

Immunohistochemical Detection and Ultrastructure of Myofibroblasts in the Stroma of Odontogenic Cysts and Ameloblastoma

F Mashhadiabbas¹, S Atarbashi Moghadam^{2*}, M Moshref¹, M Elahi¹

¹Department of oral and maxillofacial pathology, Shahid Beheshti Medical University, Tehran,
²Department of oral and maxillofacial pathology, Faculty of Dentistry, Jondishapour University of Medical Sciences, Ahvaz, Iran

Abstract

Background: Immunohistochemical phenotype, distribution and significance of proliferation of myofibroblasts (α SMA positive cells) with evaluation of ultrastructure, in dentigerous cyst, odontogenic keratocyst and ameloblastoma were analyzed.

Methods: The study included paraffin embedded blocks of ameloblastoma (n=22), odontogenic keratocyst (n=20), and dentigerous cyst (n=18). The expression of α SMA was determined by immunohistochemically stained section. The percentage of positive cells was calculated from a minimum of 1000 cells and H-score was expressed (% positive cells \times intensity of staining). For transmission electron microscopy, fresh specimens were obtained from three patients and were fixed in 2.5% glutaraldehyde. The presence of cells with the ultrastructural characteristics of the myofibroblast was recorded.

Results: The mean number of positive cells in the three groups was significantly different. The difference between odontogenic keratocyst and dentigerous cyst and also the difference between dentigerous cyst and ameloblastoma were not statistically significant. The mean number of positive cells in the odontogenic keratocyst was significantly higher than that in ameloblastoma. In ultra-structural evaluation, myofibroblasts exhibited abundant cytoplasmic microfilaments, basal lamina-like material, subsurface caveolae, pinocytotic vesicles, rough endoplasmic reticulum, and mitochondria.

Conclusions: The high frequency of stromal myofibroblast in the odontogenic keratocyst implies that myofibroblast can contribute to aggressive nature of this cyst, but between odontogenic cysts and ameloblastoma, the presence of stromal myofibroblast has no correlation with invasiveness.

Keywords: Myofibroblasts; α SMA; Odontogenic keratocyst; Dentigerous cyst; Ameloblastoma; Transmission electron microscopy

Introduction

It is now well accepted that coordinated activity of the epithelial cells with their stroma is fundamental in controlling growth and differentiation in normal and pathological situations.¹ Myofibroblasts (MF) are fibroblasts with smooth muscle-like features character-

ized by the presence of a contractile apparatus.² α SMA is commonly regarded as the most important marker for the myofibroblast. Transmission electron microscopy (TEM) remains the method of choice for diagnosis of MF.³ Ultra-structurally, it has bundles of cytoplasmic microfilaments with dense bodies running parallel to the long axis, a well developed rough endoplasmic reticulum and Golgi apparatus, a notched nucleus, pinocytotic vesicles, partial investment by basal lamina with points of plasmalemmal attachment, well developed microtendons and intercellular intermediate and gap junction.⁴

*Correspondence: Saede Atarbashi Moghadam, DDS, MS, Department of Oral and Maxillofacial Pathology, Faculty of Dentistry, Jondishapour University of Medical Sciences, Ahvaz, Iran. Tel: +98-21-22403010, Fax: +98-21-22403194, e-mail: dr.atarbashi@gmail.com
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The development of an altered stromal microenvironment is a common feature of many tumors including squamous cell carcinoma (SCC). Stromal fibroblasts become activated myofibroblasts by secretion of TGF- β 1 cytokine.⁵ Approximately 30 years ago, MF was shown to be present in the stroma of various invasive and metastatic malignant tumors characterized by hard consistency and retraction. At that time, it was assumed that this phenomenon was part of the host reaction to prevent invasion of malignant cells since there were numerous MF partially at the invasive front. However, over the past ten years, there has been an abundance of evidence that the presence of MF at the invasive front is not part of the host defense mechanism against tumor invasion, but actually promoting it.²

Several types of odontogenic lesions such as odontogenic keratocyst (OKC) and ameloblastoma have the potential for aggressive behavior and local recurrence.^{2,6,7} The aim of this study was to evaluate the role of MF in the stroma of odontogenic cysts and tumor.

