

The Diagnostic Usefulness of Immunohistochemistry for Odontogenic Lesions

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Abstract The diagnosis of odontogenic tumors can be challenging, largely due to their rarity and consequent difficulties in gaining experience in their assessment. In most cases, careful attention to morphology, in conjunction with clinical and radiological features will allow a diagnosis to be made. However, in some cases, immunohistochemical analysis of the tumor may be useful. In this review we will outline the immunohistochemical expression profile of normal developing odontogenic tissues and a range of odontogenic tumors. In many cases the immunohistochemical markers are neither specific nor sensitive enough to be of help in diagnosis, but in some cases such analysis may prove very useful. Thus we have outlined a limited number of circumstances where immunohistochemistry may be of use to the practicing diagnostic pathologist.

Keywords Odontogenic tumors · Immunohistochemistry · Ameloblastoma · Diagnosis

Introduction

Given their accepted rarity, investigation of the immunohistochemical profile of odontogenic tumors has lagged behind those of other tumor types. However, in recent years the number of publications reporting immunostaining in a range of lesions of odontogenic origin has increased. Some of these follow the increase in our understanding of

the immunophenotype of the developing tooth germ, while others are wider applications of markers, which are well understood in a number of other epithelial or mesenchymal tumors. However, most of these publications have addressed issues of pathogenesis and very few markers have been found to be useful for the diagnostic pathologist. Interestingly, in the current (2005) WHO classification, there is no mention of a useful immunophenotype for almost all of the odontogenic tumors [1].

In this review, we will outline circumstances where immunohistochemistry (IHC) may be useful in the day-to-day practice of the diagnostic pathologist. This by no means minimises the interest in, or potential usefulness of the large numbers of molecular markers whose expression has been investigated, for which clinical utility has not been established. It is undoubtedly true that for almost all odontogenic tumors there are characteristic histological features, and the diagnosis can be made with careful attention to morphology, in conjunction with radiology and other clinical features. Nevertheless, there are some problematic areas: cystic lesions, small biopsies, and the identification of malignant change for which IHC may offer some help.

In order to contextualise the use of IHC in these tumors, we will start by briefly revising the pattern of protein expression in the developing tooth germ and in the dental lamina rests from which many of these tumors arise, before dealing with each of the main tumor types in turn.

Immunohistochemical Profile of Normal Odontogenic Tissues

Tooth development is a complicated, highly coordinated process with a number of sequential morphological stages.

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These stages demonstrate variable molecular profiles that may overlap. The temporal changes in the profile of cytokeratins have been investigated throughout odontogenesis. The odontogenic epithelium expresses keratins 7, 13, 14, and 19. In particular, CK14 stains odontogenic epithelium in all stages of tooth development, including the dental lamina and stellate reticulum, while CK19 is more prominent in later stages [2, 3]. Many other molecular markers have been demonstrated during odontogenesis, particularly in relation enamel proteins and to transcription factors, which control the order and morphology of teeth [4, 5]. However, no suggestion of a clinical use in diagnosis has been explored.

The remnants of odontogenic epithelium (rest cells of Malassez and cell rests of Serres) show similar immunophenotypes [6, 7]. In dogs, calretinin has been shown to be expressed solely in odontogenic epithelium, and thus may be a useful marker to distinguish odontogenic from non-odontogenic epithelium [8]. Such investigations have not been reported in human tissues.

Hamartomas/Odontoma

There is rarely any difficulty in diagnosing either complex or compound odontoma. The small number of studies in the literature suggest that the pattern of expression of a number of molecular markers is very similar to that seen in normal tooth development [2, 9].

One area of diagnostic difficulty is the so-called odontogenic gingival epithelial hamartoma (OGEH), which has a differential diagnosis of peripheral ameloblastoma. The case series addressing these lesions are small, and it is not yet clear if these lesions are truly hamartomas or if they are, as some suggest, an earlier form of odontogenic tumor [10]. Molecular markers that may allow these distinctions to be made would be most welcome, but as yet, none have been suggested.

