Vimentin Reactivity in Renal Oncocytoma: Immunohistochemical Study of 234 Cases

Ondrej Hes, MD, PhD; Michal Michal, MD, PhD; Naoto Kuroda, MD; Guido Martignoni, MD; Matteo Brunelli, MD, PhD; Yi Lu, MD; Brian P. Adley, MD; Isabel Alvarado-Cabrero, MD; Ximing J. Yang, MD, PhD

In renal cell carcinoma (RCC), vimentin is often considered one of the diagnostic features. It is expressed in RCC but not in clear cell RCC. The presence of vimentin in renal cell tumors is significant as it distinguishes RCC from other subtypes of RCC. The authors studied vimentin in a large series of 234 renal onc cytomas

Context—The expression of vimentin in renal oncocytomas has been controversial. However, this is of clinical significance because immunostains may be used to differentiate renal tumors. Using different staining and analysis methods, we studied vimentin immunoreactivity in renal oncocytomas with a special emphasis on the Immunoreactivity patterns.

Objective—Immunohistochemical expression of vimentin has been used in the differential diagnosis of renal epithelial neoplasms. Although typically expressed in most renal cell carcinomas, the immunoreactivity of this intermediate filament in renal oncocytomas has been controversial.

Design—We studied vimentin immunoreactivity in a large series of 234 renal oncocytomas using 2 staining methods as well as manual and automated imaging analysis.

Results—We found that the focal vimentin immunoreactivity can be seen in most renal oncocytomas with vimentin-positive tumor cells usually found in the edge of a central scar or in small clusters scattered throughout the tumor. Computer-aided imaging analysis confirmed the difference in vimentin immunoreactivity between oncocytoma and other renal neoplasms.

Conclusions—Our study of vimentin immunohistochemistry in a series of renal oncocytomas, which to our knowledge is the largest ever published, showed focal vimentin positivity detected in most oncocytomas. The vimentin staining patterns in renal oncocytomas are different from those seen in clear cell or papillary renal cell carcinomas, we consider vimentin staining to be helpful in the differential diagnosis of oncocytoma from other renal tumor mimics. Furthermore, strong vimentin positivity in a renal cell neoplasm does not exclude the diagnosis of renal oncocytoma, particularly in a limited biopsy specimen.

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registry were finally included in our study. In addition, 50 renal oncocytomas were retrieved from the surgical pathology files of Northwestern Memorial Hospital in Chicago, III, including 10 cases with conventional large sections and 40 cases from tissue microarrays (TMAs) containing 1.0-mm cores. For comparison, 50 cases of chromophobe RCC identified from the files of Northwestern Memorial Hospital, including 20 cases with conventional large sections and 30 cases with TMAs, were evaluated. For automatic imaging analysis, vimentin-stained conventional sections of 2 oncocytomas (included), 2 chromophobe RCCs (included), and 2 additional cases of papillary RCC were selected to compare with nonneoplastic kidneys.

**Immunohistochemistry**

For immunohistochemical studies, formalin-fixed, paraffin-embedded tissue sections were stained with an antibody against vimentin Ab-2 (clone V9, monoclonal, 1:800; Neomarkers, Fremont, Calif). In brief, 5-μm-thick sections were deparaffinized. Sections were microwave-pretreated in 10mM citrate buffer solution (pH 6.0) at 750 W for a 3-minute cycle repeated 3 times. The sections were treated with 0.3% hydrogen peroxide in methanol for 30 minutes and washed with phosphate-buffered saline solution. Endogenous biotin was blocked using a biotin blocking kit (Vector Laboratories, Burlingame, Calif). Thereafter, the sections were exposed to primary antibody overnight (approximately 12 hours) at 4°C. Sections were cooled in phosphate-buffered saline buffer in each step of immunohistochemistry. All slides were visualized using a supersensitive streptavidin-biotin system (BioGenex, San Ramon, Calif).

