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Fascin Expression in Ameloblastoma, Odontogenic Keratocyst and Dentigerous Cyst

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ABSTRACT

Objective: The purpose of this study was to assess and compare fascin expression in 4 lesions which differ in aggressiveness: odontogenic keratocyst, dentigerous cyst and two types of ameloblastoma (solid and unicystic), and to find out whether fascin expression is associated with aggressiveness of these lesions or not.

Material and Method: Nine solid ameloblastomas , 12 unicystic ameloblastomas, 13 odontogenic keratocyst and 12 dentigerous cyst were assessed in this study. The slides were examined at x400 magnification. Finally the lesions were divided into two groups based on microscopic examination, "low expression" and "high expression".

Results: There were no significant differences between the lesions, except that fascin expression was slightly higher in unicystic ameloblastomas in comparison to other groups in intensity and count of the immunostaining cells.

Conclusion: The results of this study suggest that local aggressiveness does not result in fascin expression. We suggest more studies with more samples, assessing expression of different proteins be done in the future.

Key Words: Ameloblastoma, Dentigerous cyst, Fascin, Odontogenic keratocyst

INTRODUCTION

Fascin is a known 55-kDa globular actin-bundling protein which is mainly expressed in cell membrane protrusions like microspikes called filopodia. This protein has three isoforms in mammals: Fascin-1 (referred to from here on as Fascin) which is highly expressed in neural and mesenchymal tissues during embryogenesis and in brain, endothelium and testis in adults, fascin-2 in retina and fascin-3 in testes (1-3). Fascin is mainly expressed in neurons, dendritic cells and pericytes which have striking large filopodia and which are highly mobile (4-6). Also this protein is expressed in endothelial cells and fibroblasts (7, 8). Fascin expression has been found to be low or absent in the majority of normal adult epithelial tissue varying origin. However, in stratified squamous cells of skin, fascin is expressed at a very low intensity and only in the basal layer (9,10). Frequent and increased fascin expression in healthy, although tumor-adjacent, epithelial tissue has previously been reported (10).

Epithelial cell junctions are mostly dependent on cell-cell or cell-matrix interactions which help to stabilize the epithelial cells and prevent their migration. In epithelial malignancies, invasion to basal lamina and metastasis occurs due to elimination of these junctions. Fascin is considered in the migration and invasion of carcinoma cells since this actin-cross-linking protein is known to be responsible for controlling the guiding system of cell migration.

Many studies have reported the upregulation of fascin expression in various tumors. Moreover it has been reported that high expression of fascin is associated with high motility of cells, poor differentiation, advanced grade and stage and metastasis (11,12). Fascin helps to stabilize the actin bundles in protrusions and it therefore seems to be used by cancerous cells to assemble stable and durable invasive protrusions (called invadopodia) that help invasion into the matrix (13,14).

Ameloblastoma is slow growing and locally aggressive odontogenic epithelial tumor with a low tendency to metastasize and a high recurrence rate (15).

Odontogenic keratocyst (OKC), formerly known as keratocystic odontogenic tumor (KCOT), is a common

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odontogenic cyst that originates from the dental lamina or from its residue; occurring at any age, and is more common in men than women with a 2:1 ratio (16). Because of the high proliferation rate, aggressiveness and recurrence rate of epithelial cells of this cyst, in 2005 WHO reclassified odontogenic keratocyst as benign intra-osseous neoplasia, called KCOT (17). However, this odontogenic lesion is now listed as odontogenic keratocyst in the 2017 classification of developmental odontogenic cysts (16).

Dentigerous cyst (DC) is the second most common odontogenic cyst (following radicular cyst) which is typically seen as a radiolucency surrounding the crown of an impacted tooth. DC is not as aggressive as ameloblastoma or OKC (18).

In this article, we assessed and compared fascin expression in 4 lesions which differ in aggressiveness: OKC, two types of ameloblastoma (solid -which is believed to be more aggressive- and Unicystic) and DC to find out whether the fascin expression is associated with aggressiveness of these lesions or not.

