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## Molecular and Genetic Aspects of Odontogenic Lesions

### Abstract

In this article we outline the molecular findings of select odontogenic tumors. In each section, we briefly review selected the clinicoradiographic, histologic, immunologic features, focusing on the molecular findings and their applications in practice. The understanding of molecular pathobiology at various other organ sites has developed quite rapidly in recent years, however much remains unknown about the genetic profile of odontogenic tumors. Improved understanding of mutations in odontogenic tumors may clarify classification schema and elucidate targets for novel therapies. Molecular testing will no doubt improve our understanding of odontogenic tumor pathogenesis and will likely be, someday, an important component of routine clinical practice and its role will only increase in the coming years.

### Keywords

Odontogenic tumors, Molecular pathology, Translocation, Mutation, EWSR1, BRAF, PTCH

### Disciplines

Dentistry

## Molecular and Genetic Aspects of Odontogenic Lesions

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**Abstract** In this article we outline the molecular findings of select odontogenic tumors. In each section, we briefly review selected the clinicoradiographic, histologic, immunologic features, focusing on the molecular findings and their applications in practice. The understanding of molecular pathobiology at various other organ sites has developed quite rapidly in recent years, however much remains unknown about the genetic profile of odontogenic tumors. Improved understanding of mutations in odontogenic tumors may clarify classification schema and elucidate targets for novel therapies. Molecular testing will no doubt improve our understanding of odontogenic tumor pathogenesis and will likely be, someday, an important component of routine clinical practice and its role will only increase in the coming years.

**Keywords** Odontogenic tumors · Molecular pathology · Translocation · Mutation · EWSR1 · BRAF · PTCH

In this article we outline the molecular findings of select odontogenic tumors. Arising within the jaws or tooth-

bearing areas of the oral mucosa, odontogenic tumors comprise a heterogeneous group of lesions encountered with variable frequency and typically in a subspecialist setting. These lesions are derived from epithelial, ectomesenchymal, and/or mesenchymal elements of the tooth forming apparatus [1]. While the understanding of molecular pathobiology at various other organ sites has developed quite rapidly in recent years, much remains unknown about the genetic profile of odontogenic tumors. Improved understanding of mutations in odontogenic tumors may clarify classification schema and elucidate targets for novel therapies. In each section, we briefly review selected the clinicoradiographic, histologic, immunologic features, focusing on the molecular findings and their applications in practice.

### Ameloblastic Fibroma, Ameloblastic Fibro-Odontoma, and Ameloblastic Fibrosarcoma

Clinicoradiographic, Histologic,  
and Immunophenotypic Features

Ameloblastic fibroma (AF), ameloblastic fibro-odontoma (AFO), and ameloblastic fibrosarcoma (AFS) are uncommon, closely related, odontogenic neoplasms of mixed epithelial and ectomesenchymal origin [2]. AF and AFO are benign lesions, while AFS exhibits a malignant mesenchymal component which may arise de novo, or within a pre-existing AF or AFO [3]. Most tumors in all three categories occur in the mandible [3, 4]. AF and AFO tend to develop more frequently in the first or second decade of life while the mean age for AFS is 27.5 years [4]. AF and AFO are typically asymptomatic, or may cause swelling, whereas pain and

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swelling are characteristic of AFS. Radiographically, AF and AFO frequently surround the crown of an unerupted tooth, presenting as a well-defined radiolucent (AF) or mixed radiolucent-radiopaque (AFO) lesion [4]. AFS, on the other hand, often shows a destructive appearance. The histologic hallmark of these mixed lesions is the combination of mesenchymal and epithelial elements. AF and AFO usually show thin, elongated islands of odontogenic epithelium set in a myxoid mesenchymal stroma reminiscent of the primitive dental papilla. AFO also show dental hard tissue formation. In contrast, AFS contains benign epithelial islands scattered within a cellular background of hyperchromatic and pleomorphic spindle cells. Numerous mitotic figures are typically present. Lesions that arise from a pre-existing benign neoplasm will demonstrate features recognizable as AF or AFO [4].

