**Immunohistochemical Expression of Integrins and CD44 in Ameloblastomas**

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To clarify the role of cell adhesion molecules in epithelial odontogenic tumors, expression of integrin $\alpha_2$, $\alpha_3$ and $\beta_4$ subunits and the standard form of CD44 was immunohistochemically examined in 22 ameloblastomas as well as in 1 clear cell odontogenic tumor (CCOT). Integrin $\alpha_2$ and $\alpha_3$ subunits were expressed on the cell membrane of peripheral columnar or cuboidal cells in ameloblastomas. Most tumor cells in basal cell ameloblastomas and the CCOT were positive for these subunits. These features suggest that CCOT possesses characteristics similar to those of peripheral cells in main type ameloblastoma or neoplastic cells in basal cell ameloblastoma. Integrin $\beta_4$ subunit was found along the basement membrane in ameloblastomas and the CCOT. The expression of these representative epithelial integrins at the epithelial-mesenchymal interfaces suggests that they might mediate parenchymal-stromal cell interactions in epithelial odontogenic tumors. CD44 was detected in most tumor cells of ameloblastomas and the CCOT, but keratinizing areas in acanthomatous ameloblastomas showed decreased CD44 expression. These features suggest that CD44 is involved not only in cell adhesion but also in cellular differentiation in epithelial odontogenic tumors.

Key words: ameloblastoma, CD44, clear cell odontogenic tumor, immunohistochemistry, integrin

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**Introduction**

Odontogenic epithelium is responsible for tooth development under physiologic conditions and can give rise to tumors or cysts in the jaws (1-3). Ameloblastoma is the most frequently encountered tumor arising from odontogenic epithelium and is characterized by a benign but locally invasive behavior with a high tendency to recur (2, 4). Histologically, ameloblastoma shows considerable variation, including follicular, plexiform, acanthomatous, granular cell, basal cell and desmoplastic types (4). Clear cell odontogenic tumor (CCOT) is an unusual intraosseous tumor histologically characterized by odontogenic epithelial cells with clear vacuolated cytoplasm (4, 5). Although the 1992 World Health Organization (WHO) histological classification of odontogenic tumors newly classified CCOT as a benign tumor of the epithelial odontogenic apparatus (4), this tumor has been reported to include both benign and malignant varieties (6). These epithelial odontogenic tumors histologically resemble the epithelial odontogenic apparatus, such as enamel organ or dental lamina, in some respects; however, the detailed mechanisms of oncogenesis and cytodifferentiation remain unknown.

Cell-to-cell adhesion is indispensable for the regulation of cellular behavior, and a number of cell adhesion molecules mediating many kinds of adhesion have been identified (7, 8). Integrins are a family of cell surface glycoproteins that mediate cell-cell and cell-extracellular matrix adhesion (9, 10). Each integrin is a heterodimer comprising two non-covalently linked subunits, $\alpha$ and $\beta$ (7, 10). There are at least 18 $\alpha$ subunits and 8 $\beta$ subunits, and more than 20 different combinations have been identified and classified into several subfamilies sharing a common $\beta$ subunit (10). Integrins can bind multiple ligands, such as different types of collagens, fibronectins and laminins, and play an important role in regulating cellular interactions during growth, development and differentiation (7, 10-13). Changes in integrin expression have been found in many neoplastic tissues and are implicated in tumor cell invasion and metastasis (11, 14-17). CD44 is a family of cell surface glycoproteins that play a role in cell-cell and cell-extracellular matrix adhesion and interactions (18, 19). The CD44 gene contains at least 20 exons (19). The standard form of CD44 (CD44s)
is encoded by a sequence composed of exons 1 through 5 and exons 15 through 20, while variant isoforms of CD44 (CD44v) arise from alternative splicing of combinations of exons 6 through 14 into the polypeptide backbone of CD44s (19-21). CD44 functions as a principle receptor for hyaluronic acid, a major glycosaminoglycan of the extracellular matrix (18, 19). Altered CD44 expression, especially that of CD44v, has been detected in various neoplasms, and CD44 is thought to play a part in tumor cell differentiation, progression and metastasis (20-25). Recently, expression of integrins and CD44 has been studied in tooth germ tissues, and these molecules might have specific roles in cell interactions during tooth development (26-31).

Our previous study revealed expression of an epithelial cell adhesion molecule, E-cadherin, and its associated protein, β-catenin, in epithelial odontogenic tumors, suggesting that these molecules are associated with cytodifferentiation or malignancy of transformed odontogenic epithelium (32). In the present study, immunohistochemical expression of representative integrins expressed in epithelial cells, including integrin α2 (CD49b), α3 (CD49c) and β4 (CD104) subunits, and CD44s was examined in ameloblastoma as well as in CCOT to clarify the role of these cell adhesion molecules in epithelial odontogenic tumors.

