Claudin-7 Immunohistochemistry in Renal Tumors
A Candidate Marker for Chromophobe Renal Cell Carcinoma Identified by Gene Expression Profiling

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The differential diagnosis of chromophobe renal cell carcinoma (RCC) and renal oncocytoma can be difficult by light microscopy. In particular, chromophobe renal cell carcinoma (RCC) is difficult to distinguish from oncocytoma. This differential diagnosis is important because chromophobe RCC is malignant, whereas oncocytoma is benign. Furthermore, chromophobe RCC has distinct malignant potential and prognosis compared with eosinophilic variants of other RCC subtypes. Immunohistochemistry is useful for distinguishing chromophobe RCC from other subtypes of renal carcinoma, but no expression marker reliably separates chromophobe RCC from oncocytoma.

Objective.—In a previous gene expression microarray analysis of renal tumor subtypes, we found the distal nephron markers claudin-7 and claudin-8 to be overexpressed in chromophobe RCC versus oncocytoma and other tumor subtypes. We have confirmed similar findings in independent microarray data and validated differential claudin-7 protein expression by immunohistochemistry.

Design.—Immunohistochemical analysis of claudin-7 in 36 chromophobe RCCs, 43 oncocytomas, 42 clear cell RCCs, and 29 papillary RCCs.

Results.—Membranous claudin-7 expression was detected in 67% chromophobe RCCs, compared with 0% clear cell RCCs, 28% papillary RCCs, and 26% oncocytomas (P < .001).

Conclusions.—Based on microarray and immunohistochemical data, we propose claudin-7 to be a candidate expression marker for distinguishing chromophobe RCC from other renal tumor subtypes, including the morphologically similar oncocytoma. The clinical utility of claudin-7 should be validated in independent studies of renal tumors, possibly in combination with additional targets in a multiplex immunohistochemical panel.

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oncocytoma. However, the former subtype did appear to overexpress a small number of gene products.7 Among these, claudin-7 and claudin-8 were considered candidate markers for chromophobe RCC,22,23 Claudin family proteins are localized in epithelial tight junctions and are dysregulated in a variety of carcinomas.24–26 Claudin-7 and claudin-8 are expressed normally in distal nephron epithelium,22,23 whereas chromophobe RCC and oncocytoma overexpress several distal nephron markers,7,8,18 possibly indicating their relationship to intercalated cells of the cortical collecting duct.27,28 In the current study, we validated microarray data for claudin-7 at the protein level, using a commercial antibody suitable for immunohistochemistry on formalin-fixed, paraffin-embedded tissue.

MATERIALS AND METHODS

Experimental Specimens

Immunohistochemistry was performed on deidentified, formalin-fixed, paraffin-embedded tumors from the pathology departments of Emory University (Atlanta, Ga) and Loyola University (Maywood, Ill). In total, 150 renal tumor specimens were analyzed in this study (36 chromophobe RCCs, 43 oncocytomas, 42 clear cell RCCs, 29 papillary RCCs). Specimens included tissue sections of 1 cm² area and 5 μm thickness (29 chromophobe RCCs, 36 oncocytomas, 9 clear cell RCCs, 10 papillary RCCs), as well as tissue microarray samples described in our previous study (7 chromophobe RCCs, 7 oncocytomas, 33 clear cell RCCs, 19 papillary RCCs).7 Tumors were diagnosed by light microscopy using published histopathologic criteria consistent with the World Health Organization classification system.2–4 Ancillary methods such as cytogenetics, fluorescence in situ hybridization, or Hale colloidal iron were not used for diagnosis. This human subjects research was approved by the Emory University institutional review board.

Microarray Data Analysis

We obtained claudin-7 expression data from our previously published microarray study and used the Oncomine Cancer Profiling Database to extract claudin-7 results from an independent microarray analysis.20 Normalized relative expression of claudin-7 was compared in the samples of chromophobe RCC and oncocytoma. Our previous study included 4 chromophobe RCCs and 5 oncocytomas, whereas Higgins et al used cDNA microarrays. In each study, claudin-7 was overexpressed in the samples of chromophobe RCC versus oncocytoma. CHR indicates chromophobe renal cell carcinoma; ONC, oncocytoma.