### Molecular Findings

The molecular features of AF, AFS, and AFO are not well studied. A very limited study examining loss of heterozygosity (LOH) at tumor suppressor gene loci on 3p, 9p, 11p, 11q, and 17p of AF, AFO, and AFS revealed a higher mean fraction of allelic loss (FAL) in AFS (74.6 %) compared to AF and AFO combined [5]. Furthermore, the FAL average was higher in AFO (36.6 %) than AF (13.2 %). Overall, for both benign and malignant lesions, LOH of 17p13.1 loci involving the *p53* and *CHRNBI* genes were most frequently encountered (62 % for *p53* and 55 % for *CHRNBI*).

Diffuse positivity for p53 and c-kit also has been reported in the sarcomatous component of an AFS ex AF, although molecular analysis failed to show genetic mutations in the *KIT* gene in exons 9, 11, 13, and 17 [6]. These observations could indicate a possible role of p53 and c-kit in malignant progression of AF to AFS.

While only 2 AF and 1 AFO cases have been tested, early data has shown the *BRAF* V600E mutation present in these tumors [7].

### Applications in Practice

Current molecular findings are of little utility in routine diagnosis. Overexpression of p53, Bcl-2, Ki-67, PCNA and c-kit may be observed in AFS and might be useful in distinguishing between benign and malignant neoplasms. AFS that overexpress c-kit might respond favorably to the tyrosine kinase inhibitor imatinib (Gleevec), although the usefulness of this pharmaceutical agent in the treatment of AFS has yet to be demonstrated [6].

## Ameloblastoma

### Clinicoradiographic, Histologic, and Immunophenotypic Features

The central solid or multicystic ameloblastoma is a slow growing but aggressive benign neoplasm with a high recurrence rate [1]. The mean age of presentation is 36 with an even gender distribution, and a predilection for the posterior mandible. Small tumors may be asymptomatic, while larger lesions may show significant expansion of the jaw and tooth resorption. Radiographically, ameloblastoma may present as a unilocular or multilocular radiolucency, which may surround the crown of an unerupted tooth [1, 4]. While different histologic patterns have been described, tumors typically contain epithelial structures reminiscent of the enamel organ, with tall prominent peripheral cells exhibiting reverse nuclear polarity and central stellate reticulum-like tissue [4]. SOX2, a transcription factor involved in ectodermal development, is involved in the development of odontogenic epithelium [8]. Immunohistochemistry has shown a stronger staining pattern for SOX2 in ameloblastic carcinomas than ameloblastomas [9]. EGFR reactivity is common in ameloblastomas [10]. EGFR overexpression is correlated with SOX2 expression in ameloblastic carcinomas.

### Molecular Findings

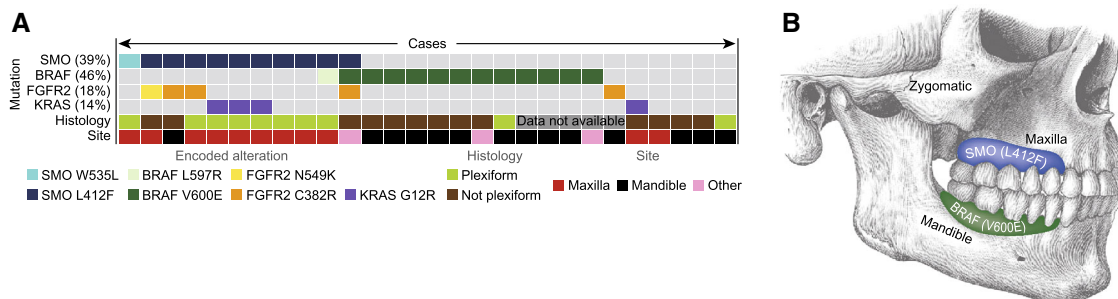
EGFR-targeted therapy has been shown to be effective in diminishing cellular proliferation in ameloblastoma cell cultures. In the presence of *BRAF* V600E mutations, a statistically significant difference was seen in diminished sensitivity to the EGFR tyrosine kinase inhibitors [11]. In one study, 63 % (15 of 24) of mandibular ameloblastomas were found to be positive for a *BRAF* V600E mutation using immunohistochemistry [11]. Another series of 28 cases, including both maxillary and mandibular ameloblastomas, detected somatic mutations in the Hedgehog and mitogen-activated protein kinase (MAPK) pathways. 39 % (11/28) had *SMO* mutations and 46 % (13/28) had *BRAF* mutations (Fig. 1a, b). Interestingly, there was a significant correlation between the mutation and location as well as histologic subtype. *SMO* mutation was present in 9/11 maxillary tumors while only present in 1/13 mandibular cases, as well as 8/10 ameloblastomas with a plexiform pattern [12]. A series of 84 ameloblastomas and 40 various other odontogenic tumors were tested for MAPK pathway mutations. Via polymerase chain reaction (PCR) 62 % (31/50) of ameloblastomas were found to have *BRAF* V600E mutations. A 100 % concordance was found between molecular detection and immunohistochemical analysis of

