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Role of Desmoplakins in Oral Squamous Cell Carcinoma - An Immunohistochemical Analysis

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Abstract

Context: Tumor metastasis is initiated by disaggregation of invasive cells from the primary tumor – a step that requires breakdown of intercellular junctions. Desmosomes are strong intercellular adhesion complexes that are found in a range of tissues including most epithelia. They play a critical role in the development and maintenance of epithelial tissue integrity. Desmoplakins I and II is a pair of proteins derived from Desmoplakins gene.

Aims: To understand the correlation between Desmoplakins expression with the degree of differentiation in oral squamous cell carcinoma.

Materials and Methods: The study group consisted of 21 well differentiated, 20 moderately differentiated and 12 poorly differentiated oral squamous cell carcinoma and immunohistochemical staining was done using Desmoplakins I and Desmoplakins II antibodies. All sections were scored based on their degree and intensity of staining.

Results: The normal mucosa yielded maximum degree and intensity of staining while poorly differentiated squamous cell carcinoma had the least degree and intensity of staining.

Conclusions: There was statistically significant (p=0.0022) correlation between decrease in staining for Desmoplakins and loss of tumor differentiation suggesting the loss of intercellular junctions in the higher grades of oral squamous cell carcinomas.

Keywords: Desmogleins, Desmoplakins, Intercellular junctions, Metastasis, Oral squamous cell carcinoma

Key Messages: Desmoplakins are downregulated in oral squamous cell carcinoma and decreased staining is directly proportional to loss of differentiation and degree of invasion.

Introduction

Squamous cell carcinoma is a locally invasive tumor, with frequent metastasis to both regional and distant organs. [1] Tumor metastasis is initiated by disaggregation of invasive cells from the primary tumor – a step that requires breakdown of intercellular junctions. [2]

Desmosomes are strong intercellular adhesion complexes that are found in a range of tissues and play a critical role in the development and maintenance of epithelial tissue integrity.[3,4]

The plaque region of desmosomes is thought to link the intermediate filaments to the cell surface. The major plaque associated proteins are Desmoplakins and plakoglobin. Desmoplakins I and II are a pair of proteins derived from Desmoplakins gene. Desmoplakins I is present in all desmosome-bearing tissues while Desmoplakins II is variably expressed.[3,12]

Several studies on carcinomas of cervix, breast and oropharynx have suggested that a reduction in desmosomal adhesion may be associated with invasive behavior.[1,4,5]The present study was intended to understand the correlation between Desmoplakins expression with the degree of differentiation in oral squamous cell carcinoma.

Materials and methods

The sample consisted of fresh biopsy tissue and archived tissue sections from Department of Oral Pathology and Microbiology, People's college of Dental Sciences and Research Centre, Bhopal and from the cases retrieved from the archives of Department of General Pathology, Jawaharlal Nehru cancer hospital, Bhopal and Department of Oral Pathology and Microbiology, (Sawangi) Wardha. Our study group consisted of 21 well differentiated, 20 moderately differentiated and 12 poorly differentiated oral squamous cell carcinoma.

Table 1:

Inclusion Criteria	Exclusion Criteria
• Carcinoma involving buccal mucosa and gingiva only were taken into consideration	• Carcinomas involving other mucosal surfaces were excluded.
• Only cases with detailed case history with TNM staging were included.	• Cases which did not have a detailed case history and TNM staging
• Diagnosis of tissue samples once obtained was assessed first with hematoxylin and eosin and only then was subjected for immunohistochemistry	• Cases in which tumour front was not evident
• Cases in which invasive tumor front was evident in tissue sections.	

As controls, 11 normal mucosal tissues obtained from gingiva and buccal mucosa during minor oral surgical procedures for which ethical clearance was obtained.

