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Extracellular matrix changes in oral squamous cell carcinoma using special stains

Pratijya Raj, Veda Hegde, Wasim Raja Mumtaz

Department of Oral Pathology and Microbiology, SDM College, Dharwad, Karnataka, India

Keywords
Early invasion, extracellular matrix, oral squamous cell carcinoma, periodic acid Schiff's, Van Gieson

Correspondence
Dr. Pratijya Raj, Department of Oral Pathology and Microbiology, SDM College Dharwad, Karnataka, India.
E-mail: drpratijyaraj@gmail.com

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Abstract

Background: Squamous cell carcinoma (SCC) is the most frequent malignancy of the oral cavity which is known to have a high mortality rate. During the invasion, tumor cells break through the basement membrane, penetrate the connective tissue, are embedded in or surrounded by the extracellular matrix (ECM), producing reactive changes in stroma. It is an established fact that tumor stroma plays a vital role in tumor progression. The ECM has an important role in tissue organization and function.
Aims: The aim of the study is to assess whether special stains are useful in detecting ECM changes early invasive and well-established oral SCC (OSCC) using special stains.
Materials and Methods: This retrospective study constitutes a total of 30 cases examined to study changes in ECM at early invasive and well-established OSCC cases. Two sections each was obtained from above study groups and were subjected to Van Gieson and Periodic Acid Schiff's (PAS) stains respectively, and were compared with routine hematoxylin and eosin stain. Interpretation of staining intensity was carried out and statistically analyzed.
Results: Matrix changes were noted using special stains in both early invasive and well-established OSCC as seen in their staining characteristics.
Conclusion: PAS and Van Gieson are indeed useful in detecting changes in glycoprotein and collagen in well-established OSCC and early invasive OSCC.

Introduction

Squamous cell carcinoma (SCC) is the most frequent malignancy of the oral cavity, which is known to have a high-mortality rate, characterized by invasion and metastasis. During invasion, tumor cells break through the basement membrane, penetrate the connective tissue, and interact with extracellular matrix (ECM), producing reactive changes in stroma.[1]

ECM is a dense lattice network of collagen and elastin, embedded in a viscoelastic ground substance composed of proteoglycans and glycoproteins.[2] It is an essential part of the milieu of a cell which is surprisingly dynamic, versatile, and influences fundamental aspect of the cell biology. ECM proteins have role in scaffolding to support tissue architecture and integrity. It also facilitates attachment for cell surface receptors and act as a reservoir of signalling capabilities that modulate host defense response.

In current study, special stains were used in an attempt to evaluate connective tissue changes in early invasive and well established OSCC.

Materials and Methods

This retrospective study constituted a total of 30 cases, 15 cases each of early invasive OSCC and well-established cases of OSCC, were retrieved and Microbiology, SDM College of Dental Sciences and Hospital, Dharwad.

Two sections from paraffin-embedded tissue blocks of each case were taken which constituted a total of 60 sections of the above study. 30 sections (15 sections of early invasive and 15 sections of well established OSCC) were stained with PAS stain and the other 30 sections (15 sections of early invasive and 15 sections of well established OSCC) were subjected to Van Gieson stain. The tissue sections were then analyzed for staining property of collagen using Van Geison and glycoproteins using PAS stain in early invasive and well established OSCC cases. Interpretation of staining intensity was done and statistically analyzed.

Exclusion criteria

Inadequate epithelium and connective tissue depth, folded tissues, necrotic tissues, recurrent cases of OSCC, and

cases with inadequate patient details were excluded from the study.

Results

In this study, the study participants were predominantly males (n = 28; 93.3%), ranging from 40 to 60 years of age. The lesional tissue were taken from buccal mucosa (n = 18; 60%), alveolus (n = 6; 20%), floor of the mouth (n = 3; 10%), and retromolar (n = 3; 10%). Although there are various techniques available to detect ECM changes in the connective tissue, special stains such as PAS and Van Gieson stains are inexpensive, less technique sensitive and can be performed under regular laboratory set up. Thus, the aim of present study is to assess alteration in the ECM, glycoprotein, and collagen in at early invasive front and well-established cases of OSCC. On comparing the staining characteristic of PAS stain in well established OSCC & early invasive OSCC it was noted that out of 15 cases only 1 case showed positive staining whereas out of 15 cases of well-established OSCC, 7 cases showed positive staining at the invasive front.

Van Gieson stain was used to analyze collagen in ECM for early invasive and well-established cases of OSCC. In the present study, only 7 cases showed positive staining for Van Gieson in early invasive OSCC and all cases of well-established OSCC showed negative staining.

