Carbonic Anhydrase IX Expression in Renal Neoplasms

Correlation With Tumor Type and Grade

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Abstract

Carbonic anhydrase IX (CAIX), a hypoxia-induced protein, is expressed in some renal tumors. We evaluated its immunohistochemical expression in 317 primary and 42 metastatic renal neoplasms (186 clear cell, 52 papillary, 35 chromophobe, 47 unclassified, and 15 Xp11.2 translocation renal cell carcinomas [RCCs]; 26 oncocytomas; 2 metanephric adenomas; 1urothelial carcinoma; 1 mixed epithelial and stromal tumor; and 1 angiomyolipoma); 7 neoplasms were unknown as to whether they were primary or metastatic. We also correlated expression with tumor type and grade. Variable staining was seen in clear cell, papillary, unclassified, and Xp11.2 translocation carcinomas. One chromophobe carcinoma had focal expression. No staining was seen with other tumors. An association was found between high expression and clear cell vs non-clear cell carcinomas with all cases (P < .01) and primary (P < .01) cases. An association between CAIX expression and grade (P < .01) in primary clear cell carcinomas was found. CAIX expression is more common in clear cell RCC than other renal tumor types and is associated with grade.

Renal cell carcinomas (RCCs) account for the majority (90%) of epithelial neoplasms of the kidney and 3% and 4% of new cancer cases in women and men, respectively.1,2 Of all RCCs, the clear cell subtype is the most common and accounts for the majority of RCC metastases.3 At diagnosis, up to 30% of patients will have metastases, and of patients who undergo nephrectomy for organ-confined disease, metastases will later develop in approximately 30% to 50%.2,4-6 Approximately, 13,000 patients die of the disease each year in the United States.2 The wide spectrum of histologically different tumor types and limited therapeutic options for systemic disease present distinctive challenges to physicians in terms of proper diagnosis and classification of these neoplasms and management of advanced disease.

Renal epithelial neoplasms are unique in that their morphologic features are highly variable in terms of growth pattern and cytologic features, and there is morphologic overlap among the tumor types. Even within individual tumor types, especially clear cell RCC and papillary RCC, the morphologic features of one tumor to the next can be quite different. Nevertheless, routine H&E-stained sections are usually sufficient to correctly classify renal neoplasms. In some circumstances, however, the proper classification of primary renal tumors and distinguishing metastatic RCC from tumors that arise elsewhere can be problematic. Further compounding the issue are core biopsy specimens, which are not infrequently small and fragmented. Biopsies may be performed on tumors at metastatic sites or on renal tumors of patients who are not surgical candidates.

In recent years, multiple immunohistochemical markers have been studied and offered as tools to distinguish...
the various renal neoplasms from each other and from morphologically similar nonrenal tumors. No one marker has been found to be entirely specific for RCC in general or for any specific type of RCC. Carbonic anhydrase IX (CAIX) is one such marker that shows expression in RCC. CAIX is a hypoxia-induced protein that has a role in regulating intracellular and extracellular pH. Liao et al first reported expression of CAIX in clear cell RCC. Several years later, Bui et al found high expression of CAIX in clear cell RCC and, furthermore, reported that decreased levels of expression of CAIX were independently associated with poor outcome in advanced RCC. This latter observation has been refuted by other investigators. Atkins et al, among others, found that CAIX shows promise as a marker for selecting patients with advanced disease who would benefit from certain specific systemic agents, specifically interleukin-2 (IL-2).

We undertook this study to assess the expression of CAIX in a variety of benign and malignant primary renal neoplasms and in RCC metastases. We sought to determine if CAIX could be used as an immunohistochemical marker to reliably distinguish among different tumor types and if its expression correlated with RCC grade.

## Materials and Methods

All research involving human subjects was conducted on anonymized tissues obtained from patients during the course of their therapy. This research was approved by the Dana Farber/Harvard Cancer Center (DF/HCC) and the Beth Israel Deaconess Medical Center (BIDMC) institutional review boards (both Boston, MA). The study group consisted of primary and metastatic renal neoplasms mostly retrieved from the surgical pathology files at 3 institutions, including Brigham and Women’s Hospital, Boston; the BIDMC; and The Johns Hopkins Medical Institutions, Baltimore, MD. In addition, tumors resected at other institutions and stored at the DF/HCC Kidney Center SPORE tumor bank were also analyzed.

