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**Original Article**

## CYTOKERATINS EXPRESSION OF CONSTITUTING CELLS IN AMELOBLASTOMA

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### Abstract

The purposes of this study were to investigate the distribution of cytokeratins in the different tissue types of ameloblastoma and to discuss the histogenesis of this tumor. CK19 and CK8, which are markers for odontogenic epithelium, reacted positively to the constituting cells in all types of ameloblastoma. This suggests that all types of ameloblastoma derive from odontogenic epithelium. However, the desmoplastic type diminished the odontogenic characteristics because the basal cells are negative to CK19. Immunoreactions of five kinds of cytokeratin revealed similar results in plexiform, follicular, acanthomatous, and granular cell types. The plexiform type is probably the original type of ameloblastoma; the other types have the characteristics of squamous epithelium, and the follicular, acanthomatous, and granular cell types can develop due to the differentiation of cells of the plexiform type into squamous epithelium.

Key words: Ameloblastoma—Cytokeratin—Immunohistochemistry—Histogenesis

### INTRODUCTION

Ameloblastoma is the most common type of the odontogenic tumor, but it is known that this tumor has a many histopathologic patterns<sup>5,8,10-12,17,18,23,25</sup>. According to the WHO (1992), this tumor is classified mainly into two types; plexiform and follicular, and is subclassified into acanthomatous, granular cell, desmoplastic, and basal cell types based on the constituting cells. In the acanthomatous type, squamous epithelium with keratinization is prominent. The granular cell type is characterized by the proliferation of granules in the cytoplasm. Fibrous stroma is distinc-

tively evident in the desmoplastic type<sup>8</sup>). The basal cell type consists of only basal cells without any other type of cell differentiation.

Although several hypotheses for the histogenesis of ameloblastoma have been proposed<sup>18,24</sup>), it is generally accepted that this tumor originates directly from odontogenic epithelium in the developmental phase<sup>3,5,25</sup>).

It has been demonstrated that the morphological characteristics and cytokeratin distribution of tumor cells in ameloblastoma are similar to those of cells constituting the enamel organ and to ameloblasts<sup>5-7,21</sup>). A study showed that nucleic acid and polysaccharide are well preserved in ameloblastoma and stained simi-

larly to the enamel organ<sup>23</sup>). Matsuda<sup>10</sup> reported that the ultrastructure of cells in ameloblastoma are analogous to those of oral mucosa or enamel organ. However, it is still an open question why this tumor derived from odontogenic epithelium has the variable histopathologic patterns described above.

There are over twenty molecular types of cytokeratin that form the cytoskeleton of epithelial cells. These molecular types vary with epithelial cell types and differentiation phases, they are subsequently utilized as epithelial cell markers during epithelial differentiation and malignant transformation. It is also known that intercellular localization of cytokeratins differs, depending on the tissue types of the tumor<sup>2,13,14</sup>. Polyclonal and mixed antibodies to cytokeratin have been employed to elucidate the histogenesis of ameloblastoma<sup>3,11,12,25</sup>. However, the identification of respective cytokeratins in the tissues of this tumor have not been fully clarified. Only a few studies of the localization of cytokeratins have used antibodies against multiple molecular types<sup>5,6,17</sup>.

The purpose of this study was to investigate the expression of cytokeratins in plexiform, follicular, acanthomatous, granular cell, and desmoplastic ameloblastomas, using PK, KL1, CK8, CK19, and CK13 antibodies in order to examine the relationship among the tissue types of ameloblastoma.

## MATERIALS AND METHODS

### 1. Materials

The seventy three cases used in this study were histopathologically diagnosed as ameloblastoma at Department of Pathology, Tokyo Dental College Hospital from 1974 to 1996. In these, forty-three cases were the plexiform type, eighteen cases were the follicular type, six cases were the acanthomatous type, three cases were the granular cell type, and three cases were the desmoplastic type. As controls, either normal oral mucosa or odontogenic epithelium which were contained in the operative material of the patients who suffered from oral cancer were used.

### 2. Methods

All materials were fixed in 10% neutral formalin buffer solution, dehydrated by a graded alcohol series and embedded in paraffin. Then, paraffin sections approximately 5 $\mu$ m in thickness were cut and stained with hematoxylin and eosin (HE stain).

