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*Original Article*

# Expression of Claudin-7 in Benign Kidney and Kidney Tumors

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**Abstract**: Claudins, a family of tight junction-related transmembrane proteins, have been implicated in the pathogenesis of various human neoplasms. Expression of claudin-7 was increased in chromophobe renal cell carcinoma in a recent oligonucleotide microarray study. We studied the expression of claudin-7 in benign and neoplastic kidneys by immunohistochemical staining. Distal nephron (distal convoluted tubule and thick ascending limb of Henle's loop) epithelium showed strong membranous staining in 100% (174/174) of the cases. Chromophobe renal cell carcinoma was positive for claudin-7 expression in 100% (36/36) of cases, while papillary renal cell carcinoma, oncocytoma and clear cell renal cell carcinoma were positive in 90% (71/80), 45% (21/47) and 7% (7/98) of the cases, respectively. Differential expression of Claudin-7 in different types of renal cell neoplasms can be useful in their differential diagnosis, particularly when used in a panel of markers. In addition, results from this study support previous reports of distal nephron origin for chromophobe renal cell carcinoma and oncocytoma. The data also suggest that, as far as claudin-7 expression is concerned, papillary renal cell carcinoma may be more closely related to the distal nephron, rather than the proximal nephron.
**Key Words**: claudin-7, kidney, neoplasm, clear cell, chromophobe, papillary, oncocytoma

## Introduction

Renal cell carcinoma (RCC) accounts for 85% of all malignant tumors of the kidney and is the 7th leading malignancy among men and the 12th among women [1]. In the United States, there are around 36,000 new cases and 12,000 deaths each year [1-2]. A quarter of the patients present with advanced disease with local invasion or metastasis. A third of the patients who undergo resection of localized disease will have recurrence. Median survival for patients with metastatic disease is about 13 months [1].

RCC is a heterogeneous group of malignant tumors arising from renal epithelium and their classification has been evolving over the years. The major subtypes of RCC include clear cell RCC, papillary RCC, and chromophobe RCC. Because different subtypes carry different prognoses, accurate classification is of great importance in the management of patients and prognosis. Although classification is usually straightforward, there are overlapping histologic features that make it very difficult to distinguish these tumors with certainty in a significant number of cases. More importantly, a relatively common benign tumor, renal oncocytoma, cannot be easily distinguished from RCCs with eosinophilic cytoplasm, particularly chromophobe RCC, based on histologic features alone [3-8].

Gene expression profiling has identified novel molecular markers that are differentially expressed in different subtypes of renal epithelial neoplasms, including α-methyl acyl CoA racemase [9-10], C-Kit [11-13], E-cadherin [14], parvalbumin [15] and Ron [16]. Another marker, claudin-7, has been found to be overexpressed in chromophobe RCC, but not in oncocytoma [17] by oligonucleotide microarray analysis.

To study the potential utility of claudin-7 in the differential diagnosis of different subtypes of renal epithelial neoplasms, we conducted a comprehensive immunohistochemical study in a large number of cases of benign and malignant renal epithelial tumors as well as normal kidney tissue.

## Material and Methods

*Selection of Cases*

This study was approved by the Research Subject Review Board of the University of Rochester Medical Center. All identifiers were removed to protect patient confidentiality. Two hundred sixty one cases of renal epithelial neoplasms were retrieved from the files of the Department of Pathology of two large tertiary hospitals. Fifty four cases of benign kidney were also used in the study. The histology was reviewed by 2 pathologists independently and each case classified according to the 2004 WHO classification [3]. Papillary RCCs were subclassified as type 1 or type 2 following the reported criteria [18]. The nuclear grade of clear cell RCC was assessed following the Fuhrman grading system [19]. One hundred and nine cases were built into tissue microarray (TMA) with 4 cores from each case. H&E stained slides from each TMA block were reviewed for quality control after construction. Regular histology sections from the remaining 152 cases were stained with the identical method to validate results obtained from the TMA studies.