Materials and methods

Tissue preparation

Specimens were surgically removed from 23 patients with epithelial odontogenic tumors at the Department of Oral and Maxillofacial Surgery, Tohoku University Dental Hospital. The tumors were divided into several parts. The first part was fixed in 10% buffered formalin for 1 to several days and embedded in paraffin. The tissue blocks were sliced and stained with hematoxylin and eosin for histological diagnosis according to the WHO histological typing of odontogenic tumors (4). A total of 22 ameloblastomas and 1 CCOT were studied. The ameloblastomas comprised 13 follicular and 9 plexiform types, including 8 acanthomatous (6 follicular and 2 plexiform types) and 2 basal cell (1 follicular and 1 plexiform types) subtypes. The second part of each tumor was embedded in Tissue-Tek OCT Compound (Miles Laboratories, Elkhart, IN, USA), quick-frozen in a mixture of acetone and dry ice, and stored at -80˚C until immunohistochemical examination.

Immunohistochemistry

Serial cryostat sections 6 μm thick were obtained from each frozen block. The sections were fixed in cold acetone for 10 min and washed in cold phosphate-buffered saline (PBS). After treatment with normal rabbit serum for 30 min, the sections were incubated with primary antibodies at 4˚C overnight. The applied antibodies are listed in Table 1. The standard streptavidin-biotin-peroxidase complex method was performed to bind the primary antibodies with the use of a Histofine SAB-PO kit (Nichirei, Tokyo, Japan). Reaction products were visualized by immersing the sections in 0.03% diaminobenzidine solution containing 2 mM hydrogen peroxide for 1 to 3 min. Nuclei were lightly counterstained with methylgreen. For control studies of the antibodies, the serial sections were treated with PBS and mouse antidesmin monoclonal antibody (Nichirei; subclass IgG1) instead of the primary antibodies and were confirmed to be unstained.

Results

Immunohistochemical reactivity for integrin α2 and α3 subunits was detected in pericellular distribution around the tumor cells in ameloblastomas and the CCOT (Fig. 1 & 2). Follicular and plexiform ameloblastomas showed positive reactions for integrin α2 and α3 subunits in peripheral columnar or cuboidal cells, but not in central polyhedral or keratinizing cells (Fig. 1a & 2a). No distinct difference in reactivity was seen between these two main types of ameloblastomas. In basal cell ameloblastomas and the CCOT, most tumor cells were positive for integrin α2 and α3 subunits (Fig. 1b & 2b). Expression of integrin β4 subunit was found in linear distribution at the interface between the epithelial parenchyma and connective tissue stroma in ameloblastomas and the CCOT (Fig. 3). There was no distinct difference in reactivity for integrin β4 subunit among the variants of ameloblastomas.

Immunohistochemical reactivity for CD44 was detected in pericellular distribution around the tumor cells in ameloblastomas and the CCOT (Fig. 4). Follicular and plexiform ameloblastomas showed positive reactions for CD44 in peripheral columnar or cuboidal cells and central polyhedral cells (Fig. 4a). No distinct difference in reactivity was seen between these two main types. Expression of CD44 was low in keratinizing cells in acanthomatous ameloblastomas (Fig. 4b). In basal cell

Table 1: Monoclonal antibodies used

<table>
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<th>Antibody</th>
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<td>DF1485 (mouse IgG1)</td>
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Fig. 1: Immunohistochemical reactivity for integrin \( \alpha_2 \) subunit. (a) Follicular ameloblastoma showing reactivity on the cell membrane of peripheral cuboidal cells. (190) (b) Basal cell ameloblastoma showing reactivity on the cell membrane of most tumor cells. (170)

Fig. 2: Immunohistochemical reactivity for integrin \( \alpha_3 \) subunit. (a) Follicular ameloblastoma showing reactivity on the cell membrane of peripheral columnar cells. (135) (b) Clear cell odontogenic tumor showing reactivity on the cell membrane of most tumor cells. (135)

Fig. 3: Immunohistochemical reactivity for integrin \( \beta_4 \) subunit in plexiform ameloblastoma (a) and clear cell odontogenic tumor (b). Reactivity was found along the basement membrane zone. (a: 100, b: 160)

Fig. 4: Immunohistochemical reactivity for CD44. (a) Acanthomatous ameloblastoma showing reactivity on the cell membrane of most tumor cells and reduced reactivity in a keratinizing area. (220) (b) Clear cell odontogenic tumor showing reactivity on the cell membrane of most tumor cells. (210)
ameloblastomas and the CCOT, most tumor cells were positive for CD44. Expression patterns of these integrins and CD44 in ameloblastomas and the CCOT are summarized in Table 2.

