

Original Research Article

Role of Calretinin in the Pathogenesis of Odontogenic Cysts and Tumors

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Abstract: Calretinin, a calcium-binding protein, is expressed primarily in certain subtypes of neurons. It has also been shown to be present in mesotheliomas and few other tumors. The aim of the study was to determine the expression of calretinin in selected odontogenic cysts and tumors. Immunohistochemically study for calretinin was performed on 10 odontogenic tumors ameloblastomas (2 solid ameloblastomas and 2 unicystic ameloblastomas) and 2 adenomatoid odontogenic tumors, 2 keratocystic odontogenic tumours and 2 cystic calcifying ghost cell odontogenic tumors), 4 odontogenic cysts (2 dentigerous cysts and 2 radicular cysts) and 2 dental follicle tissues. The distribution, intensity, pattern and localization of immunoreactive cells were determined by conventional light microscopy. All 4 ameloblastomas and 2 cystic calcifying ghost cell odontogenic tumours showed intense immunopositivity with a diffuse distribution pattern. None of the other cysts and tumours showed reactivity with calretinin. Considering that ameloblastomas and cystic calcifying ghost cell odontogenic tumours, in contrast to the other studied cysts and tumours were consistently reactive for calretinin, this protein may have a role in the pathogenesis of these tumours.

Keywords: Calretinin; Immunohistochemistry; Odontogenic tumours; Odontogenic cysts

INTRODUCTION

Calretinin is a calcium-binding protein of 29 kilo Dalton (kDa), is a member of the large family of EF-hand proteins to which S 100 protein also belongs. EFhand proteins are characterized by a peculiar amino acid sequence that folds up into a helix-loop-helix which acts as the calcium-binding site. The EF-hand motif consists of two α -helices joined by a Ca²⁺ binding loop. Calretinin contains six EF-hand sequences [1]. It is widely expressed in central and peripheral neural tissues particularly in the retina and neurons of sensory pathway [2]. The precise biological behavior of calretinin is unknown, but possible roles as a calcium buffer and/or calcium sensor and regulator of apoptosis have been postulated [3,4]. In 1996, Doglioni *et al.* [5], demonstrated that calretinin is expressed in human mesothelial cells and mesothelioma. Since then, several other studies have confirmed the utility of calretinin expression in the differential diagnosis between mesothelioma and adenocarcinoma [6]. Other neoplasms found to be positive for calretinin include sex cord-stromal tumors, mesonephric cervical

adenocarcinoma, Wolffian adnexal tumor, synovial sarcoma and ameloblastoma [7-9].

Odontogenic cysts (OCs) and odontogenic tumours (OTs) are a group of a lesions arising from the tooth-producing apparatus or its remnants. They may originate from odontogenic epithelium and/or ectomesenchyme with varying degrees of inductive tissue interaction [10]. The expression of calretinin has been documented in odontogenic epithelium during tooth development in rats and has also been reported in both unicystic and solid ameloblastomas [9,11]. However, this protein has not been demonstrated in other odontogenic cysts and tumours. The aim of this study was to assess the expression of calretinin in a number of odontogenic cysts and tumours, in order to gain a better understanding of the nature of these cysts and neoplasms.

MATERIALS AND METHOD

Sixteen formalin-fixed paraffin-embedded blocks of OCs, OTs and dental follicle tissues (DFTs)

were retrieved from the Oral Pathology archives of Dr D Y Patil Dental College and Hospital, Pune, during 2004 -2010. Reevaluation of all cases was performed according to the World Health Organization (WHO) histological typing of OCs and OTs based on light microscopy using haematoxylin and eosin sections [12]. The sample consisted of 2 follicular ameloblastomas (FAB), 2 unicystic ameloblastomas (UABs, Type 3), 2 adenomatoid odontogenic tumours (AOTs), 2 cystic calcifying ghost cell odontogenic tumours (CCGCOT), 2 keratocystic odontogenic tumours (KCOT), 2 radicular cysts (RCs) and 2 dentigerous cysts (DCs). DFTs associated with impacted mandibular third molars were also included in this study. Serial sections of 5 microns thickness were cut from the paraffin blocks and processed for immunohistochemical examination.

