Claudin expression and tight junction protein localization in the lining epithelium of the keratocystic odontogenic tumors, dentigerous cysts, and radicular cysts

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Objective. The aim of this study was to evaluate the expression and localization of tight junction proteins (TJPs) or claudins in the keratocystic odontogenic tumor (KCOT) and to correlate with its biological behavior.

Study Design. Five claudins (-1, -3, -4, -5, and 7) were examined immunohistochemically in 25 KCOTs and compared with 10 dentigerous cysts (DCs) and 10 radicular cysts (RCs).

Results. Marked claudin-3 loss of expression in KCOT basal layer (n = 24/25; 96%) compared with DCs (n = 1/10; 10%) and RCs (n = 5/10; 50%) (P < .05) suggests that claudin-3 downregulation may indicate altered or loss of basal cell polarity and impaired barrier function of KCOT lining epithelium and this might contribute indirectly to its biological behavior. In contrast, claudins-1, -4, -5, and -7 distribution patterns were less distinctive in all three entities, suggesting that these TJP molecules probably play limited roles in influencing their different growth potentials.

Conclusion. Present findings suggest that differential claudin expressions in the lining epithelium of KCOTs, DCs, and RCs probably reflect their neoplastic or nonneoplastic nature. (Oral Surg Oral Med Oral Pathol Oral Radiol 2013;115:652-659)

Claudins, the major component of tight junctions, consist of a 24-member family of integral transmembrane proteins, which are essential for structural tissue integrity and barrier function of epithelia and endothelia.1 These protein molecules were first purified and identified in 1998 by two Japanese researchers, Mikio Furuse and Shoichiro Tsukita.1,2 The name claudin comes from the Latin word “claudere,” which means “to close,” thus highlighting the barrier role of these proteins. Claudins are believed to play regulatory roles in the electrical resistance and paracellular ionic selectivity of both epithelia and endothelia.3 Their expression and distribution may vary with tissue types and sites.4

The keratocystic odontogenic tumor (KCOT) is defined by the World Health Organization (WHO) as a benign uni- and multicystic, intraosseous tumor of odontogenic origin, with a characteristic lining of parakeratinized stratified squamous epithelium and a potential for aggressive, infiltrative behavior.5 This term is believed to reflect better the neoplastic nature of this lesion. In this new classification, cystic lesions lined by orthokeratinized epithelium do not form part of the spectrum of KCOT.6,7 On the other hand, dentigerous cysts (DCs) and radicular cysts (RCs) represent nonneoplastic odontogenic cysts with an innocuous behavior and seldom recur after surgical removal.8 Although KCOT presents with a cystic structure similar to DC and RC, the evolution of these lesions and their biological behaviors are distinct.8,9

In disease, aberrant claudin activity has been implicated in the biological behavior of a variety of tumors.10 Selective gain or loss of expression for claudin purportedly influences disease outcome in various cancer types.11-13 In the jaws, claudins-1, -4, -5, and -7 distribution in the developing human14 and rat15 tooth germs has been investigated. Altered expressions of these molecules have also been characterized in ameloblastomas and ameloblastic carcinomas.14 However, to date, the immunophenotypic features of these tight junction proteins in the KCOT remain unclarified. The aim here was to determine their distribution patterns in the lining epithelium of KCOTs and to compare with the lining epithelium of DCs and RCs, taking into

Statement of Clinical Relevance

Immunophenotypic characterization of claudin proteins in KCOT lining epithelium is of clinical relevance because (i) claudin might serve as a potential prognostic marker and (ii) claudin data might be useful in designing molecular targeted therapies.
consideration their differential expression in the constitutive layers of the lining epithelium (basal, intermediate, and surface zones) and to relate these findings to their biological behaviors.

MATERIALS AND METHODS

Samples
Twenty-five cases of KCOTs, 10 cases each of DC and RC were obtained from the archives of the Department of Oral Pathology, Oral Medicine and Periodontology, Faculty of Dentistry, University of Malaya, Kuala Lumpur, Malaysia. Cases were reviewed by a qualified oral pathologist (C.H.S.) and selected according to the criteria of the WHO Histological Classification of Tumours. Patient characteristics, namely age at presentation, gender, ethnicity, anatomic location, treatment, and case summaries were recorded.

