

## Immunohistochemical Study of Collagen Type IV Alpha Chains and MMP-2, -9 at the Basement Membrane in Oral Carcinogenesis

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**Abstract:** Destruction of basement membrane is an important element in invasion of cancer cells. Type IV collagen, the major component of basement membrane, has six distinct  $\alpha$  chains. Matrix metalloproteinases (MMPs) are enzymes that resolve extracellular matrix. Oral squamous cell carcinoma is occurred after precancerous lesions, epithelial dysplasia and carcinoma in situ. We investigated localization of six  $\alpha$  chains and MMPs in normal oral mucosal tissue, precancerous lesions, early squamous cell carcinoma immunohistochemically. In normal oral mucosal tissue and epithelial dysplasia,  $\alpha 1$  (IV),  $\alpha 2$  (IV),  $\alpha 5$  (IV) and  $\alpha 6$  (IV) chains were detected continuously along basement membrane. In carcinoma in situ and early squamous cell carcinoma, either  $\alpha 5$  (IV) and  $\alpha 6$  (IV) chains or all  $\alpha$  chains were not stained. In contrast, MMP-2 and MMP-9 that are members of type IV collagenase were stained at the parts of disappearance of  $\alpha$  chains. This study suggested that the disappearance of  $\alpha$  chains is an important element in carcinogenesis of oral squamous cell carcinoma.

### Introduction

Oral squamous cell carcinoma occurs through a stage dependent pathway from precancerous lesions, epithelial dysplasia and carcinoma in situ. Once the invasive cancer appears, cancer cells destroy the connective tissues through destruction of basement membrane.

Type IV collagen is the major component of basement membrane, and includes six distinct  $\alpha$  chains. Matrix metalloproteinases (MMPs) are enzymes that resolve extracellular matrix. We investigated localization of six  $\alpha$  chains and MMPs in normal oral mucosal tissue, precancerous lesions, early squamous cell carcinoma immunohistochemically.

### Materials and Methods

#### Specimens

Folmalin-fixed, paraffin embedded sections from 10 early squamous cell carcinomas, 10 carcinoma in situs, 10 epithelial dysplasias and 10 normal tissues were obtained from archival paraffin blocks at Okayama University Hospital. For electron microscopic observation, tissue samples were fixed in 2% glutaraldehyde-2% paraformaldehyde, and postfixation was performed with a 1% osmium tetroxide solution. After dehydration, the tissues were embedded in epon. Ultrathin sections were cut with a diamond knife and doubly stained with uranyl acetate and lead citrate.

#### Immunohistochemistry

Sections of 4  $\mu$ m in thickness were dewaxed in xylene and rehydrated in alcohol, then heated to 121°C in an autoclave for 5 minutes. The endogenous peroxidase activity was suppressed by a solution of 0.3% hydrogen peroxide in methanol for 30 minutes. After being rinsed 3 times in Tris-buffered saline (TBS), the sections were treated for 18 hours with rat monoclonal antibodies ( $\alpha 1$ -6, Shigei Medical Research Institute, Okayama, Japan) and mouse monoclonal antibodies (MMP-2, -9, Fuji Chemical,

Toyama, Japan) at 4°C. After washing three times in TBS, the sections were treated with ABC kit (Vector Lab.) for 30 minutes at room temperature (RT). The slides were then washed in TBS and developed in 0.05M tris HCl (pH 7.6) containing 0.01% 3-3' diaminobenzidine at RT. The sections were counterstained in Mayer's hematoxylin and mounted.

### Results

#### Normal oral mucosa

The normal oral mucosa showed no atypical cells with uniform epithelial structure. The basement membrane could also be clearly seen separating the epithelium from the underlying connective tissue. (figure 1a)

The basement membrane was positive to  $\alpha 1$ ,  $\alpha 2$ ,  $\alpha 5$  and  $\alpha 6$  chains without disruption. The basement membrane of the vessels was likewise positive to  $\alpha 1$  and  $\alpha 2$  chains. (figure 1b-e)

#### Epithelial dysplasia

In H. E. section, epithelial cells had mild to moderate atypia, and the basement membrane beneath the basal cells was observed clearly. (figure 2a)