Selected sections were placed on 3-aminopropyl-triethoxysilane-coated slides overnight, at room temperature. Polyclonal rabbit anticalretinin (Seemed Laboratories Inc., San Francisco, CA, USA) was used for immunohistochemistry employing the streptavidin-biotin complex immunoperoxidase technique (StreptABCComplex/HRP Duet, mouse/rabbit; Dako, Glostrup, Denmark).

Deparaffinized sections (xylene, 2x5 min) were microwaved (800W, medium power) in 0.01 M citrate buffer (pH 6.0) for 2x5 min. following 20 min of cooling; they were immersed in 3% methanol-hydrogen peroxide for 30 min, washed in water and rinsed in phosphate-buffered saline (PBS) (pH 7.6). All sections were then incubated with normal goat serum (1:5) for 20 minutes and treated with the primary antiserum (1:25) at room temperature for 1 hour. Incubation with the biotinylated secondary antibody was performed for 30 minutes after washing with PBS; all specimens were

treated with Strep ABC for 30 minutes at room temperature and washed with PBS. A brown reaction detected using 3,3' -diaminobenzidine hydrochloride (Sigma, St Louis, MO, USA) for 5 min. Meyer's hematoxylin (5minutes) was utilized for counterstaining. All reagent dilutions were prepared with 3% bovine serum albumin factor V (Bustranger Mannheim, Mannheim, Germany). Human normal pleura lining was used as the positive control for negative controls the primary specific antibodies were substituted with non-immune serum. Immunoreactivity was evaluated with regard to intensity, distribution and localization of immunoreactivity cells using conventional light microscopy. Immunopositivity was classified as diffuse (when involving more than 50% of the cells) or focal (less than 50%). Intensity of immunopositivity was scored as 0 to 3 as - 0, no staining; 1, weak; 2, moderate and 3, strong.

RESULTS

In all immunopositive cases, reactivity was observed in both nucleus and cytoplasm and was of strong intensity. The 2 cases of FABs, 2 cases of UABs and 2 cases of CCGCOTs were immunopositive for calretinin and showed positivity of the amyloplastic epithelium with a diffuse distribution pattern (fig. 1-3). Immunoreactivity was restricted to the stellate reticulum-like epithelium and was intense in all tumors.

Interestingly weak expression of calretinin was observed in ghost cells in CCGCOTs (fig. 4). All other OCs, OTs, 2 adenomatous odontogenic tumours (AOTs), 2 cystic calcifying ghost cell odontogenic tumors (CCGCOT), 2 Ceratocystis odontogenic tumours (KCOT), 2 radicular cysts (RCs) and 2 dentigerous cysts (DCs) and DFTs were completely negative for calretinin (fig. 5-9).

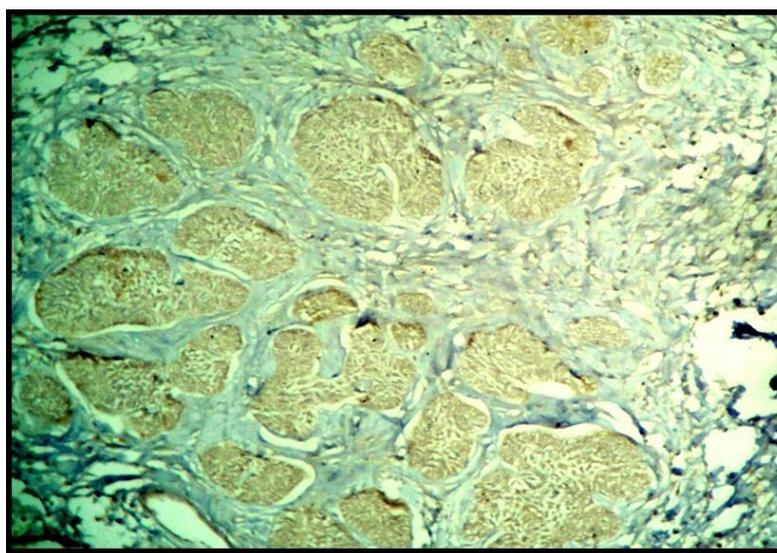


Fig-1: Ameloblastoma: Calretinin immunoreactivity restricted to the stellate reticulum-like epithelium.