MATERIAL and METHODS

Due to the retrospective nature of this study, it was granted an exemption in writing by the institutional review board of Tehran University of Medical Sciences (TUMS). For this descriptive analytic study, 82 biopsy-proven samples were retrieved from the archives of Departments of Oral and Maxillofacial Pathology of Tehran and Qazvin University of Medical Sciences. A total of 36 cases were excluded because of quantitatively inadequate available tissue in paraffin blocks, and recurrent or inflammatory lesions.

Thus, 46 paraffin blocks including 9 solid ameloblastomas (SA), 12 unicystic ameloblastomas (UA), 13 OKCs and 12 DCs were assessed in this study.

After obtaining sections with a thickness of 4µm, immunohistochemical staining was carried out with the monoclonal anti-fascin antibody (Clone 55K-2 Dako, Glostrup, Denmark) diluted 1:50. For each case, a section with the primary antibody omitted was used as negative control and the cytoplasm of endothelial cells was used as internal positive control.

In microscopic assessment, the slides were examined at x400 magnification (Figure 1A-F) by an oral and maxillofacial pathologist who was blinded to the clinical characteristics of the samples. Immunoexpression of fascin in tumor cells was evaluated in terms of extent and intensity. The extent of immunostaining was scored according to Terence K. Lee (19): 1-negative: less than 10% of cells, 2-weak: 11-50% of cells, 3-moderate: 51-80% of cells and 4-strong: more that 81% of cells. Moreover, the staining intensity of the samples was categorized according to the cytoplasmic staining of cells into: 1-faint, 2-moderate and 3-severe. Finally, to determine the expression level of fascin, a Combined Immunoreactivity Score (CIS) was calculated by adding the score of extent to the score of intensity for each case. The samples were further classified into 4 groups: Negative expression: staining in less than 10% of cells regardless their staining intensity, weak expression: CIS equal to 3, moderate expression: CIS equal to 4 or 5, strong expression: CIS equal to 6 or 7.

In statistical analyses, the first 2 groups were merged as the "low expression" group, and the groups 3 and 4 merged as the "high expression" group. Research data were analyzed using the Statistical Package for Social Sciences (SPSS), version 18. The Kruskal-Wallis and two by two Dunn Tests were used. P values less than 0.05 were considered significant.

RESULTS

The scores of stained-cell counts, staining intensities and CIS are shown in Table I. Ten samples from 12 unicystic ameloblastoma (83.3%) showed a score of 3 in intensity and also 10 samples showed a score of 4 in the count of immunostaining cells (Table I). As mentioned formerly, these four groups were merged into low expression (group 1&2) and high expression (group 3&4). No significant difference was found between these groups ($P>0.05$). All the samples of the four groups (DC, OKC, SA and UA) were classified in the high expression group except one sample of OKC (7.7%) that was classified in the low expression group (Table I). In the two by two comparison test, although a significant P value was seen in UA comparison with other lesions, there was no significant difference between the groups when other criteria were considered in the statistical tests.

DISCUSSION

Four different tumors based on aggressiveness of their clinical behavior were studied in this study. The aggressive behavior of OKC was close to ameloblastoma as in many studies which investigated the expression of markers such as p53, p63, Ki67, PCNA, AgNors and Ipo (20-23) and concluded that the proteins expressed on epithelial cells of OKC is more alike to that of ameloblastoma than DC.

In order to assess aggressiveness of lesions, some studies have examined the extra cellular matrix and connective tissue proteins such as fibronectin, laminin, tenascin, RANK, RANKL, and OPG in these lesions. Many of them found significant differences in the expression levels of these proteins among DC, ameloblastoma and OKC (24-27).