Neoplastic Odontogenic Epithelium

Ameloblastoma (and Tumors with Ameloblastoma-Like Tissue)

A number of investigators have reported patterns of cytokeratin expression in ameloblastoma similar to normal odontogenic tissues. In general, ameloblastomas express cytokeratins 5/6, 13, 14 and 19, although expression may vary in some subtypes (Fig. 1a–c) [2, 11, 12]. In solid/multicystic ameloblastomas and unicystic ameloblastomas, CK13 is preferentially expressed in the stellate reticulum-like cells, CK14 in peripheral cells and CK19 in all cells,

including areas of acanthomatous or granular cell differentiation [12, 13]. This pattern does not hold for some peripheral ameloblastomas or desmoplastic ameloblastomas [12]. The expression of CK13 in stellate reticulum-like cells is interesting as CK13 is not expressed in the stellate reticulum of the developing tooth germ [2].

Variations in proliferation rate in the various ameloblastoma subtypes have been reported, with both peripheral and desmoplastic ameloblastoma showing lower Ki-67 labelling indices [13–16]. Overall, however, all ameloblastomas have been shown to have a very low proliferative index (Fig. 1f, reviewed in Gomes et al. [17]), and it is possible that proliferation markers may be useful to help diagnose malignant ameloblastomas, especially in small biopsies [18]. However, this issue requires further exploration to determine its clinical usefulness.

In conjunction with CK13, CK14, and CK19, CD56 (expressed in peripheral cells) and calretinin (expressed in stellate reticulum-like cells), may be of use in small biopsies or biopsies of cystic lesions. CD56 (or NCAM) is expressed in the peripheral cells of the tumor islands in all types of ameloblastoma (Fig. 1e) [19, 20], while calretinin is reciprocally expressed in the stellate reticulum-like cells [21, 22], including in most unicystic ameloblastoma (although less frequent than in solid ameloblastoma [21, 23]). However, both markers are expressed, to a much lesser extent, in odontogenic keratocyst/KCOT, thus using markers in isolation to distinguish a cystic ameloblastoma from odontogenic keratocyst (OKC)/keratocystic odontogenic tumor (KCOT) may still result in diagnostic uncertainty [20, 24].

Odontogenic Keratocyst (OKC)/Keratocystic Odontogenic Tumor (KCOT)

There is little evidence that IHC is helpful in the diagnosis of OKC. The lesion has very typical and almost pathognomonic features, which make diagnosis based on histological examination quite straightforward. Although there have been many publications reporting the immunophenotype of OKC, these have all been directed at elucidating the pathogenesis or in an attempt to determine the putative neoplastic nature of the lesion. The profile of cytokeratin expression in OKC is similar to that found in other odontogenic cysts (high molecular weight CKs and CK19 are common) [25], although in addition, OKC will express CK1 and CK10, which are markers of cornification. However, these are not needed for diagnostic purposes since this is evident on H&E staining. An area of diagnostic difficulty is in distinguishing inflamed OKC from other cyst types, especially in small biopsies, but in these cases the keratinisation pattern is lost and the keratin profile is of no value.