From the archival formalin-fixed, paraffin-embedded tissue blocks of these cases, new 5-μm thick sections were prepared for staining with hematoxylin–eosin and for immunohistochemistry.

Immunohistochemistry
Five commercially available antibodies; namely rabbit polyclonal antibody to claudin-1 (AB15098), claudin-4 (AB15104), claudin-5 (AB53765), and claudin-7 (AB27487), (Abcam, Inc., Cambridge, MA, USA), and goat monoclonal antibody to claudin-3 (AB15102), claudin-1, claudin-4 (Abcam, Inc.) were obtained.

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The Envision technique was used. Briefly, deparaffinized sections of 5 μm thickness were pretreated for antigen retrieval by microwave (99°C) in 10 mM of citrate buffer (pH 6, 20 min). These sections were then immersed in 0.3% methanol containing 3% hydrogen peroxide for 20 min to block endogenous peroxidase and rinsed in 0.05 M Tris-buffered saline (5 min, 2 times) before immersing in blocking solution (Dako Corporation, Carpinteria, CA, USA) for 20 min at room temperature. The sections were then incubated with the following optimally diluted primary antibodies: claudin-1 [1:100], claudin-3 [1:50], claudin-4 [1:200], claudin-5 [1:200], and claudin-7 [1:400] for 30 min at room temperature. Immunoreactions were performed using the Envision Kit (Dako Corporation).

The level of expression of claudins-1, -3, -4, -5, and -7 was quantified according to the percentage of immunoreactive epithelial cells present: (−) negative when none of the epithelial cells were positively stained in the cytoplasm or membrane; (+), <25% cells positive; (++), 25%–50% cells positive; and (+++), >50% cells positive. The percentage of stained cells was subjectively scored in relation to the whole field of view. Discordant results were reviewed by both investigators and a consensus was reached. Areas of inflamed epithelium were avoided.

Immunohistochemical analysis
Claudin distribution patterns and levels of staining in all KCOTs, RCs, and DCs were evaluated using descriptive and semiquantitative methods. In the latter, analysis of the immunohistochemical reactions in the epithelial lining cells of KCOTs, DCs, and RCs was carried out by dividing their constitutive layers into basal, intermediate, and surface zones. Digitized images (Olyvia DotSlide Virtual Slide System; Olympus Imaging, Inc., Tokyo, Japan) of all tissue sections were reviewed by two investigators and representative sections were selected. From these representative sections, 5 hot spots or fields in each epithelial layer were randomly selected at ×200 magnification. The level of expression for claudins-1, -3, -4, -5, and -7 was quantified according to the percentage of immunoreactive epithelial cells present: (−) negative when none of the epithelial cells were positively stained in the cytoplasm or membrane; (+), <25% cells positive; (++), 25%–50% cells positive; and (+++), >50% cells positive. The percentage of stained cells was subjectively scored in relation to the whole field of view by two independent investigators who were calibrated. Discordant results were reviewed by both investigators and a consensus was reached. Areas of inflamed epithelium were avoided.

Statistical analysis
Differences in the percentages of cases with different immunoreactivity levels were analyzed by the Fisher exact or Mann–Whitney U test for differences between two groups or the Kruskal–Wallis test for differences among ≥3 groups. Statistical level of significance was set at P < .05.

RESULTS

Patients’ characteristics
The demographic and clinical information of patients with KCOT, RC, and DC are summarized in Table I.

