Localization of Type IV Collagen Alpha Chains in the Basement Membrane of Ameloblastoma, Tooth Germ and Oral Mucosa by Using Indirect Immunofluorescence.

Hirokuni Kou1,2, Silvia Susana Borkosky2, Hitoshi Nagatsu2, Ryo Tamamura2, Keisuke Nakano2, and Noriyuki Nagai2.

1) Japan Institute for Advanced Dentistry, Osaka, 2) Okayama University, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences.

Introduction
Type IV collagen is a major structural component of basement membrane (BM) and acts as a scaffold for other BM constituents. It is a heterotrimeric molecule that exists in six genetically distinct forms, α1(IV) - α6(IV).

The ameloblastoma is the most frequently encountered odontogenic epithelial tumor. The BM zone of the ameloblastoma remains a subject of research interest primarily because of increasing evidence of its mediatory role during oncogenesis. However, the expression pattern of specific collagen α(IV) chains in the ameloblastoma BM has not been previously reported. In this preliminary study, indirect immunofluorescence was utilized to localize α1(IV) - α6(IV) chains in the BM of two

Materials and Methods
Preparation of tissues
Surgical samples of two ameloblastomas from mandible and autopsy specimens of four human tooth germ and human oral mucosas were prepared for frozen section. One set of the cryosections was stained routinely with hematoxylin and eosin.

Immunohistochemistry
Immunolocalization of type IV collagen α chains was performed by using rat monoclonal antibodies: H11, H22, H31, H43, M54, and M69 (provided by Dr. Naito, I., and Dr. Sado, Y.), recognizing type IV collagen α1, α2, α3, α4, α5, and α6 chains, respectively.

Results
In the two ameloblastoma studied, the BM surrounding the neoplastic epithelial islands (follicular pattern), and interlacing strands (plexiform pattern) showed positive expression for all but α3(IV) chains. α1(IV), α2(IV), α5(IV), and α6(IV) were intensively expressed whereas α4(IV) chain expression was rare and irregular in its distribution. Oral mucosa BM also expressed α1(IV), α2(IV), α5(IV), and α6(IV) chains.

In the human tooth germ, the BM associated with the inner enamel epithelium was positive only for α1(IV), α2(IV), and α4(IV) chains whereas in the outer enamel epithelium, it was positive for

![Fig. 1. Immunofluorescence localization of type IV collagen α chain in the basement membrane zone of plexiform ameloblastoma (A-D), follicular ameloblastoma (E-H) and oral mucosa (I-J). Strong expression for collagen (IV) α1 (B, F, I) and α5 (D, H, J) are shown in the BM of both types of ameloblastoma and oral mucosa, respectively. Immunoreactivity for α6(IV) chain is rare in both types of ameloblastoma (C, G). (A,E) H&E; (A-J) x250.](image-url)
Fig. 2. Immunofluorescence localization of type IV collagen α chain in the basement membrane zone of developing tooth germ (A-F). The BM of the inner enamel epithelium (iie) was positive for collagen (IV) α1 (B, E), and α4 (C, F) chains whereas in the outer enamel epithelium (oee), it was positive for collagen (IV) α1 (B) with limited expression for α5 chains (D). In the cuspal region (E, F), where enamel (e) and dentin formation have occurred, there is loss of collagen (IV) α chain expression. (A) H&E, x125; (B-F) x250.

Discussion

The similar expression patterns of α1(IV), α2(IV), α5(IV), and α6(IV) in the BM zone of ameloblastoma and oral mucosa suggests that the ameloblastoma tumor cells with this expression pattern are more mature, whereas tumor areas showing α4(IV) chain expression may represent a more primitive phenotype. The collagen IV molecular composition in the ameloblastoma BM suggests that these BM constituents play an important role in tumor cytodifferentiation and progression.