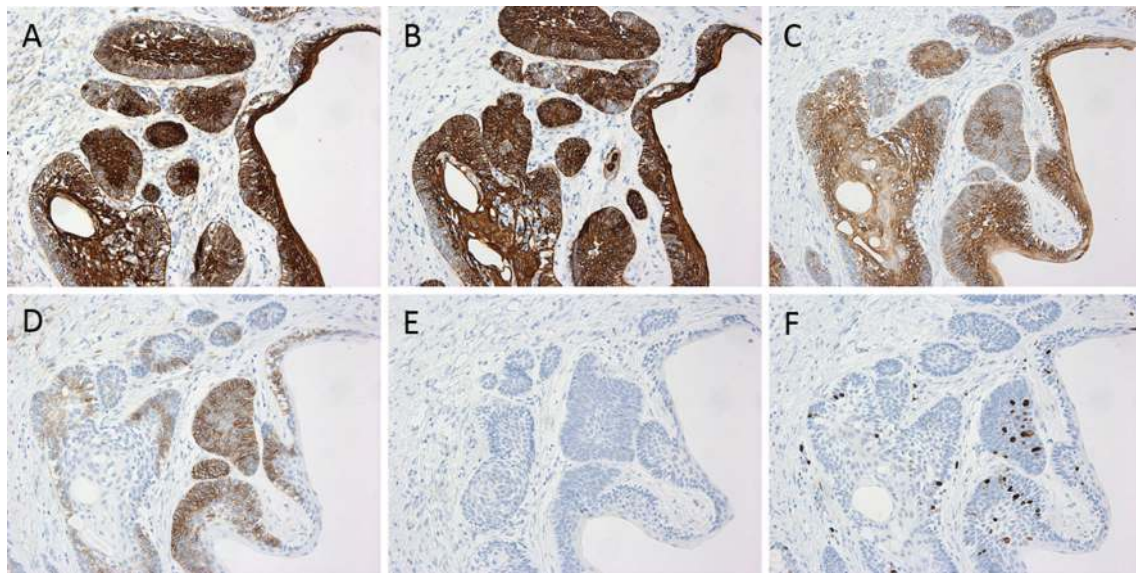


Fig. 1 Photomicrographs of immunohistochemical staining of a solid/multicystic ameloblastoma, showing expression of CK5/6 (a), CK14 (b), CK19 (c), CD56 (d), calretinin (e), and Ki-67 (f), showing

the variability of staining seen even within a small area in one tumor. Total magnification $\times 200$

Proliferation markers, most often Ki-67, have often been used to demonstrate a higher proliferation rate in OKC compared to other cyst types. OKC show increased mitoses and Ki-67 expression compared to other cyst types with expression above the basal layers being characteristic. This finding and evidence of PTCH gene expression has been widely used as evidence of a neoplastic origin for this lesion [26]. Discussion of this issue is not a subject of this review, but suggestions that reduced PTCH protein expression may be a marker for OKC, may provide evidence of neoplastic origin, or may distinguish syndromic from non-syndromic cysts have not been borne out. Immunohistochemical studies have shown that PTCH protein expression is similar in OKC, cystic ameloblastomas and other types of odontogenic cysts [27]. There is some evidence emerging that anti-apoptotic markers, including bcl-2 and BAX, may be specifically increased in OKC, but this needs confirmation and its diagnostic value has not been considered [27, 28].

Calcifying Epithelial Odontogenic Tumor (CEOT)

Detailed immunohistochemical studies of CEOT are not plentiful in the literature. The small number of studies show expression of CK14 and CK19 (variable) by the epithelial cells [2, 29]. Other investigations have demonstrated a CEOT gene expression signature, the usefulness of which has not been tested in a large cohort [30]. One potentially diagnostically useful feature is the presence of amyloid-like material in many tumors, which may become calcified. Morphology and histochemical stains (Congo red

or Thioflavin-T) are currently more likely to be of help than IHC in its assessment. However, recent investigation of a number of odontogenic epithelia associated proteins, such as odontogenic ameloblast-associated protein (ODAM), may yet yield useful biomarkers [31].

Recently a small series of CEOT was compared with a number of cases of dental follicle, which contained CEOT-like proliferations [29]. Classic CEOT showed a higher Ki-67 index and expression of mini chromosome maintenance (MCM) proteins, but this will require further investigation before its usefulness is established.

Adenomatoid Odontogenic Tumor (AOT)

The immunophenotype of AOT is not clear with contradictory results. In small case series, Angiero et al. showed expression of CK5/6, CK17, and CK19 while others only showed CK14 expression [2, 32]. The one large case series, which presented a cohort with extensive overlap with CEOT (36 of 39 showed CEOT-like areas), demonstrated expression of CK5, CK14, and CK19 [33]. Interestingly, a number also expressed CK7, although this was not in the duct-like areas. No expression of calretinin has been shown and a number of studies have pointed out that expression of Ki-67 is very low or absent [32, 34, 35]. However, there are few diagnostic difficulties presented by AOT and on the whole, IHC does not seem to be helpful. The overlap between AOT and CEOT seen in a minority of cases can raise problems, but no immunohistochemical marker has been reported which reliably distinguishes those that may be hamartomas from neoplasms. Similar diagnostic

difficulties present in differentiating AOT from adenoid ameloblastoma with dentinoid in small biopsies [36] and immunohistochemical markers to distinguish these entities would be welcome.

Squamous Odontogenic Tumor (SOT)

The literature related to the SOT is largely that of individual case reports, and although these were collated in a comprehensive review, the immunophenotype is not well established [37].

