The expression of cathepsin-D in odontogenic cysts and tumors: Immunohistochemistry study

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Abstract

Background: Cathepsin-D, a protease, which is an invasion promoter and plays a central role in solid tumors including oral cancer. Our aim of the study was to look for their expression pattern in epithelium and stroma of odontogenic cysts and tumors and correlate their aggressiveness to the staining intensity.

Materials and Methods: To elucidate the expression patterns of this marker, we examined immunohistochemically on formalin-fixed, paraffin-embedded sections of 24 odontogenic cysts and 10 odontogenic tumors, which are received for histopathologic examination in the Department of Oral Pathology, The Oxford Dental College and Hospital, Bengaluru.

Results: The epithelium of granular cell ameloblastoma (GCA) and odontogenic keratocyst (OKC) showed maximum staining with spillage of stained material in the connective tissue wall and at the separation of epithelium to capsule in OKC compared to other cysts and tumors.

Conclusions: Cathepsin-D could be one of the enzymes important in separation of epithelium and connective tissue in OKC which helps in recurrence and intense expression in GCA with spillage into stroma, compared to other odontogenic tumors may explain its aggressive behavior, recurrence, and metastatic potential. To further validate our findings, it is suggested to use more sample size and monoclonal antibody for cathepsin-D.

Keywords

Cathepsin-D, immunohistochemistry, odontogenic cysts, odontogenic tumors

Introduction

Odontogenic cysts and tumors constitute an important aspect of oral and maxillofacial pathology. Odontogenic cysts are encountered relatively common in dental practice and tumors by contrast are uncommon lesions. These lesions are of clinical significance because of their biological behavior. Various attempts to categorize morphological features to relate the biological activity have been made over the years.¹ It is well-established that the cysts of histogenic labeling of odontogenic keratocyst (OKC) are more aggressive tendency to behave more like a sub-malignant tumor.² It has also been suggested that cysts other than OKC showing keratinization if not more locally aggressive tend to have a predisposition to neoplastic change.³ There have been attempts to correlate follicle size with aggression in ameloblastoma, and morphologically different granular cell variant has been known to be more clinically aggressive, showing metastatic potential.⁴ Numerous studies on the enzyme histochemistry of odontogenic cysts and tumors have been conducted over the years for the expression of oxidative enzymes NADH2 and NADPH2, G6PD, glutamate dehydrogenase, acid phosphates, leucine amino peptidase, and ATPase.⁵ The epithelial lining of all the varieties of cysts showed a weak reaction for leucine amino peptidase a lysosomal protease, but there was a strong positivity in the lamina propria of OKC. Similar studies on follicular ameloblastoma (FA) have showed ATPase activity in the peripheral and central cells of the follicle.⁶ Based on these we made an attempt to study the expression of cathepsin-D in odontogenic cysts and tumors, by grouping them into locally
aggressive and non-aggressive based on their clinical and radiographic features.

Cathepsin-D is a proteolytic enzyme that belongs to a family known as aspartic proteases. Many homologies in the amino acid sequence have been shown to exist among the members of this group of enzymes, which includes the pepsin, gastrin, and rennin. Like other enzymes, cathepsin-D has been shown to be synthesized in a precursor form. The enzyme itself is a glycoprotein of approximate molecular weight 52 KD and has an optimum pH of 3.5. Cathepsin-D was present in many of the normal tissue including epithelium, fibroblast, and macrophages. The physiologic role of cathepsin-D is believed to be involved in self-destruction of senescent or damaged epithelial cells. As cathepsin-D is an intracellular lysosomal aspartic aspartic protease apart from its role in protein catabolism through the degradation of the endocytosed protein. Cathepsin-D has attracted clinical attention because of its overexpression in a variety of diseases. Increased levels of these enzymes have been reported to be an indicator of aggressive behavior in human tumors including oral squamous cell carcinoma.