**Discussion**

Integrin $\alpha_2$ and $\alpha_3$ subunits are combined with $\beta_1$ subunit to form integrin $\alpha_2\beta_1$ (very late antigen (VLA)-2) and integrin $\alpha_3\beta_1$ (VLA-3) cell adhesion molecules, respectively, which belong to the integrin $\beta_1$ subfamily (7, 10). Integrin $\alpha_2\beta_1$ mainly mediates cell adhesion to collagens, while integrin $\alpha_3\beta_1$ mainly binds laminins (10). Decreased expression of these $\beta_1$ subfamily integrins has been linked to tumor invasion, metastasis or both in many tumors (11, 13-16). During tooth development, the $\beta_1$ integrins are expressed chiefly in the basement membrane zone of epithelial components (27, 30). In the present study, integrin $\alpha_2$ and $\alpha_3$ subunits were detected uniformly in peripheral columnar or cuboidal cells of ameloblastomas and were considered to be preserved in ameloblastomas as well as in tooth germs. Scattered epithelial cells attached to the basement membrane proliferate in tooth germs and ameloblastomas (33-35). These features suggest that expression of the $\alpha_2$ and $\alpha_3$ subunits might be associated with the cellular proliferative activity of odontogenic epithelium. Basal cell ameloblastoma shows diffuse immunoreactivity for bel-2 and bcl-x proteins, as well as high cyclin D1 expression, indicating a high potential for cell survival or proliferation (35-37). CCOT is classified as a benign epithelial odontogenic tumor, but is more aggressive than ameloblastoma (4, 6). This tumor is histologically characterized by biphasic cellular elements of clear oval cells and amphophilic basaloid cells (6). In the present study, basal cell ameloblastomas and the CCOT were similarly positive for integrin $\alpha_2$ and $\alpha_3$ subunits in most tumor cells, suggesting that CCOT cells possess characteristics similar to those of neoplastic cells in basal cell ameloblastoma.

Integrin $\alpha_4$ cell adhesion molecule is only a member of the integrin $\alpha_4$ subfamily (7, 10). This cell adhesion molecule binds a recently discovered laminin isomer, laminin-5, and focal or extensive loss of the molecule has been recognized and correlated with invasion or metastasis in some tumors (15, 16). In developing teeth, integrin $\alpha_4$ subunit has been found on the basal cell surfaces facing the basement membrane of dental epithelial structures (30). Recently, expression of laminin-5 in ameloblastomas has been linked to progression potential of tumor cells (38). In our study, expression of integrin $\alpha_4$ subunit in the epithelial odontogenic tumors was distributed similarly to that in developing teeth (30) and corresponded to the distribution of laminin-5 expression in ameloblastomas (38), suggesting that integrin $\alpha_4$ subunit is associated with progression potential in neoplastic odontogenic epithelium. By binding to matrix proteins, integrins mediate signal transduction from the extracellular milieu to the cytoplasm and nucleus, leading to alterations in cell function and behavior (10, 39, 40). Ligand proteins of integrins, such as collagens, laminins and fibronectins, have been detected in several odontogenic tumors (38, 41-44). We found epithelial integrins along the basement membrane zones of epithelial odontogenic tumors, suggesting that these integrins might participate in tumor proliferation and migration by mediating parenchymal-stromal cell interactions.

CD44 is expressed in a wide variety of cell types, including hematopoietic cells, fibroblasts, macrophages, epithelial cells, muscle cells and glial cells (19). This molecule functions as a major receptor for hyaluronic acid, and is involved in adhesion, movement and activation of normal and transformed cells (18, 19, 22, 24). Changes in CD44 expression are associated with progression and metastasis in a variety of human tumors (20, 21, 23, 25). Expression of hyaluronic acid and CD44 has been recognized in dental epithelial structures of tooth germs, suggesting that these molecules play a role in morphogenesis and differentiation during tooth development (26, 31, 45). In the present study, CD44 was expressed widely in tumor cells of ameloblastomas and one CCOT, but keratinizing areas in acanthomatous ameloblastomas showed decreased expression of CD44. These results suggest that CD44 might be involved not only in cell adhesion but also in tumor progression and cellular differentiation of epithelial odontogenic tumors.

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*:positive in basement membrane zone; "*:slightly positive
References

35. Kumamoto H, Kiki K and Ooya K. Detection of cell cycle-


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