For immunohistochemical study, Histofine SAB-PO (MULTI) kit (Nichirei Co., Ltd., Tokyo, Japan) was used in the streptavidin-biotinyl immunoperoxidase method. Paraffin sections were dipped in 0.3% methanolic hydrogen peroxidase for 30 min in order to hamper endogenous peroxidase. Then, for activating the antigen, they were treated in proteolytic enzyme for 30 min at 37°C in 0.1% trypsin (GIBCO) tris-HCl buffer (pH 7.6), or treated with microwaves for 20 min at 60°C in 0.01 M citrate buffer (pH 6.0). After they were cooled to room temperature, they were cleansed for 5 min in phosphate buffered saline (PBS) three times. Next, in order to prevent a non-specific reaction, they were placed in 10% of normal goat serum for 10 min. Afterward, the primary antibody was applied at room temperature for one hour to react. Table 1 shows the antibodies used in this study. As a negative control, PBS was used instead of the primary antibody. After the primary antibody reaction and rinsing three times for 5 min in PBS, the secondary antibody (biotinylated anti-mouse IgG and anti-rabbit IgG) was reacted at room temperature for 30 min. After three more 5 min rinses in PBS, peroxidase-conjugated streptavidine was applied. Then after three more rinses in PBS for 5 min, 3,3'-diamino-benzidinetetrahydrochloride Tris-HCl buffer (pH 7.6) was used to visualize the reaction. Finally, sections were stained by hematoxylin and were observed with light microscope.

### 3. Evaluation method

To evaluate the immunohistochemical staining, we divided the constituent cells into basal, suprabasal, and inner layers in each tissue type of ameloblastoma. The plexiform type consisted of two layers: a basal layer (BL) and a suprabasal layer (SBL). The follicular type,

Table 1 Molecular weight and dilution of the primary antibodies used in this study

Antibody	Molecular weight	Company	Dilution
KL1	55–57KD	Immunotech	1: 50
CK13	54KD	Progen Biotechnik	1: 10
CK19	40KD	Progen Biotechnik	1: 10
CK8	52.5KD	DAKO	1: 30
PK	mainly 56KD & 64KD	DAKO	1:100

acanthomatous type, granular cell type, and desmoplastic type consist of three layers: a basal layer (BL), a suprabasal layer (SBL) and an inner layer (IL). Staining characteristics were also investigated; a positive ratio was calculated (positive ratio (%) = number of positive cases of each tissue type/number of cases of each tissue type  $\times$  100) based on the distribution of positive reactions in the cytoplasm of the cells constituting the tumor.

## RESULTS

### 1. Control group

The cells of the stratified squamous epithelium of oral mucosa consist of two functional populations: a progenitor population are situated in the basal layer and a maturing population located above the basal layer in the prickle-cell layer, granular cell layer, and keratinized layer. CK19 was only positive in the progenitor population (Fig. 1), and KL1 and CK13 (Fig. 2) reacted positively with all the maturing population. PK was positive for both the progenitor and maturing populations. However, no cells of oral epithelium were positive for CK8.

Odontogenic epithelium in the connective tissue was seen as cell clusters that stained for PK and CK19 (Fig. 3), but not for KL1, CK13 (Fig. 4), or CK8.

### 2. Follicular type

In the follicular type, tumor cells were arranged in island like or follicular forms. Cubic or columnar cells lined the basal side and polygonal cells resembling the stellate

reticulum of the enamel organ were located inside. Immunostaining for cytokeratins in BL showed that KL1 was positive in the peripheral cytoplasm, and that CK19 (Fig. 5), CK8 and PK were uniformly positive in the cytoplasm. CK13 was negative in BL (Fig. 6). In SBL and IL, KL1, CK13, CK19, CK8, and PK positively reacted uniformly all over the cytoplasm. In intensity of immunoreaction, CK19 was stronger than CK8. Cytokeratin stained positively in the portion where numerous IL cells could be observed around the cyst. This finding was also evident for KL1 and CK13, although it was fluctuated.

The positive ratios of cytokeratin for KL1 were 9/18 (50%) in BL, 17/18 (94%) in SBL, and 18/18 (100%) in IL. For CK13, the ratios were 11/18 (61%) in SL and 15/18 (83%) in IL. For CK19, they were 17/18 (94%) in BL, 18/18 (100%) in SBL and 15/18 (83%) in IL. For CK8, these ratios were 10/18 (56%) in BL, 13/18 (72%) in SBL and 8/18 (44%) in IL. For PK, they were 16/18 (89%) in BL, 15/18 (83%) in SBL and 18/18 (100%) in IL.