Discussion

SCC is the most frequent malignancy of the oral cavity which is known to have a high-mortality rate. The concept of a step-wise development of cancer in the oral mucosa, i.e., the initial presence of a precursor (pre-malignant/pre-cancerous) lesion subsequently developing into cancer is well established.

Cancer is the loss of tissue organization and aberrant behaviour of the cellular components. The ECM is the non-cellular component present within all tissues and organs which acts as scaffold that binds the cells and tissues to one another.[2,3] It has been reported that tissue produced by the altered cells differs from that produced by the normal cells as the invading tumor cell induces abundant collagenous, or desmoplastic stroma.[4] ECM has an important role in tissue organization and function. Thus, it is important to study the changes occurring in ECM.

Patankar *et al.*, in his study showed that tumor cells and inflammatory cells alter ECM by release of certain substances.[1] Tumor cells break the basement membrane invading into the connective tissue stroma. During invasion, some molecular events occur such as secretion of proteases and collagenases which causes destruction of ECM. Damage causes recruitment of lymphocytes to the site as a part of host immune response. Lymphocytes secrete cytokines which further causes destruction of collagen and also promotes angiogenesis.[4]

ECM contains glycoproteins such as tenascin and fibronectin. Tenascin is an adhesive glycoprotein of ECM which facilitates cell–cell adhesion, cell migration as well as cell adhesion close to matrix through fibronectin binding to proteoglycans. When this protein is produced by malignant cells, there is an increase in proliferation and migration, because of the fact that it has anti-adhesive properties, as it blocks binding of fibronectin to cells. It has been demonstrated that its expression is increased in many neoplastic lesions.

Expression pattern of many proteins in ECM, such as laminin, Collagen I and IV, fibronectin, and tenascin in lower lip and tongue SCC with varied histology grading was analyzed by Pereira *et al.*[5] Fibronectin was immunomarked in all studied cases and tenascin expressed intensively on epithelial basal membrane in most cases. Similarly as a whole, glycoproteins are altered in ECM of neoplastic cells. Therefore to assess the alterations in ECM for glycoproteins, PAS satin may be of use.

George *et al.*, all grades of SCC showed a bright staining of PAS around the tumor islands, which were probably the secretion of basement membrane components by the tumor cells.[6] In the present study, glycoprotein detected using PAS stain in well-established OSCC [Figures 1-3] was probably because of the secretion of basement membrane components and glycoproteins like tenascin by the tumor cells.

Rathore *et al.* used Van Gieson stain to observe changes in collagen of the in the connective tissue of OSCC and found abundant collagen distribution in the stroma of well established OSCC, attributed to the increased deposition of the thick bands of collagen fibers at the site of tumor cell invasion. However, in poorly differentiated carcinomas, the disintegration of collagen

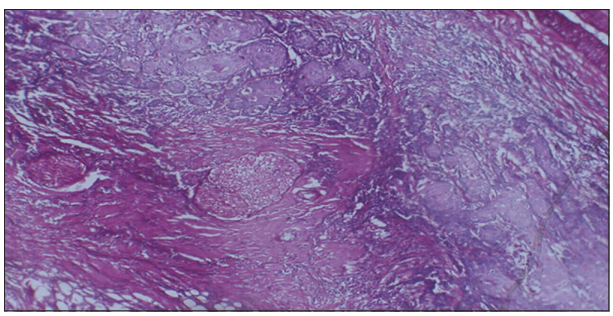

Figure 1: Well-established oral squamous cell carcinoma showing neural tissue surrounded by intensely stained extracellular matrix component (Periodic Acid Schiff's, ×10)

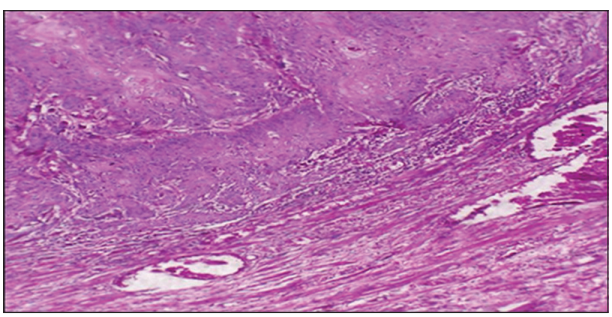

Figure 2: Characteristic staining of extracellular matrix at the invasive tumor front (Peiodic Acid Schiff's stain, ×40)

