STM JOURNALS

International Journal of
Cell Biology and Cellular Functions

Volume 2, Issue 1, 2024
January–June
DOI: 10.37591/IJCBCF

https://journals.stmjournals.com/ijcbcf

Research IJCBCF

An Immunohistochemical Study to Assess the Role of Myofibroblasts in Diagnosing and Predicting the Outcome of Oral Squamous Cell Carcinoma

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Abstract

Background: *Squamous cell carcinoma (SCC) accounts for approximately 94% of all oral malignancies, hence establishing oral squamous cell carcinoma (OSCC) as one of the top 10 most prevalent malignant tumors. Cells with several functions, such as macrophages and myofibroblasts, play a vital role in the biological behavior of tumors. This study aimed to assess and evaluate the prevalence of myofibroblasts (MF) and macrophages in SCCs occurring in the oral region.* ***Methodology:*** *Samples comprising 15 experimental subjects diagnosed with well-differentiated oral squamous cell carcinoma (WDOSCC), moderately differentiated oral squamous cell carcinoma (MDOSCC), and poorly differentiated oral squamous cell carcinoma (PDOSCC) were included in the study. While 15 healthy subjects were taken as a control group. The tissue samples were divided into sections that were 4 micrometers thick. These sections were subsequently subjected to both conventional staining using hematoxylin and eosin, as well as immunohistochemistry (IHC) staining using α-smooth muscle actin (α-SMA). The comparative analysis of the expression levels of microRNAs was conducted across different stages of OSCC. Statistical analysis was conducted on all the outcomes.* ***Results:*** *The findings revealed that the average final staining index score for patients with WDOSCC was 9.23, while it was 8.98 for those with MDOSCC and 6.54 for individuals with PDOSCC. In contrast, the control group displayed nil MF cellular expressions with statistically significant differences from different grades of OSCC.* ***Conclusion:*** *The present investigation's results indicate that MFs play a significant role in the pathogenesis of OSCCs, and their evaluation may serve as a valuable tool for predicting the invasive characteristics of these malignancies. Therefore, our study suggests the utilization of myofibroblasts (MF) as a stromal marker to aid in identifying invasion and progression in OSCC.*

Keywords: Alpha-smooth muscle actin, myofibroblast, squamous cell, carcinoma, oral cancer

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Received Date: February 15, 2024
Accepted Date: February 20, 2024
Published Date: March 11, 2024

Citation: Priyanka Raghav, Abhiraj Raghav. An Immunohistochemical Study to Assess the Role of Myofibroblasts in Diagnosing and Predicting the Outcome of Oral Squamous Cell Carcinoma. International Journal of Cell Biology and Cellular Functions. 2024; 2(1): 13–17p.

INTRODUCTION

Oral cancer comprises a spectrum of malignant diseases arising from the surface tissues of the oral cavity, posing significant challenges in diagnosis, treatment, and management, and necessitating comprehensive approaches for effective patient care. Squamous cell carcinomas (SCCs), primarily derived from keratinocytes, constitute 90–95% of head and neck malignancies, followed by basal cell carcinomas, mesenchymal malignancies, and melanomas [1].

The involvement of myofibroblasts (MF) in diagnosing and predicting the outcome of oral squamous cell carcinoma (OSCC) is still under

research [2]. These specialized cells, characterized by their enlarged and irregular morphology, play a crucial role in the tumor microenvironment. Myofibroblasts exhibit distinctive features such as well-developed interactions with the extracellular matrix and intracellular gap junctions, along with the presence of α-smooth muscle actin (α-SMA) in their cytoskeletal structures, resulting in enhanced contractile abilities [3].

Research [4] suggests that myofibroblasts may serve as valuable diagnostic markers for OSCC. Their presence and abundance within the tumor stroma can provide important information regarding the aggressiveness and progression of the disease. Additionally, the contractile forces exerted by myofibroblasts contribute to the remodeling of the tumor microenvironment, influencing tumor growth, invasion, and metastasis [5, 6].

Furthermore, the density and distribution of myofibroblasts have been associated with prognostic outcomes in oral squamous cell carcinoma patients with OSCC. Higher levels of myofibroblast infiltration have been correlated with a poor prognosis, including an increased risk of recurrence, and decreased overall survival. Therefore, assessing the presence and activity of myofibroblasts in the tumor microenvironment may aid in predicting clinical outcomes and guiding treatment decisions for patients with OSCC [7–9].

Thus, this study aimed to evaluate the significance of myofibroblasts in the diagnosis and prediction of outcomes in OSCC.

MATERIAL AND METHODS

The current study took 15 samples with well-differentiated oral squamous cell carcinoma (WDOSCC), moderately differentiated oral squamous cell carcinoma (MDOSCC), poorly differentiated oral squamous cell carcinoma (PDOSCC), and 15 controls to evaluate the expression of myofibroblasts (MFs) using immunohistochemistry (IHC) utilizing a SMA antibody. Our study sample included 15 patients with WDOSCC, MDOSCC, and PDOSCC, confirmed through histological examination. Additionally, the study included 15 tissue samples from histologically confirmed normal mucosa. The control group consisted of histological sections taken from the enucleated 3rd molars dental follicular tissues representing the normal mucosa. Two slices, each 4 m thick, were obtained from each tissue block. One tissue segment was stained using the conventional hematoxylin and eosin (H&E) method, whereas the other was subjected to immunohistochemical examination using the SMA marker. H&E stained slides were utilized as reference slides to evaluate and confirm cases of OSCC.

