

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

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### 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,  $\alpha 1(\text{IV})$  -  $\alpha 6(\text{IV})$ .

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  $\alpha$  (IV) chains in the ameloblastoma BM has not been previously reported.

In this preliminary study, indirect immunofluorescence was utilized to localize  $\alpha 1(\text{IV})$  -  $\alpha 6(\text{IV})$  chains in the BM of two

mucosas were prepared for frozen section. One set of the cryosections was stained routinely with hematoxylin and eosin.

### Immunohistochemistry

Immunolocalization of type IV collagen  $\alpha$  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  $\alpha 1$ ,  $\alpha 2$ ,  $\alpha 3$ ,  $\alpha 4$ ,  $\alpha 5$ , and  $\alpha 6$  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  $\alpha 3(\text{IV})$  chains.  $\alpha 1(\text{IV})$ ,  $\alpha 2(\text{IV})$ ,  $\alpha 5(\text{IV})$ , and  $\alpha 6(\text{IV})$  were intensively expressed whereas  $\alpha 4(\text{IV})$  chain expression was rare and irregular in its distribution. Oral mucosa BM also expressed  $\alpha 1(\text{IV})$ ,  $\alpha 2(\text{IV})$ ,  $\alpha 5(\text{IV})$ , and  $\alpha 6(\text{IV})$  chains.

In the human tooth germ, the BM associated with the inner enamel epithelium was positive only for  $\alpha 1(\text{IV})$ ,  $\alpha 2(\text{IV})$ , and  $\alpha 4(\text{IV})$  chains whereas in the outer enamel epithelium, it was positive for

### Materials and Methods

#### Preparation of tissues

Surgical samples of two ameloblastomas from mandible and autopsy specimens of four human tooth germ and human oral

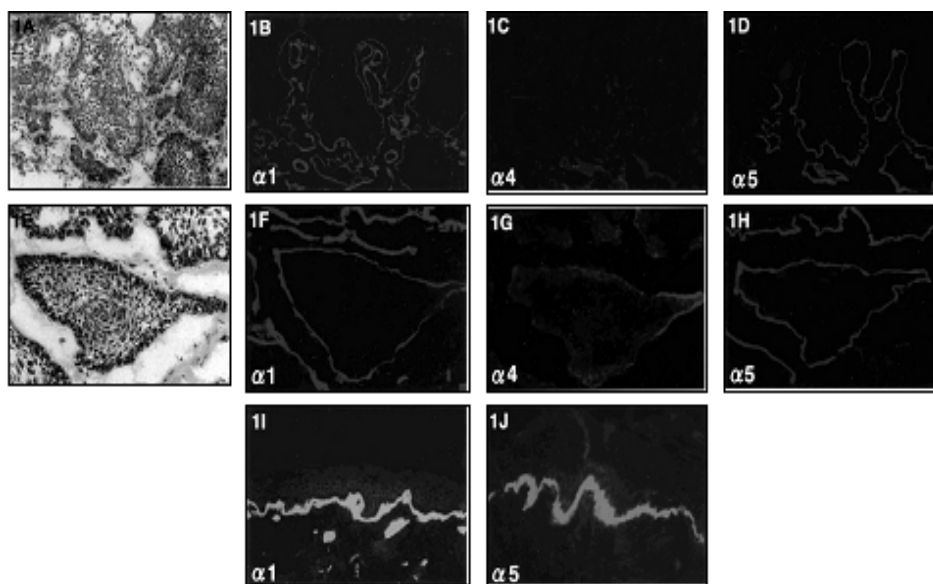


Fig. 1. Immunofluorescence localization of type IV collagen  $\alpha$  chain in the basement membrane zone of plexiform ameloblastoma (A-D), follicular ameloblastoma (E-H) and oral mucosa (I-J). Strong expression for collagen (IV)  $\alpha 1$  (B, F, I) and  $\alpha 5$  (D, H, J) are shown in the BM of both types of ameloblastoma and oral mucosa, respectively. Immunoreactivity for  $\alpha 4(\text{IV})$  chain is rare in both types of ameloblastoma (C, G). (A,E) H&E; (A-J) x250.

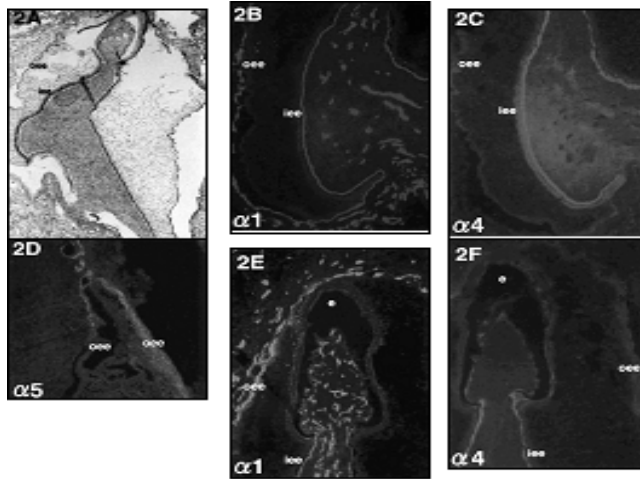


Fig.2. Immunofluorescence localization of type IV collagen  $\alpha$  chain in the basement membrane zone of developing tooth germ (A-F). The BM of the inner enamel epithelium (iee) was positive for collagen (IV)  $\alpha$ 1 (B, E), and  $\alpha$ 4 (C, F) chains whereas in the outer enamel epithelium (oee), it was positive for collagen (IV)  $\alpha$ 1 (B) with limited expression for  $\alpha$ 5 chains (D). In the cuspal region (E, F), where enamel (e) and dentin formation have occurred, there is loss of collagen (IV)  $\alpha$  chain expression. (A) H&E, x125; (B-F) x250.

only  $\alpha$ 1(IV),  $\alpha$ 2(IV),  $\alpha$ 5(IV), and  $\alpha$ 6(IV) chains. Near the cuspal region no immunoreactivity for collagen  $\alpha$  chains was detected.

#### Discussion

The similar expression patterns of  $\alpha$ 1(IV),  $\alpha$ 2(IV),  $\alpha$ 5(IV), and  $\alpha$ 6(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  $\alpha$ 4(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