Materials and Methods

Tissue used in the study was biopsy material submitted to Department of Oral Pathology, The Oxford Dental College, Hospital and Research Centre, Bengaluru. Total sample size taken was from 34 patients which comprised of 9 ameloblastoma (1 plexiform unicystic ameloblastoma [UA]), 7 OKC, 1 adenomatoid odontogenic tumor (AOT), 11 Radicular cysts (RC), and 6 Dentigerous cysts (DC) which were grouped into locally aggressive and non-aggressive based on their clinical and radiologic features such as size and extent of lesion, peripheral cortication, scalloping, and root resorption.

| Locally aggressive | Non aggressive |
|--------------------|---------------|
| Ameloblastoma (9)   | Radicular cysts (10) |
| Odontogenic keratocyst (7) | Dentigerous cysts (2) |
| Radicular cyst (1)* | Adenomatoid odontogenic tumor (1) |

*This particular radicular cyst was an extensive lesion extending from the maxillary canine to the third molar extending into and destroying the maxillary sinus and had caused root resorption from canine to second molar without causing any bony expansion. The initial clinical impression was that of a malignancy arising in the maxillary sinus

Methodology

Formalin-fixed paraffin embedded sections of odontogenic cysts and tumors were stained by hematoxylin and eosin stain, the serial sections of the same were studied by Immunohistochemistry procedure using cathepsin-D and observed under the microscope for the intensity of cathepsin-D staining expression or non-expression. Controls were prepared by omitting the primary antibody.

A grading system for the intensity of expression was devised and used.

Antibody used:
1. Polyclonal rabbit anti-human primary cathepsin-D, 7ml ready to use (DAKO Corporation N162S). Denmark
2. Biotinylated anti-mouse, anti-rabbit, anti-goat Igs, LINK/secondary antibody, 15 ml ready to use. (DAKO LSAB+ system, K0679).
3. Streptavidin conjugated to horseradish peroxidase. (DAKO LSAB+ system, K0679).
4. Liquid Diamino benzidine chromogen.

Observation and Results

All odontogenic cysts and tumors were observed for intensity of cathepsin-D stain in epithelium and stroma/connective tissue capsule by categorized into mild, moderate, and marked staining. Statistical analysis was done using Student’s t-test. Table 1 shows the number of cases in which cathepsin-D shows mild, moderate, and marked staining in various epithelial layers and stroma. Table 2 shows statistical relation of staining intensity of cathepsin-D in each layer and stroma/capsular wall between each odontogenic cysts. Table 3 shows statistical relation of staining intensity of cathepsin-D in each layer and connective tissue stroma between each odontogenic tumors.

Discussion

Cathepsin-D is a proteinase which causes collagenolytic activity, bone resorption and is closely involved in biological mechanism of tumor progression which has been reported to be an indicator of aggressive behavior in human tumors including oral squamous cell carcinoma by its ability of extracellular matrix digestion. Collagenolytic activity was demonstrated in homogenates of OKC and RC walls. It seemed probable that the activity of collagenase in tissue was controlled by a complex regulatory system which in cyst tissue might exert effects on collagenase activity and thus influence the expansion cysts within bone.

The idea of immunohistochemistry staining for a lysosomal protease cathepsin-D in odontogenic cysts and tumors of varying biological behavior pattern was with the hope that it could contribute to a better understanding of metabolic processes that are responsible for that behavior. Traditionally we have always focused on the epithelium in odontogenic cysts and epithelial tumors. Much like the mesmerizing effect of giant cells in giant cell lesions, the epithelium in odontogenic cysts and epithelial tumors has held a magnetic quality for research workers. The epithelial component dictates the diagnosis, but the role of connective tissue wall and the stromal cells in tumors has not always been given due consideration. The epithelium is not always at the advancing front of these lesions as is especially seen in the case of cysts. In this study, in addition to the epithelium, we also looked at the expressivity of cathepsin-D in the connective tissue and stromal cells.