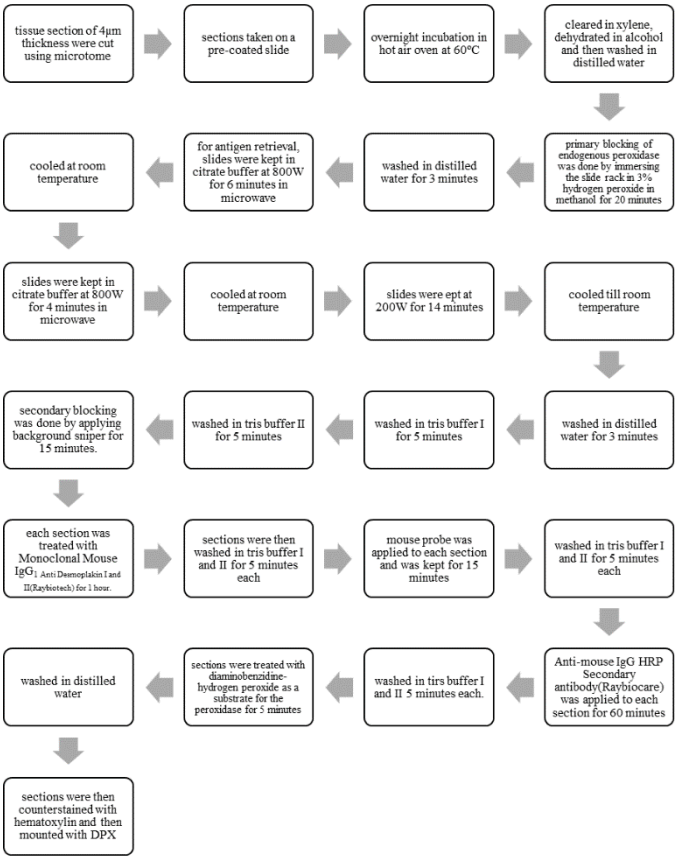
Preparation Of Tissue Sections

All specimens were fixed in 10% formalin for 24 hours, dehydrated in increasing concentrations of ethanol, cleared in xylene, impregnated in paraffin wax using hot air oven and paraffin embedded tissue blocks were prepared. Tissue sections of 4 µm thickness were cut using a soft tissue microtome from the tissue blocks. The

sections obtained were stained with Harris hematoxylin and Eosin.

The diagnosis of squamous cell carcinoma was reconfirmed and sections were obtained for immunohistochemistry.

Immunohistochemical Staining



As a negative control, primary antibody was not applied to one of the sections of normal mucosa. The slides were then visualized under microscope for scoring the extent and intensity of staining.

Scoring

The semiquantitative analysis of degree and intensity of staining was done.

The entire tissue section was observed for extent of staining. Membranous staining for Desmoplakins was considered. Areas of tissue section showing nuclear staining were considered abnormal. Degree of staining was scored as per Hiraki etal[5].

Table 2:

Score	Degree of Staining
3+	extensive staining of the tumour including the invasion front towards the connective tissue
2+	more than 50% of positive staining
1+	less than 50% of positive staining
0	almost negative

The intensity of staining was also assessed according to Narayana et al. Staining was evaluated upon comparison with control tissue.

Table 3:

Score	Intensity of Staining
3	intense cell border and relatively weak cytoplasmic signal
2	moderate cell border and cytoplasmic signal
1	overall weak staining intensity
0	staining intensity similar to background levels

All sections were scored by two independent observers with no prior knowledge of clinical data. In cases, where scores differed, the sections were scored by a third independent observer and the majority decision was adopted.

The statistical analysis between degree and intensity of staining and its relationship with the different grades of squamous cell carcinoma was carried out by chi square test and to rule out the interobserver variability, sign test was done. Significant differences between the different groups was determined by Kruskal-wallis ANOVA multiple comparisons.

Results

Degree of Staining

Degree of Staining of Desmoplakins In Normal Oral Mucosa

The monoclonal antibody yielded positive signals in surface epithelia, but not in connective tissues. Labelling occurred only in the cell membranes, showing a typical punctuate lines on cell-to-cell boundaries, which is peculiar for normal mucosa. Staining was strongest in stratum spinosum and was moderate in basal cell layers. 81% of cases of normal mucosa showed extensive staining (score 3+) while 19% of the cases had more than 50% of positive staining (score 2+). Relationship between degree of staining of Desmoplakins in different histological grades of Oral squamous cell carcinomas

Staining for Desmoplakins in well differentiated squamous cell carcinoma was strong in 57% of the cases (score 3+) (Image 4) and similar to that of normal mucosa. 43% of the cases showed moderate to weak staining {score 1+ (Image 2) to 2+(Image 3)}. In case of moderately differentiated squamous cell carcinomas, 45% of cases were stained with a score of 2+, while only 30% of the cases had staining similar to that of well differentiated carcinomas (score 3+) and rest 25% had scored 1+ regarding the degree of staining of Desmoplakins I and II. 50% of poorly differentiated squamous cell carcinomas had a score of 1+, with only one case showing almost negative staining {score 0(Image 1)} for Desmoplakins I and II and 41% of the cases had more than 50% of positive staining (score 2+). There was statistically significant (P=0.0022) correlation between decrease in staining for Desmoplakins and loss of tumor differentiation suggesting the loss of intercellular junctions in the higher grades of oral squamous cell carcinomas. Results were also statistically significant (P= 0.0290) for the loss in the degree of staining between the groups of oral squamous cell carcinoma (TABLE 2).

