,-8,-10,-13,-17,-21[9]/74,<4N>,XX,-X,-X,idemx2[6]/46,XX[7]	-
11	37-43,XY,(1)(q10),-3,-8,-14,-15,-16,+mar1[cp11]	-

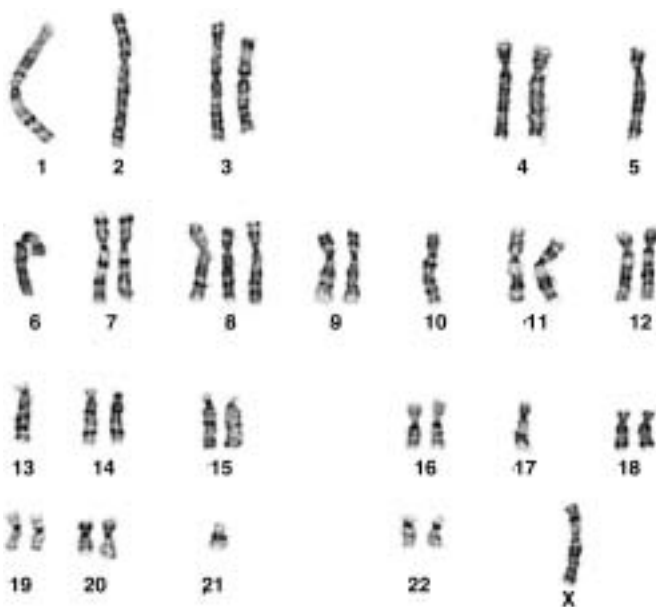
an additional marker to aid in the distinction between ChCs and other tumors, especially the granular variant of CRCC.

PAX2 is a transcription factor that is essential for kidney development, promoting the transition of mesenchyme to epithelium. In the normal adult kidney, PAX2 protein expression is seen predominantly in the distal nephron—the distal tubule and collecting ducts.<sup>19</sup>

Our results demonstrating frequent expression of PAX2 in CRCC and rare or absent expression in ChC are similar to those in previous reports.<sup>18,20</sup> Daniel et al<sup>20</sup> demonstrated expression of PAX2 in more than 90% of CRCCs and in 100% of PRCCs but in only 1 of 3 oncocytomas and 1 of 4 ChCs. In

addition, in a recent study, Mazal et al<sup>18</sup> showed expression of PAX2 in 88% of CRCCs but in only a few PRCCs, ChCs, and oncocytomas, despite including tumors with fewer than 10% positive cells as positive, whereas the present study included only tumors with greater than 10% positive cells (corresponding to “++” and “+++” of Mazal et al<sup>18</sup>). Daniel et al<sup>20</sup> used only frozen sections and had only 3 oncocytomas. Mazal et al<sup>18</sup> used the same antibody and antigen retrieval as in the present study, diluted to 1:100 (as opposed to 1:50 in the present study). It is unclear which avidin-biotin system was used by Mazal et al.<sup>18</sup> We used a highly sensitive commercial streptavidin peroxidase system. Subtle differences in antigen preservation or retrieval or in signal detection can cause significant alterations in staining rates, an inherent limitation of immunohistochemical procedures performed on routinely processed tissue. The Fuhrman grade, noted by Mazal et al<sup>18</sup> to correlate with PAX2 expression *only* for CRCC, is an unlikely explanation for these differences. Our samples incorporated a broad range of Fuhrman nuclear grades with no relationship of staining to grade.

Overall, the 4 major expression profiles “correctly” classified 72 (79%) of 91 cases. Three CRCCs exhibited the CK7-/KIT+/PAX2+ phenotype characteristic of oncocytomas. Two CRCCs were CK7+/KIT-/PAX2+, a profile shared with PRCC. Although CRCC is generally easily distinguished from oncocytoma and PRCC, it is useful to note that CRCC may express KIT and/or CK7. Of the 91 cases, 14 (15%) did not fit into the aforementioned predominant phenotypes. These included 6 CRCCs, 1 ChC, 4 oncocytomas, and 3 PRCCs (Table 2). Of these, the expression profiles of the 8 CK7-/KIT-/PAX2- neoplasms (6 CRCCs, 1 oncocytoma, and 1 PRCC) and 2 CK7-/KIT+/PAX2- oncocytomas are clearly distinct from the usually CK7+ ChC. The 2 CK7+/KIT-/PAX2- PRCCs potentially overlap immunophenotypically with ChC but are generally easily distinguished morphologically from them. This leaves 1 CK7+/KIT+/PAX2+



**Image 2** Representative karyotype of chromophobe renal cell carcinoma (Table 3, case 5) showing multiple chromosome losses, including chromosome 10, and t(3;8) translocation.

ChC and 1 CK7+/KIT+/PAX2+ oncocytoma as the 2 cases with a truly confusing expression pattern with this panel. These cases highlight the limitations of diagnostic immunohistochemical analysis. The significance of immunostaining results should be interpreted only in the context of morphologic evaluation. In addition, the use of other markers such as RCC marker, expressed by CRCC but not ChC or oncocytomas,<sup>15</sup> or parvalbumin, expressed by ChC but not CRCC or oncocytoma,<sup>21</sup> may be of value in the proper setting.

PAX2 is encoded on chromosome 10. Monosomy of chromosome 10 is common in ChC.<sup>22</sup> Cytogenetic data were available for all 11 ChCs, of which 6 showed chromosome 10 losses. The 1 ChC case with PAX2 expression had no loss of chromosome 10. The number of cases is too small for statistical analysis. However, these findings raise a question of a possible relationship between chromosome 10 loss and absence of PAX2 expression. Evaluation of a larger number of ChCs is needed to definitively address this question.

Our results demonstrate definite differences in CK7, KIT, and PAX2 staining patterns of the major histologic subtypes of renal neoplasms, indicating that this panel of immunostains may be of value in the differential diagnosis of renal neoplasms. In addition, we demonstrated loss of PAX2 expression in ChC and raised the question of whether this loss of PAX2 expression is related to loss of chromosome 10.

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