Table I. Patients’ characteristics

<table>
<thead>
<tr>
<th>Findings</th>
<th>KCOT</th>
<th>Dentigerous cyst</th>
<th>Radicular cyst</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (N)</td>
<td>25 cases</td>
<td>10 cases</td>
<td>10 cases</td>
</tr>
<tr>
<td>Gender, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>10 (40.0)</td>
<td>7 (70.0)</td>
<td>5 (50.0)</td>
</tr>
<tr>
<td>Female</td>
<td>15 (60.0)</td>
<td>3 (30.0)</td>
<td>5 (50.0)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>28.9</td>
<td>39.6</td>
<td>38.7</td>
</tr>
<tr>
<td>Range</td>
<td>11-52</td>
<td>17-67</td>
<td>27-62</td>
</tr>
<tr>
<td>Ethnicity, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malays</td>
<td>7 (28.0)</td>
<td>2 (20.0)</td>
<td>1 (10.0)</td>
</tr>
<tr>
<td>Chinese</td>
<td>8 (32.0)</td>
<td>6 (60.0)</td>
<td>9 (90.0)</td>
</tr>
<tr>
<td>Indians</td>
<td>9 (36.0)</td>
<td>2 (20.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Others</td>
<td>1 (4.0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Locations, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maxilla</td>
<td>5 (20.0)</td>
<td>4 (40.0)</td>
<td>8 (80.0)</td>
</tr>
<tr>
<td>Mandible</td>
<td>19 (76.0)</td>
<td>6 (60.0)</td>
<td>2 (20.0)</td>
</tr>
<tr>
<td>Maxilla and mandible</td>
<td>1 (4.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Briefly, in KCOT patients, the most common presenting complaint was swelling (n = 17/25; 68%) followed by pain (n = 10/25; 40%) and pus discharge (n = 2/25; 8%). Most cases (n = 19/25; 76%) were preoperatively diagnosed as most likely representing KCOT or ameloblastoma (n = 6/25; 24%). The treatment of choice was surgical enucleation. In DC patients, an impacted or missing tooth was the most common presenting complaint (n = 8/10; 80%). Six cases were associated with the crowns of impacted mandibular third molars, 3 with impacted maxillary canines, and 1 with a supernumerary tooth. All cases were treated by surgical enucleation of the cyst and removal of the impacted tooth. In RC patients, the salient complaint was swelling (n = 5/10; 50%) and pus discharge (n = 2/10; 20%) and nonvital tooth associated with a periapical radiolucency (n = 10/10; 100%). Surgical enucleation of cyst with apicectomy and retrograde root filling of the affected tooth were the standard procedures carried out. No follow-up records were available to determine their recurrence rates.

### Immunohistochemical findings

Results on immunostaining and semiquantitative analysis of claudins are detailed in Table II and illustrated in Figures 1-3.

**KCOTs (n = 25).** Claudin-1 was strongly expressed in the lining epithelium of all 25 KCOT cases. A polarized distribution pattern was generally observed where claudin-1 was preferentially expressed either in the surface (Figure 1A and B) and/or intermediate zone (Figure 1C and D) of the lining epithelium. Protein localization was circumferential membranous producing a honeycomb appearance (Figure 1D) and/or cytoplasmic. This honeycomb pattern extended variably into the basal layer (Figure 1C). Intracellular granular spotty claudin-1 positivity in both parabasal and basal epithelial cells was also observed (Figure 1B).

Staining for claudin-3 was negative in all epithelial layers of the 10 KCOT cases (40%). Loss of expression for claudin-3 was observed in the basal epithelial layer (Figure 1E and F) of nearly all KCOT cases (n = 24/25; 96%). In the remaining KCOT cases that expressed claudin-3, immunoreactivity was generally weak and focal and mostly seen in the surface or intermediate zones.

Claudin-4 was detected in all KCOT cases. Its positive reaction was concentrated along the superficial epithelial layers, reducing gradually in intensity toward the basal cell layer (Figure 1G). As with claudin-1, a honeycomb pattern of expression and cytoplasmic granular deposits were variably identified (Figure 1H). Claudin-4—positive staining of the nucleus was also occasionally seen (Figure 1H). On the other hand, claudin-5 was nonreactive in all epithelial layers of 4 KCOTs (16%). In the positive, a gradual decrease in the intensity of expression from the surface toward the basal layer was generally observed. Claudin-5 protein localization was circumferential membranous, producing a honeycomb appearance (Figure 1I). In the adjacent connective tissue wall, the vascular endothelium also stained positive for claudin-5 proteins.