Lesions Containing Ghost Cells

The main lesions that contain ghost cells include the calcifying cystic odontogenic tumor (CCOT) and solid variants (dentinogenic ghost cell tumor, DGCT), however, ghost cells can be present focally in a wide range of other odontogenic tumors. In CCOT, cytokeratins 14 and 19 have been identified in the epithelial component, with CK6 expression in both the epithelium and ghost cells [38]. The proliferation fraction, as assessed by Ki-67, is very similar to ameloblastoma, particularly in those lesions where the lining is obviously ameloblastoma-like [39, 40]. The solid lesions show a similar immunophenotype [41].

Neoplastic Odontogenic Mesenchyme (With or Without Epithelium)

Ameloblastic Fibroma (AF)

The mesenchymal component of the ameloblastic fibroma resembles primitive dental pulp, and rarely will require immunohistochemical analysis. Occasional mesenchymal cells express S100, with juxta-epithelial GFAP expression in ameloblastic fibrodentinoma associated with induction of hard tissue formation [42]. Other studies have examined various odontogenic tumors including AF; however, because of the small number of cases examined, it is not clear how representative these results are for AF as a whole.

Odontogenic Fibroma (OF)

One study of reasonable size is present in the literature, presenting the immunophenotype of 14 OFs, almost all of which were epithelium-rich (formerly WHO-type). These authors confirmed that the epithelial component had a very similar pattern of cytokeratin expression as seen in other odontogenic epithelia, namely expressing AE1/AE3, CK5, CK14, CK19, and 34 β E12 and negative for CK1 and CK18. There were two cases weakly positive for CK7 and

CK8 [43]. The mesenchyme expressed vimentin and occasional cells expressed SMA. The main diagnostic issue in OF is that of the epithelium-poor type, where IHC may be used to find or confirm the presence of epithelium, to differentiate the lesion from desmoplastic fibroma or fibromyxoma. However, careful attention to the morphological features with appropriate radiology is far more useful.

Odontogenic Myxoma

The odontogenic myxoma may present diagnostic difficulties due to morphological overlap with a number of non-odontogenic lesions, from which it should be distinguished. The mesenchymal component contains spindle-shaped cells most of which express vimentin (Fig. 2a, b), with a sub population in some tumors expressing actins [44]. An epithelial component is unusual, but when present expressed CK14, and showed a very low proliferation fraction. There is disagreement in the literature over expression of CK19 [44, 45]. The main issue in the differential diagnosis of odontogenic myxoma is distinction from myxoid variants of other tumors, including myxoid neural (e.g. schwannoma, neurofibroma), myofibroblastic (myxoid nodular fasciitis), muscle (myxoid rhabdomyosarcoma) and lipomatous tumors (myxolipoma), amongst others [46]. Occasionally there is need for distinction from myxoid change in an enlarged dental follicle. In this regard, an odontogenic myxoma should ideally not express S100 or CD34 (except in vasculature) or any of the muscle markers, or CD68. However, S100 expression has been reported, a finding which may compound difficulties in distinction from myxoid peripheral nerve sheath tumors [45]. Furthermore, it has been suggested that the identification of S100 expressing small nerve fibres may indicate an enlarged dental follicle rather than an odontogenic myxoma [45]. In many cases, a clear origin within the alveolus is very helpful, but IHC may be warranted in more difficult sites such as the posterior maxilla, where it is more difficult to be certain of an origin within the tooth-bearing portion of the jaws.

Malignant Odontogenic Tumors

An area of particular difficulty in the diagnosis of odontogenic tumors is that of malignancy, particularly when it arises in a pre-existing benign lesion. A number of investigators have shown that the cytokeratin profile characteristic of ameloblastoma (e.g. CK14 and CK19 expression) is largely similar in ameloblastic carcinoma and has no diagnostic utility (Fig. 3a–e) [16]. Many other reports centre on the proliferation fraction as a means of