*BRAF* V600E. 88 % of ameloblastomas were found to have a *BRAF*, *RAS*, or *FGFR2* mutation and these were found to be mutually exclusive [7].

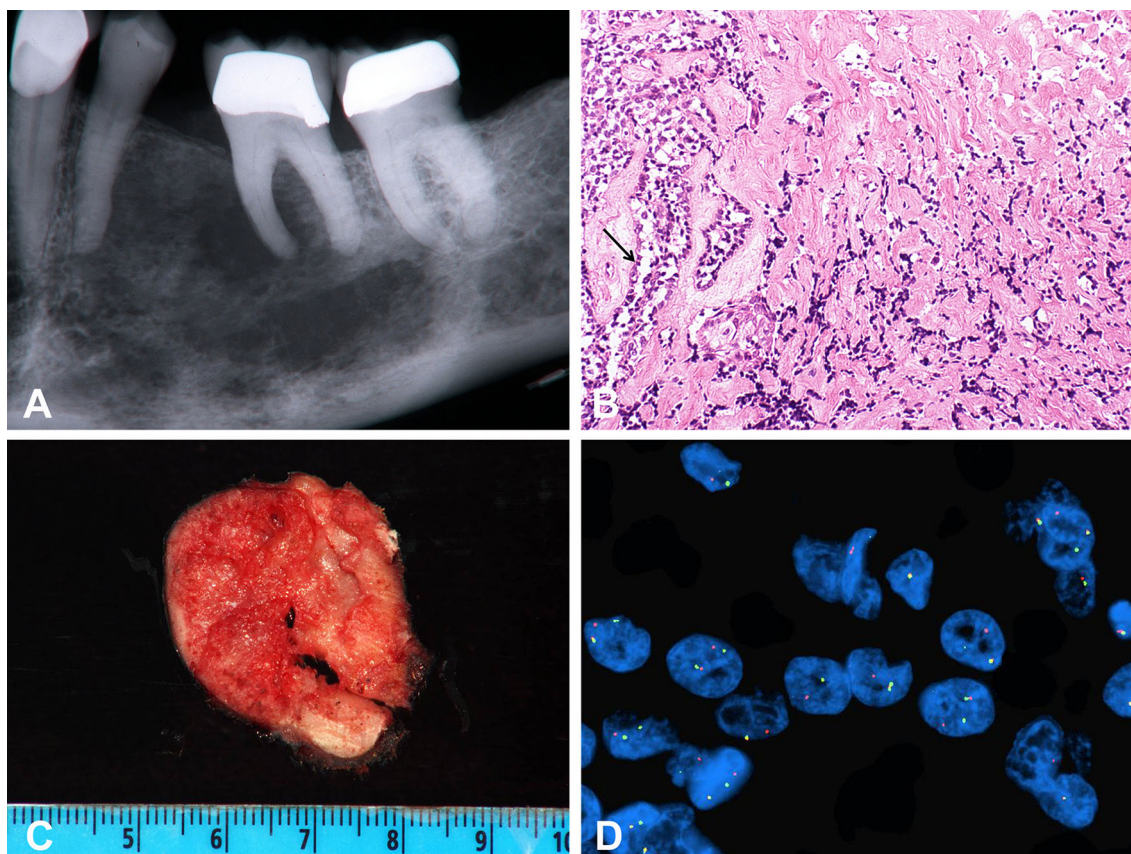
### Applications in Practice

The presence of *BRAF* V600E mutation in a high proportion of mandibular ameloblastomas as well as the

identification of other mutations has the potential to change our perspectives, classification, and even treatment of ameloblastoma. These mutations may correlate with clinical behavior and provide targets for drugs and allow for more personalized therapy to reduce surgical morbidity. Much of the research in the molecular pathology of ameloblastomas is recent, and there is a need for additional studies in this area.



**Fig. 1** Reprinted by permission from Macmillan Publishers Ltd: Nature Genetics, Sweeney et al. [12]. **a** Ameloblastoma mutation status for four genes correlated with histology and site. **b** Graphical representation of ameloblastoma distribution and tumor mutation



**Fig. 2** Clear cell odontogenic carcinoma. **a** Aggressive tumor presenting as an ill-defined radiolucency of the mandible, **b** cross section of fresh resection specimen, **c** H&E,  $\times 20$ ; cords and nests of cell in a hyalinized stroma. Focal peripheral palisading is seen

(arrow). **d** Fluorescence in situ hybridization dual-color break apart probe indicated the presence of *EWSR1* (22q12) gene rearrangement in clear cell odontogenic carcinoma by the split red and green color signal



## Clear Cell Odontogenic Carcinoma

### Clinicoradiographic, Histologic, and Immunophenotypic Features

Clear cell odontogenic carcinoma (CCOC) is a locally aggressive low grade malignancy found in the gnathic region, presenting as an ill-defined lucency of the jaw, most commonly the mandible with a slight female predilection (Fig. 2a, b) [13]. Clinically, about one-third of tumors recur locally and metastases to distant sites, most commonly the lungs, have been reported. The tumor morphologically resembles salivary (hyalinizing) clear cell carcinoma (CCC) and is comprised of sheets, cords, or nests of polygonal cells separated by a hyalinized to fibrous stroma. Clear cells, which may predominate, are PAS positive and diastase sensitive, demonstrating the presence of intracytoplasmic glycogen [14]. A vague histologic feature generally seen in CCOCs but not CCC of salivary gland origin is peripheral palisading, though only 58.8 % CCOCs exhibit this feature (Fig. 2c) [13].

CCOCs are classically considered to be of odontogenic origin. As with many odontogenic lesions, CCOC are phenotypically squamous and like CCC, express high molecular weight keratins and p63. They may also show focal keratin pearl formation, and ultrastructurally demonstrate tonofilaments [15].

### Molecular Findings

In addition to sharing morphologic and immunophenotypic features with salivary CCC, CCOC also appear to share *EWSR1-ATF1* translocation, which may imply that CCOC is better considered a 'central' CCC. In one study, 83.3 % of CCOCs harbor a *EWSR1* rearrangement (Fig. 2d) and one case was tested and positive for the *ATF1* rearrangement [16]. *EWSR1-ATF1* has also been reported subsequently in a CCOC case report [17]. To date, specific clear cell tumors found to be negative for this translocation include other primary clear cell salivary, odontogenic, and squamous neoplasms [16].

### Applications in Practice

*EWSR1* testing is readily available using breakapart fluorescence in situ hybridization (FISH) methodology used for most types of *EWSR1* rearrangements. If nucleic acid of sufficient quality for RT-PCR testing can be obtained from a specimen, assessment for *EWSR1-ATF1* fusion transcript is plausible as well. As noted above, the presence of this translocation can distinguish CCOC from other clear cell lesions. Specific clear cell tumors tested to date and

negative for this translocation include: clear cell calcifying epithelial odontogenic tumor, clear cell squamous carcinoma, epithelial–myoepithelial carcinoma, sinonasal renal cell-like adenocarcinoma, and clear cell mucoepidermoid carcinoma.

