Extracellular matrix in oral squamous cell carcinoma: Friend or foe?

Sangeeta R Patankar, Divyesh P Wankhedkar, Nidhi S Tripathi, Sanya N Bhatia, Gokul Sridharan

ABSTRACT

Background: Oral squamous cell carcinoma (OSCC) primarily spreads through direct invasion and/or lymphatic route. During the invasion, tumor cells break through the basement membrane, penetrate the connective tissue to interact with the extracellular matrix (ECM). An attempt was made to evaluate the connective tissue changes in different grades of OSCCs and their influence in predicting the biological behavior of these tumors.

Materials and Methods: A total of 30 histologically proven cases comprising 5 normal mucosa, 10 well-differentiated OSCC’s, 10 moderately differentiated OSCC’s, and 5 poorly differentiated OSCC’s were examined for the presence of any ECM changes by using special stains. Interpretation of staining intensity was carried out and statistically analyzed.

Results: Van Gieson stain showed abundant thick collagen fibers, dispersed collagen fibers, thin few dispersed collagen fibers in well-, moderately- and poorly-differentiated OSCC’s, respectively. Verhoeff’s Van-Gieson showed negative staining for elastic fibers around tumor islands in different grades of OSCCs. PAS stain showed moderate staining for glycoprotein in well-differentiated OSCC and negative in moderately and poorly differentiated cases. Picrosirius red stain showed Type 1 collagen fibers in well and moderately differentiated OSCC cases and Type 3 collagen fibers in poorly differentiated cases.

Conclusion: The observations of this study revealed altered staining reactions of the collagenous stroma and glycoproteins suggesting that tumor cells may release certain enzymes that play a role in the manipulation of ECM to enhance their own survival.

Key words: Extracellular matrix, oral squamous cell carcinoma, special stains
In this study, an attempt was made to evaluate if correlation exists between the connective tissue changes in different grades of OSCCs and whether these changes have an influence in predicting the biological behavior of these tumors.

MATERIALS AND METHODS

Formalin-fixed paraffin-embedded tissues were retrieved from the archives of Department of Oral Pathology and Microbiology. Histologically proven cases comprising 5 normal mucosa (Group 1), 10 well differentiated OSCC (Group 2), 10 moderately differentiated OSCC (Group 3), and 5 poorly differentiated OSCC (Group 4) were included in the study. Cases showing distant metastasis, recurrent OSCCs, patients undergoing chemotherapy or radiotherapy and patients with presence of any systemic illness were excluded from the study. Cases of normal mucosa were used as controls. Five sections, each of 5 μ thickness, from paraffin-embedded tissues of Groups 1, 2, 3, and 4 were obtained. Five sections of each group were stained with hematoxylin and eosin and with special stains (Van Gieson, PAS, Verhoff Van Gieson and picrosirius red stain). All the three grades of OSCC were evaluated for staining intensity of each component around tumor islands. A total of five areas in each slide were randomly selected and evaluated. To eliminate subjective bias two observers independently evaluated all the slides. Staining intensity around tumor islands was scored as, strongly positive = 2, moderately positive = 1, weakly positive or negative = 0. Statistical analysis was performed using Chi-square test.

RESULTS

In the current study, staining intensity around tumor islands for collagen fibers in well differentiated OSCC cases showed abundant thick collagen fibers around tumor islands [Figure 1]. Moderately differentiated OSCC cases showed dispersed collagen fibers around tumor islands [Figure 2]. Poorly differentiated OSCC cases showed thin weakly stained few dispersed collagen fibers in the connective tissue stroma [Figure 3]. On application of Chi-square test, significant “P” value ($\chi^2 = 27.083, P < 0.0001$) was noted in staining of collagen fibers with Van-Gieson stain in all the different grades of OSCCs [Table 1 and Graph 1].