For comparison, another staining method using a biotin-free detection system was used as follows. Antigen retrieval was carried out in citrate buffer (10mM, pH 6) for 15 minutes at 100°C in a microwave oven. The slides were incubated with a primary mouse monoclonal anti-vimentin antibody (clone Vm 3B4, Dako, Carpinteria, Calif) at 1:100 dilution for 1 hour at room temperature. Sections were then incubated with the secondary anti-mouse immunoglobulin G antibody for 30 minutes. A subsequent reaction was performed with biotin-free horseradish peroxidase enzyme-labeled polymer from EnVision + detection system (Dako). A positive reaction was visualized with diaminobenzidine solution followed by counterstaining with hematoxylin. Immunohistochemical vimentin positivity in the vascular walls was used as an internal positive control. Only cases with strong vimentin staining were counted as positive. For conventional sections, the positive staining was graded as focal (greater than 1%, less than or equal to 5% cells) or diffuse (greater than 5% cells). For the TMA sections, positivity was defined as any case with positive tumor cells. Tumors with weak focal or weak diffuse staining were counted as negative.

**Automatic Imaging Analysis**

An automated Cellular Imaging System II (ACISII, ChromaVision Medical System Inc, San Juan Capistrano, Calif) was used to compare the intensity and percentage of vimentin staining in 2 cases of renal oncocytoma with that of adjacent benign renal cortical and medullary tissue in the same section. In addition, 2 papillary RCCs (positive controls) and 2 chromophobe RCCs (negative controls) were studied for comparison. With ACISII, positive staining was calculated by applying 2 thresholds with one recognizing blue background (hematoxylin-stained) cells and another recognizing brown positive cells. The percentage of positivity was the area detected by the brown threshold (positive cells) divided by the sum of the area detected by the brown and blue thresholds (total positive and negative cells). The intensity was calculated by masking out all areas not selected by the brown threshold and calculating the integrated optical density of brown within the remaining area. This value was divided by the area in pixels of the brown mask to calculate an average intensity of a selected area. The mean values from nonneoplastic kidneys (22 areas), oncocytomas (75 areas), chromophobe RCCs (27 areas), and papillary RCCs (20 areas) were obtained and analyzed by using the ChromaVision ACISII.

**RESULTS**

Each tumor was classified according to the predominant histologic growth pattern identified on hematoxylin-eosin-stained sections. There was no difference in the vimentin staining patterns when comparing the biotin-based method (77.1% positive) or the biotin-free method (75% positive) (Table 1). Among the 234 cases examined, 64 (27.4%) renal oncocytomas were entirely negative for vimentin. The remaining 170 (72.6%) tumors showed focal vimentin positivity representing a small subset of tumor cells. Among the conventional sections, 77% (157/204) of oncocytomas showed focal vimentin positivity, whereas among the TMA sections, 43% (13/30) of oncocytomas showed vimentin positivity. We evaluated the vimentin positivity in 7 different growth patterns of oncocytomas. The vimentin-positive rates ranged from solid-alveolar pattern (91/129, 70.5%), small cell pattern (4/5, 80%), solid tubular pattern (10/12, 83.3%), tubular pattern (23/24, 95.8%), prominent stromal pattern (12/12, 100%), and pseudopapillary pattern (2/2, 100%). Essentially, all the patterns may show vimentin positivity in more than 70% of cases. Results are summarized in Table 2.

Three staining patterns of vimentin were identified: clustered, tubular, and mosaic. The clusters of vimentin-positive oncocytoma cells were often seen in areas next to fibrotic and hyalinized scars of the tumors (Figure 1, A through D). Vimentin-positive tumor cells forming small tubules were also noted in the central scars or in viable parenchyma of the tumors (Figure 2, A and B). The mosaic pattern was characterized by scattered tumor cells with vimentin-positive cytoplasmic staining and negative nuclear staining (Figure 2, C and D). Sometimes vimentin immunoreactivity was observed in

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**Table 1. Focal Vimentin Immunoreactivity in Oncocytomas**

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Total Cases, No.</th>
<th>Focal Vimentin Positivity No. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large sections 1</td>
<td>184</td>
<td>142</td>
</tr>
<tr>
<td>Large sections 2</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>TMA</td>
<td>30</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>234</td>
<td>170</td>
</tr>
</tbody>
</table>

* TMA indicates tissue microarray. Positivity of large sections was defined as 1% to 5% of positive tumor cells. Positivity of TMA sections was defined as any positive tumor cells. Large sections 1 obtained from Charles University Hospital, Pilsen, Czech Republic. Large sections 2 and TMA sections obtained from Northwestern Memorial Hospital, Chicago, Ill.