*Immunohistochemical Study*

Immunohistochemical study was performed on formalin-fixed, paraffin-embedded tissue sections using a mouse monoclonal antibody against Claudin-7 (Zymed/Invitrogen Immunodetection, San Francisco, CA 94080, Cat. 18-7391, used at 1:200 dilution). Paraffin blocks were sectioned at 5 μm thickness and floated on distilled water at 45°C. Sections were mounted on chemically charged slides followed by drying at room temperature until opaque and placed in the oven at 57°C overnight. Sections were deparaffinized according to established procedures and quenched with 3% hydrogen peroxide for 6 minutes. They were then cleared in running water followed by TBS (50 mM Tris-hydrogen chloride, 150 mM sodium chloride, and 0.05% Tween 20 at pH 7.6).

Antigen unmasking was performed by pre-heating the slides at 95-99°C in Dako antigen retrieval solution (EDTA Buffer pH 8.8, Dako, Carpentaria, CA) in a Black and Decker steamer (Shelton, CT, Model HS800) for 30 minutes followed by a 15 minute cool down period. The slides were then rinsed with TBS for 5 minutes. The slides were mounted in the DAKO Autostainer and covered with fresh TBS to prevent drying of sections during mounting. Blocking for Avidin/Biotin was performed according to manufacturer's instruction for 15 minutes (Vector Laboratories, Inc., Burlingame, CA). Sections were washed with TBS and then incubated with anti-Claudin-7 antibody at room temperature for 60 minutes, followed by 30-minute incubation each with horse anti-mouse IgG-Biotin (Vector Laboratories, Inc. Burlingame, Ca.) and Streptavidin-horseradish peroxidase (Jackson Labs). Slides were developed with AEC+ substrate chromogen (Dako, Carpenteria, CA) for 10 minutes, rinsed in running distilled water, counterstained in Modified Mayer's Hematoxylin, blued in 0.3% ammonia water followed by a tap water rinse. Slides were then mounted using an aqueous media and viewed with a light microscope.

Both staining intensity (0 to 3+) and percentage of cells stained were recorded. A case was considered positive for claudin-7 when there was 2+ or higher staining intensity in 10% or more of the cells. Fisher exact test was used for statistical analysis.

## Results

*Expression of Claudin-7 in Non-Neoplastic Kidney*

In all 54 normal kidney tissue cores from TMA and all 120 cases of non-neoplastic kidneys from nephrectomy specimens for renal tumors, positive staining for claudin-7 was present in distal convoluted tubules (DCT), cortical collecting tubules (CCT), and thick ascending limb of the Henle's loop. In these structures, a strong staining in the cytoplasmic membrane was observed (**Figure 1**). In contrast, the proximal convoluted tubules (PCT), thin limb of the Henle's loop, Glomeruli and stromal cells showed negative to minimal staining for claudin-7 (**Figure 1**).

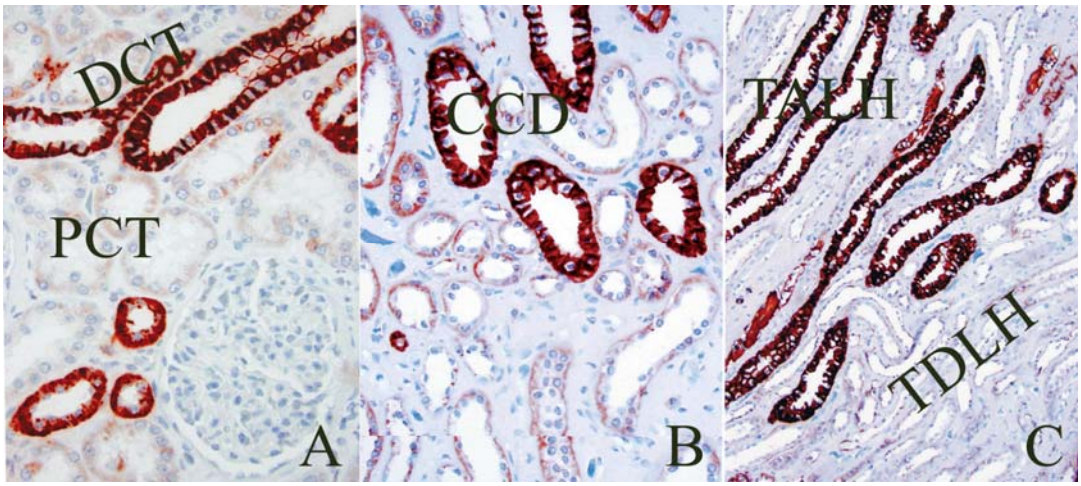


**Figure 1** Immunohistochemical study for the expression of claudin-7 in non-neoplastic kidney tissue. Positive staining is detected in thick ascending limb of Henle's loop (TALH), distal convoluted tubule (DCT), and cortical collecting tubule (CCT); but not in proximal convoluted tubule (PCT) and thin descending limb of Henle's loop (TDLH) (original magnifications x 200).