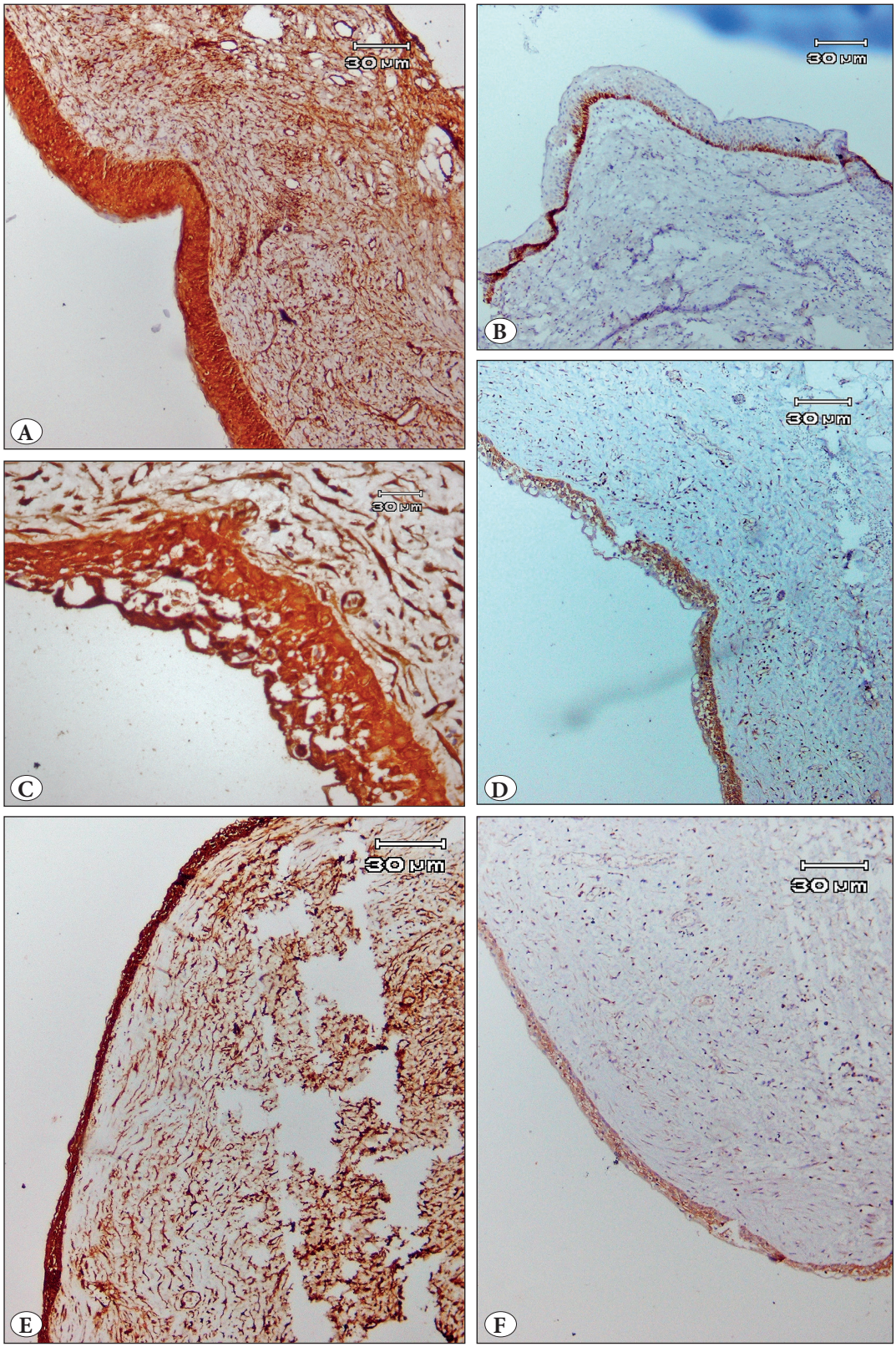

Figure 1: A) High expression of fascin in basal and suprabasal layers in odontogenic keratocyst. Note immunoactivity of stomal fibroblasts and endothelial cells as positive internal control (IHC; x100). **B)** Low expression of fascin only in basal layer in odontogenic keratocyst (IHC; x100). **C)** High expression of fascin in basal and suprabasal stratum reticulum layers in unicyctic ameloblastoma (IHC; x400). **D)** Low expression of fascin in unicyctic ameloblastoma. Note immunoactivity of stromal fibroblasts and endothelial cells even in low epithelial lining reaction (IHC; x100). **E)** High expression of fascin in dentigerous cyst (IHC; x100). **F)** Low expression of fascin in dentigerous cyst (IHC; x100).