**Table 2. Histologic Patterns and Vimentin Positivity in Renal Oncocytomas**

<table>
<thead>
<tr>
<th>Pattern of Growth</th>
<th>Cases, No.</th>
<th>Positive Cases No. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solid-alveolar</td>
<td>129</td>
<td>91</td>
</tr>
<tr>
<td>Tubular</td>
<td>24</td>
<td>23</td>
</tr>
<tr>
<td>Solid-tubular</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>With prominent stromal component</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Small cell</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Pseudopapillary</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>
Figure 1. Vimentin staining in oncocytoma. Oncocytoma stained with hematoxylin-eosin (original magnifications ×40 [A] and ×400 [C]) and vimentin (original magnifications ×40 [B] and ×400 [D]). Vimentin reactivity in the vascular networks in scar tissue is highlighted (B). In the area next to the hyalinized central scar (A), there are clusters of vimentin-positive tumor cells (B). The clusters of oncocytoma cells (D) show strong vimentin staining in the cytoplasm.

the neoplastic cells showing multinucleation and degenerative nuclear changes that can occur in renal oncocytomas. Vimentin usually stained the cells with copious cytoplasm. We also observed scattered foci of vimentin-positive tumor cells without any relation to hyalinization and fibrous changes or degenerative changes especially in the peripheral parts of the tumors.

Other stromal elements associated with the neoplastic epithelial cells showed vimentin positivity including delicate vascular networks and fibrous tissue and were used as internal positive controls.

Forty-eight of 50 chromophobe RCCs tested for comparison were vimentin negative. The 2 vimentin-positive chromophobe RCCs demonstrated diffuse but weak vimentin staining, different from the strong but focal vimentin reactivity observed in a portion of oncocytomas.

The mean vimentin staining intensities and percentages of immunoreactive cells measured using ACISII were as follows. Nonneoplastic kidneys showed intensities of 150.5 and 143.7 and percentages of 61.2% and 54.0%, respectively. Oncocytomas were divided into 2 groups: one with focal vimentin reactivity (1A and 2A intensities = 159.1 and 156.7, percentages = 89% and 83.7%, respectively) and one without vimentin reactivity (1B and 2B intensities = 31.6 and 33.5, percentages = 0.1% and 0.1%). The papillary RCCs demonstrated intensities of 158.3 and 157.1 and percentages of 81.7% and 98.4%, and the chromophobe RCCs demonstrated intensities of 31.8 and 32.3 and percentages of 0.1% and 0.1%. The results of vimentin immunoreactivity represented by the product of intensity and percentage (intensity × percentage) are summarized in Table 3.

**COMMENT**

Renal oncocytoma and chromophobe RCC may share several overlapping morphologic and immunophenotypic features. Efforts to distinguish difficult cases of renal oncocytoma and chromophobe RCC using different immunohistochemical markers have been made.11–14 Tickoo et al15 reported the utility of antimitochondrial antibody 113-1 reactivity in distinguishing renal oncocytoma and chromophobe RCC from granular variants of clear cell RCC and papillary RCC.15 However, in some cases of renal tumors that had overlapping morphologic features between renal oncocytoma and chromophobe RCC, the immunohistochemical staining for antimitochondrial antibody...
Figure 2. Other vimentin staining patterns in oncocytomas. The vascular networks in renal stromal tissue provide a positive internal control. Tubular patterns of vimentin positivity observed in the center of an oncocytoma (original magnification ×100 [A]) and within the hyalinized central scar of another oncocytoma (original magnification ×100 [B]). Mosaic patterns of vimentin positivity seen in areas with scattered tumor cells showing cytoplasmic staining in a background of tumor cells with negative nuclear staining (original magnifications ×400 [C] and ×100 [D]).

Table 3. Vimentin Immunoreactivity in Renal Tumors and Renal Tissue Controls Measured by Using Automatic Imaging Analysis (ChromaVision ACISII)*