### 3. Plexiform type

The plexiform type was characterized histologically by the irregular funicle form or plexiform arrangements of cubic or columnar cells of parenchyma. In the latter arrangement, structures resembling the stellate reticulum of enamel organ were definite. Staining results for the plexiform type were similar to those of the plexiform type.

Immunohistochemistry for cytokeratin in BL revealed that KL1 reacted positively in the peripheral cytoplasm and that CK19 (Fig. 7), CK8, and PK were positive uniformly all over

the cytoplasm. However, CK13 was negative in BL (Fig. 8). In SBL, immunoreactions for KL1, CK13, CK19, CK8 and PK were obvious in the cytoplasm. In positivity, CK19 was more intense than CK8.

Positive ratios of cytokeratin for KL1 were 21/43 (49%) in BL and 39/43 (91%) in SBL. For CK13 antibody, it was 34/43 (79%) in SBL. For CK19 antibody, the ratios were 41/43 (95%) in BL and 43/43 (100%) in SBL. For CK8 antibody, they were 20/43 (47%) in BL and 36/43 (84%) in SBL. For PK antibody, it was 43/43 (100%) in both BL and SBL.

#### 4. Acanthomatous type

Histologically, tumor cells of this type were characterized by an extensive squamous metaplasia with keratin formation. Tumor cell nests revealed island-like or follicular form that were lined by cubic or columnar cells at the basal side. Immunostaining results of the acanthomatous type were analogous to those of the plexiform and follicular types. KL1 was positive in the peripheral areas of cytoplasm in BL, and CK19 (Fig. 9), CK8, and PK were uniformly positive in the cytoplasm. In both SBL and IL, KL1, CK13 (Fig. 10), CK19, CK8, and PK were uniformly stained in the cytoplasm. The positive reaction for CK19 was more intense than those for the others. Cytokeratin is definitely found in the region where numerous IL cells could be detected around the cyst formation. Although this characteristic was not stable, it was obvious in KL1 and CK13.

Positive ratios for KL1 were 4/6 (67%) in BL, 6/6 (100%) in SBL, and 6/6 (100%) in IL. For CK13, they were 5/6 (83%) in SBL and 6/6 (100%) in IL. The ratios for CK19 were 6/6 (100%) in BL, SBL, and IL. For CK8 were 5/6 (83%) in BL, 4/6 (67%) in SBL, and 3/6 (50%) in IL. For PK, they were 6/6 (100%) in BL, SBL, and IL.

#### 5. Granular cell type

The tumor cells of granular cell types were histologically characterized by a granular transformation of the epithelial cells of inner layer. The cells were large, and cuboidal, columnar

or round in shape. Their bulky cytoplasm contained eosinophilic granules. At the basal side of the tumor cell nest, cubic or columnar cells could be found.

In BL, immunostainings for CK19 (Fig. 11) and PK were uniformly positive in the cytoplasm. In SBL and IL, KL1 which were mainly positive for granular cells, CK13 (Fig. 12), CK19, CK8, and PK weakly reacted positively in the cytoplasm. Peripheral areas of cells in the granular cells were intensely stained.

Positive ratios for KL1 were 3/3 (100%) in both SBL and IL. For CK 13, they were 2/3 (67%) in SBL and 3/3 (100%) in IL. The ratios for CK19 were 2/3 (67%) in BL, SBL, and IL. For CK8, it was 1/3 (33%) in SBL and 2/3 (67%) in IL. For PK, they were 1/3 (33%) in BL and 3/3 (100%) in both SBL and IL.

#### 6. Desmoplastic type

In the desmoplastic type, cellular elements were scarce. Hyperplasia of fibrous elements was definite in the interstitium, parenchyma had small circle-like or irregular island-like structures, the basal side was lined by cubic or short columnar cells, and polygonal or squamous epithelium-like cells could be observed to have formed inside.

Immunostaining for cytokeratins in BL revealed that KL1, CK8, and PK were positive in the cytoplasm. In SBL and IL, KL1, CK19 (Fig. 13), CK13 (Fig. 14), CK8, and PK were positively reacted in the cytoplasm.