Table 4: Relationship between degree of staining and grade of differentiation.

Degree Of Staining	Normal (N=11)	Grade Of Differentiation			Chi Squ	P Value
		Well (n=21)	Moderate (n=20)	Poor (n=12)		
3+	9	12	6	0	25.741	0.0022 (S)
2+	2	7	9	5		
1+	0	2	5	6		
0	0	0	0	1		

Kruskal- Wallis ANOVA

Grp1 n=65 Mean Rank=110.3077

Grp2 n=80 Mean Rank=97.3750

Grp3 n=50 Mean Rank=83.0000

Kruskal-Wallis H=7.0782 df=2 p=0.0290

Fig.1: Histopathological picture showing degree of staining of score 0.

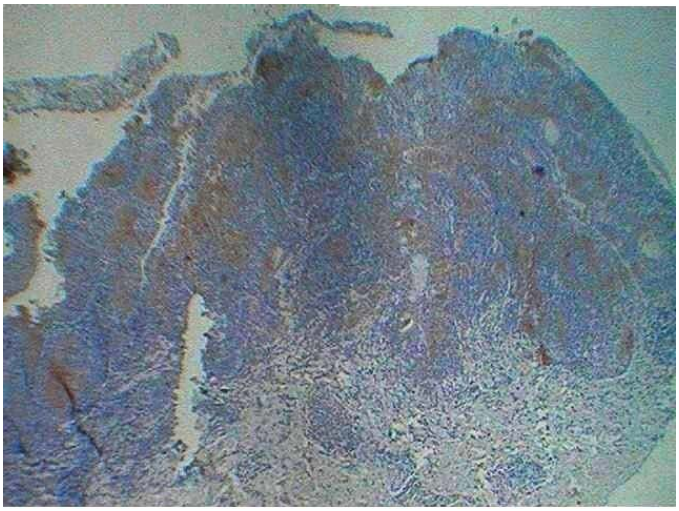


Fig. 2**:** Histopathological picture showing degree of staining of score 1+.

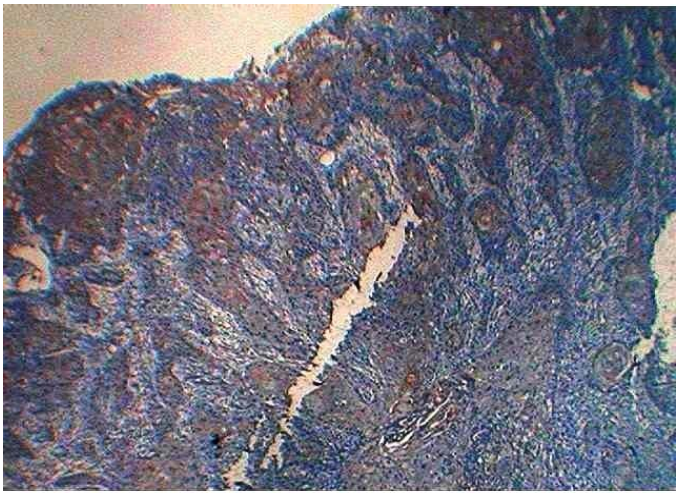


Fig. 3**:** Histopathological picture showing degree of staining of score 2+.