RESULTS

In our previous microarray analysis of renal tumors, claudin-7 mRNA was overexpressed in chromophobe RCC, compared with oncocytoma, clear cell RCC, papillary RCC, and angiomyolipoma.7 Focusing only on data from the former 2 subtypes, the 4 cases of chromophobe RCC had above-average expression (log₂[relative expression] ranging from 1.6 to 3.9), whereas the 3 cases of oncocytoma had below-average expression (log₂[relative expression] ranging from −0.3 to −0.9). Using analytical tools in the Oncomine Cancer Profiling Database,29 we identified similar trends in normalized data from an independent cDNA microarray study,20 with log₂[relative expression] in 3 cases of chromophobe RCC ranging from 0.8 to 2.0 and in 2 cases of oncocytoma from −1.0 to −1.1 (Figure 1). Statistical significance could not be established in either microarray study because of limited case numbers.

In the current immunohistochemical analysis, membranous claudin-7 protein expression was detected in more than 5% of tumor cells in 24 (67%) of 36 chromophobe RCCs, compared with 0 (0%) of 42 clear cell RCCs, 8 (28%) of 29 papillary RCCs, and 10 (23%) of 43 oncocytomas (P < .001, chromophobe RCC vs all other tumors). The chromophobe RCC specimens included 10 of 36 cases with predominantly eosinophilic histopathology (Figure 2, a). The remaining 26 of 36 cases exhibited a predominance of typical pale tumor cells with flocculent cytoplasm and distinct cell borders or contained mixtures of pale and eosinophilic tumor cells (Figure 2, b). Positive cases of chromophobe RCC displayed strong membranous staining for claudin-7 on most tumor cells. Within limits of this analysis, similar frequencies of claudin-7 positivity were observed for eosinophilic or typical chromophobe RCC (Figure 2, a and b). In addition, no correlation was seen between claudin-7 expression and Fuhrman nuclear grade or pathologic stage in this RCC subtype. In contrast to chromophobe RCC, most oncocytoma specimens were uniformly negative, although a minority expressed claudin-7 on a fraction of neoplastic cells (Figure 2, c and d).
Figure 2. Immunohistochemical analysis of claudin-7 in chromophobe renal cell carcinoma (RCC) and oncocytoma. Claudin-7 protein was detected by immunoperoxidase reactions, using diaminobenzidine as the chromogenic peroxidase substrate and hematoxylin as the nuclear counterstain. Representative images are shown for each renal tumor subtype. Claudin-7 was detected in 24 of 36 chromophobe RCCs. Most cases exhibited strong, membranous expression on the majority of tumor cells, in both the eosinophilic and typical pale-staining histopathologic variants. In contrast, membranous claudin-7 expression was detected in only 10 of 43 oncocytomas \( (P < .001) \). Positive oncocytomas tended to be focally positive, whereas most were negative in all tumor cells. a, Chromophobe RCC with eosinophilic tumor cells, positive for claudin-7 expression. b, Chromophobe RCC containing pale-staining tumor cells, positive for claudin-7 expression. c, Oncocytoma with focal claudin-7 expression. d, Oncocytoma negative for claudin-7 expression (immunoperoxidase, original magnifications \( \times200 \)).

Most papillary RCC cases were negative for claudin-7. When positive, expression was localized on the basal membrane of tumor cells at the interface with stromal papillary cores and thus could easily be distinguished from expression patterns in chromophobe RCC (Figure 3, a and b). All clear cell RCC cases were uniformly negative on tumor cells (Figure 3, c). In nonneoplastic kidney, membranous staining was restricted to tubular epithelium that was morphologically consistent with distal nephron (Figure 3, d).

For the specific differential diagnosis between chromophobe RCC and oncocytoma, claudin-7 immunoreactivity was 67% sensitive and 77% specific for chromophobe RCC, with positive and negative predictive values of 71% and 73%, respectively \( (P < .001) \). These predictive values did not change if we adjusted the arbitrary positivity cut-off from reactive in 5% to 30% of tumor cells.