Claudin-7 was detected in all KCOT cases. Its positive reaction was concentrated along the superficial epithelial layers, reducing gradually in intensity toward the basal cell layer (Figure 1G). As with claudin-1, a honeycomb pattern of expression and cytoplasmic granular deposits were variably identified (Figure 1H). Claudin-4—positive staining of the nucleus was also occasionally seen (Figure 1H). On the other hand, claudin-5 was nonreactive in all epithelial layers of 4 KCOTs (16%). In the positive, a gradual decrease in the intensity of expression from the surface toward the basal layer was generally observed. Claudin-5 protein localization was circumferential membranous, producing a honeycomb appearance (Figure 1I). In the adjacent connective tissue wall, the vascular endothelium also stained positive for claudin-5 proteins.

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### Table II. Distribution of claudins-1, -3, -4, -5, and -7 in KCOTs and in dentigerous and radicular cysts

<table>
<thead>
<tr>
<th>Variables</th>
<th>Claudin-1</th>
<th>Claudin-3</th>
<th>Claudin-4</th>
<th>Claudin-5</th>
<th>Claudin-7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3+ 2+ 1+ 0</td>
<td>3+ 2+ 1+ 0</td>
<td>3+ 2+ 1+ 0</td>
<td>3+ 2+ 1+ 0</td>
<td>3+ 2+ 1+ 0</td>
</tr>
<tr>
<td><strong>KCOT (n = 25)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface zone</td>
<td>2 4 16 3 0</td>
<td>0 11 14 5 0</td>
<td>0 11 14 5 0</td>
<td>0 11 14 5 0</td>
<td>0 11 14 5 0</td>
</tr>
<tr>
<td>Intermediate zone</td>
<td>12 6 6 1 0</td>
<td>1 14 10 19 2</td>
<td>4 0 0 4 0</td>
<td>1 5 19 2 6</td>
<td>10 7 6 4 1</td>
</tr>
<tr>
<td>Basal zone</td>
<td>2 1 8 14 0</td>
<td>0 0 1 24 0</td>
<td>0 5 5 15 0</td>
<td>1 1 5 19 2</td>
<td>6 10 7 5 1</td>
</tr>
<tr>
<td><strong>Dentigerous cyst (n = 10)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface zone</td>
<td>4 3 3 0 2</td>
<td>1 7 0 0 3</td>
<td>7 0 1 6 3</td>
<td>0 2 2 6 0</td>
<td>4 0 0 5 5</td>
</tr>
<tr>
<td>Intermediate zone</td>
<td>4 3 3 0 2</td>
<td>1 7 0 0 3</td>
<td>6 1 1 6 2</td>
<td>1 2 2 6 0</td>
<td>0 0 0 5 5</td>
</tr>
<tr>
<td>Basal zone</td>
<td>4 2 4 0 2</td>
<td>1 6 1 0 0</td>
<td>1 1 5 9 0</td>
<td>0 0 6 4 0</td>
<td>0 5 5 5 0</td>
</tr>
<tr>
<td><strong>Radicular cyst (n = 10)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface zone</td>
<td>6 3 1 0 0</td>
<td>0 3 5 2 4</td>
<td>2 0 0 4 0</td>
<td>1 5 3 2 1</td>
<td>7 0 6 1 0</td>
</tr>
<tr>
<td>Intermediate zone</td>
<td>6 3 1 0 0</td>
<td>0 3 5 2 4</td>
<td>0 2 0 4 0</td>
<td>1 4 2 1 6</td>
<td>1 0 0 5 5</td>
</tr>
<tr>
<td>Basal zone</td>
<td>4 3 3 0 0</td>
<td>0 0 5 5 0</td>
<td>0 1 9 0 1</td>
<td>0 0 0 9 0</td>
<td>5 5 0 5 0</td>
</tr>
</tbody>
</table>

**KCOT**, keratocystic odontogenic tumor; 0, negative staining; 1+, mild staining; 2+, moderate staining; 3+, strong staining.
Claudin-3 was detected in all cases of DCs and 8 RCs (80%). Their expression levels were heterogeneous in both cysts (Figure 2C and D and Figure 3B). Protein localization occurred predominantly as intracytoplasmic granular deposits. Loss of expression for claudin-3 along the basal cell layer was detected in a case of DC (10%) and 5 RCs (50%).