## Glandular Odontogenic Cyst Versus Central Mucoepidermoid Carcinoma

### Clinicoradiographic, Histologic, and Immunophenotypic Features

The term glandular odontogenic cyst (GOC) was coined by Gardner in 1988 [18], although the lesion was described initially by Padayachee and Van Wyk as a sialo-odontogenic cyst [19]. Radiologically they present as either unilocular or multilocular well defined lesions, 80 % of which arise in the mandible, usually anteriorly [20]. They are somewhat aggressive lesions, often causing cortical perforation and less commonly root displacement or resorption. They occur across a wide age range with the mean age at presentation in the 5th decade [20]. Recurrence rates range from 30 to 50 % [20, 21] with larger, multicystic lesions treated by curettage having higher risk for recurrence [22]. Five major histologic criteria that have been proposed for GOCs are: (1) a squamous lining with a flat interface to the adjacent tissue, (2) plaque-like thickenings with whorled epithelium (3) cuboidal eosinophilic hobnail cells (4) mucous or goblet cells and (5) duct-like structures. Other features include papillae, ciliated cells, multicystic architecture, and clear cells in the basal or spinous layer [20, 23]. The histologic differential diagnosis of GOC includes a dentigerous cyst with mucous prosoplasia, and central mucoepidermoid carcinoma. In contrast to GOC and cMEC, the lining of dentigerous cysts is usually overall thinner without whorled plaques. While mucous prosoplasia and cilia may be present, they are not prominent.

Central mucoepidermoid carcinomas (cMECs) share a number of histologic features with GOCs. They can also have a squamous (epidermoid) component, cystic areas, and intracellular mucin making distinction from GOC challenging on small biopsies. These tend to be more architecturally complex with pericyclic sclerosis in comparison to cMEC. Although cMECs represent only 2–3 % of mucoepidermoid carcinomas they are the most common intra-osseous salivary type malignancy. It is unclear whether they arise from heterotopic salivary rests or represent salivary analogues of odontogenic origin [24, 25]. Interestingly, earlier literature cited an association with odontogenic cysts [26], complicating the diagnosis.

## Molecular Findings

For the diagnosis of more challenging MEC cases, FISH may be performed to identify the t(11;19)(q21;p13) translocation, which results in a *CRTC1-MAML2* fusion. *CRTC1* activates CREB mediated transcription, whereas *MAML2* is involved in Notch signaling pathways. Two recent series have shown the absence of the *CRTC1-MAML2* translocation in all GOCs analyzed (n = 9 and n = 21, respectively) [27, 28]. Interestingly, there has been a claim that half of dentigerous cysts with mucous cell prosoplasia showed a subpopulation of *MAML2* rearranged cells [29]. However, this fraction is so low that most laboratories would consider it negative, and this study did not validate this finding by another methodology.

## Applications in Practice

In clinical practice, FISH for *CRTC1-MAML2* can be useful in assisting in the discrimination of a cMEC and an architecturally complex GOC or a small biopsy sample of GOC. Future studies are needed to substantiate the findings of *MAML2* rearrangement in odontogenic cysts with mucous prosoplasia.