Verhoeff’s Van-Gieson showed negative staining ($\chi^2 = 1.562, P = 0.458$) for elastic fibers around tumor islands in all the different grades of OSCCs [Table 2 and Graph 2]. PAS staining showed moderate staining of glycoproteins around tumor islands in well-differentiated cases [Figure 4] and negative staining around tumor islands in case of moderately and poorly differentiated OSCC [Figures 5 and 6]. On application of Chi-square test, significant “P” value ($\chi^2 = 11.213, P = 0.004$) was noted in staining of glycoproteins in all the different grades of OSCCs [Table 3, Graph 3]. Picrosirius red staining showed Type 1 collagen fibers (thick, mature) in the connective tissue stoma in well and moderately differentiated cases [Figures 7 and 8] and Type

![Figure 1: Well differentiated oral squamous cell carcinoma showing abundant thick collagen fibers around tumor islands using Van-Gieson stain (×10)](image1)

![Figure 2: Moderately differentiated oral squamous cell carcinoma showing dispersed collagen fibers around tumor islands using Van-Gieson stain (×10)](image2)

![Figure 3: Poorly differentiated oral squamous cell carcinoma showing thin weakly stained few dispersed collagen fibers using Van-Gieson stain (×10)](image3)
Extracellular matrix in oral squamous cell carcinoma

Table 1: Staining intensity of oral squamous cell carcinoma with Van-Gieson stain

|                | Well differentiated OSCC (n=10) | Moderately differentiated OSCC (n=10) | Poorly differentiated OSCC (n=05) | Chi-square test |
|----------------|-------------------------------|--------------------------------------|----------------------------------|-----------------|
|                | n                             | Percentage                           | n                                | Percentage      | $\chi^2$ | $P$       |
| Weakly positive/negative | 0                              | 0                                    | 5                                | 50              | 4        | 80        |
| Moderately positive     | 0                              | 0                                    | 5                                | 50              | 1        | 20        |
| Strongly positive       | 10                             | 100                                  | 0                                | 0               |          |           |

OSCC=Oral squamous cell carcinoma

Table 2: Staining intensity of oral squamous cell carcinoma with Verhoff’s Van-Gieson stain

|                | Well differentiated OSCC (n=10) | Moderately differentiated OSCC (n=10) | Poorly differentiated OSCC (n=05) | Chi-square test |
|----------------|-------------------------------|--------------------------------------|----------------------------------|-----------------|
|                | n                             | Percentage                           | n                                | Percentage      | $\chi^2$ | $P$       |
| Weakly positive/negative | 9                              | 90                                   | 10                               | 100             | 05       | 100       |
| Moderately positive     | 1                              | 10                                   | 0                                | 0               | 0        | 0         |
| Strongly positive       | 0                              | 0                                    | 0                                | 0               | 0        | 0         |

OSCC=Oral squamous cell carcinoma

Graph 1: Staining intensity of oral squamous cell carcinoma with Van Gieson Stain

Graph 2: Staining intensity of oral squamous cell carcinoma with Verhoff’s Van Gieson Stain

Figure 4: Well differentiated oral squamous cell carcinoma showing moderate staining of glycoproteins around tumor island using PAS stain (×10)

Figure 5: Moderately differentiated oral squamous cell carcinoma showing negative staining of glycoproteins around tumor island using PAS stain (×10)

Clinical correlation

Well-differentiated OSCC’s 10 cases - 6 cases no lymph node involvement seen. These histopathologically showed thick abundant, brightly stained collagen fibers around tumor islands. However in 4 cases showed Level I nodes were involved, the histopathological picture showed dispersed brightly stained collagen fibers around tumor islands.

Moderately differentiated OSCC’s 10 cases - 6 cases Level I nodes involved showed dispersed moderately stained collagen fibers around tumor islands, 4 cases showed 3 fibers (thin, immature) in poorly differentiated cases [Figure 9] ($\chi^2 = 15.349$, $P < 0.0001$) [Table 4 and Graph 4].
Poorly differentiated OSCC’s 5 cases - All cases showed Levels I and II nodes involvement and histologically showed thin weakly stained collagen fibers.

