Significance of podoplanin expression in keratocystic odontogenic tumor

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BACKGROUND: The most important clinical features of the keratocystic odontogenic tumor (KCOT) are its potential for locally destructive behavior, a tendency to recur, and its origin in the odontogenic epithelium. The clinical features of KCOT are similar to those of ameloblastoma (AM). Histologically, KCOT is distinguished from jaw cyst with keratinization (orthokeratinized odontogenic cyst; OOC). However, current scientifically based clinical parameters cannot predict any potential for neoplastic behavior, or aggressive and localized invasiveness, in patients with KCOT. We have shown that podoplanin, a lymphatic endothelial marker, is highly expressed in AM. The purpose of this study was to determine the usefulness of podoplanin for reclassification of the odontogenic keratocyst (OKC) from cyst to tumor status.

METHODS: Paraffin-embedded tissue specimens of 57 OKCs (46 KCOTs and 11 OOCs) and 15 dentigerous cysts (DCs) were immunohistochemically examined using antibody against podoplanin.

RESULTS: Immunohistochemical reactivity for podoplanin was detected in the cell membrane and cytoplasm of most of the basal and suprabasal layer, areas of budding basal cell proliferation, epithelial nests and peripheral cells of daughter cysts in the stromal connective tissue in KCOTs. In the case of OOC and DC, only cases associated with inflammation were positive for podoplanin.

CONCLUSION: Podoplanin is strongly expressed in KCOTs in comparison with OOCs. The pattern of staining for podoplanin in KCOT could be related to its neoplastic nature, and suggests a role of the protein in tumor invasiveness.

Keywords: immunohistochemistry; keratocystic odontogenic tumor; orthokeratinized odontogenic cyst; podoplanin

Introduction

In the new World Health Organization (WHO) classification (1), the odontogenic keratocyst (OKC), originally described by Philipsen in 1956 (2), is categorized as a benign odontogenic tumor under the name of keratocystic odontogenic tumor (KCOT) (2). KCOT is defined 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 (1). The WHO recommends the term KCOT, as it better reflects its neoplastic nature (1). By contrast, a cystic lesion in which the whole lining epithelium shows orthokeratinization is referred to as orthokeratinized odontogenic cyst (OOC) and should be separated from KCOT because it rarely shows recurrence (1, 3). However, evidence-based current clinical parameters cannot predict the potential for neoplastic behavior, or aggressive and localized infiltration, in OOC and KCOT. The problem seems to lie in the fact that there is no suitable immunohistochemical marker that can be used to evaluate both odontogenic cysts and tumors.

Podoplanin, a transmembrane glycoprotein, is expressed specifically by lymphatic endothelial cells (4, 5). Recent studies have shown that the protein is expressed in a variety of normal as well as neoplastic tissues, and that its expression might be related to cell migration and invasion (6–8).

In this study, we examined whether podoplanin expression is correlated with a neoplastic character and is a useful parameter for assessment of odontogenic tumors.

Materials and methods

Paraffin-embedded tissue specimens of 57 cases previously diagnosed as OKC, and 15 dentigerous cysts (DCs) were examined immunohistochemically using a primary antibody against podoplanin (D2–40). The study protocol was reviewed and approved by the Research Ethics Committee of Meikai University School of Dentistry (A0832). The 57 cases were re-diagnosed as...
46 cases of KCOT (Fig. 1A), including recurrence of two cases, and 11 cases of OOC (Fig. 1B).

**Collection of samples**
The tissue samples of 46 KCOTs, 11 OOCs, and 15 DCs were obtained from the files of the Division of Pathology, Department of Diagnosis and Therapeutic Sciences, Meikai University School of Dentistry, and matching data for patient age, gender and lesion site were obtained from information submitted with the surgery request forms.

**Immunohistochemistry**
Each sample of KCOT, OOC, and DC embedded in paraffin wax was sectioned and mounted on glass microscope slides. Deparaffinized sections were immersed in absolute methanol containing 0.3% H$_2$O$_2$ for 15 min at room temperature to block endogenous peroxidase activity. After washing, the sections were immersed in 0.01 M citrate buffer, pH 6.0, and heated in a microwave oven for 5 min at high voltage and then for 15 min at low voltage. An appropriately diluted mouse monoclonal anti-human D2–40 (anti-podoplanin) antibody (Dako North America, Inc., Carpinteria, CA, USA) was applied to the sections for 1 h at room temperature, followed by a pre-diluted anti-mouse IgG antibody conjugated with peroxidase (Nichirei, Tokyo, Japan) for 1 h at room temperature. The sections were immersed for 8 min in 0.05% 3,3'-diaminobenzidine tetrahydrochloride (DAB) in 0.05 M Tris–HCl buffer (pH 8.5) containing 0.01% H$_2$O$_2$, and then counterstained with Mayer’s hematoxylin. Immunohistochemical reactivity for podoplanin was evaluated and classified into three groups: (−) negative, (+) weakly to moderately positive, and (++) strongly positive.

**Results**
A positive immunoreaction product for podoplanin was found in lymphatic endothelial cells in the submucosal connective tissue of mucosal epithelium (Fig. 2). Immunohistochemical results for KCOTs, OOCs, and DCs are summarized in Table 1.

Expression of podoplanin in KCOTs was strongly positive in the cell membrane and cytoplasm of most of the cells in the basal and suprabasal layers (Fig. 3A), areas of budding basal cell proliferation and epithelial

![Figure 1](image1.png) (A) Hematoxylin and eosin (HE) staining of KCOT. (B) HE staining of OOC.

