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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CONTENTS

	PAGE
ACKNOWLEDGMENTS	vii-viii
ABSTRACT	x-xii
LIST OF FIGURES	xiii-xix
LIST OF TABLES	xx-xxiii
PUBLICATIONS	xxiv
CHAPTER ONE INTRODUCTION	1-5
CHAPTER TWO LITERATURE REVIEW	6-64
2.1. Introduction	7-10
2.2. Basal lamina components	10-21
2.2.1 Type IV collagen	10-13
2.2.2 Laminin	13-16
2.2.3 Heparan sulfate	16-17
2.2.4 Fibronectin	18-19
2.2.5 Entactin/Nidogen	19
2.2.6 Bullous pemphigoid antigen	19-20
2.2.7 Tenascin	20-21
2.3. Non-basal lamina extracellular matrix components	21-27
2.3.1 Other collagens	22-27
2.3.2 Other proteoglycans	27
2.4. Changes in basal lamina components in pathological situations	27-33
2.4.1 Non-neoplastic conditions	28
2.4.2 Neoplasms	28-33

2.5.	Changes to other extracellular matrix components in neoplasms	33-34
2.6.	Malignant neoplastic invasion-associated enzymes	34-37
2.6.1.	Collagenases	35-37
2.6.2.	Other enzymes	37
2.7.	Summary	38
2.8.	Immunoelectron microscopic techniques	39-64
2.8.1	Tissue preparation	40-58
2.8.2	Immunostaining	58-62
2.8.3	Quantitation and immunogold techniques	62-63
2.8.4	Summary	63-64
CHAPTER THREE	ESTABLISHMENT OF OPTIMUM METHODS FOR THE ULTRASTRUCTURAL LOCALIZATION OF BASAL LAMINA LAMININ AND TYPE IV COLLAGEN IN RAT ORAL MUCOSA	65-149
3.1.	Introduction	66-69
3.2.	Materials and methods	70-74
3.3.	Effect of different fixatives on the morphology of lingual mucosa and immunolabelling of laminin and type IV collagen	75-80

3.4.	Effect of fixative concentration on the morphology of lingual mucosa and immunolabelling of laminin and type IV collagen	81-84
3.5.	Effect of fixation additive on the morphology of lingual mucosa and immunolabelling of laminin and type IV collagen	85-91
3.6.	Effect of buffer system on the morphology of lingual mucosa and immunolabelling of laminin and type IV collagen	92-95
3.7.	Effect of fixation osmolarity on the morphology of lingual mucosa and immunolabelling of laminin and type IV collagen	96-100
3.8.	Effect of dehydration condition on the morphology of lingual mucosa and immunolabelling of laminin and type IV collagen	101-104
3.9.	Effect of polymerization temperature for L.R.White resin on the morphology of lingual mucosa and immunolabelling of laminin and type IV collagen	105-111

	3.10. Investigation of causes of poor labelling and high non-specific background staining in immunolabelling for type IV collagen	112-123
	3.11. Establishment of controls for immunolabelling of laminin and type IV collagen	124-127
	3.12. Discussion	128-149
CHAPTER FOUR	ULTRASTRUCTURAL IMMUNOLOCALIZATION OF BASAL LAMINA LAMININ AND TYPE IV COLLAGEN IN NORMAL RAT TONGUE MUCOSA AND INDUCED ORAL CARCINOMAS	150-174
	4.1. Introduction	151-152
	4.2. Materials and methods	153-158
	4.3. Ultrastructural observation of basal lamina and the distribution of laminin and type IV collagen in normal rat tongue mucosa and induced oral carcinomas	159-163
	4.4. Immunocytochemical controls	164-165
	4.5. Discussion	166-174
CHAPTER FIVE	ULTRASTRUCTURAL MORPHOMETRY OF BASAL LAMINA LAMININ AND TYPE IV COLLAGEN IN NORMAL RAT TONGUE MUCOSA AND INDUCED ORAL CARCINOMAS	175-232

5.1.	Introduction	176-177
5.2.	Materials and methods	178
5.3.	Sampling	178-182
5.4.	Determination of an optimum magnification of electronmicrographs	183
5.5.	Selection of an appropriate measurement grid for estimating the surface area of basal lamina on sections	184-185
5.6.	Analysis of appearances of basal lamina and hemidesmosomes in different given planes	186-190
5.7.	Establishment of structural criteria for the ultrastructural morphometry of basal lamina laminin and type IV collagen in normal rat tongue mucosa and induced oral carcinomas	191-193
5.8.	Determination of the optimum number of electronmicrographs for the ultrastructural morphometry of basal lamina laminin and type IV collagen in normal rat tongue mucosa and induced oral carcinomas	193-198

	5.9. Quantitative analysis of the expression of basal lamina laminin and type IV collagen in normal rat tongue mucosa and induced oral carcinomas	199-216
	5.10. Test of reproducibility of results in this study	217
	5.11. Discussion	218-232
CHAPTER SIX	SUMMARY AND CONCLUSIONS	233-239
CHAPTER SEVEN	SUGGESTIONS FOR PROSPECTIVE STUDIES	240-242
REFERENCES		243-267
APPENDIX		268

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.