Immunohistochemical assessment of odontogenic tumors using smooth muscle actin - A short study

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ABSTRACT:
Context: Odontogenic tumors constitute an unusually diverse group of lesions. They are usually benign, though occasionally locally invasive, slow-growing neoplasms, exhibit various biological behaviors. The biological behavior of the odontogenic tumors is contributed by many factors of which stromal myofibroblasts (MF) have the potential to facilitate progression of odontogenic tumors which could contribute to the same. The presence of alpha smooth muscle action (αSMA) positive myofibroblasts in odontogenic tumors has not been thoroughly investigated. Only a few studies in odontogenic tumors are available in English literature. The present study is focused on the same, assessing the presence of stromal myofibroblasts in odontogenic tumors by expressing SMA immunohistochemically.

Aims: To assess the expression of αSMA, immunohistochemically, in different odontogenic tumors namely follicular ameloblastoma, ameloblastic fibro-odontoma, adenomatoid odontogenic tumor, calcifying epithelial odontogenic tumor and odontogenic myxoma; and correlate it to their aggressive biological behavior.

Materials and Methods: Formalin fixed paraffin wax embedded tissue from four follicular ameloblastomas (FA), four ameloblastic fibro-odontomas (AFO), four adenomatoid odontogenic tumors (AOT), four calcifying epithelial odontogenic tumors (CEOT) and four odontogenic myxomas (OM) were selected randomly. 4µm sections were made and were assessed immunohistochemically for αSMA.

Results: Immunohistochemically, reactivity to αSMA was restricted around the blood vessels in FA and AFO cases whereas moderate reactivity was exhibited in AOT and CEOT cases; and intense reactivity in OM cases.

Conclusion: The high frequency of stromal MF in known aggressive odontogenic tumors implies that MF can contribute to the biological behavior of these odontogenic lesions. The agents that control stromal MF can be used as an aid to reduce extensive and mutilating surgery in cases of remarkably aggressive odontogenic tumors.

Key words: Odontogenic tumors, myofibroblasts, αSMA.
Introduction

Odontogenic tumors comprise a complex group of lesions with diverse histopathological types and clinical behaviors. These lesions are derived from the tooth-producing tissues or their remnants that remain entrapped either within the jawbones or into the adjacent soft tissues. From a biological point of view, some of these lesions represent hamartomas with varying degrees of differentiation, while the rest are benign or malignant neoplasms with variable aggressiveness and potential to develop metastasis. They are usually benign, though occasionally locally invasive, slow-growing neoplasms, exhibit various biological behaviors.1,2 The biological behavior of the odontogenic tumors is contributed by many factors of which stromal myofibroblasts have the potential to facilitate progression of odontogenic tumors which could contribute to the same.3

Myofibroblasts (MF) are modulated fibroblasts that have acquired the capacity to neoexpress alpha-Smooth Muscle Actin (αSMA) which is a key contractile protein found in smooth muscle cells. It is now well accepted that the MF is a key cell for the connective tissue remodeling which takes place during wound healing and fibrosis development. MFs are capable of remodeling connective tissue but also interact with epithelial cells and other connective tissue cells and may thus control such phenomena as tumor invasion and angiogenesis. Presence of myofibroblasts at the invasion front is not part of the host defense mechanism against tumor, but actually promotes it.3,4

The presence of αSMA positive myofibroblasts in odontogenic tumors has not been thoroughly investigated. Only a few studies in odontogenic tumors are available in English literature. The present study is focused on the same, assessing the presence of stromal myofibroblasts in odontogenic tumors namely follicular ameloblastoma (FA), ameloblastic fibro-odontoma (AFO), adenomatoid odontogenic tumor (AOT), calcifying epithelial odontogenic tumors (CEOT) and odontogenic myxomas (OM), by expressing αSMA Immunohistochemically and correlate it to their aggressive biological behavior.