Materials and Methods

Formalin fixed and paraffin embedded blocks from cases of dentigerous cyst (DC), OKC and ameloblastoma were obtained from the archives of the Department of Oral and Maxillofacial Pathology, Shahid Beheshti University of Medical Sciences. These included 60 cases of DC (n=18), OKC (n=20) and ameloblastoma (n=22). Histopathological diagnosis was confirmed by an experienced pathologist using H&E stained sections. Clinical data were recorded.

The expression of α -SMA was determined by immunohistochemistry on paraffin sections from tissue blocks using a microwave antigen retrieval method. The monoclonal antibody used was mouse anti-human antibody α -SMA (DAKO, N158430, Denmark), dilution 1:100. The sections were deparaffinized in xylene in 5% hydrogen peroxide for 15 min. Following antigen retrieval procedures (10 mM citrate buffer, pH 6.0, 100°C for 15 min in a microwave generator, 750 W), the sections were kept in a container for 30 min to reach room temperature. After being washed in distilled water (1 min) and PBS (1 min), the sections were incubated in blocking milk buffer (5% dry skimmed milk in PBS, pH 7.6 for 30 min) with primary antibody at 4°C overnight. DAB was used as chromogen and hematoxylin stained the background.

Some representative fields were randomly selected in each immunohistochemically stained section. Ten

fields were chosen for each section. The percentage of positive cells was calculated (In HPF) from a minimum of 1000 cells and the H-score was expressed (% positive cells \times intensity of staining): 1, no staining or weak; 2, moderate; 3, strong.

The intensity of inflammatory cells was estimated on the basis of the H&E stain and graded as 1, without inflammation or weak; 2, moderate; 3, severe. For transmission electron microscopy, the biopsies were obtained from three patients: DC (n=1), OKC (n=1) and ameloblastoma (n=1)). Fresh specimens were fixed in 2.5% glutaraldehyde. Semithin and ultrathin sections were done. The presence of cells with the ultra-structural characteristics of the myofibroblast was recorded.

SPSS (Statistical Package for Social Science) software (Version 11.5, Chocago, IL, USA) was used to analyze the data. Differences in the mean number of α -SMA positive cells per HPF among all types of the lesions and the differences among the group of odontogenic cysts were analyzed, using one-way ANOVA test. Multiple comparisons were done by Bonefroni test. Considering inflammation, Factorial ANOVA test was done to calculate H-score. Statistical significance was at $p < 0.05$.

Results

The Mean 95% CI for MF was 0.371 \pm 0.173 in OKC group, 0.246 \pm 0.165 for DC group, and 0.145 \pm 0.235 for ameloblastoma. There were significant differences between the three groups ($p=0.002$). The difference between OKC and dentigerous cyst was not statistically significant ($p=0.164$). The difference between dentigerous cyst and ameloblastoma was also not statistically significant ($p=0.344$). The mean number of positive cells in OKC was significantly higher than that in ameloblastoma ($p < 0.001$).

For precise evaluation, H-score was calculated and the results were demonstrated again. Mean 95% CI for H-score for OKC group was 0.821 \pm 0.570, for DC group 0.433 \pm 0.402, and for ameloblastoma group 0.385 \pm 0.708. There were significant differences among the three groups ($p=0.037$). In general, five cases of OKC (Figure 1), two cases of DC and three cases of ameloblastoma (follicular type) (Figure 2) were stained strongly. H-score was calculated while considering inflammation. Fifty five percent of OKC, 33.3% of DC and 95.5 % of ameloblastoma demonstrated mild inflammation. There were significant

differences among the three groups for inflammation ($p=0.001$), but inflammation did not affect the results.

