Investigation On Metastatic And Non-Metastatic Oral Squamous With Cancer-Associated Fibroblast (CAF)

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Abstract - **Carcinogenesis is supported by the stroma of tumors. Previous research has linked myofibroblast expression in stromal fibroblast (cancer-associated fibroblast, CAF) to a poor clinical outcome. The expression of alpha smooth muscle actin (-SMA) in non-metastatic and metastatic oral squamous cell carcinoma (OSCC) was evaluated and compared. A retrospective investigation was carried out on 50 formalin-fixed, paraffin-embedded tissue blocks, 25 of which were metastatic and 25 of which were not. The proportion and intensity of SMA expression in the tumors stroma were measured. From non-metastatic to metastatic OSCC, -SMA expression increases dramatically. In metastatic OSCC, there was statistically significant expression of -SMA (p0.001). The presence of -SMA in tumors stroma shows that myofibroblasts play a beneficial role in tumors growth and could be used as a therapeutic target.**

Keywords: Cancer-associated fibroblast, Tumors progression and myofibroblast, oral squamous cell carcinoma

1. INTRODUCTION

Oral squamous cell carcinoma (OSCC) is one of the most frequent cancers in humans, with a 5-year survival rate of 50-60%. Cancer Associated Fibroblasts (CAF), Immune cells, blood vessels, and other members of the tumors microenvironment (TME) all have a role in cancer progression and metastasis [1]. OSCC development is influenced not only by rapid cell divisions, but also by the reaction of non-cancerous cells and other stoma components. Fibroblast is the most common stromal cell found in connective tissue, and it performs a variety of tasks such as collagen fiber release, chemical mediators of inflammation, and growth factor signaling [2]. This fibroblast transdifferentiates into myofibroblasts or CAF in the presence of tumor-associated cytokines and growth factors in malignancy [3]. Myofibroblasts are smooth muscle-like fibroblasts that play a role in wound healing by reorganizing the extracellular matrix and contracting the tissue [4]. Several studies have shown that the interaction between epithelial and mesenchymal cells is critical in tumors progression and development [(5,8)]. The transdifferentiated myofibroblast contributes to epithelial proliferation in cancer by secreting growth hormones and inflammatory mediators, and hence plays an important role in tumors growth, progression, and invasion [(9)]. The presence of CAF in metastatic and non-metastatic OSCC is assessed in this study using the expression of the -SMA marker. As a result, it is easier to comprehend CAF's role in carcinogenesis, as it is a critical regulator of tumors growth, progression, and metastasis.

2. MATERIALS AND METHODS

The Department of Oral Pathology and Oral Microbiology at Vinayaka Mission's Sankarachariyar Dental College in Salem provided a total of 100 histopathologically verified cases of OSCC. The study covered conventional OSCC that developed in any location of the oral cavity with or without regional lymph node metastases, but not variations of oral squamous cell carcinoma. This study was authorized by the ethical committee of Vinayaka

Mission's Sankarachariyar Dental College, Salem (VMDC/IEC/Approval No 80) on October 13, 2017.

Study design: Immunohistochemical staining was performed on 50 metastatic and 50 non-metastatic instances of OSCC formalin-fixed and paraffin embedded blocks. The study included 20 well differentiated squamous cell carcinoma (WDSCC), 20 moderately differentiated squamous cell carcinoma (MDSCC), and 10 poorly differentiated squamous cell carcinoma (PDSCC) cases from a total of 50 metastatic and non-metastatic cases. The slices were stained with Mayer's hematoxylin and treated with a ready-to-use mouse monoclonal -SMA antibody. According to Kellerman et al 10 in 2008, score 1 indicates that nil or 1% of cells are stained, score 2 indicates that 1-50 percent of cells are stained, and score 3 indicates that >50 percent of cells are stained with -SMA.

Statistical analysis: SPSS software version 11.5 was used to analyse the final data (SPSS Inc., Chicago,IL). The Chi Square test is used to examine the major differences between the groups. Statistical significance was defined as a P value of less than 0.05.

Results: α-SMA expression was found between the tumors islands in immunohistochemical investigation of metastatic and non-metastatic OSCC. In almost all lesions, SMA expression was positive for endothelium lining blood vessels. There was a substantial difference between the different grades of OSCC in metastatic and non-metastatic OSCC.
60 percent of WDSCC shows score 2 -SMA expression, 65 percent of MDSCC shows score 2 expression, and 60 percent of PDSCC shows score 3 -SMA expression among non-metastatic patients; the findings were statistically significant with a p value of 0.05. (0.005). In metastatic OSCC, 55 percent of WDSCC expresses score 2 -SMA, 55 percent of MDSCC expresses score 3, and 100 percent of PDSCC expresses score 3; the findings are statistically significant with a p value of 0.05. (0.010). The expression of -SMA in the histological grades of metastatic and non-metastatic OSCC, as well as WD and PD metastatic OSCC, was statistically significant. (See Figs. 1a and 1b.)