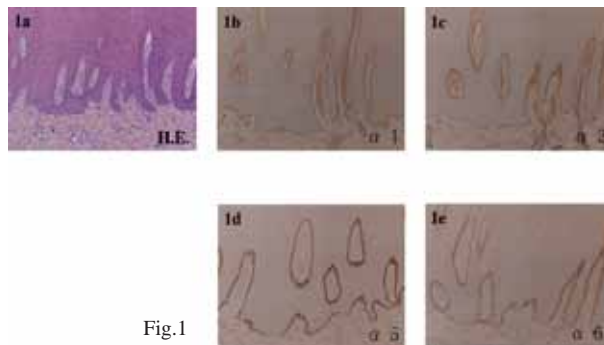


Fig.1

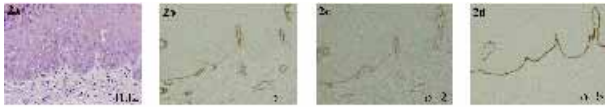


Fig.2

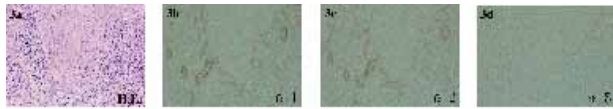
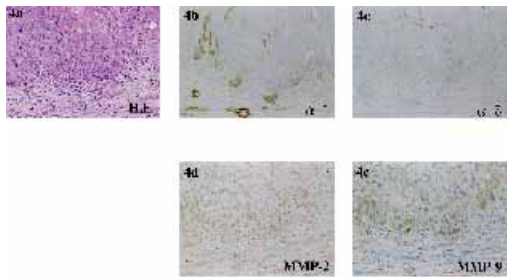


Fig.3



Fig.4



$\alpha$ 1,  $\alpha$ 2,  $\alpha$ 5 and  $\alpha$ 6 chains were stained continuously along the basement membrane. (figure 2b-e)  
MMP-2 and MMP-9 were negative. (figure 2f-g)

**Carcinoma in situ**

Atypical cells were seen in all epithelial layers. An increase in the number of lymphocytes both within the epithelium and connective tissue was likewise observed. (figure 3a)  
The basement membrane was only partially positive to  $\alpha$ 1,  $\alpha$ 2,  $\alpha$ 5 and  $\alpha$ 6 chains. The segments of the basement membrane not stained by  $\alpha$ 5 and  $\alpha$ 6 chains were wider compared to  $\alpha$ 1 and  $\alpha$ 2 chains. (figure 3b-e)  
MMP-2 and MMP-9 were positive in atypical cells and fibroblasts. (figure 3f-g)

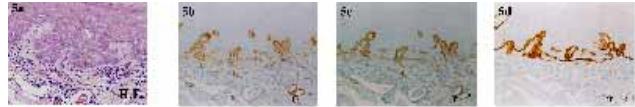


Fig.5

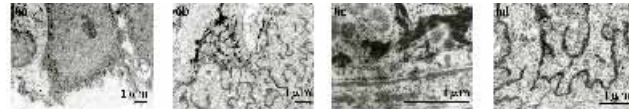


Fig.6

**Early squamous cell carcinoma**

The cancer cells with strong atypia invaded into the connective tissue. The epithelial structure was broken. Few inflammatory cells, as part of the stromal reaction was also observed. The basement membrane could no longer be clearly distinguished. (figure 4a)

Almost all  $\alpha$  chains were not stained. (figure 4b-c)  
Cancer cells were positive to both MMP-2 and MMP-9, but MMP-9 was stronger compared to MMP-2. (figure 4d-e)

**Hypertrophic area of basement membrane in epithelial dysplasia**

In some H. E. sections, we could see eosinophilic, irregular and thick band-like structure under the epithelium. (figure 5a)  
The thick band-like structure was continuously positive to all  $\alpha$  chains. (figure 5b-e)  
MMPs were stained weakly in the cytoplasm of atypical cells. (figure 5f-g)  
Under the electron microscope, we could observe a piling up of wavy lamina densa that projected into collagen fibers. (figure 6b)  
In high magnification, we could see stratified lamina densa together with the epithelial cell that projected into the connective tissue. We could also see lots of anchoring fibers, but hemidesmosome in between epithelial cell and lamina densa was not observed. (figure 6d)

**Conclusion**

Basement membrane of the oral mucosa consists of  $\alpha$ 1,  $\alpha$ 2,  $\alpha$ 5 and  $\alpha$  6 chains.  
Basement membrane disappears with the progress of carcinogenesis, and the disappearance of basement membrane is caused by MMPs. The resolution of  $\alpha$ 1 and  $\alpha$ 2 chains occurs after that of  $\alpha$ 5 and  $\alpha$ 6 chains.  
The disappearance of  $\alpha$  chains is an important element in the carcinogenesis of oral squamous cell carcinoma.