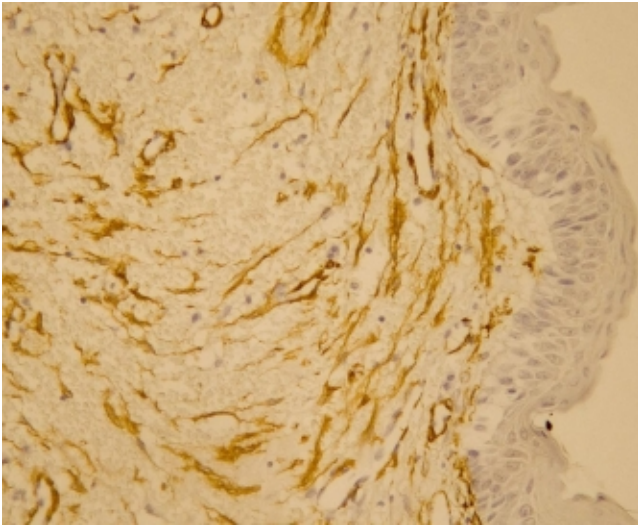


Fig. 1: photomicrograph illustrates considerable α SMA expression by myofibroblasts in OKC

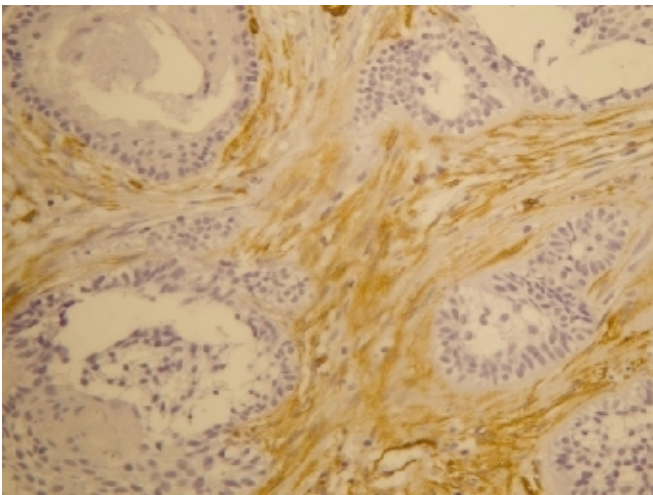


Fig. 2: Photomicrograph shows epithelial islands of ameloblastoma surrounded by myofibroblasts

The α -SMA positive cells within blood vessel served as positive control. The distribution pattern of MF in DC and OKC was in the subepithelial and through the cyst wall. But the distribution in cases of ameloblastoma was in the connective tissue close to the islands and in the stroma far from the islands. The degree of myofibroblast proliferation was inversely related to the density of lymphocytic infiltration. Among these three groups, OKC had the highest mean number of α -SMA positive cells per field

($p<0.05$) while ameloblastoma had the lowest. In the ultra-structural evaluation (TEM), myofibroblasts exhibit abundant cytoplasmic microfilaments, basal lamina-like material, subsurface caveolae, pinocytic vesicles, rough endoplasmic reticulum and mitochondria (Figure 3).

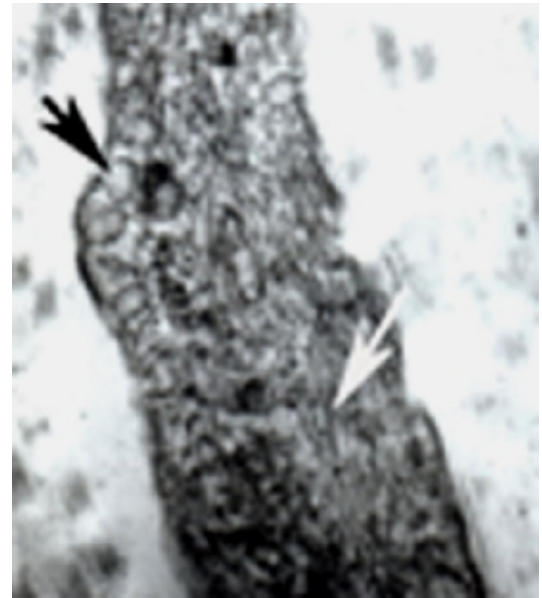


Fig. 3: Transmission electron micrograph demonstrates multiple subsurface caveolae and myofibrils