DISCUSSION

Oral cancer is the 6th most common cancer in India and more common among the lower socioeconomic groups.
Extracellular matrix in oral squamous cell carcinoma

Apart from habits such as tobacco, alcoholism, and betel-quid chewing, the other etiologic factors can be poor oral hygiene, ill-fitting prosthesis and chronic irritation. The histopathological diagnosis helps the surgeon to plan further treatment according to the grade of tumor. Hence, it is of importance to study the changes occurring in tumor microenvironment.[1]

The ECM has an important role in tissue organization and function. The ECM acts as scaffold that binds the cells and tissues to one another. The ECM macromolecules are known to alter cellular events such as adhesion, migration, proliferation and differentiation. Tumor cells cause proteolysis of ECM, which modifies the structure of ECM and facilitates tumor cell migration.[2]

The role of inflammation in human carcinogenesis is often regarded as a double-edged sword wherein the body’s immune system is known to nullify the harmful effects of cancer cell proliferation whereas it may also promote tumor growth. The inflammatory cells are known to release various chemokines that promote angiogenesis and also cause degradation of the ECM scaffold. Data available in the literature provides evidence towards the dual action of the inflammatory cells.

In this study, the connective tissue stroma around the tumor islands showed some observable changes in the host response in different histological grades of OSCCs. Among the fifty fields observed in ten different cases of well-differentiated OSCCs showed brightly stained collagen fibers scaffolding around the tumor islands. On the other hand fifty fields of ten cases of moderately differentiated OSCCs showed moderate staining for collagen fibers, which did not border the tumor islands and were dispersed around them. The poorly differentiated OSCCs showed weak or negative staining for collagen fibers indicating paucity of the collagen fibers.

Studies on the ductal infiltrating carcinoma of the breast indicated that there is an over-deposition of collagen fiber bundles at the invasive front of the tumor.[4] In a disease condition such as cancer, there is a persistent secretion of collagen-destroying enzymes (collagenases, proteinases) by cancer cells causing the destruction of the surrounding collagen.[5] As a result, the surrounding collagen fibers are dissolved or immature, facilitating tumor growth, and metastasis.

In this study, elastic fibers showed negative staining around the tumor islands in all the grades of OSCCs. However they were stained positive within the connective tissue. Elastic fibers are destroyed by secretion of proteininas and elastases secreted by the tumor cells. Elastic fibers are designed to maintain elastic function of the ECM throughout the life in normal conditions. However, various enzymes (matrix metalloproteinases and serine proteases) can cleave elastic fiber molecules causing loss of these fibers in diseased state.[6]

In this study, relationship between the collagenous component in the tumor stroma and invading tumor cells showed some observable changes in different grades of OSCC’s. In well differentiated and moderately differentiated cases deposits of collagen showed reddish orange birefringence, with picrosirius red stain around tumor islands. This indicates that there is deposition of thick collagen fiber bundles in these cases. Poorly differentiated cases showed greenish yellow birefringence in picrosirius red stain, indicating weak fibers in these cases.

Thick fibers are Type 1 collagen fibers and exhibit red, orange birefringence and Type 3 are weak fibers exhibiting green birefringence in polarizing microscopy.[7,8] Sharf et al. from their study on physical aggregation of the collagen fibers revealed a color profile of orange to red, which corresponded to the well-packed fibers and the green to greenish yellow to poorly packed fibers.[9]

The origin of these types of collagen around the tumor islands can be from stromal cells, and play a role in walling off the invading tumor cells.[10] This is supported by the studies done on cell lines which were derived from various neoplasms, to synthesize different types of collagen, predominantly the Types 1, 3 and 4 collagen.[11-13]
The difference in the type of collagen at the invasive front may be due to the action of enzymes such as collagenases or the metalloproteinases secreted by tumor cells or proliferation of the tumor cells with the secretion of their abnormal matrix.\textsuperscript{[14]} Stenback et al. reported the presence of delicate meshwork of Type 3 collagen at the invading front of tumor island in skin cancer.\textsuperscript{[15]}