The classification of the tumors from Brigham and Women’s Hospital, BIDMC, and the DF/HCC Kidney Cancer SPORE tumor bank was recorded from review of H&E-stained slides by 2 pathologists (E.M.G. and S.S.) or from pathology reports when all slides were not available for review. The Fuhrman grading system was used to grade RCCs. A coinvestigator from The Johns Hopkins Medical Institutions contributed 15 cases of Xp11.2 translocation RCC.

CAIX immunostaining was performed on 1 representative section of tumor from each case in a DAKO autostainer system (DAKO, Carpinteria, CA). Sections were deparaffinized, soaked in alcohol, and, after microwave treatment in antigen unmasking solution for 10 minutes, incubated in 3% hydrogen peroxide for 15 minutes to inactivate endogenous peroxidase. Slides were then incubated with the mouse monoclonal antibody MN-75 (1:10,000 dilution), and detection was performed using the DAKO LSAB+ detection kit (DAKO). Semiquantitative assessment of the antibody staining for each slide was performed by 2 pathologists (E.M.G. and S.S.) who were blinded to the clinicopathologic variables of each case. Each specimen was scored based on the percentage of positive cells. As previously described, specimens in which more than 85% of tumor cells stained for CAIX were labeled as high-CAIX-expressing tumors, whereas those in which 85% or fewer tumor cells stained for CAIX were labeled as low-CAIX-expressing tumors.

Fisher exact tests were used to determine the association between CAIX expression and tumor type and Fuhrman grade. Statistical significance was set at a P value of .05 or less.

## Results

We retrieved 366 cases for analysis. Table I presents the distribution of the cases by tumor type, grade, stage, and CAIX staining. There were 186 cases (50.8%) of clear cell RCC, and the remaining 180 (49.2%) were non–clear cell RCC or were unclassified, unknown, or more than 1 tumor type. The distribution of non–clear cell tumor type was as follows: 52 papillary RCCs, 35 chromophobe RCCs, 47 unclassified RCCs, 15 Xp11.2 translocation RCCs, 26 oncocytomas, 2 metanephric adenomas, 1 urothelial carcinoma,
1 mixed epithelial and stromal tumor, and 1 classic angiomylipoma. There were 317 (86.6%) primary renal tumors, 42 (11.5%) metastatic RCCs, and 7 cases (1.9%) with unknown site (unknown if primary or metastatic tumor). The distribution of the tumor grades (in 237 tumors) was as follows: 13 grade 1, 96 grade 2, 80 grade 3, and 48 grade 4. Immunohistochemical analysis for CAIX was performed on all 366 cases, but only 356 cases were evaluated and classified, using a cutoff score of 85%, into high-expressing (n = 142 [39.9%]) and low-expressing (n = 214 [60.1%]) categories. The remaining 10 cases, which represented biopsy specimens containing limited tumor tissue, were excluded from further analysis.

Variable cytoplasmic membrane immunoreactivity for CAIX was seen in clear cell RCC, papillary RCC, unclassified RCC, and Xp11.2 translocation RCC. Of the cases, 184 clear cell RCCs demonstrated immunoreactivity for CAIX, with most (71.2%) being high-expressing tumors (ie, >85% positive tumor cells). In contrast, high CAIX expression was seen in only 4 (8%) of non–clear cell RCCs (papillary RCCs). The majority (47/51 [92%]) of papillary RCCs also expressed some CAIX; however, they were largely low-expressing tumors (ie, ≤85% positive tumor cells), and 11 Xp11.2 translocation RCCs had focal (low) expression of CAIX. Some but not all of the CAIX positivity in these 2 RCC subtypes was adjacent to areas of tumor necrosis. One chromophobe RCC showed focal weak staining, with the remainder of these tumors being completely negative. While the majority (33/40 [83%]) of unclassified RCCs expressed low CAIX (ie, ≤85% positive tumor cells), 7 tumors demonstrated high levels of CAIX. Among the 33 unclassified RCCs categorized as low CAIX expressors, 21 did not show any CAIX immunoreactivity. CAIX expression was not seen with any other tumor type. The distribution of CAIX+ staining was most often diffuse in clear cell RCCs compared with the other tumor types that expressed CAIX for which the staining was focal or patchy.