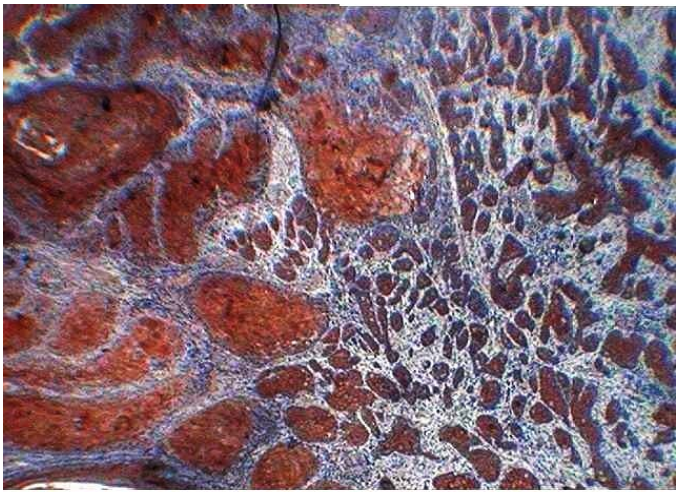


Fig. 4**:** Histopathological picture showing degree of staining of score 3+.

Intensity of Staining

Relationship between the intensity of Desmoplakin staining and normal oral mucosa: Desmoplakin staining in normal mucosa samples scored highest (score 3) with 90% of the samples showing an intense cell border and weak cytoplasmic staining. Only 10% of the cases had a staining intensity of score 2.

Relationship between the intensity of Desmoplakin staining and the different grades of Oral squamous cell carcinoma

Desmoplakins staining in oral squamous cell carcinomas scored significantly lower as compared to normal mucosa. 62% of well differentiated tumors had an intensity similar to that of normal mucosa (score 3) (Image 8), with 38% of the cases showing a lower score of intensity (score 1 to 2). In case of moderately differentiated squamous cell carcinomas, the intensity of staining was score 3 in 40% of the cases and score 2(Image 7) in the same percentage of cases. 59% of the cases in poorly differentiated squamous cell carcinomas had lower staining intensity {score 1(Image 6)} with only 33% of the cases with moderate intensity (score 2). Rest had a staining intensity similar to background levels {score 0(Image 5)}. There was statistically significant (P=0.0022) correlation between decrease in staining intensity for Desmoplakins and loss of tumor differentiation. These data suggest that loss of anti-Desmoplakins immunoreactivity is detectable during the transition from normal to carcinoma in the oral cavity. Results were also statistically significant (P=0.0290) for the loss in the intensity of staining of desmoplakin between the groups of oral squamous cell carcinoma (TABLE 5).

Table 5: Relationship between intensity of staining and grade of differentiation.

Intensity Of Staining	Normal (N=11)	Grade Of Differentiation			Chi Square	P Value
		Well (n=21)	Moderate (n=20)	Poor (n=12)		
3	10	13	8	0	31.8485	0.00020845 (<0.001)
2	1	7	8	4		
1	0	1	4	7		
0	0	0	0	1		

Kruskal- Wallis ANOVA

Grp1 n=65 Mean Rank=110.3077

Grp2 n=80 Mean Rank=97.3750

Grp3 n=50 Mean Rank=83.0000

Kruskal-Wallis H=7.0782 df=2 p=0.0290

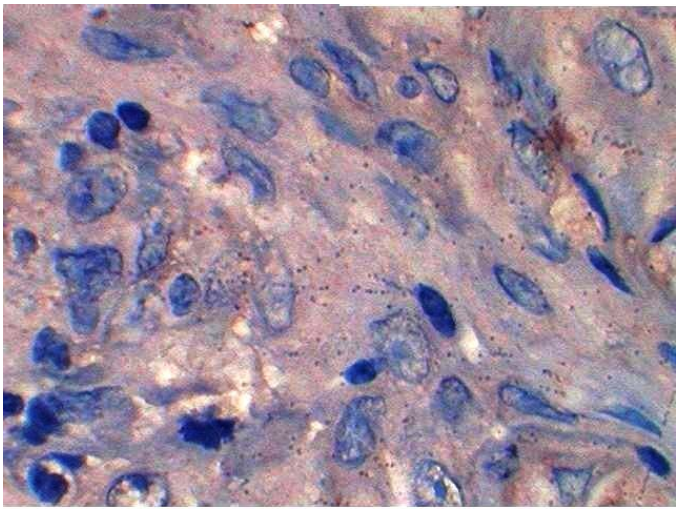


Fig 5: Histopathological picture showing intensity of staining of score 0.