*Expression of Claudin-7 in Renal Epithelial Neoplasms*

One hundred and nine cases of renal epithelial tumors in TMAs were studied by immunohistochemical staining using an anti-claudin-7 antibody. The results were validated by staining regular histologic sections in another 152 cases. The two sources gave very similar results and thus the cases were combined and summarized in **Table 1**.

Of the 98 cases of clear cell RCC, positive staining for claudin-7 was observed in 7 cases (7%), while the remaining 91 cases (93%) were negative (**Figure 2A** and **Table 1**). Interestingly, the few positive cases were all of lower nuclear grade but overall, we do not believe Fuhrman nuclear grade significantly influences the expression of claudin-7 in these tumors. In addition, there were 5 cases in which clear cell RCC co-existed with another type of renal epithelial neoplasm (4 papillary RCC and 1 oncocytoma). In all 5 cases, staining for claudin-7 was negative in the clear cell RCC component.

**Table 1** Differential expression of claudin-7 in renal epithelial neoplasms

| | Total cases | Positive cases | %Positive |
|---|---|---|---|
| Clear cell RCC | | | |
| Nuclear grade | | | |
| 1 | 4 | 1 | |
| 2 | 77 | 6 | |
| 3 | 10 | 0 | |
| 4 | 7 | 0 | |
| Subtotal | 98 | 7 | 7% |
| Chromophobe RCC | 36 | 36 | 100% |
| Papillary RCC | | | |
| Type-1 | 43 | 41 | |
| Type-2 | 28 | 23 | |
| Type-1+2 | 9 | 8 | |
| Subtotal | 80 | 72 | 90% |
| Oncocytoma | 47 | 21 | 45% |

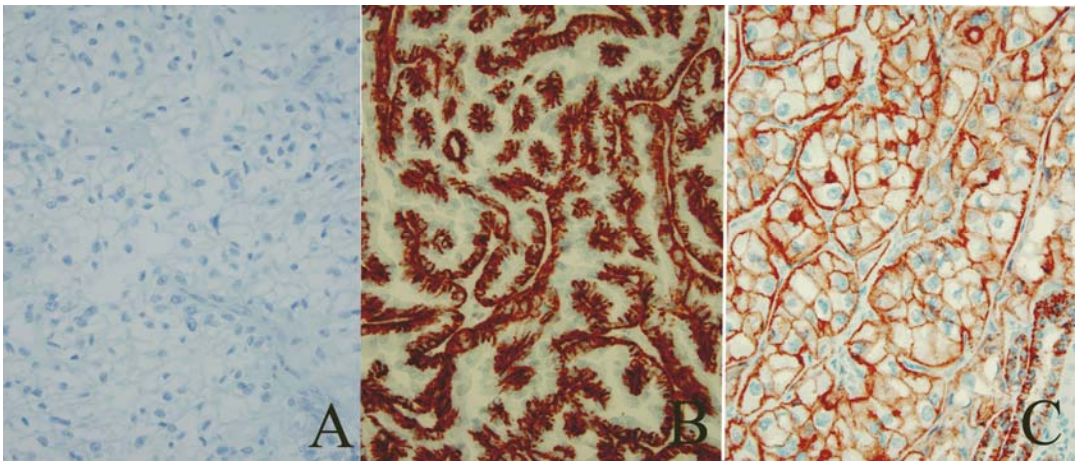


**Figure 2** Immunohistochemical study for claudin-7 expression in renal cell carcinomas. Clear cell renal cell carcinoma does not express claudin-7 (**A**), while papillary renal cell carcinoma (**B**) and chromophobe renal cell carcinoma (**C**) express claudin-7 in a diffuse, strong membranous pattern (original magnifications x 200).