Table 2 presents the distribution of tumor types by CAIX expression categories. A statistically significant correlation between CAIX positivity (high vs low) and tumor type (clear cell RCC vs non–clear cell tumors) was found when all cases were analyzed (P < .01). Moreover, a significant association was found between CAIX expression and tumor type when the primary tumors were analyzed separately (P < .01). However, the association between CAIX expression and tumor types did not reach statistical significance when the metastatic cases were analyzed separately (P = 1.00).

Table 4 presents the association between CAIX expression and tumor grade for primary clear cell and primary papillary RCC. There was a significant association between CAIX expression levels and grade of primary clear cell RCC (P < .01) with high CAIX expression in the lower grades

Table 2

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>High Expressors</th>
<th>Low Expressors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear cell RCC (n = 184)</td>
<td>131 (71.2)</td>
<td>53 (28.8)</td>
</tr>
<tr>
<td>Non–clear cell† (n = 132)</td>
<td>4 (3.0)</td>
<td>128 (97.0)</td>
</tr>
<tr>
<td>Primary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clear cell RCC (n = 158)</td>
<td>112 (70.9)</td>
<td>46 (29.1)</td>
</tr>
<tr>
<td>Non–clear cell RCC (n = 131)</td>
<td>3 (2.3)</td>
<td>128 (97.7)</td>
</tr>
<tr>
<td>Metastatic†</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Clear cell RCC (n = 26)</td>
<td>19 (73)</td>
<td>7 (27)</td>
</tr>
<tr>
<td>Non–clear cell RCC (n = 1)</td>
<td>1 (100)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

CAIX, carbonic anhydrase IX; RCC, renal cell carcinoma.
† Data are given as number (percentage). High-expressing tumors, >85% expression of CAIX; low-expressing tumors, ≤85% expression of CAIX.
‡ Fisher exact test.
‡ Includes oncocytoma, metanephric adenoma, urothelial carcinoma, mixed epithelial and stromal tumor, and classic angiomylipoma.

Table 3

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>High</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clear cell RCC (n = 184)</td>
<td>131</td>
<td>53</td>
</tr>
<tr>
<td>Non–clear cell† (n = 132)</td>
<td>4</td>
<td>128</td>
</tr>
<tr>
<td>Primary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clear cell RCC (n = 158)</td>
<td>112</td>
<td>46</td>
</tr>
<tr>
<td>Non–clear cell RCC (n = 131)</td>
<td>3</td>
<td>128</td>
</tr>
<tr>
<td>Metastatic†</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Clear cell RCC (n = 26)</td>
<td>19</td>
<td>7</td>
</tr>
<tr>
<td>Non–clear cell RCC (n = 1)</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

CAIX, carbonic anhydrase IX; RCC, renal cell carcinoma.
† Data are given as number (percentage). High-expressing tumors, >85% expression of CAIX; low-expressing tumors, ≤85% expression of CAIX.
‡ Fisher exact test.
‡ Non–clear cell tumors: papillary RCC, chromophobe RCC, oncocytoma, metanephric adenoma, urothelial carcinoma, mixed epithelial and stromal tumor, and classic angiomylipoma.

Discussion

The lack of immunohistochemical markers with fairly high specificity for RCC in general, as well as its specific subtypes, and the lack of effective treatment for systemic disease continue to be diagnostic and therapeutic issues. RCCs as a group are unique in that the morphologic features of the tumors are highly variable, and cytogenetic studies have found that many of the tumors have distinctive profiles. As such, it would seem improbable that 1 marker could be used to define all types of RCC. Similarly, finding 1 therapeutic agent
**Table 4**

Association of CAIX Expression With Tumor Grade of Primary Clear Cell and Papillary RCC

<table>
<thead>
<tr>
<th>Fuhrman Grade</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAIX Expression</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary clear cell RCC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤85% (low)</td>
<td>1/12 (8)</td>
<td>10/65 (15)</td>
<td>10/41 (24)</td>
<td>21/36 (58)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>&gt;85% (high)</td>
<td>11/12 (92)</td>
<td>55/65 (85)</td>
<td>31/41 (76)</td>
<td>15/36 (42)</td>
<td></td>
</tr>
<tr>
<td>Primary papillary RCC</td>
<td>0 (0)</td>
<td>18/19 (95)</td>
<td>25/26 (96)</td>
<td>3/4 (75)</td>
<td>.28</td>
</tr>
<tr>
<td>≤85% (low)</td>
<td>0 (0)</td>
<td>1/19 (5)</td>
<td>1/26 (4)</td>
<td>1/4 (25)</td>
<td></td>
</tr>
<tr>
<td>&gt;85% (high)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CAIX, carbonic anhydrase IX; RCC, renal cell carcinoma.