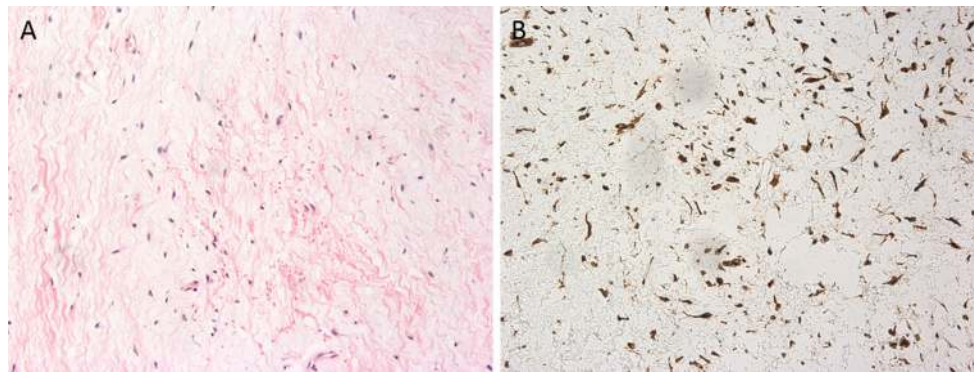


Fig. 2 H&E stained section of odontogenic myxoma (a) with immunohistochemical staining for vimentin expression in the same tumour (b). Total magnification $\times 200$

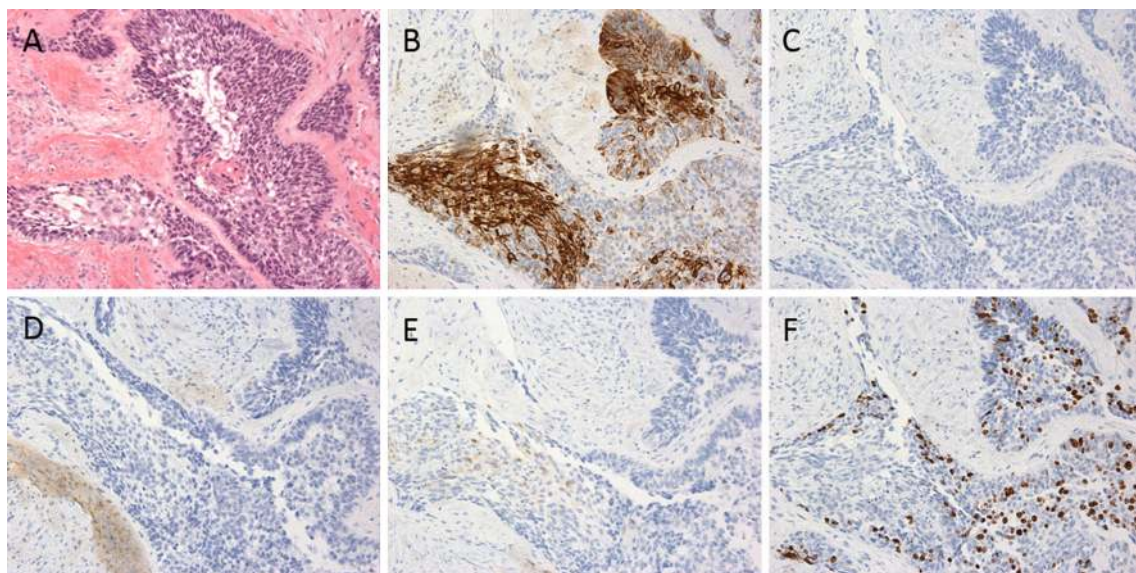


Fig. 3 An H&E stained section (a) and immunohistochemical staining of a case of ameloblastic carcinoma, showing expression of CK14 (b), CK19 (c), CD56 (d), calretinin (e), and Ki-67 (f), showing the variability of staining seen even within a small area in one tumor.

CK19 and CD56 expression have been lost with calretinin only staining a few cells in the centre of the tumor nests. Total magnification $\times 200$

distinguishing these two entities. However, while all show a higher Ki-67 proliferation fraction in ameloblastic carcinoma than in ameloblastoma, the reported rates vary from 2.9–14.9 % in ameloblastoma and 8–48.7 % in ameloblastic carcinoma (compare Figs. 1f, 3f) [14–16]. This means that while a focal area of increased Ki-67 expression in a given tumor may suggest progression to malignancy, it is not possible to give even an estimate of a cut-off percentage to help make a diagnosis of ameloblastic carcinoma, except if the fraction is uniformly very high (e.g. over 20 %). Thus, morphological features including the cytological features of malignancy and a prominent infiltrative and destructive growth pattern are likely to be more useful [16]. However, a number of newer immunohistochemical markers have been suggested, such as

nuclear SOX2 expression [47], and these may prove useful markers to identify malignant ameloblastic tumours.