RESULTS

The present study involved the enrolment of 15 cases each of WDOSCC, MDOSCC, PDOSCC, and 15 controls. The investigation was carried out across three grades of OSCC. Immunohistochemical analysis was conducted on the tissues using SMA markers. The findings revealed that the average final staining index score for patients with WDOSCC was 9.23, while it was 8.98 for those with MDOSCC and 6.54 for individuals with PDOSCC. In contrast, the control group did not show myofibroblast (MF) cell expression. Intergroup comparison of the final staining index scores across different stages of OSCC did not reveal statistically significant differences ($P>0.05$) (Table 1). Similarly, analysis of myofibroblast expression across different grades of OSCC showed uninteresting findings. There was a statistically significant difference ($P<0.05$) in the final staining index score when comparing instances of OSCC with normal controls (Table 2).

DISCUSSION

Oral squamous cell carcinoma is the most prevalent form of oral malignancy globally and is associated with a high mortality rate [10]. Traditionally, carcinoma progression has been linked to a stepwise accumulation of genetic alterations within the target epithelium [11].

Table 1. Comparison of final staining index score between different grades of oral squamous cell carcinoma.

Groups	P-value
Well-differentiated oral squamous cell carcinoma versus moderately differentiated oral squamous cell carcinoma	0.123
Well-differentiated oral squamous cell carcinoma versus poorly differentiated oral squamous cell carcinoma	0.101
Moderately differentiated oral squamous cell carcinoma versus poorly differentiated oral squamous cell carcinoma	0.110

Table 2. Comparison of final staining index score between different grades of oral squamous cell carcinoma and normal mucosa.

Groups	P-value
Normal control versus well-differentiated oral squamous cell carcinoma	0.033
Normal control versus moderately differentiated oral squamous cell	0.021
Normal control versus poorly differentiated oral squamous cell carcinoma	0.022

This molecular progression is evident in the oral mucosa, initially manifesting as precursor lesions characterized by epithelial hyperplasia and dysplasia, eventually culminating in the development of overt carcinoma, accompanied by a surge in genetic mutations within the epithelium. However, there has been a recent shift in focus, acknowledging the significant contribution of the microenvironment to tumor progression.

In conjunction with the alteration from normal epithelial tissue to precancerous epithelium and carcinoma, stromal tissues also undergo a transition from normal to activated or advanced malignant states. Tumor cells initiate remodeling of the extracellular matrix (ECM), termed "stromagenesis," while stromal cells orchestrate this process. Within this milieu, fibroblasts play a pivotal role in driving tumor progression. Myofibroblasts, a distinct subset of cells exhibiting phenotypic characteristics intermediate between those of smooth muscle cells and fibroblasts, have gained attention [12]. In addition to their traditional role in tissue homeostasis and repair, changes in the quantity and activity of myofibroblasts have been linked to conditions characterized by increased extracellular matrix (ECM) accumulation and subsequent fibrosis [13]. Recently, researchers have begun to unravel their involvement in cancer. Myofibroblasts exert their influence on the tumor stroma by secreting a diverse array of factors, including chemokines, growth factors, and matrix-degrading enzymes such as matrix metalloproteinases (MMPs). Myofibroblasts are a prominent feature of the tumor stroma in many, though not all, OSCCs [13–15]. Therefore, the primary objective of this study was to investigate the significance of myofibroblasts as vital prognostic indicators of OSCC.

The present study involved the enrolment of 15 cases each of WDOSCC, MDOSCC, PDOSCC, and 15 controls. The investigation was carried out across three grades of OSCC. Immunohistochemical analysis was conducted on the tissues using SMA markers. The findings revealed that the average final staining index score for patients with WDOSCC was 9.23, while it was 8.98 for those with MDOSCC and 6.54 for individuals with PDOSCC. In contrast, the control group displayed no MF cellular expression. The comparison between different stages of OSCC did not show any statistically significant difference in the final staining index score ($P>0.05$). However, a statistically significant difference ($P<0.05$) was observed in the final staining index score when comparing instances of OSCC with normal controls.

In a similar study by Gandhi et al. [15], the difference in staining index scores and expression of myofibroblasts (MFs) among different grades of OSCC was not statistically significant. However, in accordance with our study, they also found a high statistical significance in the comparison of the final staining index scores between OSCC and normal controls.

In a similar study investigating the presence of myofibroblasts in different grades of OSCC using immunohistochemistry with α-SMA antibodies. Prasad et al. [14] examined a total of 50 biopsy specimens. Immunohistochemical staining was performed using monoclonal anti-human α-SMA and myofibroblast distribution was assessed following the method described by Etemad-Moghadam et al. In their study, the mean percentage of myofibroblasts for WDOSCC and poorly differentiated OSCC (PDOSCC) was found to be 2.88 and 2.92, respectively. Additionally, the mean staining intensity scores in WDOSCC and PDOSCC were 2.88 and 2.55, respectively. Their research also revealed significant findings by comparing the final staining index score between the OSCC and normal control groups. Similar to our study, they also observed no significant correlation when comparing the mean staining index score between WDOSCC and PDOSCC.

These findings suggest that the malignant epithelium might stimulate the adjacent stromal tissue to generate myofibroblasts. Given their involvement, these specialized cells are promising therapeutic targets for OSCC treatment.

CONCLUSION

The findings of the present study indicate that myofibroblasts (MFs) play a critical role in OSCC pathogenesis. Furthermore, the assessment of MFs may serve as a valuable tool for predicting the invasive behavior of OSCCs. Hence, we advocate the use of MFs as a stromal marker in patients with OSCC to facilitate the visualization of invasion and progression.

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