* Data are given as the number/total (percentage) of cases. CAIX expression is the percentage of tumor cells positive for CAIX.

† Fisher exact test.

**Image 1** Low-grade clear cell renal cell carcinoma predominantly constituted by cells with clear cytoplasm (A, H&E, ×10). CAIX expression is detected in the vast majority of tumor cells (B, ×10).

**Image 2** High-grade clear cell renal cell carcinoma predominantly constituted by cells with eosinophilic cytoplasm (A, H&E, ×4). CAIX expression is detected in a small percentage of tumor cells (B, ×4).
that can target all varieties of RCC seems unlikely. As such, as biomarkers for the various RCCs are identified and new classifications of RCC are proposed, it becomes even more crucial to properly classify these tumors.

Multiple immunohistochemical markers, including epithelial membrane antigen, vimentin, C-kit (CD 117), cytokeratin 7, CD10, RCC, TFE-3, p504S (racemase), peanut lectin, ulex lectin, and PAX-2 have been identified as markers to assist in classifying various benign and malignant renal neoplasms. While no one marker is typically used alone to define a neoplasm, when used in combination as a panel of antibodies, markers can often help in classifying diagnostically challenging neoplasms. CAIX, a hypoxia-induced protein, is another marker with reported expression in RCC, predominantly in the clear cell type. Indeed, most (approximately 60%-80%) clear cell RCC cases are characterized by biallelic inactivation of the von Hippel-Lindau gene, which leads to stabilization of the α subunit of hypoxia inducible factor and subsequent induction of various target genes, including CAIX. It is important to note that it has also been shown that CAIX may not only have diagnostic usefulness, but also may have a role in the treatment of patients with advanced metastatic disease and be a predictor of outcome. Several published reports have looked at the expression of CAIX principally in clear cell RCC with a few more recent reports describing CAIX immunoreactivity in different types of renal neoplasms.

In this study, we evaluated the expression of CAIX in 356 primary and metastatic renal tumors and correlated it with tumor type and grade (of RCC). Biomarker expression can be affected not only by the antibody used, but also by the area of tumor sampled. Given the variable morphologic features that can be present within any individual renal neoplasm, expression of molecular markers could also potentially be variable throughout a tumor. The smaller the piece of tissue used for analysis, such as core biopsy specimens or tissue microarrays, the less likely a truly representative section of tumor will be studied. Ideally, although not practical, several areas of a tumor could be sampled. In an effort to evaluate more representative tumor samples, in the present study CAIX expression was assessed only on whole tissue sections of tumors, and core biopsy specimens were excluded from analysis.

We found that clear cell RCCs, papillary RCCs, unclassified RCCs, and Xp11.2 translocation RCCs all had some degree of cytoplasmic membrane immunoreactivity for CAIX; however, clear cell RCCs more often and more consistently demonstrated high (>85%) expression than any other tumor type. For clear cell RCCs, the association with high CAIX expression was not only limited to the tumor type, but also correlated with the grade of the neoplasm. As the grade of clear cell RCC increases, the expression of CAIX decreases.

Papillary RCCs and Xp11.2 translocation RCCs were similar in that immunoreactivity for CAIX, when present, tended to be focal or patchy with overall low expression levels (<85%). Only 1 chromophobe RCC demonstrated focal positivity for CAIX, but the staining intensity was very weak. Our results for clear cell RCC, papillary RCC, chromophobe RCC, and oncocytomas seem to be similar to those of a recently published study by Gupta et al, who also evaluated the expression of CAIX in a variety of renal tumors. We found a higher percentage of Xp11.2 translocation RCCs had focal or patchy immunoreactivity for CAIX than in the study by Gupta et al; however, the level of expression was low. The majority of unclassified tumors in our study showed low levels of expression.
CAIX expression (i.e., ≥85% positive tumor cells), although 7 cases showed high expression.

