ULTRASTRUCTURAL LOCALIZATION AND QUANTITATION OF BASAL LAMINA LAMININ AND TYPE IV COLLAGEN IN NORMAL RAT TONGUE MUCOSA AND INDUCED ORAL CARCINOMAS

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Thesis submitted for the degree of
Doctor of Philosophy
at
The University of Adelaide
(Faculty of Dentistry)

1993

Awarded 1994
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ABSTRACT

In this study, special methods for the ultrastructural localization of basal lamina laminin and type IV collagen in animal oral mucosa were developed in a series of experiments aimed at determining optimum methods for tissue fixation, dehydration, embedding and immunoincubation. Furthermore, the distribution of laminin and type IV collagen in normal rat tongue mucosa and induced carcinomas was characterized. Quantitative descriptions of basal lamina laminin and type IV collagen in normal rat tongue mucosa and experimentally induced oral carcinomas were also established. The results of these studies provide a tool enabling further understanding of the molecular organization of normal oral mucosal basal lamina and basal lamina in squamous cell carcinomas.

To establish optimum tissue preparation and immunostaining protocols for the ultrastructural demonstration of basal lamina laminin and type IV collagen in rat tongue tissues, post-embedding (L.R.White resin) immunogold techniques were employed as basic methods to investigate the effect of a number of variables in tissue preparation and in immunostaining relative to morphological preservation and antigen retention. The variables investigated included:

1). Different fixatives (glutaraldehyde, paraformaldehyde and glutaraldehyde-paraformaldehyde mixture).
2). Variable fixative concentrations.
3). Different fixation additives (picric acid, polyvinylpyrrolidone and sucrose).
4). Different buffer systems (phosphate buffer and phosphate buffered saline).
5). Fixation osmolarity.
6). Dehydration methods.
7). Temperature of resin polymerization.
8). Primary antibody and gold-complex variables.
9). Blocking agents.

The results of this study indicate that the antigen expression of basal lamina laminin and type IV collagen is related to fixative used, fixative concentration, additive types and the temperature of resin polymerization. The choice of primary antibody and gold-complex, also, in some cases, affects immunostaining. The morphological preservation of tissue is associated with fixative used, fixation concentration, additive types, buffer system, fixation osmolarity, dehydration and the temperature of resin polymerization.

Observations on the distribution of laminin and type IV collagen in normal rat tongue mucosa and experimentally induced oral carcinoma were carried out by correlating gold particle distribution with morphological detail. It was shown that laminin and type IV collagen were essentially confined to the lamina densa of epithelial basal lamina in normal tissues and induced oral carcinomas, and that some fibroblasts in normal tissues and
induced oral carcinomas and carcinoma cells also expressed laminin. Laminin appeared also to be distributed in the stroma of neoplasms.

Quantitative analyses of basal lamina laminin and type IV collagen in normal rat tongue mucosa and experimentally induced oral carcinomas were undertaken using morphometric methods combined with immunogold techniques. Prior to the formal establishment of quantitative data, a pilot study was performed to establish a specimen sampling pattern, the determination of optimum magnification, the selection of a measurement grid, the establishment of structural criteria and the determination of sample size. Statistical analysis of quantitative data obtained in this study indicates that laminin is significantly increased in tumour basal lamina; whereas type IV collagen is significantly decreased in tumour basal lamina.