The expression profiles of acidic epithelial keratins in ameloblastoma

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Objective. To characterize the subtypes of ameloblastoma by differentiation markers.
Study Design. Expression of 9 major acidic epithelial keratins was immunohistochemically examined in 28 ameloblastomas.
Results. Keratin 15 (K15) expression patterns corresponded to histological variants: follicular, plexiform and acanthomatous. Tumor nests comprising K15 expressing basal cells mimicked oral epithelium or dental lamina, and tumor nests comprising K15 negative basal cells mimicked outer enamel epithelium. Keratin 19 (K19) was consistently expressed in solid/multicystic ameloblastoma and unicystic ameloblastoma, while peripheral ameloblastoma and desmoplastic ameloblastoma contained K19 negative cells.
Conclusions. The 4 current subtypes had unvaried expression patterns within each group. However, they could be divided into 2 groups by K19 expression pattern: solid/multicystic and unicystic versus extraosseous/peripheral and desmoplastic. K15 expression pattern represented various types of differentiation for tumor nests mimicking tooth germ and oral epithelium. The results clarify the homogeneity and heterogeneity of ameloblastoma cell lineage and differentiation. (Oral Surg Oral Med Oral Pathol Oral Radiol 2013;115:523 531)

Ameloblastoma is a benign but locally invasive tumor that arises in the jaws. Ameloblastoma has histological features that are reminiscent of epithelial components of tooth germ, suggesting that it arises from odontogenic epithelium such as dental lamina or enamel epithelium. There are 2 major histological patterns: a follicular type consisting of tumor nests resembling enamel organs, and a plexiform type consisting of branching cord-like nests. In addition, there are other histological variants, including acanthomatous, basal cell, and granular cell types.[1]

Although the recurrence rates appear to differ between the follicular and the plexiform types, and between the follicular and the acanthomatous types,[2] it is believed that this histological subtyping has little clinical relevance.[1] Considering this, the 2005 edition of World Health Organization (WHO) classification of odontogenic tumors categorized ameloblastoma into 4 types solid/multicystic, unicystic, extraosseous/peripheral and desmoplastic so that the classification reflects differences in tumor behaviors including predilections by age and site, clinical manifestations and recurrence rates.[1] Solid/multicystic type is a solid/multicystic ameloblastoma, into which most cases are categorized regardless of histological variation (e.g. follicular and plexiform).[2] Unicystic ameloblastoma is manifested as a monocystic lesion whose lumen is lined with an epithelium composed of ameloblastic cells.[3] Extraosseous/peripheral ameloblastoma occurs in the soft tissue of the maxilla and mandible, mostly in the gingiva.[4-7] Desmoplastic ameloblastoma is characterized by unique histology including irregularly shaped epithelial islands embedded in dense fibrous stroma.[8,9]

The gold standard of tumor diagnosis has long been represented by histological evaluation, but a new trend has emerged, and expression of specific proteins is often used to help make the diagnosis. This expression-based method has been invaluable, for example, in the diagnosis of lymphoma or breast cancer, where expression patterns of specific proteins have uncovered distinct subgroups that were correlated to tumor behavior and prognosis.[10,11] These diagnostic markers

Statement of Clinical Relevance

This study improves our understanding on the pathogenesis of ameloblastoma, and suggests the use of markers to assess cell differentiation, which facilitates the objective diagnosis of the subtypes of ameloblastoma based on differentiation marker expression.

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include 1) proteins that have a direct role in tumorigenesis (for example, C-kit in gastrointestinal stromal tumor), 2) proteins that are expressed in the cells of a specific lineage and differentiation (for example, desmin in rhabdomyosarcoma), and 3) proteins that are commonly expressed in different tumors, although contribution of those proteins to the pathogenesis is unknown (for example, CD99 in Ewing's sarcoma). We speculate that the subtypes of ameloblastoma are represented by the expression pattern of certain proteins, and that there are other subgroups to be unveiled by the expression pattern.