Materials & Methods

The study included a total of 20 odontogenic tumors that were selected randomly from the archives of Department of Oral & Maxillofacial Pathology, Saveetha Dental College & Hospital, Chennai. The selected tumors were four cases of follicular ameloblastomas (FA), four of ameloblastic fibro-odontomas (AFO), four of adenomatoid odontogenic tumors (AOT), four of calcifying epithelial odontogenic tumors (CEOT) and four of odontogenic myxomas (OM). The histopathological diagnosis was done in conformity with criterions established in 2005 by IARC nominated work group for odontogenic tumors within WHO.5 All the tumor tissues were processed by common histopathological technique using 10% formalin fixation, paraffin embedding.

Immunohistochemical analysis was performed on serial sections of about 4µm thickness, where the sections were placed on gelatin coated glass slides and the slides were immersed in a plastic jar of citrate buffer pH 6.0 and incubated for 5 mins for antigen retrieval. As antigen retrieval, pressure cooker technique was used at 15 lbs for 20 minutes. Sections were then left to cool to room temperature and rinsed in 0.05 M Tris buffer at pH 7.4. Sections were then immunostained for αSMA using the avidin-biotin complex technique. The marker used was a monoclonal antibody raised against the human wild type SMA protein. Sections were incubated for 90 mins with the primary antibody. DAB (diaminobenzidine tetrahydrochloride) substrate solution was used for the visualization and counter staining was performed with haematoxylin.6,7

Immunohistochemical Assessment

The criterion for a positive reaction confirming the presence of αSMA protein was a dark, brownish, intranuclear precipitate. An arbitrary, semiquantitative evaluation of the immunoreactivity to the αSMA marker was assessed using the following:

- None of the cells revealed positivity for αSMA marker.
- + mild: <5% positive αSMA cells (MF)
- ++ moderate: <5% - 50% positive αSMA cells (MF)
- +++strong/intense: >50% positive αSMA cells (MF)

RESULTS

The study included 20 cases of odontogenic tumors that belonged to patients aged between 27-
71 years, predominantly males. All the cases were intraosseous. Expression of positive αSMA immunoreactivity was present in 12 of the 20 cases of odontogenic tumors.

In follicular ameloblastomas and ameloblastic fibro-odontoma cases there was focal reactivity to αSMA around the blood vessels (figure 1 & 2). The stroma didn't contain abundant myofibroblasts and the immunoreactivity was restricted around the blood vessels.

In adenomatoid odontogenic tumor cases, positivity to αSMA marker was evident in about 38% of the cells per high power field. There was moderate reactivity (++) in the epithelial cells, the ductal cells and in the cell rich zone in three cases whereas there was moderate to intense reactivity in one case (figure 3).

In calcifying epithelial odontogenic tumor cases, positivity to αSMA marker was evident in about 42% of the cells per high power field. In all the four cases there was moderate reactivity (++) in the stromal cells, near the tumor islands (figure 4).

In odontogenic myxoma cases, αSMA was detected in the population of stellate and spindle cells, in about 85% of the cells per high power field. There was moderate to intense reactivity (+++) in almost all the tumor cells. Intense reactivity was seen in the myxoid stroma (figure 5).

Discussion

Odontogenic tumors constitute an unusually diverse group of lesions. They are usually benign, though occasionally locally invasive, slow-growing neoplasms, exhibit various biological behaviors. The biological behavior of the odontogenic tumors is contributed by many factors of which stromal myofibroblasts (MF) have the potential to facilitate progression of odontogenic tumors which could contribute to the same. The present study had focused on assessing the presence of stromal myofibroblasts in odontogenic tumors by expressing αSMA Immunohistochemically and thereby determining their biological behavior and/or aggressiveness.

In the present study, expression of positive αSMA immunoreactivity was restricted around the blood vessels in follicular ameloblastomas and ameloblastic fibro-odontoma cases due to the presence of pericytes. In adenomatoid odontogenic tumor cases, moderate reactivity to αSMA marker was evident in about 38% of the cells per high power field in three cases and moderate to intense reactivity in one case. In calcifying epithelial odontogenic tumor cases, moderate reactivity to αSMA marker was evident in about 42% of the cells per high power field. In odontogenic myxoma cases, intense reactivity to αSMA was detected in the population of stellate and spindle cells, in about 85% of the cells per high power field. The positivity could be due to the presence of myofibroblasts containing αSMA protein that could contribute to the biological behavior of the odontogenic tumors and in turn to their aggressiveness. The more the immunoreactivity to αSMA marker in the odontogenic tumors the more the aggressiveness of the tumor could be. In the present study, odontogenic myxoma could be more aggressive followed by CEOT, AOT, FA and AFO respectively. This nature could be attributed to the presence of myofibroblasts containing αSMA protein.