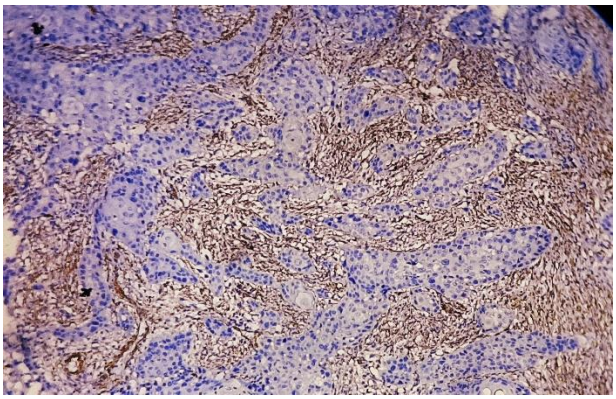

Fig.1.a. The low power photomicrograph(10x 10=100x) shows intense staining of α-SMA in metastatic squamous cell carcinoma- more than 50%

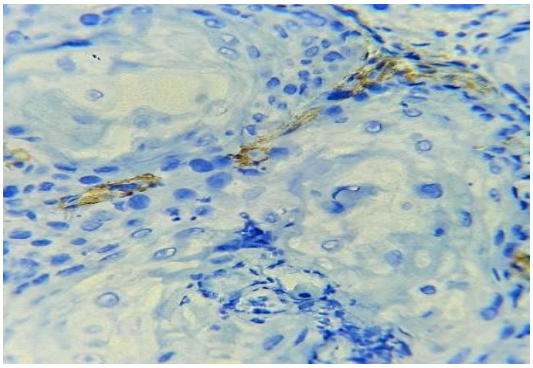

Fig.1.b. Photomicrograph (10 x40= 400x) shows mild expression of α-SMA in non metastatic well-differentiated squamous cell carcinoma.

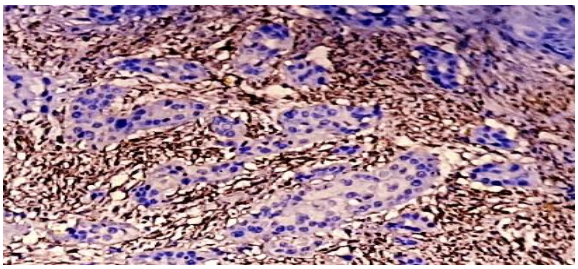

Fig.1.c. Photomicrograph (10x40=400x) shows severe expression of α-SMA in metastatic poorly differentiated squamous cell carcinoma.

When the overall expression of α- SMA was compared between metastatic and non-metastatic

tumors, metastatic tumors had a significantly greater value with a significant p value (0.004).

3. DISCUSSION

One of the most important components of the tumors stroma is cancer-associated fibroblast, which is involved in the remodeling of the extracellular matrix. CAF population is still poorly identified in tumors due to a lack of clarity in origin, heterogeneity in biology, and a lack of markers (11,12). CAF displays myofibroblast differentiation by expressing -SMA [13]. Myofibroblasts are activated fibroblasts that are commonly found in wound healing. In several clinical trials, stromal expression of -SMA has been found to be inversely linked to the patient's disease-free status and overall survival [14,15,16]. In this study, myofibroblastic differentiation was found in 82 percent of non-metastatic OSCC cases and 96 percent of metastatic OSCC cases. The findings were comparable to those published by Vered et al in 2010 [17], who found that MF positive was found in 98 percent of tumors stroma. Similar findings were found by Sridgara et al in 2013[18] and Smitha et al in 2018 [3], who found a rise of positive MF in metastatic OSCC compared to non-metastatic OSCC. In contrast to previous studies conducted by Etemad Moghadam et al in 2009[9] and Jeyaraj et al in 2015[5], -SMA showed a significant difference in histological grading SCC. MF positive is higher in well differentiated and poorly differentiated SC in metastatic OSCC, which is similar to the findings of Kellerman et al in 2008[10] and Smitha et al in 2018[3], who found that MF expression is elevated in poorly differentiated SC. Kellerman et al. showed in 2007 that an increase in myofibroblast expression influences the tumor's aggressiveness and proliferative potential. Vered et al. concluded in 2005 that elevated stromal myofibroblast expression is linked to the aggressiveness of odontogenic lesions. Jeyaraj et al. compared OSCC to possibly malignant illnesses in 2015, finding a significant difference between the research groups and concluding that myofibroblasts play a critical role in tumour growth. Smitha et al. concluded in 2018 that when the disease progresses from epithelial dysplasia to cancer, myofibroblast expression increases. According to the findings, myofibroblasts create an ideal environment for tumour growth, progression, and invasion. In addition, as the grade of OSCC increases, the expression of CAF with myofibroblastic differentiation rises.

4. CONCLUSION

CAF has emerged as a key player in stromal changes and cancer progression in recent years, as evidenced by numerous contemporary research. Our research also found that SMA expression was higher in metastatic and poorly differentiated tumors, but there is still a big sample size and novel markers to investigate for further stromal changes caused by CAF. All of this will aid cancer biologists in developing new molecular targeted therapeutics for CAF and its released chemicals.

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