Positive ratios for KL1 were 1/3 (33%) in BL and 3/3 (100%) in SBL and IL. For CK 13, they were 3/3 (100%) in SBL and 2/3 (67%) in IL. The ratios for CK19 were 3/3 (100%) in both SBL and IL. For CK8, they were 1/3 (33%) in BL and 3/3 (100%) in SBL and IL. For PK, the ratios were 3/3 (100%) in BL, SBL, and IL.

## DISCUSSION

Cytokeratin, which is contained in various types of epithelial cells to form cytoskeleton, is an intracytoplasmic fibrous constituent and is classified as one of intermediate filaments.



Fig. 1 CK-19 immunohistochemical findings in normal oral tissue ( $\times 198$ ). CK19 was positive only in basal cells.



Fig. 2 CK-13 immunohistochemical findings in normal oral tissue ( $\times 198$ ). CK13 reacted positively in all the maturing population.



Fig. 3 CK-19 immunohistochemical findings in odontogenic epithelium ( $\times 277$ ). CK19 was positive in all the cells.

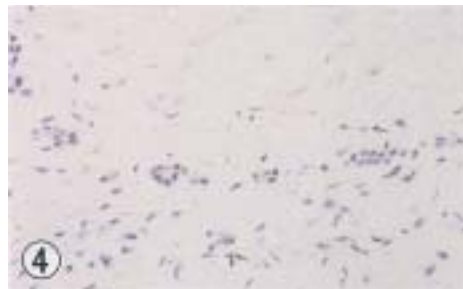


Fig. 4 CK-13 immunohistochemical findings in odontogenic epithelium ( $\times 277$ ). CK13 was negative in all the cells.

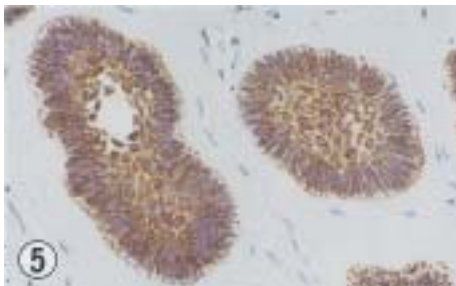


Fig. 5 CK-19 immunohistochemical findings in follicular type ( $\times 231$ ). CK19 was positive uniformly in the cytoplasm in BL.

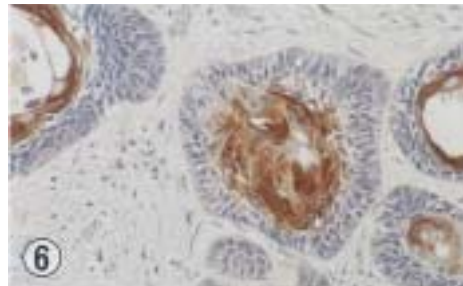


Fig. 6 CK-13 immunohistochemical findings in follicular type ( $\times 317$ ). CK13 was positively reacted uniformly all over the cytoplasm.

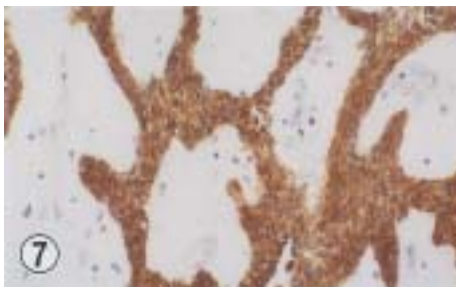


Fig. 7 CK-19 immunohistochemical findings in plexiform type ( $\times 231$ ). CK19 was positive uniformly all over the cytoplasm.



Fig. 8 CK-13 immunohistochemical findings in plexiform type ( $\times 187$ ). CK13 was negative in BL but were positive in the cytoplasm in SBL.

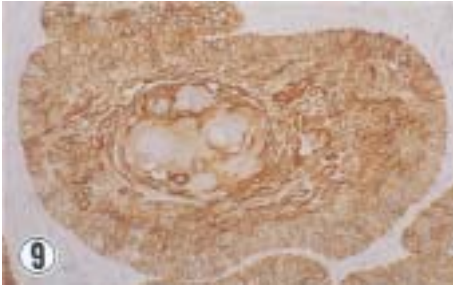


Fig. 9 CK-19 immunohistochemical findings in acanthomatous type ( $\times 224$ ). CK19 was uniformly positive in the cytoplasm in BL.