In granular cell ameloblastoma (GCA), we observed marked staining pattern in the cytoplasm of the granular cells, often
Table 1: Comparison of the total number of odontogenic cysts and tumors showing the intensity of staining pattern in each layer and stroma/capsule

| Staining intensity | OKC | DC | RC | UA | FA | AOT | GCA |
|--------------------|-----|----|----|----|----|-----|-----|
| MILD (+) Epithelium | 1   | 10 |    |    |    |     |     |
| Basal layer        |     |    |    |    |    |     |     |
| Suprabasal layer   | 1   | 6  |    |    |    |     |     |
| Superficial layer  | 1   | 10 |    | 1  | 1  | 1   | 1   |
| Stromal staining   | 1   | 6  | 10 |    |    |     |     |
| MODERATE(++) Epithelium | 1   |     |    |    |    |     |     |
| Basal layer        | 1   | 1  |    |    |    |     |     |
| Suprabasal layer   | 11  | 2  | 1  |    |    |     |     |
| Superficial layer  | 2   | 1  |    |    |    |     |     |
| Stromal staining   | 6   | 1  |    | 1  |    |     |     |
| MARKED(+++) Epithelium | 1   |     |    |    |    |     |     |
| Basal layer        | 1   | 1  |    |    |    |     |     |
| Suprabasal layer   | 11  | 2  | 1  |    |    |     |     |
| Superficial layer  | 2   | 1  |    |    |    |     |     |
| Stromal staining   | 6   | 1  |    | 1  |    |     |     |

Total number of cases: 7 6 11 2 6 1 1

Intensity of the staining refers to color of the DAB chromogen stain in various layers and stroma. Stromal staining refers to extra-cellular spillage of immunoreactive material.

OKC: Odontogenic keratocyst, DC: Dentigerous cyst, RC: Radicular cyst, UA: Unicystic ameloblastoma, FA: Follicular ameloblastoma, AOT: Adenomatoid odontogenic tumor, GCA: Granular cell ameloblastoma. All the inflammatory cells showed marked staining.

Table 2: Statistical significance of cathepsin-D stain in each layer and stroma/capsule of various odontogenic cysts using Student's t-test

| Intensity of stain | Layers | Student's t-test | Inference |
|--------------------|--------|------------------|-----------|
| Mild               | Basal layer | P=0.05          | RC showed maximum stain compared to OKC and DC |
|                    | Suprabasal layer | P=0.05          | DC showed more stain compared to OKC and DC |
|                    | Superficial layer | P=0.05          | OKC and DC showed more stain compared to RC |
|                    | Stromal staining | P=0.05          | RC and DC showed maximum stain compared to OKC and DC |
| Moderate           | Basal layer | P=0.05          | RC showed maximum stain compared to OKC and DC |
|                    | Suprabasal layer | P=0.05          | RC showed maximum stain compared to OKC and DC |
|                    | Superficial layer | P=0.05          | RC showed maximum stain compared to OKC and DC |
|                    | Stromal staining | P=0.05          | OKC showed more stain compared to RC and DC |
| Severe             | Basal layer | P=0.05          | OKC showed more stain compared to RC and DC |
|                    | Suprabasal layer | P=0.05          | OKC showed more stain compared to RC and DC |
|                    | Superficial layer | P=0.05          | None showed |
|                    | Stromal staining | P=0.05          | None showed |

P<0.05: Not significant, P=0.05: Significant, OKC: Odontogenic keratocyst, DC: Dentigerous cyst, RC: Radicular cyst

Table 3: Statistical significance of cathepsin-D stain in each layer and stroma of various odontogenic tumors using Student's t-test

| Intensity of stain | Layers | Student's t-test | Inference |
|--------------------|--------|------------------|-----------|
| Mild               | Columnar ameloblast-like cells | P=0.05          | Not shown in any tumors |
|                    | Stellate reticulum-like cells | P=0.05          | Only in UA |
|                    | Other cells | P=0.05          | Only in UA |
|                    | Stromal staining | P=0.05          | Was the maximum in UA compared to other tumors |
| Moderate           | Columnar ameloblast-like cells | P=0.05          | Showed only in UA |
|                    | Stellate reticulum-like cells | P=0.05          | More in FA compared to others |
|                    | Other cells | P=0.05          | More in GCA compared to others |
|                    | Stromal staining | P=0.05          | More in GCA compared to others |
| Severe             | Columnar ameloblast-like cells | P=0.05          | Only FA and GCA showed maximum stain |
|                    | Stellate reticulum-like cells | P=0.05          | Only FA and GCA showed maximum stain |
|                    | Other cells | P=0.05          | None showed |
|                    | Stromal staining | P=0.05          | None showed |