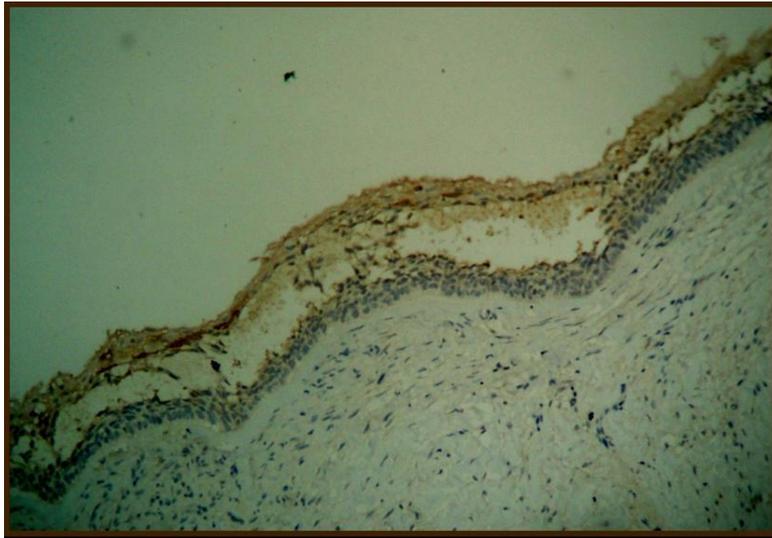


Fig-2: Unicyclic-ameloblastoma: Calretinin immunoreactivity restricted to the stellate reticulum-like epithelium.

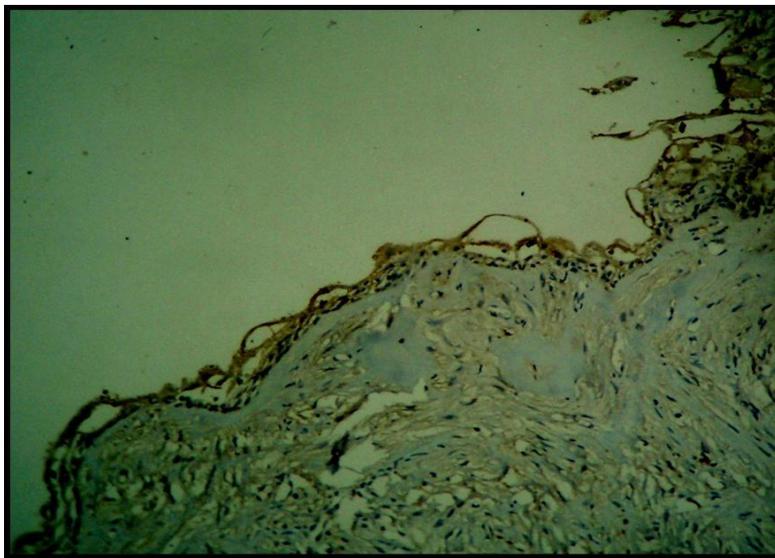


Fig-3: Cystic Calcifying Ghost Cell Odontogenic Tumor: Calretinin immunoreactivity restricted to the stellate reticulum-like epithelium.