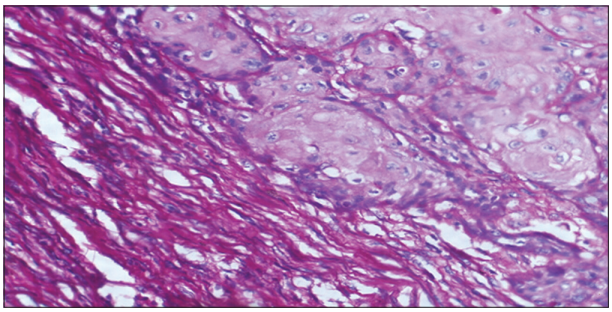

Figure 3: Early invasive oral squamous cell carcinoma showing intense staining at the invasive tumor front (periodic acid Schiff's stain, ×40)

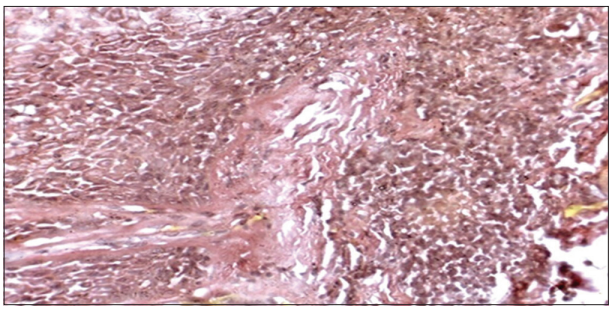

Figure 4: Early invasive squamous cell carcinoma showing pale collagen staining (Van Gieson stain, ×10)

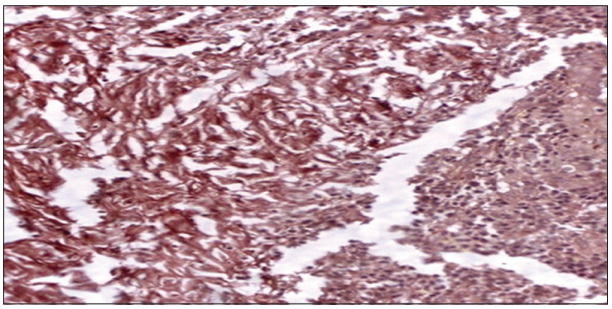

Figure 5: Early invasive squamous cell carcinoma shows red collagen (Van Gieson stain, ×10)

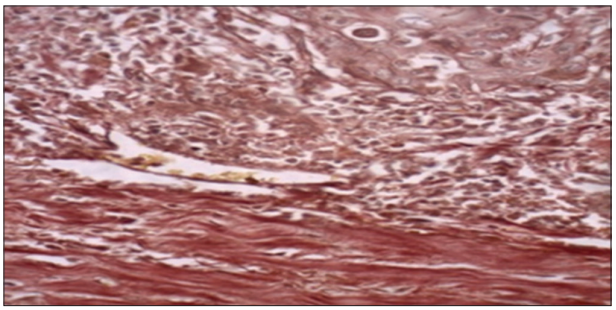

Figure 6: Early invasion case showing thick band of collagen near the tumor island (Van Gieson stain, ×40)

fibers was noted. In the current study, collagen was detected only in 7 cases with the help of Van Gieson stain in early invasive OSCC [Figures 4-6] cases and no sections of well-established cases of OSCC was stained. This difference in the staining pattern as seen in our case could be due to extensive destruction of collagen fibers in later group compared to the former. The collagen around the tumor cells play a role in walling off the invading cells which was not seen in the well-established OSCC.[7-9] This would thus bestow invasive property to the tumor cells as seen in our study.

Therefore, in the present study, notable changes in the ECM as seen in the differences in the staining characteristic with PAS & Van Gieson stain in early invasive and well established OSCC suggest that tumor cells do alter the connective tissue stroma facilitating tumorigenesis and these ECM changes can influence the tumor progression.

Conclusion

Tumor cells modify ECM by release of certain substances which alter the surrounding stroma. IHC and electron microscopy, though specific and sensitive in identifying ECM changes, their technique, cost, and special laboratory requirements makes them a less likely choice in routine laboratories. An alternative method is the use of special stain as we have used, PAS and Van Gieson to identify changes in glycoprotein and collagen respectively, in the ECM. In our study, glycoproteins were altered in early invasive OSCC and collagen was altered in well-established cases of OSCC. The difference in the staining pattern in the two study groups as in our case has proved the same. It can be concluded that special stains are indeed useful in identifying matrix changes in OSCC. Although further studies with a larger sample size using these special stains will enhance our understanding. Likewise, immunohistochemistry needs to be undertaken to verify the result obtained from our study.

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