<table>
<thead>
<tr>
<th>Case</th>
<th>Fields Examined, No.</th>
<th>Mean Intensity</th>
<th>Mean Percentage</th>
<th>Immunoreactivity (Intensity × Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign kidney 1</td>
<td>11</td>
<td>150.5</td>
<td>61.2</td>
<td>92.1</td>
</tr>
<tr>
<td>Benign kidney 2</td>
<td>11</td>
<td>143.7</td>
<td>54.0</td>
<td>77.6</td>
</tr>
<tr>
<td>Oncocytoma 1A</td>
<td>20</td>
<td>159.1</td>
<td>89.0</td>
<td>141.6</td>
</tr>
<tr>
<td>Oncocytoma 1B</td>
<td>27</td>
<td>31.6</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>Oncocytoma 2A</td>
<td>22</td>
<td>156.7</td>
<td>83.7</td>
<td>131.6</td>
</tr>
<tr>
<td>Oncocytoma 2B</td>
<td>6</td>
<td>33.5</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>Chromophobe RCC case 1</td>
<td>12</td>
<td>31.8</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>Chromophobe RCC case 2</td>
<td>15</td>
<td>32.3</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>Papillary RCC case 1</td>
<td>10</td>
<td>158.3</td>
<td>81.7</td>
<td>129.3</td>
</tr>
<tr>
<td>Papillary RCC case 2</td>
<td>10</td>
<td>157.1</td>
<td>98.4</td>
<td>154.6</td>
</tr>
</tbody>
</table>

*RCC indicates renal cell carcinoma. Oncocytoma 1A: case 1, vimentin-positive areas; 1B: case 1, vimentin-negative areas; 2A: case 2, vimentin-positive areas; 2B: case 2, vimentin-negative areas.

113-1 gave confusing results. Castrén et al16 reported that immunohistochemical staining with cathepsin H could be used to differentiate renal oncocytomas (positive) from subtypes of RCCs (negative). However, both studies have not been substantiated by other investigators.

Many malignant tumors resemble their cell of origin, expressing only one type of intermediate filament. Some types of cancers, particularly RCC, may express more than one type of intermediate filament including vimentin as well as keratin.17 Vimentin, a 15-kd protein that is one of
the several types of intermediate filaments, is normally present in stromal cells but not in epithelial cells. Keratin, another type of intermediate filament, is normally expressed in epithelial cells but not stromal cells. It is well documented, however, that both keratin and vimentin expression can be seen in RCC and other malignant tumor cells. Extensive studies have shown that vimentin positivity is one of the diagnostic features of clear cell RCC and papillary RCC but not for chromophobe RCC (Table 4). Renal oncocytomas were generally thought to be negative for vimentin.

Several other articles described strong vimentin positivity in oncocytomas. Castrén et al.26 noted positivity in a similar pattern as presented in their illustrations. Additionally, we observed vimentin-positive areas outside of central scars and not associated with regressively changed areas of the tumors.

We believe that the discrepancy of vimentin-positive rates in oncocytoma reported in the literature was primarily caused by the definition of positivity. Using complementary DNA microarrays, Northern blotting, or Western blotting, the presence of a minor component of vimentin-positive cells in oncocytoma may not be evident because the minority of vimentin-positive oncocytoma tumor cells could easily be overwhelmed by the majority of vimentin-negative tumor cells or overlooked when sampling was limited. If the positivity was defined as more than 5% of cells showing staining, most of our cases would be considered negative because the vimentin positivity was usually very focal, representing only 1% to 5% positive cells in oncocytomas.

Several other articles described strong vimentin positivity in oncocytomas. Castrén et al.26 mentioned 13 of 16 cases of renal oncocytoma were strongly vimentin positive. In contrast to our findings, the authors noted only 1 case to be weakly positive, although they did not specify morphologic patterns of their vimentin-positive tumor cells. In our series, we excluded all cases of oncocytic tumors with atypical morphology. In our experience, these oncocytic tumors with atypical morphology may represent oncocytic papillary RCCs, especially when stained strongly and diffusely with antibody to vimentin. McNutt et al.25 suggested that renal oncocytoma and low-grade clear cell RCC seldom show positive reaction with vimentin antibody and that the expression of vimentin becomes more intense in RCC of higher grades, especially in sarcomatoid RCC. This opinion is in agreement with a published study of sarcomatoid components arising in chromophobe RCC in which vimentin stained focally in the carcinomatous components and diffusely in the sarcomatoid components. We obtained similar results in our series of 13 sarcomatoid components arising in chromophobe RCC (unpublished data).