Ninety percent (72/80) of papillary RCC were positive for claudin-7 expression and there was no significant difference in the frequency of claudin-7 expression between the two subtypes (type 1 and type 2) (**Figure 2B** and **Table 1**).

All 36 cases of chromophobe RCC were positive for claudin-7. Similar to the staining pattern of distal convoluted tubules of the non-neoplastic kidney tissue, all tumor cells of chromophobe RCC showed diffuse and strong staining for claudin-7 with a distinct membranous pattern (**Figure 2C**). The staining further accentuated the cytological features of the tumor cells such as distinct cell borders, wrinkled nuclei and perinuclear clearing. Two cases in this group contained areas of sarcomatoid transformation. Interestingly, the sarcomatoid component in both cases stained negatively for claudin-7.

Of the 47 cases of oncocytomas studied, 45% (21/47) were positive for claudin-7 (**Figure 3 and Table 1**) while 55% (26/47) were completely negative. Among the cases that were considered positive, 8 showed diffuse, strong membranous staining (**Figure 3C**) while the remaining 13 cases showed focal staining (**Figure 3B**). The cases with negative claudin-7 staining had a predominantly nested growth pattern and the tumor cells had small, bland nuclei and inconspicuous nucleoli.

Four nephrectomy specimens had coexisting clear cell RCC and papillary RCC in the same kidney and another had synchronous clear cell RCC and oncocytoma. In these cases, clear cell RCC and oncocytoma were negative for claudin-7, while papillary RCC component was strongly positive. Staining for claudin-7 was positive in 2 cases of papillary adenoma, but 2 cases of mucinous tubular and spindle cell carcinoma only showed focal and weak staining.

## Discussion

Claudins are a family of transmembrane proteins that are involved in tight junction formation between epithelial cells [20]. There are at least 24 members in the claudin family and they are expressed in an organ- and tissue-specific manner [21-23]. In the kidney, expression patterns of claudins change with development and renal segments [24-26]. Its deregulation has been associated with polycystic renal disease [27].

Aberrant expression of various claudins has been reported in a variety of human neoplasms. Claudin-2 is overexpressed in gastrointestinal tumors [20, 28] and breast carcinoma [29]. Claudin-4 is up-regulated in pancreatic adenocarcinoma and its precursor lesions [30-31]. Overexpression of claudin-3 and -4 has been reported in prostate and ovarian carcinoma [32-33]. While down-regulation of claudin-7 has been correlated with increased grade and metastasis of breast cancer and squamous cell carcinoma of esophagus [34-36], its upregulation has been

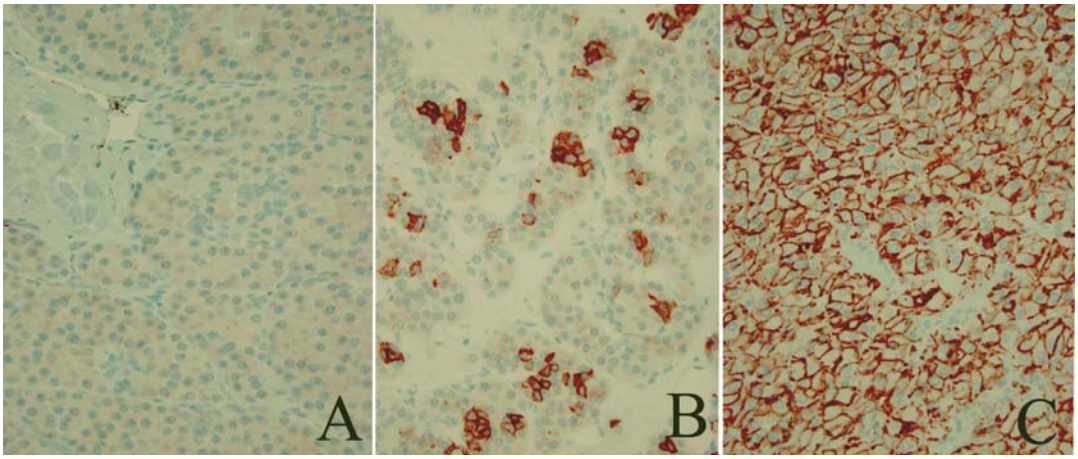


**Figure 3** Immunohistochemical study for claudin-7 expression in renal oncocytomas. These tumors show a full spectrum of staining patterns from negative staining (**A**) to focal scattered positive staining (**B**) to strong, diffuse membranous staining (**C**) (original magnifications x 200).

observed in the progression of cervical neoplasia [37] and early stage of gastric adenocarcinoma [38].