## Keratocystic Odontogenic Tumor

### Clinicoradiographic, Histologic, and Immunophenotypic Features

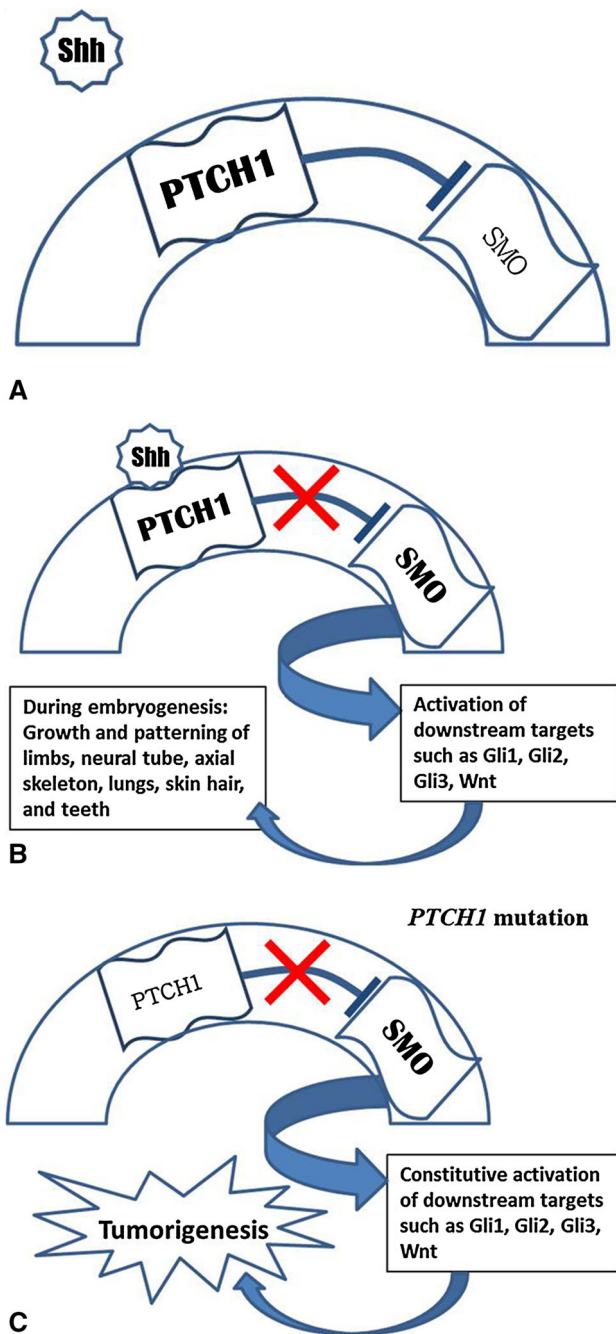
Keratocystic odontogenic tumor (KCOT) is a relatively common cystic neoplasm of the jaws which accounts for 3–11 % of all gnathic cystic lesions derived from remnants of the tooth-forming apparatus [4]. Also known as odontogenic keratocyst, it was renamed KCOT by the World Health Organization in 2005 to better account for its clonal nature and high recurrence rate [1]. KCOT favors the posterior jaws, with the mandibular posterior body and ascending ramus accounting for almost half of the cases [4, 30]. Any age group can be affected, although most sporadic KCOTs occur in young to middle-aged adults, and there is a slight male predilection [30]. Clinically, a large KCOT may cause bony expansion and result in obvious facial asymmetry, but many lesions are clinically imperceptible, owing either to the size of the lesion or to a tendency of KCOT to grow in an antero-posterior direction without altering the lateral dimension of the bone [30]. On panoramic radiograph, KCOT presents as a well-defined, frequently corticated, uni- or multilocular radiolucency. Up to 40 % of lesions develop around the crown of an impacted tooth [4]. Histologically, KCOT consists of a cystic structure lined by parakeratinized stratified

squamous epithelium. The epithelial lining is of uniform thickness, typically 6–8 cell layers thick, with prominent, hyperchromatic and palisaded basal cells [4]. The keratin layer is frequently corrugated or ‘wavy,’ and keratin scales may be found within the cyst lumen. Satellite (or daughter) cysts are commonly encountered in the adjacent connective tissue wall. This, in addition to easy fragmentation of the cyst lining during surgical manipulation, may account for KCOT’s high recurrence rate [31, 32].

