

Research Article

Collagen Type IV Expression in Well, Moderate and Poorly Differentiated Squamous Cell Carcinoma of the Oral Cavity

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ABSTRACT

Background: Squamous cell carcinoma (SCC) is the most important and common form of cancer of the oral cavity, Having a very high risk of local invasion, and nodal and distant metastases. Studying the mechanisms of invasion and metastasis of malignant cancers has had a crucial role in developing possible therapies tools. Type VI Collagen is expressed in a variety of tissues and abnormal expression is Related to the invasion and progression of different types of cancer in the body.

Aim: To assess whether this protein (type IV collagen) may be employed as an early detector of the biological behavior of altered cells (neoplastic cell), the purpose of this research was to compare the (IV collagen) in the three grades of oral squamous cell carcinoma (SCC).

Materials and Methods: Type IV collagen staining brightness intensity and distribution mode among the histological grade of oscc, and Chi-square test or Fisher's exact t-tests were employed to analyze differences between this grade of oscc statistically.

Results: Well-differentiated(w)SCC had more elevated type IV staining than poorly-differentiated (p) SCC (P = 0.003). In comparison to highly invasive SCCs, the expression appears more bright (P value = 0.002) and more continuous (P value = 0.002) in the early stage of cancer invasion. Conclusion: The findings showed a correlation between the degree of SCC cells' differentiation and type IV in a close direct manner, this correlation shows that when SCC cells differentiate less, they lose their capacity to basement membrane creation.

Keywords: Immunohistochemistry; Squamous cell carcinoma; Type IV collagen, Basement membrane; Oral mucosa.

INTRODUCTION

Head and neck carcinomas (HNCs) in the world are considered the sixth most common non-skin cancer, with an incidence near 400,000 new cases and a 50% mortality annually [1]. Squamous cell carcinoma (SCC), which may account for

about 92.8% of all oral malignancies in the body represents one of the most prevalent malignant oral cavity lesions [2]. Although alcohol abuse and tobacco are considered the primary risk factors, nowadays human papillomavirus (HPV) has been linked to several tumor types development

[1]. Migration of cancer cells to the spared at distant sites which causes the basement membrane(BM) constituents undergo to proteolytic destruction and ingredient communication like collagen (COL IV), proteoglycans, and glycoproteins this is an important feature of OSCC invasion [3]. Collagen is crucial and significant of all of them because it forms the structural framework of the (BM). The capacity of cancer cells to transcend the compartment of the tissue is a neoplastic invasion and metastases defining feature. Since it is considered the first and most important barrier, the neoplastic cells of different number types of carcinomas, as well as cancer cells in lymph nodes and metastases to another organ in the body have been linked to the loss and destruction of the (BM) [2-4]. The COLIV keeps and maintains the continuity and integrity of the BM that is affected by cancer degradation of the tissue. Several research have exhibited the appearance of COL IV in OSCC considered as an initial occasion in carcinogenesis and understanding biological behavior. A COLIV is a single and huge type of collagen family that is found just only in the BM and compensates for nearly 50% of all basement membranes [3].

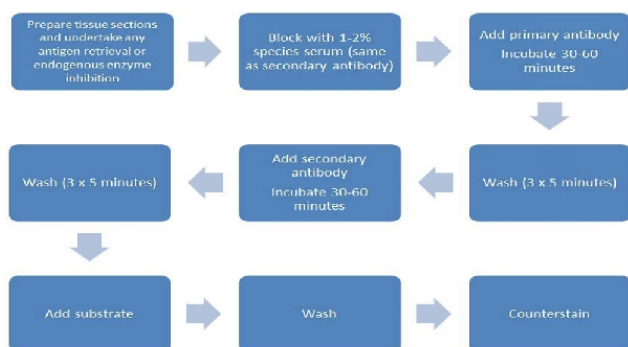
MATERIAL AND METHODS

Samples

24 formalin-fixed paraffin-embedded tissue blocks were used in this study, COLIV immunohistochemistry staining was examined in 8 cases (blocks) of each grade and one normal oral mucosa as controls.

Histology

Staining by (H&E) was used microscopically to reevaluate the grades of OSCC (Figure 1).



Immunohistochemistry

The slide was obtained from each (PEFF) block and stained immunohistochemically by A polyclonal rabbit anti-human type COL IV antibody, (Abcam) United States type, and the Indirect staining methodology was used.

Staining made by this main basic steps:

- Fixation is most important step keep tissue structure and protein.
- Antigen retrieval: to make more proteins available for identification.
- Blocking—to reduce distracting noise from the background.
- Antibody labeling and visualization to get the good pictures.