Renal epithelial neoplasms have a spectrum of biologic behavior, ranging from benign (oncocytoma), low-grade malignant (chromophobe RCC) to highly malignant (papillary RCC and clear cell RCC). A definitive subclassification is one of the most important factors in determining management of the patients and prognosis. Unfortunately, the different subtypes of renal epithelial neoplasms have overlapping histologic features, which makes differential diagnosis difficult in certain cases based on morphology alone. For example, eosinophilic cytoplasm can be seen in tumor cells of clear cell RCC, papillary RCC, chromophobe RCC and oncocytoma. Papillary structures can be seen in clear cell RCC as well as papillary RCC. Therefore, great effort has been made in identifying novel markers that may be useful in differential diagnosis. Recently, Schuetz et al [17] applied oligonucleotide microarrays on 31 adult renal tumors and found overexpression of claudin-7 expression in chromophobe RCC, suggesting that claudin-7 may potentially be useful in the differential diagnosis of renal tumors.

In the current study, we applied immunohistochemistry using an anti-claudin-7 monoclonal antibody in a large cohort of 261 cases of renal tumors. Our study showed that all chromophobe RCCs express claudin-7 but less than 50% oncocytomas do. The difference, although not as dramatic as we expected from the oligonucleotide microarray study, was statistically significant. In addition, because of the arbitrary cut-off value adopted in this study, the number of oncocytomas scored as positive for claudin-7 is probably higher than it is. Chromophobe RCCs all show strong and diffuse membranous staining. If we apply this stringent criterion (strong and diffuse membranous staining), many of the oncocytomas with focal staining would have been considered negative and only less than 20% (8/47) have staining pattern similar to that of chromophobe RCCs. Therefore, claudin-7 may be useful in the differential diagnosis of the two tumors. For example, in a difficult case with overlapping features, a negative or focal scattered positive staining for claudin-7 would essentially rule out chromophobe RCC. This marker will be even more useful when it is used in a panel of markers that are expressed with different frequencies between the two tumors.

Certain chromophobe RCCs may have clear cytoplasm while some clear cell RCCs, particularly high grade ones, have granular cytoplasm. Distinction between these two entities can be very difficult in some cases. Colloidal iron has been proposed as a specific stain for chromophobe RCC but in reality, due to the difficulty in performing and interpreting this stain, it is hardly used in daily practice. Our study has demonstrated that all chromophobe RCCs are positive for claudin-7; in contrast, clear cell RCC are rarely positive for claudin-7. Therefore, an immunohistochemical study using claudin-7 may aid in the differential diagnosis of these two tumors in difficult cases.

Because the majority of papillary RCCs are positive for claudin-7 while the majority of clear cell RCC are negative, claudin-7 may be a useful marker in distinguishing clear cell RCC with papillary features from papillary RCC, particularly type 2 papillary RCC. Similarly it may be useful to distinguish papillary RCC with focally clear cytoplasm from clear cell RCC.

The results from our study may also shed light on the origin of different types of renal epithelial tumors. We have demonstrated that in benign kidney, claudin-7 is expressed in distal nephron, which includes thick ascending limb of Henle's loop, distal convoluted tubule and portion of cortical collecting tubule [39-41]. This observation is consistent with previous reports [26, 42]. Traditionally, chromophobe RCC and oncocytoma are believed to arise from the intercalating cells of distal nephron but papillary RCC and clear cell RCC are considered to arise from the proximal nephron [43-44]. Our results showed that the majority of chromophobe RCCs and papillary RCCs express claudin-7 while only rare cases of clear cell RCC express this marker. This observation suggests that, as far as the expression of claudin-7 is concerned, papillary RCC seems to be more closely related to the distal nephron, rather than the proximal nephron. Additional studies are required to determine the significance of this surprising finding.

In conclusion, claudin-7 is highly expressed in distal nephron of the kidney and can be readily detected immunohistochemically. Their differential expression among different types of renal cell neoplasms may be useful in the differential diagnosis of difficult cases.

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