KCOT most commonly occurs as a sporadic lesion of the jaws, although there is a well-known association of KCOT with the nevoid basal cell carcinoma syndrome (NBCCS) [33]. NBCCS, also known as Gorlin syndrome, Gorlin–Goltz syndrome, and basal cell nevus syndrome, is an autosomal dominant disorder characterized by multiple basal cell carcinomas (BCCs), skeletal anomalies of the ribs and vertebrae, calcification of the falx cerebri, palmar and plantar pits, and an increased incidence of tumors, particularly childhood medulloblastoma (primitive neuroectodermal tumor) and ovarian and cardiac fibromas [33]. The presence of multiple jaw keratocysts are a major diagnostic criterion for the syndrome and are found in approximately 90 % of affected individuals. In this patient population, KCOTs typically develop at a younger age than in non-syndromic cases [4]. NBCCS-associated KCOTs exhibit the same characteristic microscopic features as those observed in sporadic cases, although satellite cysts and intramural remnants are more frequently seen in syndrome-associated lesions [4].

## Molecular Findings

Mutations in the *PTCH1* gene, mapped to 9q22.3–q31, are known to cause NBCCS and related tumors, including KCOTs [34]. *PTCH1* is the human homologue of the *Drosophila* fly *patched* gene [34]. *PTCH1* encodes for a transmembrane glycoprotein that acts as a receptor in the hedgehog signaling pathway. In vertebrates, three hedgehog genes have been identified, *Sonic (Shh)*, *Desert*, and *Indian* hedgehog [35]. Of these, *Shh* has been the most widely studied. The *Shh* pathway is most important during embryologic development, responsible for growth and patterning of the limbs, neural tube, axial skeleton, lungs, skin, hair, and teeth [35, 36]. During odontogenesis, it affects epithelial cell proliferation and might also play a role in epithelial–mesenchymal interactions [37]. *PTCH1* appears to be inactivated in various odontogenic cysts and tumors, such as dentigerous cysts, calcifying epithelial odontogenic tumors, and ameloblastomas, including one malignant ameloblastoma [38–40]. In the absence of *Shh*, the *PTCH1* transmembrane receptor acts as a gatekeeper of the hedgehog signaling pathway by inhibiting a neighboring transmembrane protein, SMO (Fig. 3a) [41, 42].



**Fig. 3** **a** PTCH1 is a transmembrane receptor which inhibits SMO in the absence of Shh. **b** Binding of Shh to PTCH1 releases the inhibitory effect on SMO, activating the Shh pathway and associated downstream targets. During embryogenesis, this leads to growth and patterning of the limbs and of various tissues. **c** Mutation of *PTCH1* results in loss of SMO inhibition. Tumorigenesis appears to result from constitutive activation of Shh-related proteins such as Gli1, Gli2, Gli3, and Wnt

Binding of Shh to PTCH1 activates the pathway and results in upregulation of downstream target genes, such as *Wnt* and genes related to bone morphogenetic proteins (Fig. 3b) [42]. The *Shh* signaling pathway might also interact

**Table 1** Specific mutations identified in KOT

Study	NBCCS versus sporadic	Mutation
Barreto et al. [48]	NBCCS	Nonsense mutation (C2760A)
	NBCCS	Missense mutation (G3499A)
	Sporadic	5-base pair deletion exon 3 (518delAAGCG)
Zedan et al. [47]	NBCCS	Tandem CC → TT substitution intron exon 2 (proximal to the immunogenic sequence)
	NBCCS	AAA deletion intron exon 11, insertion exon 14
	NBCCS	8-base pair deletion exon 3
	NBCCS	G deletion intron 17, tandem GG → TT substitution exon 17
	Sporadic	G → A substitution exon 8
	Sporadic	C insertion exon 13
	Sporadic	C insertion exon 8

and aid in activation of *bcl-2* [43]. Transcriptional activation of downstream genes is linked to tumorigenesis.