Indirect Staining Methodology

All stained slides were examined at magnifications of $\times 20$ and $\times 40$ at a light microscope. To remove subjective bias, two times went over each slide. The Chi-square test was used to make the data comparison among the numerous oral SCC grades. The within-group estimation was conducted using Fisher's exact test. When there are two small variables, the Fisher's exact test we applied $P > 0.05$ was regarded as statistically significant.

RESULTS

The control sections of normal oral mucosa in the current study displayed type IV expression at the area of interface between epithelium and connective tissue stroma as a linear continuous brown staining (Figure 2). Type IV staining was used in 24 cases of oral SCC after the normal oral mucosa examination. Three unit fields were chosen randomly in each scenario The staining brightness was rated as strong brilliant or weak in each field Around the cancer cell nests, the distribution manner as Un Interrupted and Interrupted was assessed.

In cases of early invasive Scc(wdsc), type IV immunoreactivity around tumor islands was seen to be strong brilliant intensity, with a linear staining pattern, and there was the distribution appeared uninterrupted clearly (Figure 3). While Pdsc gives weak brightness of expression in an interrupted pattern (Figure 4).

The staining brightness (P value=0.003) and distribution mode (P value=0.002) when examined by chi square test (Table 1).

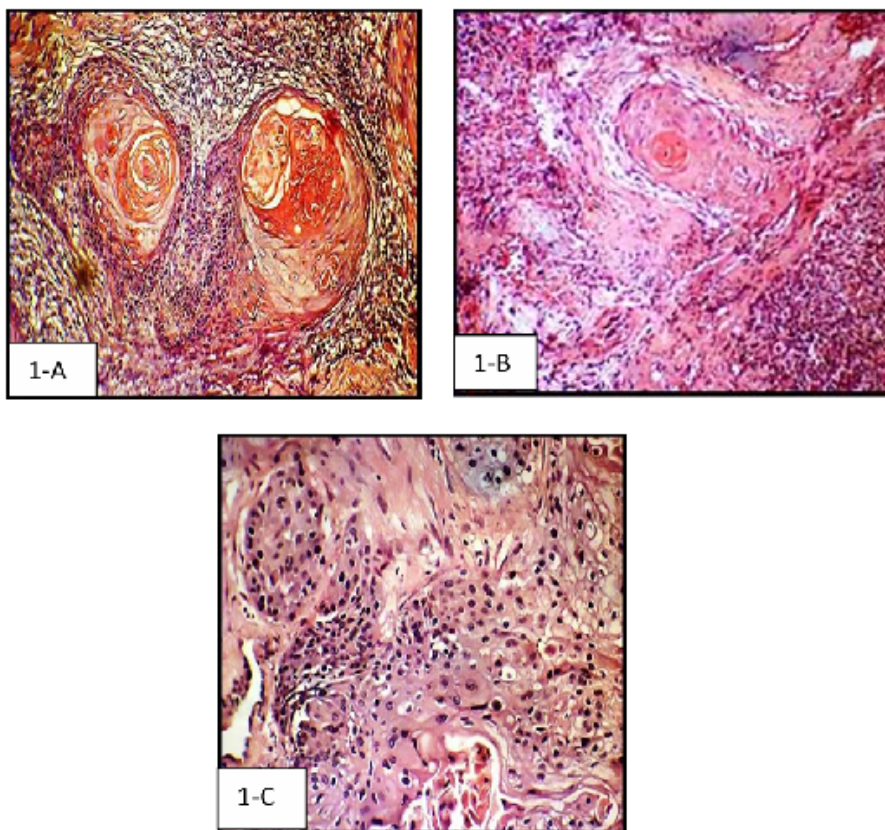


Figure (1a): H&E Moderately-Differentiated SCC(10X). Figure(1b): H&E Well-Differentiated SCC (10X). Figure (1c): H&E Poorly-Differentiated SCC (10X).

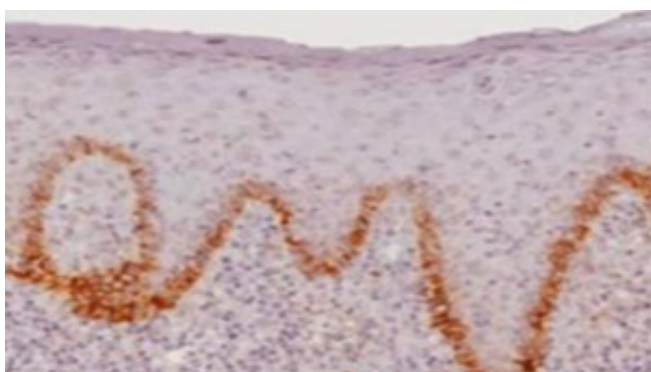


Figure 2: Control case of normal oral mucosa shows linear stain of Collagen type IV (COL IV) around the BM. (magnification ×40).