Thus, in the present study, notable color changes in collagen around tumor islands were observed which suggests that the tumor cells alter the stroma facilitating tumorogenesis. These results are supported by Brekken et al. who stated that the ECM can influence tumor progression.\textsuperscript{[16]}

CONCLUSION

The observations of the study revealed altered staining reactions of the collagenous stroma, which may indicate their role in the pathogenesis of tumor invasion. This also leads to the assumption that tumor cells may be capable of manipulating the ECM to enhance their own survival, which could be in the form of production and release of certain enzymes that alter or destroy the surrounding stroma. This paves a way for the need to develop newer therapies directed toward the modification of the stromal behavior thereby improving prognosis.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. George J, Narang RS, Rao NN. Stromal response in different histological grades of oral squamous cell carcinoma: A histochemical study. Indian J Dent Res 2012;23:842.
2. Badylak SF. The extracellular matrix as a scaffold for tissue reconstruction. Semin Cell Dev Biol 2002;13:377-83.
3. Fuentes B, Duaso J. Progressive extracellular matrix disorganization in chemically induced murine oral squamous cell carcinoma. ISRN Pathol 2012;2012:359421.
4. Luparello C. Aspects of collagen changes in breast cancer. J Carcinog Mutagen 2013;51:007.
5. Vilen ST, Salo T, Sorsa T, Nyberg P. Fluctuating roles of matrix metalloproteinase-9 in oral squamous cell carcinoma. ScientificWorldJournal 2013;2013:920595.
6. Kieltý CM, Sherratt MJ, Shuttleworth CA. Elastic fibres. J Cell Sci 2002;115(Pt 14):2817-28.
7. Aparna V, Charu S. Evaluation of collagen in different grades of oral squamous cell carcinoma by using the Picrosirius red stain – A histochemical study. J Clin Diagn Res 2010;4:3444-9.
8. Dayan D, Hiss Y, Hirshberg A, Bubis JJ, Wilman M. Are the polarization colors of picrosirius red-stained collagen determined only by the diameter of the fibers? Histochemistry 1989;93:27-9.
9. Sharf Y, Knubovets T, Dayan D, Hirshberg A, Akselrod S, Navon G. The source of the NMR detected motional anisotropy of water in blood vessel walls. Biophys J 1997;73:1198-204.
10. Montes GS, Kisztán RM, Shigihara KM, Tokoro R, Mourão PA, Junqueira LC. Histochemical and morphological characterization of reticular fibers. Histochemistry 1980;65:131-41.
11. DeClerck YA, Bogenmann E, Jones PA. Collagen Synthesis by Short-Term Explants of Pediatric Tumors. Cancer Research 1985;45:1229-38.
12. Smith BD, Martin GR, Miller EJ, Dorfman A, Swarm R. Nature of the collagen synthesized by a transplanted chondrosarcoma. Arch Biochem Biophys 1975;166:181-6.
13. Moro L, Smith BD. Identification of collagen alpha1(I) trimer and normal type I collagen in a polyoma virus-induced mouse tumor. Arch Biochem Biophys 1977;182:33-41.
14. Krieg T, Timpl R, Alitalo K, Kurkinen M, Vaheri A. Type III procollagen is the major collagenous component produced by a continuous rhabdomyosarcoma cell line. FEBS Lett 1979;104:405-9.
15. Stenbäck F, Mäkinen MJ, Jussila T, Kaupila S, Risteli J, Talve L, et al. The extracellular matrix in skin tumor development – A morphological study. J Cutan Pathol 1999;26:327-38.
16. Brekken RA, Puolakkainen P, Graves DC, Workman G, Lubkin SR, Sage EH. Enhanced growth of tumors in SPARC null mice is associated with changes in the ECM. J Clin Invest 2003;111:487-95.