COMMENT

Current trends in the management of renal tumors underscore both the difficulty and significance of diagnostic classification by histopathology. The World Health Organization classification system defines several renal tumor subtypes, which are morphologically heterogeneous and share overlapping microscopic features by hematoxylin-eosin light microscopy. Distinction of these subtypes is important because of differences in clinical behavior and prognosis. The differential diagnosis of malignant chromophobe RCC versus benign oncocytoma is especially problematic because each lesion is characterized by nests of eosinophilic tumor cells containing numerous cytoplasmic mitochondria. Renal tumor classification is further complicated by the growing use of diagnostic biopsies for early- and advanced-stage lesions. With widespread use of abdominal imaging, many renal tumors are now diagnosed incidentally at organ-confined stage and treated with laparoscopic, nephron-sparing surgical procedures. Diagnostic material from these procedures is restricted to small tumor biopsies, which provide limited histopathologic information and are prone to sampling error. Thus, diagnostic biopsies are often insufficient to rule out other renal lesions.
Figure 3. Immunohistochemical analysis of claudin-7 in clear cell renal cell carcinoma (RCC), papillary RCC, and nonneoplastic kidney. Claudin-7 protein was detected by immunoperoxidase reactions, using diaminobenzidine as the chromogenic peroxidase substrate and hematoxylin as the nuclear counterstain. Representative images are shown for each renal tumor subtype. Claudin-7 was detected in 8 of 29 papillary RCCs, with positive cases expressing protein on the basal membrane of tumor cells, at the boundary with papillary cores. However, the majority of papillary RCC cases were negative in all tumor cells. Claudin-7 expression was absent in all 42 clear cell RCCs. In all specimens included in this study, claudin-7 was detected in nonneoplastic tissues adjacent to tumor. Nonneoplastic expression was localized to epithelial cell membranes, in renal tubules morphologically consistent with distal nephron. a, Papillary RCC positive for claudin-7 expression. b, Papillary RCC negative for claudin-7 expression. c, Clear cell RCC negative for claudin-7 expression. d, Nonneoplastic kidney positive for claudin-7 expression in distal tubules (immunoperoxidase, original magnifications ×200).

out malignant potential, potentially preventing conservative management of low-grade lesions such as oncocytoma. Diagnostic biopsies may also be useful to guide debulking nephrectomy or systemic therapy for advanced-stage renal malignancies. These treatments may not be indicated for non-clear cell subtypes of RCC, such as chromophobe carcinoma, which respond poorly to systemic immunomodulatory regimens. However, confirmation of clear cell versus non-clear cell histology is difficult from biopsy material because of overlapping morphology and sampling error.

Immunohistochemical markers are emerging as important tools for renal tumor classification, complementing hematoxylin-eosin histology. To identify novel diagnostic immunomarkers, our group and others have used microarrays to characterize the unique gene expression patterns of renal tumor subtypes. This approach has led to the development of several immunohistochemical assays with clinical utility; for example, glutathione S-transferase α and adipophilin are markers for clear cell RCC. α-methylacyl CoA racemase is specific for papillary RCC, and c-Kit, β-defensin 1, and parvalbumin are markers for chromophobe RCC and oncocytoma. However, no reliable molecular targets have been identified for the critical differential diagnosis of chromophobe RCC versus oncocytoma. Recent immunohistochemical studies indicated that kidney-specific cadherin is overexpressed in chromophobe RCC, whereas RON oncogene product is overexpressed in oncocytoma, but these findings were not confirmed in subsequent analyses. More recently, S100 protein was proposed as a potential marker for oncocytoma warranting further investigation. Also, during preparation of this manuscript, a report was published using oligonucleotide microarrays to identify several candidate gene products expressed differentially between chromophobe RCC and oncocytoma. Among these gene products, claudin-8 and MAL2 were validated by immunohistochemistry as a marker overexpressed in chromo-
phobe RCC versus oncocytoma, a finding supported by our data. Despite this overall progress, identification of specific molecular markers will likely remain challenging because microarray experiments have consistently shown a high degree of concordance in the global expression profiles of chromophobe RCC and oncocytoma.\textsuperscript{7,18-20} These microarray results correlate with other similarities in molecular pathogenesis of these tumor subtypes; for example, both lesions are associated with Birt-Hogg-Dubé inherited tumor susceptibility syndrome,\textsuperscript{42} and dominant chromophobe RCC may arise in the condition of renal oncocytosis.\textsuperscript{43}

This study defines claudin-7 as a candidate immunomarker to distinguish chromophobe RCC from oncocytoma and other renal tumors. Claudin-7, and the related gene product claudin-8, were among the few mRNAs overexpressed in chromophobe RCC versus oncocytoma in our recent oligonucleotide microarray experiments,\textsuperscript{7} consistent with other recent findings.\textsuperscript{44} Claudin-7 and claudin-8 display several homologous domains, shared by claudin family proteins in general;\textsuperscript{45} however, they also exhibit several sequence-specific domains, and the commercial antibody used in our study is validated as specific for claudin-7. Claudin-7 and claudin-8 are expressed normally in distal nephron epithelium,\textsuperscript{22,23} making them strong candidate markers for chromophobe RCC. This tumor subtype (as well as oncocytoma) is related to intercalated cells of the cortical collecting duct,\textsuperscript{27,28} whereas clear cell RCC resembles proximal nephron epithelium.\textsuperscript{45}