P<0.05: Not significant, P=0.05: Significant, UA: Unicystic ameloblastoma, FA: Follicular ameloblastoma, GCA: Granular cell ameloblastoma
spilling into the connective tissue which may contribute to the aggressive nature of the lesion and its propensity for metastasis [Figure 1a and b]. As compared to the GCA other odontogenic tumor types such as FA, UA, plexiform ameloblastoma, and AOT [Figure 2a and b] showed less intense staining pattern and the staining was restricted to cytoplasm of these epithelial cells with minimal stromal staining. Apart from the GCA we could not derive any correlation between clinical behavior and cathepsin-D expression. Among the 3 cyst types, we found a characteristic epithelial staining pattern in OKC in comparison to RC and DCs. Among 7 OKC, only one case showed superficial granular staining of the epithelial cells with no separation of the epithelium from connective tissue. In all other cases, we observed granular staining through the full thickness of the epithelium, more in the basal and suprabasal layers, with intense/marked staining at the region of separation of epithelium from connective tissue with granular staining pattern in separation zone [Figure 3a and b].

In DCs, there was only superficial staining of the epithelium. The RCs showed uniform staining in the entire length of epithelium [Figure 4]. In the one RC which was clinically more aggressive; a similar pattern of staining was observed. Though the epithelial staining in RCs was almost similar to that seen in OKCs, we did not find any areas of cleavage between epithelium and connective tissue. In the OKC, the staining pattern though similar to the RCs, in the area of split the staining was very intense, and some stained material was noticed in the space between the epithelium and the connective tissue leading to the speculation that the increased expression may contribute to the split, which may have prognostic consequences in terms

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Figure 1: (a) Marked staining of cathepsin-D expression in the peripheral ameloblast-like cells with the spillage of stained material into connective tissue stroma in granular cell ameloblastoma (×10). (b) Intense staining noted in ameloblast-like cells of follicles with lighter staining of cells in other areas (×40).

Figure 2: (a) H and E picture of adenomatoid odontogenic tumor (AOT) showing duct-like structures, whirling, rosette pattern, and spindle-shaped cells arranged in sheets with outer peripheral fibrous capsule. (b) Immunohistochemistry shows mild staining of cathepsin-D in tumor cells and stroma of AOT.

Figure 3: (a) H and E picture showing parakeratotic corrugated stratified squamous epithelium and its separation from capsule in odontogenic keratocyst (×20). (b) Immunohistochemistry stain shows marked staining at the area of separation, intense staining of the superficial layer, keratin and some basal layer cells in odontogenic keratocyst (×40).

Figure 4: Immunohistochemistry stain for cathepsin-D shows moderate staining at basal layer and mild staining in superficial layers, with intense/marked staining of the underlying inflammatory cells in radicular cyst.

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of recurrence by way of cleaving of epithelium at the time of attempted enucleation or biopsies.

In addition to variations in staining patterns of the epithelial lining of the different types of cysts, their walls showed variation in staining from the epithelial end to the bony end. All the cyst types showed expressivity in the immediate sub-epithelial region as well as the bony end of the cyst wall. The intensity of staining progressively increased from the DC through the RC to the OKC. The intermediate zone showed scantly expression. This pattern of increasing expression seemed to correlate with increasing aggression. The one RC grouped in the list of aggressive lesion showed intense staining in the most peripheral areas similar to that seen in the OKC. All the inflammatory cells seen in connective tissue wall and keratin of the surface layer and granules of the granular layer of odontogenic cysts showed intense staining. Cathepsin-D is necessary for osteoclastic bone resorption, and it plays an indirect rather than a direct role. A large amount of cathepsin-D in osteoclast at the proximal growth plane of the rat femurs was demonstrated using both the avidin-biotin-peroxidase complex method for cryo-semi-thin sections and the colloidal gold-labeled IgG method for K4M ultra-thin sections indicating the role of cathepsin-D in osteoclast-mediated bone resorption.

