ISSN No:-2456-2165 https://doi.org/10.5281/zenodo.14576672

Laminin Expression as a Marker of Tumour Differentiation in Oral Squamous Cell Carcinoma: An Immunohistochemical Analysis

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Abstract:- Laminin, a readily recognized basement membrane component, can serve as a marker to ascertain if the basement membrane is intact or degraded as carcinogenesis advances. The intent of this study is to illustrate the disruption in the basement membrane's continuity and establish a correlation with the histological grades of oral squamous cell carcinoma (OSCC). Thirty six formalin fixed and paraffin embedded blocks of previously confirmed OSCC were included in the study. The stain distribution in each OSCC case was observed in the cytoplasm of the cells as well as around the basement membrane of the malignant epithelial islands. A reduced distribution of laminin from well differentiated SCC to moderately differentiated SCC to poorly differentiated SCC was revealed by statistical analysis (P value 0.011). From well differentiated SCC to moderately differentiated SCC to poorly differentiated SCC, the intracytoplasmic staining of laminin progressively increased (P value 0.017). In oral squamous cell carcinomas, a high proportion of laminin 5 γ2 should be regarded as a negative prognostic marker of malignancy and a useful marker for evaluating the histological differentiation and aggressiveness of oral cancer.

Keywords:- *Laminin, Basement Membrane,Intracytoplasmic Staining, Oral Squamous Cell Carcinoma,*

I. INTRODUCTION

Oral cancer is becoming increasingly prevalent on a global scale, and the World Health Organization predicts that the number of individuals affected by this disease will continue to increase.[1]Cancers necessitate the degradation of the basement membrane to infiltrate connective tissue, making progress crucial for metastasis.

Laminin is an easily identifiable component of the basement membrane that can be used as a marker to determine whether the basement membrane is intact or demolished during the progression of carcinogenesis. Laminin 5 gamma 2 is considered as a biochemical equivalent of the anchoring filaments which connect the basal keratinocytes to the basement membrane. [2]

The intent of this study is to illustrate the disruption in the basement membrane's continuity and establish a correlation with the histological grades of oral squamous cell carcinoma (OSCC).Discovering how laminin impacts tumor invasion and growth is essential for creating novel OSCC prognostic markers and therapeutic approaches.

II. MATERIALS AND METHODS

A. Immunohistochemical procedure

Thirty six formalin fixed and paraffin embedded blocks of previously confirmed OSCC were included in the study. The cases were collected from the archives of Department of oral pathology and Microbiology. Paraffin sections of 4µm thick were prepared, deparaffinized in xylene and rehydrated in graded series of alcohol. Endogenous peroxidase activity was blocked by immersing the sections in 3% peroxidase and the antigen was retrieved by using a pressure cooker with the sections in EDTA buffer (pH 8). The slides were incubated at room temperature for 60 min with the primary antibody laminin 5 gamma 2.The poly horseradish peroxidase reagent and DAB (3,3-Diaminobenzidine) formed the basis of the antibody detection method. Finally the sections were counterstained with Mayer's Hematoxylin.

B. Assessment of staining

The basement membranes of the muscles, blood vessels, nerves, and epitheliumwere used as internal positive control. A brown-colored final substrate was indicative of positive immunoreactivity. The stain distribution in each OSCC case was observed in the cytoplasm of the cells as well as around the basement membrane of the malignant epithelial islands.

III. RESULTS

The present study included a total of 36 confirmed cases of OSCC of which 12 cases were well differentiated SCC (WDSCC),12 cases moderately differentiated SCC (MDSCC), and 12 cases poorly differentiated SCC (PDSCC).

ISSN No:-2456-2165 https://doi.org/ 10.5281/zenodo.14558001

The immunohistochemical analysis of laminin 5γ2 was conducted using a qualitative method to find out the intracytoplasmic staining and discontinuity around basement membrane of laminin 5γ2 expression. The number of positive and negative cases in intracytoplasmic staining and continuity around basement membrane were tested for significance using the Fisher's exact test.

Table 1 shows the result of intracytoplasmic staining in three grades of OSCC. In well differentiated SCC 66.7% cases (Figure 1)showed negative and 33.3% cases was positive, in moderately differentiated SCC 58.3% cases (Figure 2), and in poorly differentiated SCC 91.7% cases (Figure 3) showed positivity and these results were statistically significant.

Table 1: Intracytoplasmic staining

Grades of OSCC	Positive	Negative	p value
Well differentiated	33.3%	66.7%	0.017*
Moderately differentiated	58.3%	41.7%	
Poorly differentiated	91.7%	8.3%	

* Fisher's exact test p value <0.05 indicates statistically significant

Table 2 shows the result of continuity around basement membrane in different grades of OSCC,where 75% cases positive for WDSCC (Figure 1),66.7% cases positive for MDSCC (Figure 2), and 16.7% cases positive for PDSCC. In PDSCC 83.3% cases were negative(Figure 3). These results were statistically significant.