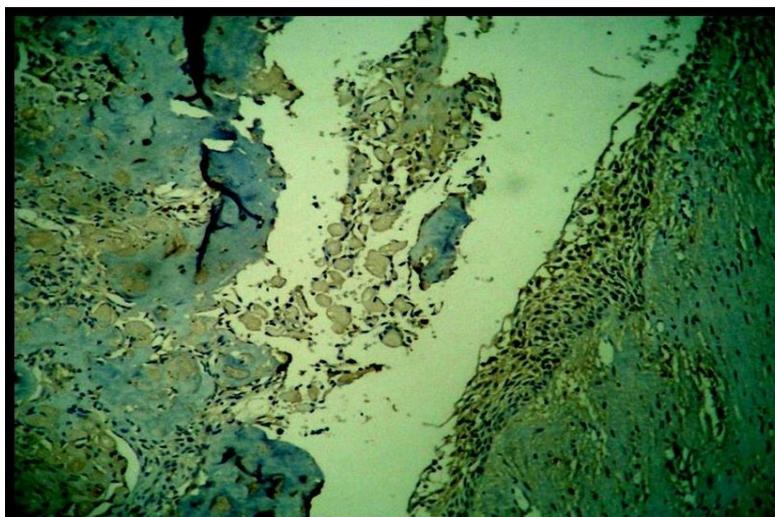


Fig-4: Cystic Calcifying Ghost Cell Odontogenic Tumor: Calretinin immunoreactivity in ghost cells.

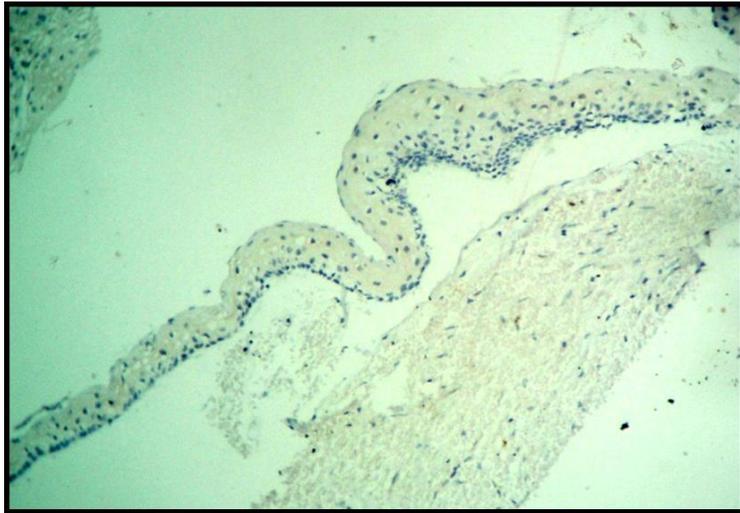


Fig-5: Keratocystic Odontogenic Tumor: Negative calretinin immunoreactivity in columnar tumour cells and small polygonal cells.

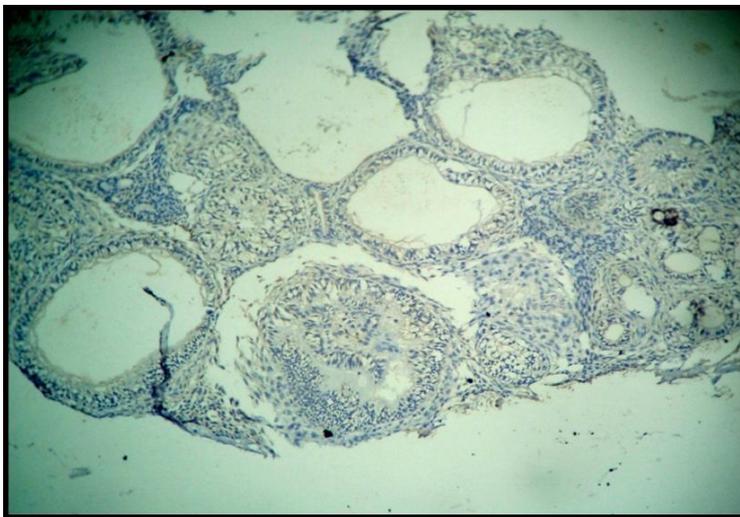


Fig-6: Adenomatoid Odontogenic Tumour: Negative calretinin immunoreactivity in columnar tumour cells and small polygonal cells between the duct-like structure