Chromophobe RCC and oncocytoma show some overlapping genetic, biochemical, and morphologic features. However, chromophobe RCC is usually vimentin negative but positive for keratin and epithelial membrane antigen, which may help to distinguish chromophobe RCC from clear cell or papillary RCC (vimentin positive and epithelial membrane antigen negative). In our study, we confirmed the vimentin-negative nature of chromophobe RCC and validated our immunostaining methods. Although occasional vimentin positivity ranging from diffuse weak to moderate staining was seen in chromophobe RCC, the patterns were different from those oncocytomas, which showed strong but focal vimentin immunoreactivity.

Other technical issues may contribute to the variation of vimentin immunoreactivity in renal tumors. It is well known that kidney cells including renal epithelial tumors contain a high level of endogenous biotin-binding proteins. Therefore, nonspecific staining using a biotin-
based immunostaining detection system may be interpreted as positive if endogenous biotin-binding activity in renal tumors is not sufficiently blocked. Therefore, the results from biotin-based staining in renal tissues would preferably be verified by using a biotin-free method. To exclude the possibility of false-positive vimentin staining in oncocytes, we compared 2 staining methods and found that vimentin immunoreactivity in oncocytes is not affected by using either of the staining methods used in this study (Table 1).

Furthermore, high-throughput TMAs are widely used to evaluate protein expression in tissues. Our study demonstrates the validation of TMA specimens in evaluating vimentin expression, although TMA specimens typically show lower sensitivity (43%) compared with that using large conventional sections (75%) when staining is focal. On the other hand, it is more difficult to be certain whether the positivity in cells represents a focal or diffuse process. Although computer-aided automatic imaging systems are believed to provide a more objective analysis, they are also operator-dependent. As demonstrated in Table 3, unlike chromophobe RCC or papillary RCC, which will be negative or positive for vimentin immunostaining, respectively, regardless of the areas of selection, oncocytes could be either positive (1A and 2A in Table 1) or negative (1B and 2B in Table 1) for vimentin depending on the areas of selection. Therefore, the results obtained using these automatic imaging analysis systems, which could also be subjective, should be interpreted with caution.

Our results indicate that focal vimentin positivity does not rule out a diagnosis of renal oncocyto, particularly when one is evaluating a small needle core biopsy of a renal mass. It is also important to note that vimentin immunoreactivity patterns in oncocytes are different from those observed in RCC, with the exception of chromophobe RCC. Other markers or additional tissue may be necessary for a definitive diagnosis. The immunohistochemical application of vimentin may be a supportive tool in distinguishing chromophobe RCC from other RCCs with the combination of other antibodies including E-cadherin, N-cadherin, or antimitochondrial antibody 113-1.30

It is important to realize the clinical implication of focal vimentin positivity in oncocyto. Currently, there is an increasing number of preoperative needle core biopsies of renal masses, because a therapeutic decision is often based on the pathologic classification of the renal mass. A diagnosis of oncocyto or RCC may lead to totally different approaches and management plans to the patient. In the case of needle core biopsy, limited material containing strong vimentin-positive cells may lead to a misdiagnosis of RCC in a patient with renal oncocyto. Several options can be considered in such an uncertain situation: (1) morphologic features as the key for distinction; (2) vimentin staining patterns for clear cell RCC (diffuse), papillary RCC (diffuse), and chromophobe RCC (negative); and (3) additional immunostaining using markers for clear cell RCC (CD10, RCC, glutathione S-transferase α), papillary RCC (cytokeratin 7, α-methylacyl CoA racemase), or chromophobe RCC (epithelial membrane antigen, c-Kit, RON, kidney-specific cadherin).31–33 Distinction of oncocyto from chromophobe RCC can still be a challenge because most markers are not highly specific.

In summary, we analyzed vimentin immunoreactivity in 234 renal oncocytes and found that 72.6% of these tumors showed focal vimentin positivity. The vimentin reactivity patterns observed in oncocytes were strong and focal, very different from those of chromophobe (negative or weakly diffuse), clear cell (diffuse and strong), or papillary (diffuse and strong) RCCs. Therefore, when focal vimentin immunoreactivity is encountered in a renal cell neoplasm, oncocyto should be considered in the differential diagnosis, particularly in the case of a limited specimen.

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References


**CAP ’08 ABSTRACT PROGRAM**

Abstract and case study submissions for the upcoming CAP ’08 meeting will be accepted beginning on February 1, 2008 through March 28, 2008. Accepted submissions will be published in the September 2008 issue of the Archives.