Myofibroblasts (MF) are modulated fibroblasts that are formed by the transformation of fibroblasts into myofibroblasts. The transformation is modulated by cancer cell derived cytokines, such as TGF-beta. Myofibroblasts are capable of remodeling connective tissue but also interact with epithelial cells and other connective tissue cells and may thus control such phenomena as tumor invasion and angiogenesis. Soluble factor secretion by stroma myofibroblasts influences tumor progression and invasion. Myofibroblastic differentiation features are the predominant cell type in different primary and metastatic epithelial tumors and play a central role in the deposition of collagen as well as in tissue remodeling phenomena that are attributed at least in part to contractile forces generated in their cytoplasm. The positivity for α-smooth muscle actin observed in mesenchymal cells surrounding noninvasive epithelial proliferation suggests that epithelial signaling may be fundamental even before the onset of invasion.

An important proportion of enzymes (eg: matrix metalloproteinase (MMP)-2 that degrades the basement membrane) is produced by stroma myofibroblasts as a host response to tumor thus allowing invasion. Myofibroblasts in tumors also expresses tissue factor, the cellular initiator of the protease blood coagulation cascade, leading to the formation of thrombin; a strong correlation between
The expression of the tissue factor by macrophages and/or myofibroblasts in close proximity to infiltrating tumor cells, and progression to invasive cancer.\textsuperscript{13}

The action of myofibroblasts in influencing tumor evolution could be exerted through at least 3 mechanisms:\textsuperscript{14}

1. Synthesis and expression of specific extracellular matrix components.
2. Mechanical remodeling of granulation tissue as well as transmission of tension to tumor cells with a mechanism involving $\alpha$-smooth muscle actin containing stress fiber isometric contraction that is mediated through Rho/Rho-kinase; this force is then transmitted by vinculin and tensin containing focal adhesions which connect myofibroblasts to the extracellular matrix and eventually to other cells.
3. Production of specific cytokines.

Along with MFs, integrin $\alpha 5\beta 1$, a classic receptor of fibronectin, also plays an important role in epithelial - mesenchymal interaction in odontogenic tumors. Binding of $\alpha 5\beta 1$ to fibronectin increases the secretion and expression of metalloproteinases which altogether causes degradation of extracellular matrix leading to invasion of the tumor.\textsuperscript{3} The more invasive a tumor is the more aggressive it is. (figure 6).

Conclusion

The myofibroblast represents a good candidate for playing an important role in such an interaction; the recently described possibility of modulating myofibroblast behavior using new tools, such as the smooth muscle actin, provides the opportunity to explore the role of this cell and of the stroma reaction in tumor evolution. The high frequency of stromal MF in known aggressive odontogenic tumors implies that MF can contribute to the biological behavior of these odontogenic lesions. The agents that control stromal MF can be used as an aid to reduce extensive and mutilating surgery in cases of remarkably aggressive odontogenic tumors. Based on this study we suggest that the myofibroblast may represent a new important target of antitumor therapy. In conclusion, the old and never fully proven possibility of epithelial/stroma reciprocal induction in many biological and pathological phenomena appears at present more and more likely.

References

Figure 1: Follicular ameloblastomas showing focal reactivity to ?SMA which is due to the presence of pericytes around the blood vessels.

Figure 2: Ameloblastic fibro-odontoma showing focal reactivity to ?SMA around the blood vessels which is due to the presence of pericytes.

Figure 3: Adenomatoid odontogenic tumor showing moderate to intense positivity to ?SMA marker.

Figure 4: Calcifying epithelial odontogenic tumor case showing moderate positivity to ?SMA marker near the tumor islands.

Figure 5: Odontogenic myxoma case showing moderate to intense reactivity to ?SMA. ?SMA was seen in the population of stellate and spindle cells.

Figure 6: Flow chart showing the sequential events for tumor invasion caused by Myofibroblasts.