Table 2 :Continuity Around Basement Membrane

Grades of OSCC	Positive	Negative	p value
Well differentiated	75.0%	25.0%	0.011*
Moderately differentiated	66.7%	33.3%	
Poorly differentiated	16.7%	83.3%	

* Fisher's exact test p value <0.05 indicates statistically significant

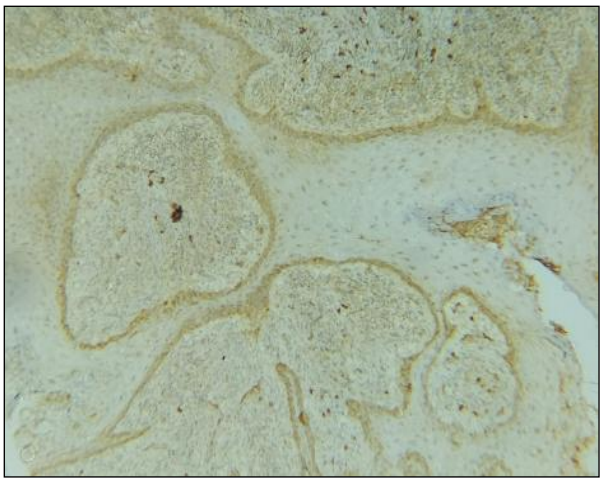

Fig 1: Negative Intracytoplasmic and Positive Basement Membrane Staining for Laminin in WDSCC (IHC, 10x)

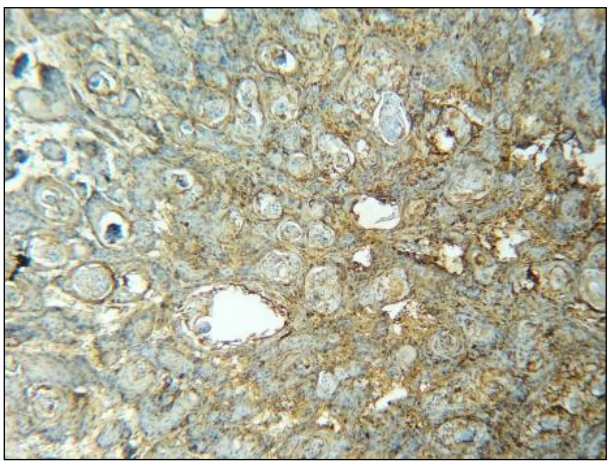

Fig 2: Positive Intracytoplasmic and Baementmembrane Staining for Laminin in MDSCC (IHC, 10x)

ISSN No:-2456-2165 https://doi.org/ 10.5281/zenodo.14558001

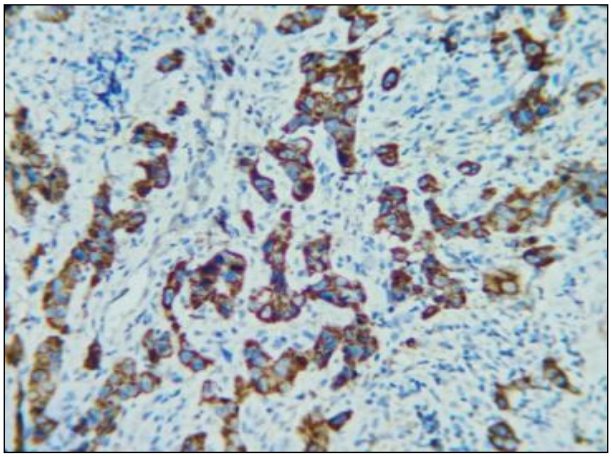

Fig 3: Positive Intracytoplasmic and Negative Basement Membrane Staining for Laminin in PDSCC(IHC, 10x)

IV. DISCUSSION

Basement membrane is a dynamic structure susceptible to qualitative as well as quantitative alteration during the evolution of OSCC, which is significant in tumour invasion. The reduction of laminin 5γ2 from the basement membrane and its elevation in the stromal region has been significantly correlated with malignant transformation.[3]

This study revealed that increased intracytoplasmic staining and multifocal breakdowns of basement membrane continuity were linked to higher grades of carcinomas. This conclusion has been supported by Jiang et al.[4] and Harada et al.[5], who noted decreased basement membrane laminin expression in metastasized malignancies. Our results were consistent with Ono et al.[6], where cytoplasmic expression of the laminin 5γ2 chain was observed in high invasive tongue carcinoma cases.

Souza LF et al.[7] found elevated expression of the cytoplasmic laminin receptor antigen is associated with poorly differentiated tumor cells that show active migration and invasive potential. Wewer et al.[8] proposed that tumor cells control the laminin receptor antigen uniquely from non-neoplastic cells. The increased cytoplasmic distribution indicates that the receptor has been internalized as an integral part of the attachment-detachment cycle of invasion.[9] This could therefore be indicative of the function of laminin in triggering cell migration and metastasis through controlling the pseudopodia production and its connections with various signaling pathways involved in invasion and metastasis.

Additionally, we discovered that 75.0% of WDSCC cases had linear staining of laminin at the tumor connective tissue interface, whereas MDSCC and PDSCC showed decreased expression of laminin. Similar finding was also noted by Souza et al.8 indicating that well-differentiated malignant cells frequently maintain the capacity to generate basement membrane components.[10]

V. CONCLUSION

A favorable environment for tumor invasion and the emergence of an invasive neoplastic cell phenotype are associated with laminin 5γ2 expression. Thus, we conclude that in oral squamous cell carcinomas, a high proportion of laminin 5 γ2 should be regarded as a negative prognostic marker of malignancy and a useful marker for evaluating the histological differentiation and aggressiveness of oral cancer.

- *Conflict of Interest*

  The authors have no conflicts of interest

- *Author Contribution*

  Every contributor has contributed adequately to the study's conceptualization, design, and intellectual substance.

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ISSN No:-2456-2165 https://doi.org/ 10.5281/zenodo.14558001

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