Ultimately, statistically significant differences were found for the brightness between wSCC and pSCC (P value =0.004) (Table 2) and for Distribution of COL IV between wSCC and pSCC (P value =0.002) (Table 3).

DISCUSSION

The antibody’s strong brilliant intensity staining in the current

research was restricted to the collections of well-organized epithelial cells, this result was to the results of a study made by (Miserocchi G et al., 2021 and Agarwal P et al., 2013) [1,2], who found a correlation between the COLIV expression that appear in the basement membrane and the differentiation level of the lesions and supposed that BM forms best guide of focal cancer differentiation grade and prevent penetration into neighboring tissues and organ. in contrast to less differentiated tumors, the well-differentiated tumor has a more noticeable basement membrane.

The current research observes that the basement membrane’s staining discontinuity increased gradually as the tumor progressed from a well-differentiated tumor grade to a poorly differentiated one resembling findings reported by [1,2] who postulated that the tumor cells were considered the primary source of Matrix metalloproteinase (MMP)-2 expression.

The reason that MMP 2 breaks down Collagen type IV (COL IV), is the amount of MMP2 in the neoplasm may be a very helpful predictor for the extension of the cancer cell to the distant region [5].

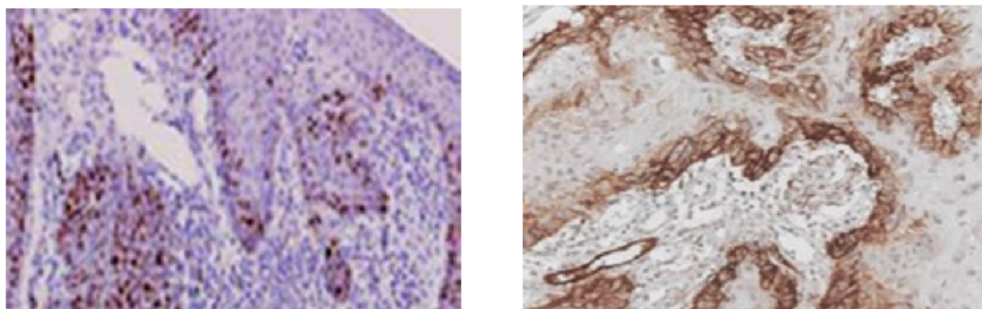


Figure 3: WdSCC gives strong brightness of expression in a linear, and uninterrupted pattern. (magnification x20).

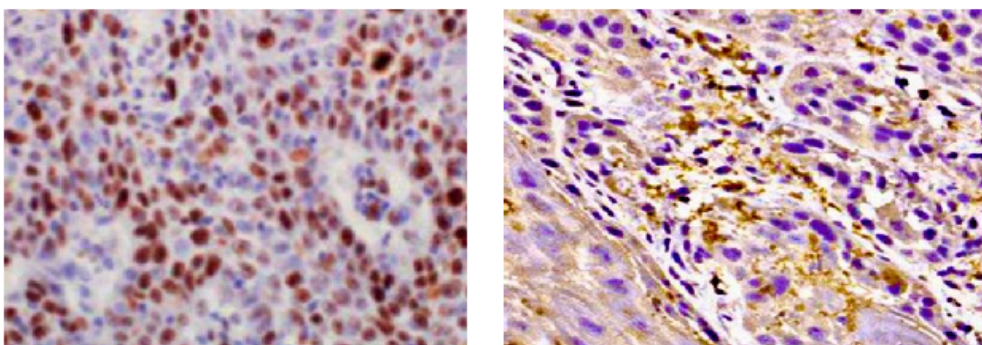


Figure 4: PdSCC gives weak brightness of expression in a interrupted pattern. (magnification x40).

Groups of Squamous cell carcinoma					
	Well differentiated SCC	Moderately differentiated SCC	Poorly-differentiated SCC	Total	
Staining brightness					
Strong	7	6	2	15	* $\chi^2=9.83$ $P=0.003$
Weak	1	2	6	9	
Total	8	8	8	24	
Distribution of COL IV					
Un Interrupted	6	5	0		** $\chi^2=10.674,$ $P=0.002$
Interrupted	2	3	8	11	
Total	8	8	8		

Table 1: Comparing the staining brightness and distribution of COL IV in three grades.

Groups					P value
	wdSCC	mdSCC	pdSCC	Total	
Staining brightness	7	6	2	15	P=2, between WdSCC and MdSCC P=0.004, between WdSCC and PSCC; P=0.082, between MdSCC and PdSCC
Strong	1	2	6	9	
Weak	8	8	8	24	
Total					

Table 2: Staining brightness within groups was compared for various grades of OSCC.