Table I: Scores of stained-cell counts, staining intensities and group classifications based on immunohistochemical assessments

			Lesion				Total
			solid ameloblastoma	odontogenic keratocyst	dentigerous cyst	unicystic ameloblastoma	
Score of Staining Intensity	1	Count	1	2	2	0	5
		% within pathology	11.10%	15.40%	16.70%	0.00%	10.90%
	2	Count	5	6	6	2	19
		% within pathology	55.60%	46.20%	50.00%	16.70%	41.30%
	3	Count	3	5	4	10	22
		% within pathology	33.30%	38.50%	33.30%	83.30%	47.80%
Score of Stained Cells Counts	1	Count	0	1	0	0	1
		% within pathology	0.00%	7.70%	0.00%	0.00%	2.20%
	2	Count	0	0	0	1	1
		% within pathology	0.00%	0.00%	0.00%	8.30%	2.20%
	3	Count	1	4	3	1	9
		% within pathology	11.10%	30.80%	25.00%	8.30%	19.60%
	4	Count	8	8	9	10	35
		% within pathology	88.90%	61.50%	75.00%	83.30%	76.10%
Classified Groups	Low Expression Group	Count	0	1	0	0	1
		% within pathology	0.00%	7.70%	0.00%	0.00%	2.20%
	High Expression Group	Count	9	12	12	12	11
		% within pathology	100.00%	92.30%	100.00%	100.00%	23.90%
Total		Count	9 (19.56%)	13 (26.08%)	12 (28.26%)	12 (26.08%)	46 (100.00%)
		% within pathology	100.00%	100.00%	100.00%	100.00%	100.00%

All mentioned markers are exclusively expressed in connective tissue cells except laminin which is expressed in epithelial tissue. In this study we assessed fascin, as another epithelial cell marker. Fascin is also known as the protein of motility (11,12). No wonder that expression of fascin can be associated with aggressive and invasive behaviors (28,29) as seen in many epithelial cancers (13). Fascin is believed to be a biomarker that indicates aggressiveness and migration of tumors. However, our results contradict this hypothesis as we observed no significant upregulation of fascin in aggressive lesions. In addition, one meta-analysis study demonstrated that fascin may have potential as a novel biomarker for early identification of aggressive and metastatic tumors (12). In our study, the only statistically significant difference among the four lesions was the increased fascin expression in unicystic ameloblastoma in comparison to the other three. There is a possibility that fascin might be more expressed in carcinomas than cyst walls or odontogenic tumors. Also since the lesions of this study were mostly intra-bony, there were no epithelial barriers to their progression. Moreover these lesions do not metastasize. Considering these, we suggest that fascin might be more involved in cell invasion and migration (as in carcinomas) than local aggressiveness. Lee et al. suggested that fascin over-expression might enhance oral squamous cell carcinoma aggressiveness, possibly by interacting with E-cadherin expression (19). There is no E-cadherin interaction in progression of odontogenic lesion versus progression of carcinomas. Therefore, according to Lee theory (19), our suggestion based on more involvement of fascin in invasion and migration than local aggressiveness in benign odontogenic lesion is justifiable. Furthermore, Li et al. showed that fascin is an integral component of invadopodia -the finger-like protrusions using by cancer cells which help them invade into and degrade extracellular matrix- and it is important for the stability of actin in invadopodia (28). Epithelial cells of benign odontogenic lesions like DC, OKC or ameloblastoma that we assessed in this study do not use invadopodia for progression, and this may be a reason for less involvement of fascin in local aggressiveness.

It is accepted that immunohistochemistry is a complex metric due to the use of different scoring systems to assess the extent of staining in different specimens but the scoring of fascin is a continuous measurement and in most publications researchers categorized specimens into high/positive or low/negative fascin based on different semi-quantitatively assessed cut-off points (12). We also

assessed the fascin expression in two groups of low and high expression as in most previous publications. However, this study has several limitations. First, we did not assess whether these lesions had perforated the cortical bone and invaded local soft tissue. Second, we just examined fascin expression and not other proteins or markers. We suggest more studies with more samples, and assessing expression of different proteins in the future.

CONFLICT of INTEREST

The authors declare no conflict of interest.

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