Keratins are epithelial-specific intermediate filament proteins that show highly selective expression depending on cell lineage and differentiation.[12] This property is beneficial for cell typing. For example, oral epithelium can be classified into 3 types according to keratin expression patterns.[13] Also, several patterns have importance in the precise classification and subtyping of tumors.[12]

Keratin expression in ameloblastoma has been examined in several studies.[14-18] Heikinheimo et al. reported that keratin (K) 8, K18 and K19, which were expressed in normal odontogenic epithelia, were expressed in ameloblastoma, and they concluded that ameloblastomas may represent a heterogenous group of tumors that arose from odontogenic epithelial cells at various differentiation levels.[16] Crivelini et al. reported that K13, K14 and K19 were expressed in ameloblastoma while K7, K8, K10 and K18 were not, and stated that K14 was a dominant keratin, while K13 and K19 represented squamous differentiation.[14] Ong'uti et al. noted the expression of K5, K6, K8, K14, K16, K18 and K19 in ameloblastoma, and suggested that K6, K16 and K19 might indicate rapid growth.[18] These results do not seem to have converged toward a definitive conclusion, and correlation between keratin expression and the current classification of ameloblastoma has not been probed. We expected that comprehensive examination of keratins would provide novel insights into both homogeneity and heterogeneity of ameloblastoma. Functional keratin filaments are formed by multimerization of basic and acidic keratins.[12] Acidic major epithelial keratins (K10-K20) usually show more varied expression patterns than their basic counterparts (K1-K9), giving them an advantage in cell typing (our unpublished observations). In this study, we conducted profiling of acidic major epithelial keratin expression in ameloblastoma.

MATERIALS AND METHODS

Tissue samples

Formalin-fixed paraffin-embedded specimens were collected from the archives of the hospital at Tokyo Medical and Dental University. They were 13 solid/multicystic, 5 unicystic, 6 extraosseous/peripheral, 4 desmoplastic ameloblastomas (Table I) plus 1 ameloblastic fibroma and 6 basal cell carcinomas of skin. The pathological diagnoses were re-evaluated according to the 2005 edition of WHO classification of odontogenic tumors.

E15-20 mouse embryos were fixed in 10% buffered formalin for 24 h, decalcified in Planch Rychlo solution for 24 h and were embedded in paraffin.

The experimental procedures were approved by the Tokyo Medical and Dental University Ethics Committee.

Immunohistochemical staining

Immunohistochemical staining was conducted as previously described.[19] For antigen retrieval, sections were heat-treated in alkaline buffer (10 mM Tris (pH 9.0) and 1 mM EDTA) at 120°C for 10 min. The primary antibodies used for human tissue staining were anti-K10 (DE-K10; Thermo Fisher Scientific), K13 (KS-1A3; Novocastra), K14 (LL002; Abcam), K15

Table I. Summary of the cases examined in this study

Case No.	*Types*	*Age, sex*	*Site*
1	SMA	13, M	Md, P, R
2	SMA	15, M	Md, P, R
3	SMA	19, F	Md, P, R
4	SMA	33, M	Md, P, R
5	SMA	35, M	Md, A, R
6	SMA	36, M	Md, A, L
7	SMA	36, M	Md, P, L
8	SMA	36, F	Md, P, R
9	SMA	37, F	Md, P, L
10	SMA	41, M	Md, P, L
11	SMA	52, M	Md, A, M
12	SMA	62, F	Md, P, R
13	SMA	84, F	Md, P, L
14	UA	16, F	Md, P, R
15	UA	19, F	Md, P, R
16	UA	22, M	Md, P, L
17	UA	27, F	Md, P, R
18	UA	32, F	Md, P, L
19	PA	51, M	Md, P, L
20	PA	61, M	Md, A, R
21	PA	63, M	Md, P, R
22	PA	67, M	Mx, P, L
23	PA	79, M	Md, A, L
24	PA	79, M	Md, A, R
25	DA	35, F	Md, A, L
26	DA	37, M	Mx, A, R
27	DA	39, M	Md, A, R
28	DA	42, F	Mx, A, R

The lesions encompassing both posterior and anterior areas were classified by the site at the center of the lesion.
SMA, solid/multicystic ameloblastoma; *UA*, unicystic ameloblastoma; *PA*, peripheral ameloblastoma; *DA*, desmoplastic ameloblastoma; *Md*, mandible; *Mx*, maxilla; *P*, posterior (molar area); *A*, anterior (incisor canine premolar area); *R*, right; *L*, left; *M*, middle.