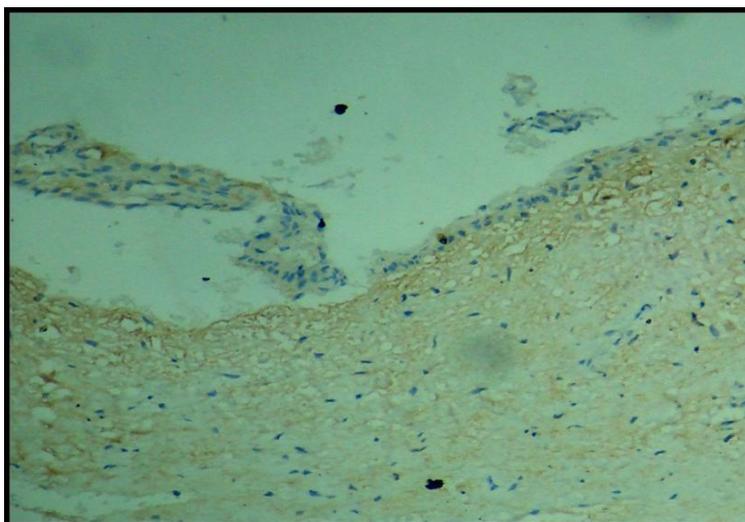


Fig-7: Dentigerous Cyst: Negative calretinin immunoreactivity in epithelial cells of the lining

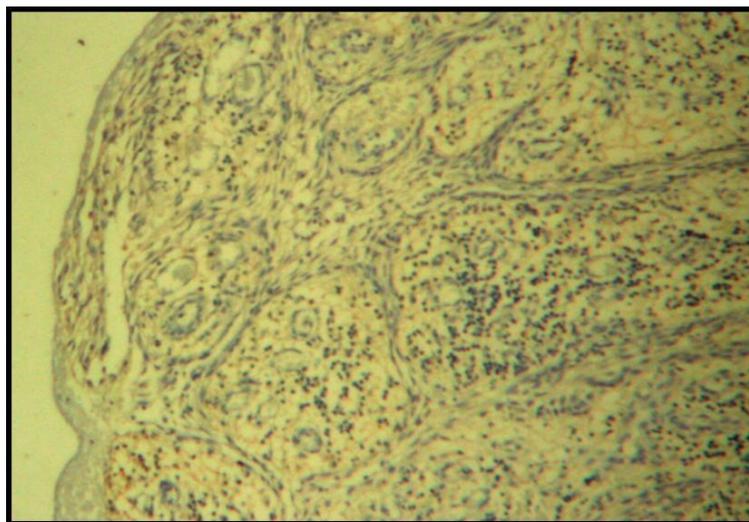


Fig-8: Radicular Cyst: Negative calretinin immunoreactivity in epithelial cells of the lining.

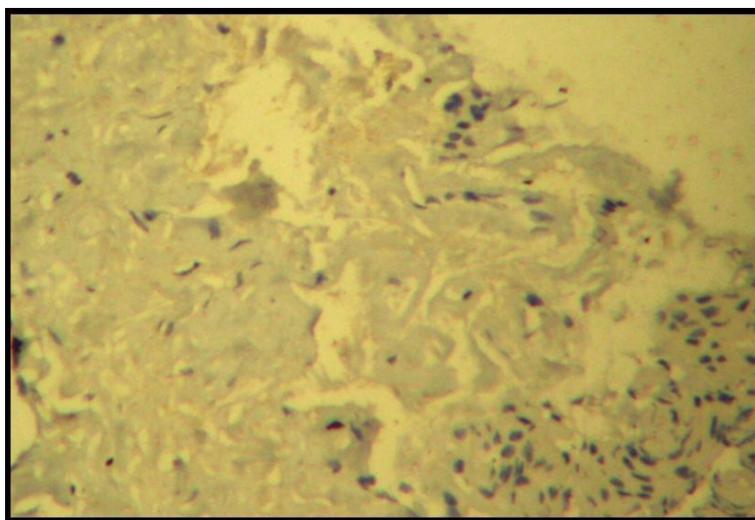


Fig-9: Dental Follicle: Negative calretinin immunoreactivity in epithelial cells.