The presence of clear cells can also create diagnostic uncertainty, given the extensive differential diagnosis such a finding can raise. However, in many cases careful attention to morphology will remove most uncertainty. There are occasions when distinction between clear cell odontogenic carcinoma (CCOC) and other carcinomas containing clear cells may require histochemical or immunohistochemical stains. CCOC will demonstrate PAS positive, but mucin negative, material in cells, and often (but not always) express CK19 (Fig. 4a, b) and calretinin [48, 49]. Other IHC which may be useful include markers for clear cell variant of melanoma (S100, Melan A), myoepithelial markers if salivary gland neoplasia is

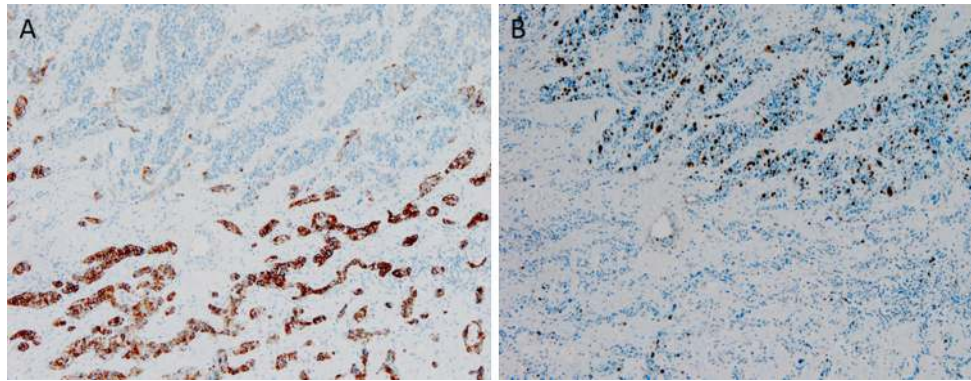


Fig. 4 Photomicrographs of immunohistochemical staining of an illustrative case of clear cell odontogenic carcinoma showing progression to higher grade tumor with loss of CK19 expression

(a) and increase in Ki-67 expression (b). Total magnification $\times 200$. Photomicrographs courtesy of Dr. Bill Barrett, UK

suspected (calponin, SMA, p63), and immuno-histochemical markers for metastases which may contain clear cells, such as renal (RCC, CD10) and prostate (PSA) carcinomas. In some cases, distinction from clear cell salivary tumors arising from minor glands associated with the jaws may not be possible [50]. More recently it has been shown that clear cell odontogenic carcinoma shows EWSR1 gene rearrangements, which are common to many other clear cell carcinomas including salivary [51]. Although this is a molecular finding, IHC for a fusion protein may become available and may allow differentiation of CCOC from clear cell variants of other odontogenic tumors.

Another area where molecular markers have proved useful is in the diagnosis of mucoepidermoid carcinomas (MEC), which have been shown to contain specific MAML2 rearrangements. Intraosseous MECs are probably of odontogenic origin and must be differentiated from glandular odontogenic cysts (GOC). GOC lack the MAML2 rearrangement and FISH can be used to confirm the diagnosis [52]. Antibodies to MAML2 fusion proteins are available and IHC may be useful for the differential diagnosis of MEC from GOC, but this has not yet been reported.

Summary: The Use of IHC in Diagnosis of Odontogenic Lesions (a Practical Guide)

From the above review of the literature it can be seen that the number of diagnostically useful IHC markers that have been demonstrated in odontogenic tumors is very small, and in most cases clinical history, radiology, and careful attention to morphology is sufficient to establish a diagnosis. It is also worth bearing in mind that most of the immunophenotypes described here are lost in areas of intense inflammation, and this has to be considered when assessing any pattern of immunohistochemical staining in these lesions. However,

careful application of what is known about the immunophenotype in the key areas of diagnostic uncertainty may be useful in the following circumstances:

- Differential diagnosis of myxoma and clear cell lesions to exclude variants and metastases from elsewhere.
- IHC for fusion proteins may be useful in clear cell lesions and to confirm or exclude an intraosseous MEC.
- A high proliferation rate (perhaps over 20 %) may be a helpful indicator of malignancy in ameloblastomas.
- Pan-cytokeratin markers may be helpful to confirm the presence of odontogenic epithelium in epithelial-poor odontogenic fibromas.
- In cystic lesions, the combination of widespread CD56 expression in peripheral cells and calretinin expression in the superficial cells supports a diagnosis of ameloblastoma over OKC/KCOT.

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