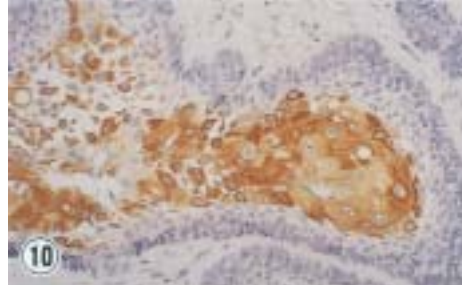


Fig. 10 CK-13 immunohistochemical findings in acanthomatous type ( $\times 187$ ). In both SBL and IL, CK13 was uniformly stained in the cytoplasm.

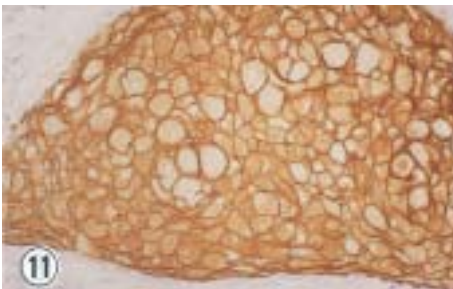


Fig. 11 CK-19 immunohistochemical findings in the granular cell type ( $\times 205$ ). CK19 was uniformly positive in the cytoplasm in BL.

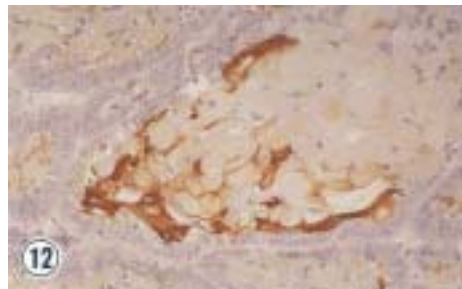


Fig. 12 CK-13 immunohistochemical findings in granular cell type ( $\times 231$ ). CK13 was positively reacted in the cytoplasm.

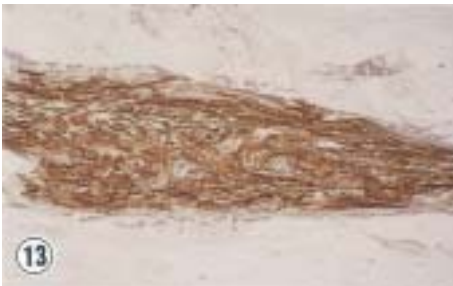


Fig. 13 CK-19 immunohistochemical findings in desmoplastic type ( $\times 218$ ). CK19 was positively reacted in the cytoplasm.



Fig. 14 CK-13 immunohistochemical findings in desmoplastic type ( $\times 218$ ). CK13 was positively reacted in the cytoplasm.

Twenty subtypes of cytokeratin, which are classified into type I and type II, are known. Type I, whose molecular weight is low, is acidic at the isoelectric point for CK9 to 20, and type II, whose molecular weight is high, is neutral or basic at the isoelectric point for CK1 to 8. It has been demonstrated that its genesis has the regularity as well as the specific character of the original tissue and cell even in a tumor<sup>2,13,14</sup>. Recently, it has been reported that

a positive reaction for cytokeratin is observed in mesothelial cells, mesothelioma, and soft tissue tumors where epithelial metaplasia has developed. This indicates that cytokeratin is useful for identifying epithelial cells as well as other cells with epithelial characteristics<sup>4,26</sup>. Thus, cytokeratin is a specific marker for epithelial cells<sup>7,9,19,22</sup>. Several investigators have demonstrated that, in the oral cavity, KL1 is detectable at the acanthomatous layer, which

is an upper portion of the basal layer in keratinized epithelium of gingiva, tongue, and palate<sup>1,21,26</sup>. CK13 and 19 can be also expressed in the analogous layer<sup>4,15,21,26</sup>.

In particular, immunostaining for CK19 is definitely specific and reacts positively mainly with simple epithelium, but it also immunostains to basal cells in gingival stratified squamous epithelium, dental lamina of odontogenic epithelium, enamel organ, and epithelial rests of Malassez<sup>4,26</sup>. These reports have suggested that CK19 can be utilized to identify either odontogenic epithelium or basal cells.

CK8, which seemed to be a simple epithelium marker, is expressed not only in epithelium of glands or secretory ducts but also in dental lamina of odontogenic epithelium and cells of enamel organ. This cytokeratin is also regarded to be useful as an odontogenic epithelium marker, but it is negative to stratified epithelium of oral cavity<sup>15,21</sup>.