![Figure 2](image2.png) Expression of podoplanin in the normal oral mucosa. A positive immunoreaction for podoplanin was found in lymphatic endothelial cells in the connective tissue. No reactivity for podoplanin was found in the mucosal epithelium.

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<th>Podoplanin</th>
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<tr>
<td>n (%)</td>
<td>(−) (+) (++)</td>
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<tr>
<td>KCOT</td>
<td>46 (100)</td>
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<td>OOC</td>
<td>11 (100)</td>
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<td>DC</td>
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Immunohistochemical reactivity: (−) negative, (+) weakly to moderately positive, (++) strongly positive. Values in parentheses are in per cent.

*Association with inflammatory cell infiltration in stromal connective tissue.
nests (Fig. 3B, C). Slight immunoreactivity for podoplanin was also found in peripheral cells of daughter cysts (Fig. 3D, E). Four of the KCOTs were negative for podoplanin.

Most of the OOCs were podoplanin-negative (Fig. 4A); however, the basal cells of OOCs showed weakly positive immunoreactivity when inflammatory cell infiltration was present in the connective tissue under the lining epithelium (Fig. 4B). In the case of DC, strong positivity for podoplanin was observed in the basal layers associated with a severe inflammatory reaction in the connective tissue (Fig. 5A), and also

Figure 3  Expression of podoplanin in the KCOT. (A) Strong expression of podoplanin was observed in the basal cells. Areas of (B) budding basal cell proliferation and (C) epithelial nests were also strongly positive. (D and enlargement E) Some peripheral cells of daughter cyst were weakly positive.
weak positivity was observed when a mild to moderate inflammatory reaction was present. Four of the DCs without an inflammatory reaction were entirely negative for podoplanin in the basal cells (Fig. 5B).

**Discussion**

Human podoplanin (T1α-2, aggrus or gp36) is a 38-kDa type-1 transmembrane sialomucin-like glycoprotein consisting of 162 amino acids, nine of which form the intracellular domain. Although the protein has been considered as a specific marker for lymphatic endothelial cells (4, 5, 9–11), its expression has also been demonstrated in various normal as well as neoplastic cells (12–16).

In normal human tissues and cells, in addition to the lymphatic endothelium, podoplanin expression has been detected in basal epithelial keratinocytes of the skin, cervix, esophagus, peritoneal mesothelial cells, osteocytes, ependymal cells, stromal reticular cells, and follicular dendritic cells of lymphoid organs (4, 5). In human neoplasms, podoplanin expression has been reported in squamous cell carcinoma (SCC) of the skin, dysgerminoma and granulose cell tumor of the ovary (5). In addition, podoplanin is reportedly associated with tumor-induced platelet aggregation and tumor metastasis (17). In oral lesions, podoplanin expression has been demonstrated in oral leukoplakia, a premalignant lesion, and its expression may serve as a marker for predicting the risk of oral cancer (18). As all these studies have detected increased expression of podoplanin in various malignant tumors, its potential role in tumor progression has been suggested.

Our present findings demonstrated that the expression of podoplanin was evidently higher in KCOTs than in OOCs, probably because KCOT has more of a neoplastic character, with progression and local invasiveness. Similarly, we have previously reported that podoplanin is expressed in limited myoepithelial elements of pleomorphic adenomas (19) and in peripheral tumor cells of ameloblastoma (AM) (20). Although pleomorphic adenoma is a common benign epithelial tumor of the salivary glands, it has a tendency to invade surrounding
tissues. Furthermore, AM is also a benign odontogenic tumor showing local invasion and recurrence. Interestingly, positive immunoreactivity for podoplanin in OOCs and DCs was found in the basal cell layer when inflammatory changes were present in the connective tissue. This phenomenon can also be seen in the inflamed gingiva (21). In the case of DC and OOC, immunoreaction for podoplanin was negative or weak in some areas of lining epithelium; while lymphatic vessels showed strong positivity for the antibody in the same lesions. Of interest, areas with inflammatory changes showed increased expression of podoplanin in the basal layer compared with areas where inflammatory changes were absent. A previous study conducted in our laboratory showed positivity for podoplanin in inflamed gingival tissue samples; however, the reason of why podoplanin expression is enhanced in the presence of inflammation remains unknown. Schacht et al. have suggested that podoplanin might play an important role in mediating cellular contractile properties and cytoskeletal reorganization (5). Importantly, it has been reported that SCCs are positive for podoplanin, and that there is an inverse relationship between its expression and tumor differentiation (5, 12–16). Podoplanin-expressing cells have been found at the invasive tumor front in human SCCs. It has been reported that podoplanin increases the activities of Rho GTPases, mainly RhoA, contributing to cytoskeletal reorganization, suggesting an important role of podoplanin in tumor invasion and metastasis (6–8).

Enhanced expression of podoplanin was especially evident in the cell membrane and cytoplasm of most of the cells in the basal and suprabasal layers, areas of budding basal cell proliferation, epithelial nests, and peripheral cells of daughter cysts in the connective tissue of KCOTs. On the other hand, OOC showed negative reaction for podoplanin in the absence of inflammation. Considering the clinical features of KCOT, including its potential for local destruction or recurrence, it seems natural to conclude that it is a cystic tumor.

To our knowledge, this is the first report that showed the difference in podoplanin expression pattern between cystic tumor and developmental cyst by immunohistochemistry. Our results suggest the possible contribution of podoplanin in the local invasiveness and the neoplastic nature of the KCOT.

References