DISCUSSION

Intracellular calcium ions are considered to be important second messengers intervening in several cellular processes, including proliferation and differentiation [13]. Calcium-binding proteins (CaBPs) act as mediators for their signal. Calretinin is a CaBP of the EF-hand family and is expressed abundantly in central and peripheral neural tissues, particularly in the retina and in neurons of the sensory pathways [1]. Investigation of the function of this protein at the neuronal level has shown that it may intervene as a buffer to absorb an abnormal intracellular increase of calcium ions [3]. This protein has recently emerged as an immunohistochemical marker with great utility for differential diagnosis in specific areas of pathology. Calretinin expression has also been investigated in many normal human tissues and other human neoplasms, but its potential role as a specific immunohistochemical marker of these tissues has yet to be fully elucidated. This protein is considered to be a definitive marker for mesotheliomas and has also been

demonstrated in some carcinomas and adenocarcinomas of the lung, breast, pancreas and ovary [5,14].

Studies in rats have demonstrated calretinin expression in neural elements of the tooth pulp, periodontal ligament and viscerosensory nerve fibres of oral and pharyngeal tissues, as well as in odontogenic epithelium during odontogenesis in molar tooth germs [11,15]. It has been shown that there is only partial correlation between immunoreactivity of normal cells and their neoplastic counterparts [5]. Therefore, further assessment of molecular aspects and immunohistochemical markers such as calretinin in normal and neoplastic tissues may provide a better understanding of the biological behaviour, influencing factors and tumorigenesis of neoplasms including OTs.

In the present study, calretinin expression was observed in all FABs, UABs and CCGCOTs. This was similar to the findings of Altini *et al.* [9] and Alaeddini *et al.* [17], which showed 29 of the 31 studied

ameloblastomas to be positive for this protein and all the 20 ameloblastomas positive for calretinin respectively. Furthermore, immunopositivity was seen almost exclusively in the stellate reticulum like cells of the studied cases, which was also in accordance with the findings of Altini *et al.* [9], Coleman *et al.* [16] and Alaeddini *et al.* [17]. To the best of our knowledge, for the first time immunohistochemical study for the expression of calretinin was done with CCGCOT. The positive expression of protein was seen with stellate reticulum like cells and ghost cells.

Mistry *et al.* studied calretinin in developing rat molars and have demonstrated that it is weakly expressed in some tooth germs at the early cap stage. As tooth development progresses the intensity of reactivity increases from weak to intense in the late bell stages. Calretinin immunoreactivity is present in the inner enamel epithelium and presecretory ameloblasts from the late cap stage onwards. In the cap and late cap stages, many of the specimens were immunopositive for calretinin in the stellate reticulum, and this number increased to more than 90% for the early and late bell stages [11]. The pattern of reactivity of the stellate reticulum observed in the present study appeared similar to the late bell stage of normal tooth development. However, calretinin immunoreactivity was not observed in the peripheral layers of the ameloblastic islands. The enamel organ has been proposed as one of the possible origins of ameloblastoma [18].