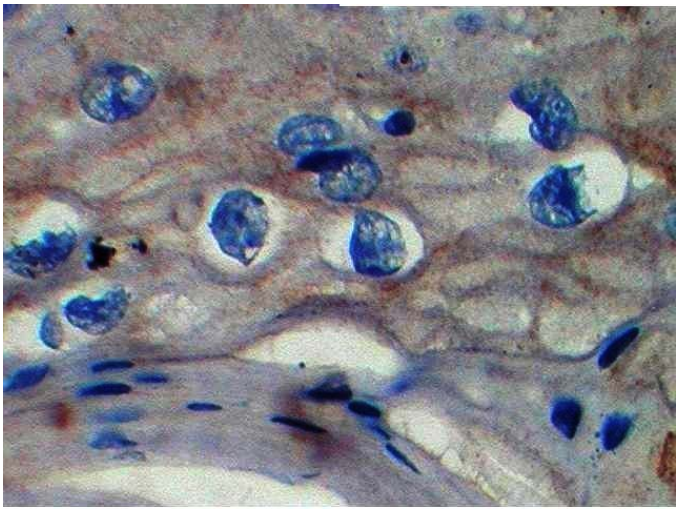


Fig 6**:** Histopathological picture showing intensity of staining of score 1+.

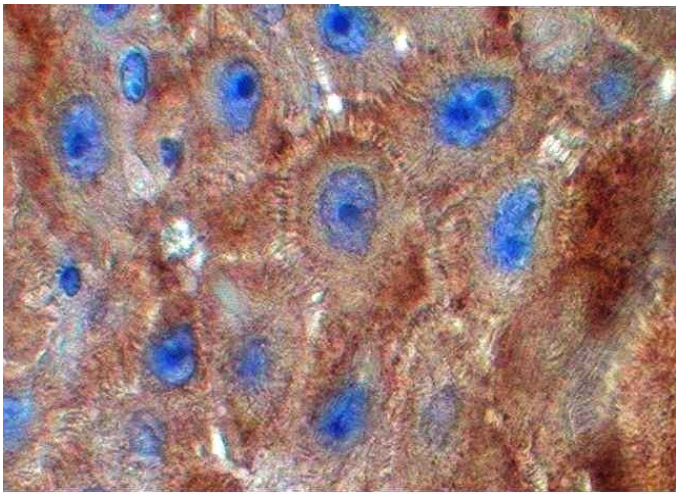


Fig 7**:** Histopathological picture showing intensity of staining of score 2+.

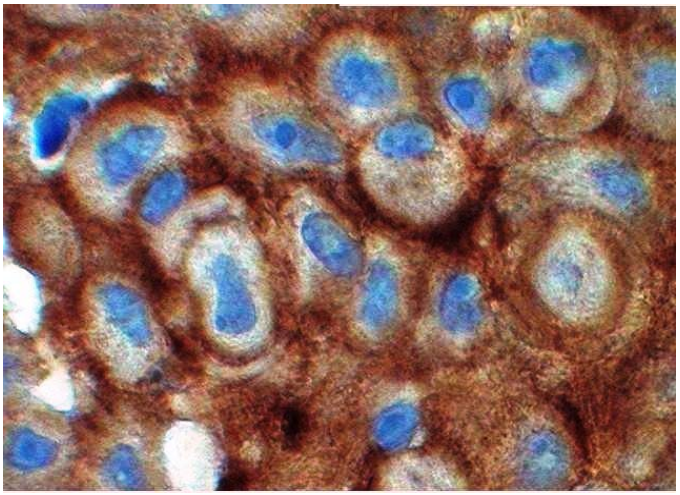


Fig 8**:** Histopathological picture showing intensity of staining of score 3+.

Discussion

Desmoplakins I and II (DPI and DPII) are the major constituent proteins of the desmosomal plaque that have been identified in epithelia from various species. Desmoplakins are localized to the inner portion of desmosomal plaque through which the intermediate filaments loop thus linking the filaments to the desmosomes.[7] Both DPI and DPII are derived from a single gene, contain three major structural domains and are homodimers consisting of globular NH_2- and COOH-terminal domains flanking a central α-helical coiled coil rod domain, which is shorter in DPII than DPI due to alternative splicing.[8]

Antibodies to these proteins allow the specific detection of desmosomes and other structures containing desmosomal plaque material (hemidesmosomes, internalized desmosomal domains), but do not react with any other type of junction. They allow semi-quantitative analysis of desmosomal densities at the light microscopic level and allows the detection of disorders and changes of desmosomal arrays on surfaces of normal and neoplastic cells.[9]

Very few studies exist correlating the degree and intensity of Desmoplakins staining with the histological grading and clinical staging of oral squamous cell carcinoma. In the present study we studied the expression of Desmoplakins I and II in oral squamous cell carcinoma and correlating its expression with the differentiation of the tumor.

