

## Immunohistochemical Evaluation of Type IV Collagen Alpha Chains in Oral Malignant Melanoma

Rosario Santos Rivera<sup>1</sup>, Hitoshi Nagatsuka<sup>1</sup>, You-Jin Lee<sup>1</sup>, Ryo Tamamura<sup>1</sup>, Naoki Katase<sup>1</sup>, Liu Yue-Heng<sup>2</sup>, and Noriyuki Nagai<sup>1</sup>

<sup>1</sup>Okayama University, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, <sup>2</sup>Beijing University, Shenzhen Hospital, China

### Introduction

The basement membrane (BM) is mainly composed of type IV collagen composed of triple combinations of 6 distinct chains ( $\alpha 1$ - $\alpha 6$ ). Invasive and metastatic melanoma cells form cluster surrounded by BM-like structures. The objective of the study was to determine the immunohistochemical pattern of type IV collagen a chain at the BM-like structures surrounding melanoma clusters.

### Materials and methods

Paraffin blocks of human tissues of 1 in situ OMM, 1 invasive OMM, 6 invasive with in situ OMM, 5 metastatic OMM to lymph nodes were sectioned. Type IV collagen  $\alpha$  chain specific rat monoclonal antibodies (provided by Dr. Naito and Dr. Sado) were used according to their specific dilutions. AEC chromogen was used to reveal the antigenic sites.

### Results

$\alpha 1$ , 2, 5 and 6 were constantly detected at the BM of the oral epithelium (Fig.1).  $\alpha 1$ , 2, 5 and 6 were intermittently detected at

the BM of the oral epithelium in in situ OMM (Fig.2).  $\alpha 1$  and 2 were intermittently detected at the BM of the oral epithelium while  $\alpha 5$  and 6 were negative in early invasive OMM (Fig.3). In invasive OMM,  $\alpha 1$  and 2 were constantly detected at the BM-like structures surrounding nodular nests (Fig.4) and were intermittently detected at the BM-like structures surrounding sheet-like nests (Fig.5). The metastatic melanoma cells likewise formed clusters and  $\alpha 1$  and 2 were constantly detected at the BM-like structures surrounding nodular nests (Fig.6) while intermittent in sheet-like nests.

### Discussion

The gradual loss of type IV collagen  $\alpha$  chains at the BM of the oral epithelium is associated with the progression of OMM. Type IV collagen  $\alpha 1$  and 2 are more stable compared to  $\alpha 5$  and 6. BMs are dynamic structures that are not only degraded but also deposited around melanoma cell clusters and the distribution pattern of type IV collagen a chains varies depending on the architecture of the nest. These suggest that type IV collagen a chains can be significant markers of oral melanocytic progression.

Fig.1

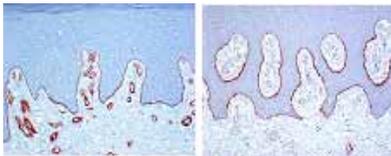


Fig.1  $\alpha 2$  and 5 were constantly detected at the BM of normal oral epithelium.

Fig.2



Fig.2  $\alpha 2$  and 5 were intermittently detected at the BM of the oral epithelium in in situ melanoma.

Fig.3



Fig.3  $\alpha 2$  was intermittently detected in early invasive melanoma while  $\alpha 6$  was negative.

Fig.4

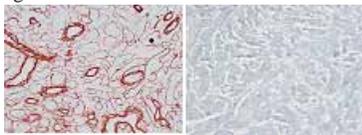


Fig.4  $\alpha 2$  was constantly detected around the nodular nests in invasive melanoma while  $\alpha 5$  was negative.

Fig.5



Fig.5  $\alpha 2$  was intermittently detected around sheet-like nests in invasive melanoma while  $\alpha 6$  was negative.

Fig.6

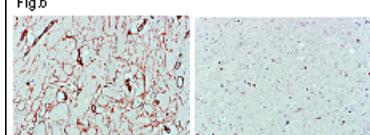


Fig.6  $\alpha 2$  was intermittently detected around sheet-like nests in invasive melanoma while  $\alpha 6$  was negative.

Table. Summary of IHC of oral epithelium in different OMM and melanoma nests in invasive and metastatic OMM.

Oral epithelium	$\alpha 1$	$\alpha 2$	$\alpha 5$	$\alpha 6$	Melanoma nest	$\alpha 1$	$\alpha 2$	$\alpha 5$	$\alpha 6$
Normal	+	+	+	+	Nodular nest	+	+	-	-
In situ OMM	$\pm$	$\pm$	$\pm$	$\pm$	Sheet-like nest	$\pm$	$\pm$	-	-
Early invasive	$\pm$	$\pm$	-	-					