It has been previously demonstrated that calretinin may act as an antiapoptotic factor [4]. Gotzos *et al.* [19] have shown increased expression of calretinin during the G1 phase of the cell cycle in WiDr cells. When treated with oligonucleotides against calretinin synthesis, these cells were blocked in the G1 phase and subsequently underwent apoptosis. Various investigations regarding the expression of apoptotic proteins in ameloblastoma exist in the literature, which indicate two relatively distinct patterns for ameloblastoma: an anti-apoptotic proliferating area in the outer layer (periphery) and a pro-apoptotic differentiating region in the inner layer (centre) [20]. The origin of AOT remains uncertain. This has posed problems in the classification of this tumour. AOT was included in the spectrum of mixed OTs in the 1992 WHO classification [18], but was considered as an epithelial OT by other investigators [12,18]. It has been suggested that this lesion may arise from post secretory amnioblasts subsequent to amylogenesis; however, the reduced enamel epithelium and the inner enamel epithelium at the pre-amyloplastic stage are possible precursor structures [21]. The AOT specimens in the present study showed no immunoreactivity for calretinin, similar to finding of Alaeddini *et al.* [17] who postulated that either calretinin's role in the development of this tumour is minimal or there is little correlation between the staining of normal pre secretory

/ secretory amnioblasts and AOT. In addition, it is conceivable that the duration of calretinin expression in the process of histodifferentiation is limited and cannot therefore be detected by conventional immunohistochemically methods.

In the current study, neither the epithelial nor the mesenchymal components of KCOT, RCs and DCs showed immunoreexpression of calretinin, which is in accordance with Eltanin *et al.* [16]. In the present study the dental follicular tissues from the impacted third molar area were included as islands of odontogenic epithelium have a potential to transform into the cyst or the tumour. But these islands were also found to be negative for calretinin expression.

A series of genetic and molecular alterations appears to promote the development and progression of tumors via multiple steps [23, 24]. Although the etiology and pathogenesis of OTs remain unknown, recent studies have identified various molecular alterations responsible for their development and progression [25]. Calretinin has been investigated in a number of no odontogenic neoplasms, but there is no general agreement on the biochemical role of this protein, especially in OTs. Ameloblastoma is characterized by locally invasive behavior with a high risk of recurrence [26]. The reason for this aggressiveness has not been determined. On the other hand, most OTs originates from the successional and accessional dental laminae, but differentiates into various entities. The mechanisms that trigger the proliferation of odontogenic epithelial rests to produce ameloblastomas, AOTs, cystic CCGCOTs, KCOT or other tumors are unknown. Various subcellular, cellular and developmentally related factors may be responsible for this differentiation [4]. Among the neoplasms studied in the present investigation, calretinin expression was observed in ABs and CCGCOTs.

This protein may have a role in the transition of the dental lamina remnants to ABs and CCGCOTs. It might also influence the difference in behaviour observed between these neoplasm and other OCs and OTs. One of the problems in the current study was the lack due to their rarity, of a sufficient number of cystic CCGCOT. Further evaluation of calretinin expression in this tumour with large sample size and their comparison with ABs may provide additional information on the behaviour and tumorigenesis of odontogenic tumours. A previous investigation has compared calretinin expression between UAB and other OCs such as RCs, DCs and KCOTs, and has reported selective expression of this marker by ameloblastic tissues and lack of immunoreactivity in other cysts. It should be noted that the dynamics of molecular and genetic interplay cannot be reflected in their entirety by immunohistochemistry at any particular point in time. Therefore, ultrastructural studies or more sophisticated methods such as cytogenetics and molecular analysis may be needed to

clarify the histogenesis and behaviour of various OTs.

In conclusion, comparing the results, of the earlier studies and our study, we can conclude that calretinin may be used as a differentiating stain in case of UAB to differentiate it from other OCs, especially the DCs. As expression of calretinin was negative in DC, the hypothesis that UAB arising from DC is kind of negated which is in favor of Shear's view. To the best of our knowledge, for the first time positive expression of calretinin was demonstrated in 2 cases of CCGCOTs. Thus calretinin may be used as a marker for CCGCOT as the calretinin expression was positive in stellate reticulum like cells and ghost cells. Result of our study indicates that calretinin may emerge as an important diagnostic aid in the differential diagnosis of cystic odontogenic lesions and odontogenic tumours. Certainly this marker may have some role in the pathogenesis and behavior of various OTs.

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