Because CAIX is not specific for renal malignancies, having also been found in carcinomas of the lung, breast, cervix, uterus, colon, and esophagus, it is not useful as a solitary marker for determining site of tumor origin. In routine tissue sections, CAIX would be useful in distinguishing clear cell RCC from chromophobe RCC and oncocytoma. CAIX in combination with other immunohistochemical markers, including cytokeratin 7, p504s (racemase), and 34βE12, could assist in distinguishing between clear cell RCC and papillary RCC in routine sections, given that CAIX expression in papillary RCC is not as diffuse as in clear cell RCC. However, with core biopsy specimens, this distinction may be more difficult to make because the CAIX positivity in a core biopsy specimen of papillary RCC may appear diffuse. While CAIX reactivity occurs in Xp11.2 translocation RCCs, these tumors seem to be low expressors of CAIX and, furthermore, typically do not have diffuse reactivity with epithelial membrane antigen or cytokeratin, which allows distinction from clear cell and papillary RCC. These translocation RCCs show reactivity with TFE-3; the problem with this latter marker is that test results are more technically difficult to perform, and the results may be difficult to interpret in suboptimally fixed tissue. In the series by Gupta et al., strong staining was found in the majority of urothelial carcinomas; the number of urothelial carcinomas in our study is insufficient for comparison. Nevertheless, based on their results, CAIX is not useful for distinguishing clear cell RCC from urothelial carcinoma.

The need to classify renal tumors as accurately as possible is important for several reasons. One of the most important reasons is that the biologic behavior of RCC is variable from one subtype to another, and, therefore, how a tumor is classified will provide information regarding the patient’s potential clinical course. Furthermore, as new classifications of RCC are proposed and markers for RCC in general, or its subtypes are found, the particular subtype that is diagnosed may determine what therapy the patient will receive. CAIX is one such biomarker that is highly expressed in clear cell RCC and potentially may be used to guide patient treatment.

Currently, the mainstay of therapy for primary RCC is surgery. For patients with metastatic disease, treatment with targeted agents has recently shown some success, but, unfortunately, not all tumors respond and responses are not durable. To date, the only therapy for advanced RCC that can result in long-term disease-free survival is high-dose IL-2. Unfortunately, long-lasting responses are very rare, and high-dose IL-2 causes significant side effects. More recently, interest has been generated in selecting specific patients who would benefit from IL-2 treatment.

Bui et al. reported that high CAIX staining was associated with a better prognosis for patients with metastatic disease at diagnosis, and patients with localized high-stage, high-grade disease had better survival than similar patients with low CAIX staining. The authors concluded that CAIX expression was independently associated with prognosis in advanced RCC. Furthermore, they found that among patients with metastases who received IL-2, the response rate to IL-2 therapy was higher (27%) in patients with the CAIX high-expressing tumors than in patients with the CAIX low-expressing tumors (14%). In a case-control study by Atkins et al., patient response to IL-2 was also found to be associated with CAIX expression. Specifically, high CAIX expression in tumors was higher in the IL-2–responding patients (78%) than in the IL-2–nonresponding patients (51%), and patients with high-CAIX-expressing tumors had better survival than with low-expressing tumors. Our study supports other observations that high CAIX expression is much more common in clear cell RCC. Additionally, we found that CAIX expression was associated with the grade of clear cell RCC. While an analysis of our subset of cases is needed in terms of response to IL-2, our data suggest that, as a group, patients with lower grade clear cell RCC might respond better to IL-2 than patients with higher grade tumors. The high expression of CAIX in clear cell RCC also raises the possibility that CAIX-targeted therapy could be developed against these tumors. However, given that CAIX is found in some nonneoplastic tissues as well, carefully controlled studies would be needed.

CAIX is most often expressed and typically highly expressed in clear cell RCCs compared with other RCC subtypes. This marker seems to be diagnostically useful in distinguishing clear cell RCCs from chromophobe RCCs and oncocytomas and potentially useful, when combined with other markers, in distinguishing clear cell RCCs from papillary RCCs. CAIX is expressed in high-grade clear cell RCC and urothelial carcinoma and, therefore, is not useful for distinguishing between these tumors. Given that CAIX is expressed in other epithelial malignancies, it is not useful for determining a tumor’s site of origin.
Anatomic Pathology / Original Article

References