The remaining 3 claudins (-4, -5, and -7) (Figure 2E-L and Figure 3C-F) were more frequently detected in the upper two-thirds of the lining epithelium (Table II). Protein localization was membranous and/or cytoplasmic. Their immunoreactions presented as membranous annular positivity and/or intracytoplasmic spotty granules. Vascular endothelium in the cystic connective tissue wall stained positively for claudin-5.

**Statistical analysis**

Mean expression scores for claudin-3 were lower than claudins-1 and -4 in KCOT ($P < .05$). Comparison of KCOT with DCs and RCs revealed that the mean expression scores for claudin-3 among these groups were significantly different ($P < .05$). For the other claudin members, no significant differences were found between the groups.

**DISCUSSION**

In recent years, considerable interest has been focused on characterizing the immunophenotypic features of KCOT specifically to elucidate the mechanisms involved in the biological behavior of this lesion. Emerging evidence implicates intraepithelial rich perlecan deposits, imbalance of receptor activator of nuclear factor $\kappa$B (RANK), receptor activator of nuclear factor $\kappa$B ligand (RANKL), and osteoprotegerin (OPG), interaction between collagen IV, matrix metalloproteinase (MMP)-9, tissue inhibitor of metalloproteinase (TIMP)-2, overexpression of osteopontin, CD44v6, podoplanin, cyclooxygenase (COX)-2, p53, Ki-67, and upregulation of angiogenesis as important factors contributing toward the neoplastic potential of KCOT. On the other hand, aside from the 2 previous studies that separately examined claudins-1, -4, -5, and -7 expression in developing human and rat tooth germs, ameloblastoma and ameloblastic carcinoma, a search of the English language literature revealed that the immunoprofile of the tight junction proteins and their impact on the biological nature of KCOT remains largely unknown. We sought to clarify this by...
examining the cellular localization of 5 claudin proteins (-1, -3, -4, -5, and -7) in KCOT and comparing these findings with DCs and RCs.

The most significant observation in this study was distinct loss of expression for claudin-3 along the basal cell layer in almost all KCOT cases compared with DCs and RCs. In addition, 10 KCOTs were completely claudin-3 negative. The implications of this finding are unclear. Moreover, the role of this tight junction protein during normal odontogenesis has not been elucidated. The role of altered cell–cell junction as a factor influencing the growth pattern of KCOT is only partially known. Significant downregulation of E-cadherin, a key adherens junction molecule, has been implicated in KCOT local aggressiveness.24 Accordingly, E-cadherin loss of expression occurred most frequently in the basal layer of KCOT lining epithelium. On the other hand, claudin-3 belongs to the 24-member family of tight junction proteins crucial for the maintenance of cell polarity and establishment of barrier function in normal epithelium.3,4 It is likely that underexpression of this molecule in the lining epithelium of KCOT may indicate disruption of these tight junction proteins resulting in altered or loss of cellular polarity and impaired paracellular barrier function. It is known that tight junction structural and functional deficiencies may induce leakiness in the epithelium and this could enhance access of growth factors and nutrients, thereby promoting cellular growth and survival.25 In contrast, the lining epithelium of all DCs and 8 RCs demonstrated positive expression for this protein molecule indicating intact claudin-3 tight junctions. These differences in findings suggest that altered claudin-3 expression might play a role in influencing their growth potentials. It is, however, not clear whether the claudin-3–low phenotype is specific for KCOT or is seen in other odontogenic epithelial neoplasms as well.

Claudin-1, the backbone of tight junctions, not only functions to maintain paracellular activity and cellular polarity but also is purportedly involved in recruiting
cell signaling proteins. During odontogenesis, claudin-1 is strongly expressed in the inner and outer enamel epithelium, stratum intermedium, ameloblasts, and newly formed enamel matrix in the developing human teeth at the late bell stage. In the present study, all KCOTs were claudin-1 positive. A polarized distribution pattern in the keratinous zones of the lining epithelium was observed. Strong claudin-1 expression may represent an attempt to maintain cell–cell attachments at the sites of cystic degeneration. However, increased claudin-1 expression can reduce permeability of these tight junctions. Unlike KCOT, claudin-1 was uniformly distributed in the lining epithelium of DCs and RCs. Claudin-1 localization in the cytoplasm and membrane corresponded with the anticlaudin polyclonal antibody directed against the intracellular carboxyl terminal epitope and the extracellular loops of this protein molecule, respectively.