Our microarray studies have repeatedly shown that distal nephron markers, such as β-defensin 1, parvalbumin, and chloride channel Kb, are overexpressed in chromophobe RCC and oncocytoma and underexpressed in clear cell RCC.\textsuperscript{7,18} Consistent with these findings, positive claudin-7 immunohistochemistry was very effective at ruling out clear cell RCC, as all cases in our study were completely negative. Most papillary RCC cases were also negative in our series; because of the limited number of positive specimens, we could not assess whether claudin-7 expression correlated with type 1 or type 2 papillary histology.\textsuperscript{2} Although papillary RCC is believed to be related to proximal nephron,\textsuperscript{10} we have shown in previous studies that a significant fraction of cases express distal nephron markers, such as β-defensin 1 and parvalbumin.\textsuperscript{46} Based on our data, we believe claudin-7 may have particular clinical value in distinguishing chromophobe RCC from oncocytoma because these lesions can already be distinguished from the other renal tumor subtypes with existing immunomarkers. In the context of this specific differential diagnosis, we report positive and negative predictive values of 71% and 75%, respectively, for chromophobe RCC using claudin-7 immunohistochemistry, which we would have obtained for a cutoff for positivity anywhere from 5% to 30% reactivity in tumor cells (we arbitrarily chose 5% reactivity as a definition of positive, although additional studies are needed to confirm our findings and establish optimal definitions of positive claudin-7 expression). Our estimates of positive and negative predictive value should be applicable to clinical practice because chromophobe RCC and oncocytoma have comparable prevalence among surgically resected renal tumors.\textsuperscript{7} However, certain limitations of this study suggest that our findings should be confirmed independently as claudin-7 is applied in general clinical settings. For example, we focused on tumors that could be diagnosed by routine histopathology. Future studies will be useful to focus on difficult specimens, such as histologically equivocal eosinophilic renal tumors or small tumor biopsies, and cases with ancillary diagnostic data such as cytogenetics, fluorescence in situ hybridization, or Hale colloidal iron.

Claudin-7 and claudin-8 are also plausible as mediators of neoplastic transformation. Claudin family proteins are key components of epithelial tight junctions and thus are important for processes relevant to carcinoma initiation and progression.\textsuperscript{24-26} For example, claudins are critical for cell-cell adhesion. They maintain cell polarity by preventing diffusion of biomolecules between apical and basolateral layers of epithelial membranes and regulate concentration gradients across epithelial sheets by preventing paracellular solute transport. Furthermore, claudins interact physically with proteins linked to intracellular signal transduction pathways. More than 20 claudin family proteins have been identified, many of which are expressed differentially in normal epithelia and carcinomas from a variety of tissues.\textsuperscript{24-26} Claudin-7 is overexpressed in squamous cell carcinoma of the uterine cervix, as well as in adenocarcinoma of the stomach and esophagus.\textsuperscript{46-48} In contrast, it is underexpressed in ductal carcinoma of the breast.\textsuperscript{49} The significance of claudin expression in cancer pathobiology is uncertain. Overexpression may dysregulate cell signaling, polarity, or adhesion via interactions with other proteins,\textsuperscript{50-52} whereas underexpression may reduce cell adhesion as neoplasms progress to an invasive or metastatic phenotype.\textsuperscript{53} Based on these hypotheses, future experiments should determine whether claudin-7 expression is reduced in metastatic chromophobe RCC.

In summary, claudin-7 is a novel immunohistochemical marker for renal tumor classification. This candidate biomarker was identified in a microarray analysis of renal tumors, which showed the gene product to be overexpressed in chromophobe RCC. Consistent with these data, chromophobe RCC could be readily distinguished from clear cell or papillary RCC by membranous immunoreactivity for claudin-7. Importantly, claudin-7 expression also supported a diagnosis of chromophobe RCC rather than oncocytoma. In our case cohort, diagnostic sensitivity and specificity was limited to 67% and 77%, respectively, for the distinction of these histologically similar tumor subtypes. Therefore, our findings must be confirmed in independent immunohistochemical studies to establish the clinical utility of claudin-7. In addition, it may be necessary to combine claudin-7 in multiplex assays (eg, immunohistochemistry for S100, claudin-8, MAL2, or other novel markers as they are discovered; cytogenetics or Hale colloidal iron when they are practical) to optimize effectiveness in the differential diagnosis of chromophobe RCC and oncocytoma.

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References