Discussion

In general, the stroma is essential for the maintenance of the epithelial tissues. Myofibroblasts are specialized fibroblasts, initially described in human granulation tissue, which develop structural features of smooth muscle cells, including nuclear indentation, abundant cytoplasmic microfilaments with dense bodies, complex intercellular junction, and a basal lamina-like material.⁸ Some investigators found that TGF β 1 upregulated the expression of α SMA, thus indicating pulpal fibroblast to acquire the myofibroblast phenotype.⁹ Although the role of inflammatory cells and endothelial cells in the tumor immunity and of neoangiogenesis in tumor progression has been described well, the role of myofibroblasts in cancer evolution has not been fully elucidated at present.^{1,10} Also, the presence of MF in odontogenic lesion has not been thoroughly investigated.²

Zidar *et al.*¹¹ describe that invasion beyond the basement membrane is necessary to evoke a myofibroblastic stromal reaction. Also, Kojc *et al.*¹² state

that disappearance of CD34-positive stromal cells and appearance of SMA-positive stromal myofibroblast are associated with transformation of laryngeal squamous intraepithelial lesions to SCC. It was reported that abundant presence of myofibroblast significantly correlated with N stage, disease stage, regional recurrence and proliferative potential of the tumor cells in oral SCC.^{13,14} Rothhouse *et al.*¹³ indicated that the stromal component of ameloblastoma is composed of myofibroblast with associated collagen and basal lamina material.

One study reported a recurrent infiltrative ameloblastoma whose stroma contained abundant myofibroblast and concluded that these cells accompany the invasive behavior of this tumor.¹⁵ Vered *et al.*² found that among the odontogenic cysts, OKC had the highest mean number of MF and DC had the lowest. Also, among the odontogenic tumor MF in ameloblastoma was significantly higher than unicystic ameloblastoma and concluded that MFs in the stroma of odontogenic cysts and tumors can contribute to variations in the biological behavior of lesions. Our results indicated that the presence of MF between OKC and DC may play a role in aggressive behavior of OKC than DC but when the three groups were compared the results did not support this hypothesis; this reveals that “when more MFs are present in the stroma, a more aggressive behavior of the odontogenic cysts and tumors can be anticipated”.

Lombardi *et al.*⁸ confirmed the presence of MF in odontogenic cysts' wall and suggested that they might be part of a homeostatic response to the distension caused by cyst enlargement. The presence of MF in the stroma of DC and OKC in our study may be

related to cystic expansion. Vered *et al.*¹⁶ investigated the correlation between MF density in aggressive and nonaggressive central giant cell granuloma (CGCG) and found that MFs were an integral component of CGCG stromal cells, but aggressive and nonaggressive lesions could not distinguished through the density of such cells. One research described that stromal MF in low grade mucoepidermoid carcinoma (MEC) was higher than that in intermediate and high grades (MEC). They suggest that inflammatory infiltrate in the MEC stroma stop MF differentiation, being indicative of a worse prognosis, as it facilitates progression of neoplastic cells.^{17,18} Also, in our study the degree of myofibroblast proliferation was inversely related to the density of lymphocytic infiltration.

The presence of MF in the stroma of invasive carcinoma such as SCC that are often concentrated at the invasive margin of the tumor suggested that proliferation of MF was seen in malignant neoplasm and because ameloblastoma is benign, locally invasive neoplasm did not show MF proliferation. In conclusion, the presence of MF in the stroma of the odontogenic lesions has no relationship with aggressive behavior. For further evaluation, working on malignant neoplasms of this family is suggested. Proliferation of MF in these lesions might have another role.

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Conflict of interest: None declared.

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