Claudin-4 is a less well-known member of the claudin family. During tooth development, claudin-4 is weakly expressed in the outer enamel epithelium and stellate reticulum of developing human teeth at the late bell stage. In the present study, all 25 KCOT cases were claudin-4 positive with an enhanced expression along the surface keratinous layers. Although the exact physiological role of claudin-4 is unclear, it is likely that overexpressed claudins, including claudin-4, are associated with abnormal tight junction function. The mechanism of claudin-4 overexpression is unclear, but reports suggest that the methylation status of claudins appears to be important. Unlike claudin-1, claudin-4 expression pattern was similar between the lining epithelium of KCOT, DCs and RCs.

At the late bell stage, claudin-5 is only detected in the vascular structures of the dental follicle and papilla. In the present study, 20% of KCOTs were claudin-5 negative. These observations suggest that claudin-5 is an uncommon tight junction protein in the developing tooth germ and their neoplastic counterparts. In the remaining KCOT cases, claudin-5 demonstrated mild to moderate coexpression with claudin-4, exhibiting the same gradual decrease in staining intensity from the surface toward the basal layer. This same distribution pattern was also encountered in DCs and RCs. It is generally known that more than 2 types of claudins can be present in individual epithelial cells. In the present study, the vascular endothelium of KCOTs, DCs, and RCs stained positively for claudin-5 and not for claudins-1, -3, -4, and -7. This observation concurred with the well-established fact that claudin-5 is the only known claudin member expressed by endothelial cells.

During tooth development, claudin-7 is strongly expressed in the inner and outer enamel epithelium, stratum intermedium, ameloblasts, and newly formed enamel matrix but is absent in odontoblasts in the developing human teeth at the late bell stage. In the present study, the expression levels for claudin-7 in
the various epithelial layers and their protein localization patterns differed between KCOT and DC and RC. These findings suggest that differences in subcellular localization of this claudin-7 may exist between the lining epithelium of KCOT and those of DCs and RCs. It was further observed that Rushton hyaline bodies present within the lining epithelium of a case of KCOT stained positively for claudin-7. This similarity in expression pattern with the lining epithelium of KCOT suggests that the latter may play a role in the formation of these bodies and that positive claudin-7 expression may indicate that these bodies still retain lining epithelium—related attribute.

In contrast to KCOT, the neoplastic potential of the lining epithelium of DCs and RCs has not been well investigated. Long-standing inflammation and keratinization of cyst lining have been alluded to as risk factors that may potentiate malignant change in odontogenic cysts. Future study comparing claudin expression profile in DCs and RCs with or without in situ neoplastic transformation may help to clarify what role, if any, the claudins may play in neoplastic transformation of these cysts.

In summary, the differential expression of claudins-1, -3, -4, -5, and -7 in KCOT, DCs, and RCs was investigated. In general, the different claudin composition observed in all 3 cystic entities may be attributed to the differentiation stage/phenotype of cells in the constitutive layers of their lining epithelia. The observed differences in expression and localization of these claudins among these lesions may contribute to differences in paracellular transport and permeability to growth factors and nutrients and indirectly contribute to different growth patterns. In specific, altered claudin-3 function may be a distinctive feature of KCOT lining epithelium and may contribute indirectly to its biological behavior. On the other hand, in DCs and RCs, differences in expression of claudins-1, -3, -4, -5, and -7 did not allow their respective lining epithelia to be characterized by their specific pattern of claudin expression. Finally, other roles of claudins, for example, recruiting signaling proteins essential for the regulation of proliferation, differentiation, and other cellular functions in these cysts was not assessed in this immunohistochemical study.

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REFERENCES


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