Desmoplakin I & II Staining In Control Samples

The pattern of staining in control samples was in accordance with the findings reported by Narayana N[6] and Alazawi WOF[3] where strong membrane positivity was noted especially in the stratum spinosum. 81% of cases of normal mucosa showed extensive staining (score 3+) with remaining 19% showing staining score of 2+. Though few areas in the control samples revealed cytoplasmic signal it was always accompanied with membranous staining.

Desmoplakin I & II Staining and Tumor Differentiation

Our results show decrease in the expression of desmoplakin I and II in progressive grades of oral squamous cell carcinoma in comparison to normal epithelium. Desmoplakin immunostaining was typically seen as punctate lines on cell-to-cell boundaries, often allowing resolution of individual desmosomes.[9] Staining for well differentiated squamous cell carcinoma was strong and was found similar in all the differentiated layers of normal stratified squamous epithelium. In contrast, none of the poorly differentiated oral squamous cell carcinoma showed score 3 (extensive staining). Whereas, in case of moderately differentiated oral squamous cell carcinoma, a large number of cases (14/20) showed focal staining (score 2 and 1). Thus, an inverse correlation was seen between the expression of desmoplakin and the increasing grades of squamous cell carcinoma which was statistically significant. Even between the groups, a statistically significant association was seen.

Similar changes have been observed in lesions of breast, cervix and oropharynx[10,4,5,6] where well differentiated tumors showed staining similar to normal structures with diminished staining seen in moderately and poorly differentiated carcinomas. Narayana N reported disrupted localization of desmoplakin in oral dysplastic lesions with further reduced or no expression in oral squamous cell carcinoma samples. However, the grade of oral squamous cell carcinoma was not specified.[6] Another study in oropharyngeal cancer revealed focal and subcellular staining in moderately and poorly differentiated carcinomas.[10]

In contrast, Alazawi WOF found no significant change in staining between normal cervical tissue, low-grade and high grade squamous intraepithelial lesions.[3]

Other desmosomal components like Desmogleins, Desmocollins, E- cadherins have also been studied with reference to tumor differentiation in various carcinoma types. Similar relationship between staining and tumor grading has been reported.

Semiquantitative scoring performed to assess the staining intensity, demonstrated an intense cell border in all the layers of normal stratified squamous epithelium. Desmoplakin immunostaining of moderate and poorly differentiated squamous cell carcinomas often revealed a decrease in overall intensity, when compared to well differentiated squamous cell carcinomas. The intensity of well differentiated squamous cell carcinoma was found to be similar to that of normal tissues.

Similar findings were reported by Papagerakis **S**.[10] These data suggest that loss of anti-desmoplakin immunoreactivity is detectable during the transition from normal to neoplastic epithelium in the oral cavity. Thus, disruption of desmoplakin adhesion may be an early event in the progression to oral squamous cell carcinoma, possibly in response to altered keratin intermediate filament attachment at sites of cell-cell contact.[6]

Conclusion

Typical punctuate lines on cell-to-cell boundaries were seen, with the strongest staining seen in stratum spinosum and moderate in basal cell layers. Results also revealed that maximum cases of normal mucosal tissue had an extensive staining with only few cases showing a staining pattern of less than 50%.

Degree of staining of different histological grades when compared with normal mucosa showed a statistically significant correlation between loss of expression of desmoplakin and tumor differentiation. Results were also significant for the loss of staining pattern between different grades of squamous cell carcinomas. This probably suggests the loss of expression of intercellular junctions in the increasing grades of oral squamous cell carcinoma.

90% of the samples of normal oral mucosa demonstrated an intense cell border with weak cytoplasmic staining in all the layers of normal stratified squamous epithelium.

Desmoplakin immunostaining of moderate and poorly differentiated squamous cell carcinomas often revealed a decrease in overall intensity, when compared to well differentiated squamous cell carcinomas, possibly due to altered keratin intermediate filament attachment at sites of cell-cell contact.

Mechanism involving mutation or suppression of this protein may be loss at chromosomal 6p21, which is a frequent event in oral squamous cell carcinoma and as the desmoplakin gene are located on chromosome 6(6pter-p21), its loss may cause decreased expression of this intercellular junction. [5,3,11]

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