KL1 strongly immunoreacts with cells located at the upper portion in all human epithelia of stratified squamous epithelium of oral cavity. One study has indicated that KL1 antibody is useful for elucidating differentiation of human epithelium<sup>27</sup>.

It is known that CK13 also reacts positively to cells located at the upper portion in parakeratinized or non-keratinized stratified squamous epithelium and is expressed in relation to stratified epithelium<sup>1,21,26</sup>.

There have been many studies discussing the histogenesis of ameloblastoma using cytokeratin immunohistochemistry. It is speculated that ameloblastoma originates from cells closely related to non-keratinized stratified squamous epithelium, because epithelial cells of the tumor are negatively react to a monoclonal antibody (RKSE 60) which is specific to keratin of keratinized stratified squamous epithelium<sup>3</sup>.

A study on cytokeratin distribution has demonstrated that CK-1 (CK6 and 18), NSE-K (Non squamous epithelium keratin: (CK8), and 19-K (CK19) immuno-react positively in the peripheral portion of cell nest in follicular type, whereas CK 1, SE-K (squamous epithelium

keratin: CK1–2, 5, 6, 10), NSE-K and 19-K are positive in the central portion of the cell nest. They pointed out that the cytokeratin distribution in ameloblastoma is similar to that in fetal oral mucosal epithelium or epithelial rests of Malassez<sup>17</sup>. Our immunohistochemical results showed that tumor cells in some portions of all cases of ameloblastoma were always positive to CK8 and CK19. Taken all together, these data may indicate that ameloblastoma originates from odontogenic epithelium in the prenatal period rather than basal layer or scarf skin of oral mucosa<sup>5,6,17</sup>.

Concerning the relationship among tissue types, a immunohistochemical study using PKK1 and KL1 antibodies proved that macro-molecular keratin is contained in the follicular and acanthomatous type, suggesting the cellular differentiation into squamous epithelium, that the character of the basal cell type is similar to that of basal cells in squamous epithelium, and that the squamous epithelium character is lacking in the plexiform type, but holds the initial phase of tumor differentiation<sup>11</sup>.

In the present study, we observed that basal layers of plexiform, follicular, and acanthomatous types were positively stained by KL1 but negatively by CK13 and that suprabasal cell and inner cells were positive for both KL1 and CK13. Sandra *et al.* (2001)<sup>20</sup> analyzed the proliferation activity of 32 ameloblastomas and concluded that there was higher proliferative activity in the solid type than the cystic and the mixed type and also that activity in outer layer cells was significantly higher than that of inner layer cells. From these, it is surmised that cells in basal layers (BL) may have the ability to differentiate into squamous epithelium, but not to differentiate into stratified epithelium; nevertheless, suprabasal and inner cells are able to differentiate into both squamous and stratified epithelium. We are tempted to conclude that plexiform, follicular, and acanthomatous types possess histologically similar characteristics because of their similarity of cytokeratin distribution.

Our immunohistochemical results for cytokeratin may indicate that the plexiform type is



most probably the original type of ameloblastoma, and other types are provided the characteristics of squamous epithelium. The and that follicular and acanthomatous types can thus be developed due to the differentiation of the cells of plexiform type into squamous epithelium.

There are a few immunohistochemical studies of cytokeratin in the granular cell type. Although de Wilde *et al.*<sup>3)</sup> and Ota *et al.*<sup>18)</sup> suggested that the granular cell type is a tumor cell which has an epithelium character, no study on its relationship to other tissue types has been reported. From the result of our study, the granular cells weakly stained for all the keratin used in this study, and the peripheral areas of cells intensely stained. This may suggest that the granules in the cytoplasm had degenerated from the stellate reticulum and were transformed from cells of the plexiform type. Furthermore, as acidophilic granules increased in the cytoplasm of these tumor cells, positive reaction became detectable mainly in the surrounding cells, suggesting that cytokeratin may distribute broadly toward the periphery of tumor cells.

There is an idea that desmoplastic type is an acanthomatous subtype or type II of the classification of Ishikawa *et al.*, who proposed and considered this type to be an ameloblastoma of comparatively less differentiation<sup>16)</sup>. The direction of the differentiation to the odontogenic epithelium may be diminished, because the cells at the basal side of the tumor cell nest negatively stain with CK19.

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