Groups					P value
	wSCC	mSCC	pSCC	Total	
Distribution of COL IV					P=2, between WdSCC and MdSCC P=0.002, between WdSCC and PSCC; P=0.01, between MdSCC and PdSCC
Un Interrupted	6	5	0	11	
Interrupted	2	3	8	13	
Total	8	8	8	24	

Table 3: Comparing the COL IV distribution within groups for various grades of OSCC.

Collagen type IV (COL IV) expression was contingent upon enzymatic activity of metalloproteinase, specifically the generation and degradation, which lead to basal lamina rupturing by Collagen type IV (COL IV) breaking and permitting cancer cell penetration to the underlying tissue. The boundary of OSCC and its very early invasion can be considered as early detection of the breaks in the basal lamina because they express Collagen type IV (COL IV) prominently, which supports cancer invasion [3-7].

According to a study done by Antonio Santos García et al., the incidence of discontinuity of collagen IV gradually increased from normal epithelium to, hyperplasic, dysplastic change, to squamous cell carcinomas [8].

Immunohistochemistry features are impacted by the activity of neoplastic cell enzymes, which lead to Collagen type IV (COL IV) lyse and degradation throughout the cancer invasion process [9].

Cancer that were well differentiated and, as a result, less invasive, expressed Collagen type IV (COL IV) markings so extremely similar to normal epithelium [10].

Six out of eight cases in this study with well-differentiated tumors had continuous Collagen type IV (COL IV) expression. This observation in this research implies that cancer cells and cells that are next to the basal membrane still can collagen secretion [6-8] According to a study by Virginia A. Jones et al., show a clear correlation between the amount of Collagen type IV (COL IV) and the cell differentiation stage. As SCC cells become poorly differentiated lose the BM components generation [11].

A related study was conducted and discovered that the early stage of invasion and metastasis of cancer is the basement membrane loss. Additionally, the Collagen type IV (COL IV) expression in cancer that has large molecular weight demonstrated Collagen type IV (COL IV) destruction [3,6,12,13].

Furthermore, Collagen type IV (COL IV) was continuously and linearly deposited in early-grade carcinoma (well-differentiated and moderately differentiated), proposing that the cancer cells remain capable of basement membrane component production. Collagen type IV (COL IV) antibody

was distributed sporadically and granularly in all micro slides of poorly differentiated squamous cell carcinoma. This result might be believed as regions of reduplication, or the compacted stromal components could be considered as the source of the deposition of collagen [3,6,14].

Before local infiltration of neoplastic cells, They can destroy the BM pass into the nearby mesenchymal component and form a new BM compartment [11].

The findings of the current study demonstrate a distinct change in Collagen type IV (COL IV) distribution and a very notable disappearance in all cases of poorly differentiated. This change is considered indicative of the emergence of structurally changeable cancer cells at the site of invasion, which reflects the most important feature that naturally determines how aggressive a cancer is [2,15].

According to another study, Collagen type IV (COL IV) was seen to be, compressed, broken up, and disintegrated, creating a pathway for tumor cells to make lamina propria infiltration. Collagen type IV (COL IV) was detected as thick sparse bands surrounding the cancer nest in (low grade) and was destroyed near tumor nests in moderately and high grade, indicating that well-differentiated grades of tumors have weak invasiveness, and a low potential for malignancy, whereas (moderate and poor) grade carcinoma shows strong invasiveness with high potential for malignancy [5,6,16,17].

The loss of Collagen type IV (COL IV) continuity as well as invasive capability for malignant neoplasia have been attached to the malignant neoplastic cell capacity to damage the basement membrane [6,18-20].

In this research, the feature of immunohistochemistry protein expression examined in the BM at the tumor invasion front of the cancer epithelial nests was jointly evaluated. This finding implied that through the process of cancer invasion, collagen type IV is broken down, facilitating the malignant neoplastic cell migration [2,21,22].

Biological traits that are linked to tumor aggressiveness include the induction of migratory features and the expression of EMT markers [1,12,21,22].

CONCLUSION

This study highlights the crucial role of Collagen type IV (COL IV) in the structural integrity of the basement membrane (BM) and its involvement in the progression and invasion of oral

squamous cell carcinoma (OSCC). The findings demonstrate a clear correlation between the differentiation of tumor cells and the continuity of Collagen type IV (COL IV) expression, with well-differentiated tumors showing a more continuous and intact Collagen type IV (COL IV) presence, whereas poorly differentiated tumors exhibit significant disruptions in Collagen type IV (COL IV). These disruptions in Collagen type IV (COL IV) not only mark the early stages of BM degradation but also reflect the invasive potential and aggressiveness of the cancer. The observed patterns suggest that monitoring Collagen type IV (COL IV) expression could serve as a valuable tool in assessing the malignancy and invasive capacity of OSCC, offering insights into the biological behavior of the tumor and potentially guiding therapeutic approaches.

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