To the best of our knowledge, this is the first study on the expression of cathepsin-D in odontogenic cysts and tumors although studies on various other lysosomal enzymes like leucine amino peptidase, etc., have been published. Hence, it may be presumptuous on our part to make claims on the role of cathepsin-D in aggressive behavior of odontogenic cysts and tumors, however that there is perceptible variation in expression would suggest that additional efforts in the area may help to understand the metabolic processes that lead to aggressive behavior. Another area open for exploration is precystic epithelium as in the case of periapical granulomas and the role of these enzymes in cystogenesis.

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References

1. Shear M. The aggressive nature of the odontogenic kerato cyst: Is it a benign cystic neoplasm? Oral Oncol 2002;38:219-26.
2. Jackson IT, Potparic Z, Fasching M, Schievink WI, Tidstrom K, Hussain K. Penetration of the skull base by dissecting keratocyst. J Cranio Maxillofac Surg 1993;21:319-25.
3. Chuong R, Donoff RB, Guralnick W. The odontogenic keratocyst. J Oral Maxillofac Surg 1982;40:797-802.
4. Worrell SF. Recurrent odontogenic keratocyst within the temporalis muscle. Br J Oral Maxillofac Surg 1992;30:59-62.
5. Brannon RB. The odontogenic keratocyst. A clinicopathologic study of 312 cases. Part II. Histologic features. Oral Surg Oral Med Oral Pathol 1977;43:233-55.
6. Ahlfors E, Larsson A, Sjögren S. The odontogenic keratocyst: A benign cystic tumor? J Oral Maxillofac Surg 1984;42:10-9.
7. Fanibunda K, Soames JV. Malignant and premalignant change in odontogenic cysts. J Oral Maxillofac Surg 1995;53:1469-72.
8. Hoke HF Jr, Harrelson AB. Granular cell ameloblastoma with metastasis to the cervical vertebrae. Observations on the origin of the granular cells. Cancer 1967;20:991-9.
9. Magnusson BC. Odontogenic keratocysts: A clinical and histological study with special reference to enzyme histochemistry. J Oral Pathol 1978;7:8-18.
10. Cutler IS, Innes DJ Jr. An electron-microscopic and cytochemical study of follicular ameloblastoma. J Oral Pathol 1983;12:502-14.
11. Reid WA, Valler MJ, Kay J. Immunolocalization of cathepsin D in normal and neoplastic human tissues. J Clin Pathol 1986;39:1323-30.
12. Marsigliante S, Biscozzo L, Resta L, Leo G, Mottaghi A, Maiorano E, et al. Immunohistochemical and immunoradiometric evaluations of total cathepsin D in human larynx. Eur J Cancer B Oral Oncol 1994;30B:51-5.
13. Brysk MM, Lei G, Adler-Storthz K, Chen Z, Horikoshi T, Brysk H, et al. Differentiation and cathepsin D expression in human oral tumors. Laryngoscope 1998;108:1234-7.
14. Lubansu A, Ruchoux MM, Brotchi J, Salmon I, Kiss R, Lefranc F. Cathepsin B, D and K expression in adamantinomatous craniofacial tumors relates to their levels of differentiation as determined by the patterns of retinoic acid receptor expression. Histopathology 2003;43:563-72.
15. Kumamoto H. Molecular pathology of odontogenic tumors. J Oral Pathol Med 2006;35:65-74.
16. Goto T, Tsukuba T, Ayasaka N, Yamamoto K, Tanaka T. Immunocytochemical localization of cathepsin D in the rat osteoclast. Histochemistry 1992;97:13-8.

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