*PTCH1* therefore appears to act as a tumor suppressor gene by inhibiting the hedgehog pathway (Fig. 3c) [34, 42]. While haploinsufficiency alone is adequate for the developmental anomalies observed in NBCCS patients, tumors are likely to occur with inactivation of the remaining allele [42]. Both familial and sporadic BCCs show LOH of *PTCH1* [34]. Similarly, LOH on chromosome 9q has been noted in both NBCCS-associated and sporadic KCOTs, although allelic loss appears to be less common in sporadic versus hereditary lesions [44]. This suggests that other mechanisms might be involved, such as point mutations in both copies of the gene or epigenetic changes, such as hypermethylation [44]. Inactivating *PTCH1* mutations have also been linked to development of primitive neuroectodermal tumors (including medulloblastoma), trichoepithelioma, esophageal squamous cell carcinoma and transitional cell carcinoma [42].

LOH of common tumor suppressor genes has been observed in KCOT. In one study, LOH of at least one tumor suppressor gene was observed in 7 of 10 sporadic KCOTs [45]. Allelic loss was observed most frequently in *PTCH*, *p16*, *p53*, and *MCC*. In another study, expression of *bcl-2* oncoprotein was significantly higher in both sporadic and hereditary KCOTs compared to other odontogenic cysts [43]. These findings support the argument that KCOTs are neoplastic. The authors also noted a significant correlation between LOH and the presence of satellite cysts. Epigenetic events have also been reported [46]. Methylation of the promoter region of the cyclin-dependent kinase inhibitor *p21* was found in 3 of 10 KCOTs, but not



in dental follicles or normal mucosa. Methylation of *p16* was noted in 50 % of KCOTs, but was similarly present in normal and follicular tissues. *P27* showed fewer epigenetic alterations in KCOTs than in non-tumor tissues. *P53* and *RBI* methylation was not seen in any of the KCOTs.

### Applications in Practice

Various *PTCH1* mutations have been described in both sporadic and hereditary KCOTs, although no hot spots have yet been identified (Table 1). Additionally, overexpression of *PTCH1* protein product has been demonstrated with the use of immunohistochemistry in all hereditary and sporadic KCOTs studied thus far, although differences in the pattern of expression were noted [47, 48]. Syndromic KCOTs showed heavy cytoplasmic staining in the basal cell layers, whereas non-syndromic cysts showed staining exclusively in the superficial epithelial cells. Increased expression of downstream *Gli-1* was also noted in most cysts and was noted to parallel the staining pattern of *PTCH1* [47]. They explain these differing patterns of expression as indicative of a more differentiated phenotype in non-syndromic KCOT.

Current experimental strategies for the treatment of BCCs SMO inhibitors (cyclopamine, CUR61414, GDC-0449, vitamin D3, LDE225, and LEQ506) and other *Shh* pathway antagonists (vismodegib, hedgehog interacting protein, MYCN inhibitors, rapamycin, statins, and itraconazole) [49, 50]. Such targeted therapies might someday be of use in the treatment of KCOT, particularly in hereditary cases, or in patients with recurrent lesions.

### Odontogenic Tumors with Ghost Cells

#### Clinicoradiographic, Histologic, and Immunophenotypic Features

Odontogenic tumors with ghost cells include calcifying cystic odontogenic tumor (CCOT) (formerly termed calcifying odontogenic cyst or Gorlin cyst) and dentinogenic ghost cell tumor, as well as some odontomas. Ghost cell odontogenic carcinoma is the malignant counterpart of CCOT. CCOTs were first described by Gorlin who described the presence eosinophilic ghost-cells, comparing them to the calcifying epithelioma of Malherbe (pilomatricoma) [51]. These ghost cells have a tendency to calcify. The simple cystic CCOT is comprised of an epithelial lining 4–10 cell layers thick with a palisaded layer of cuboidal to columnar basal cells that may exhibit reverse polarity, with areas of epithelial proliferation seen [52].

Odontomas are considered the most common odontogenic tumors, more common than all others combined. However, many pathologists consider odontomas to be hamartomatous, as the tissue from which they are comprised is morphologically relatively normally, although aberrantly arranged. Clinically, odontomas present in the jaws of children or adolescents often after radiographic evaluation for an unerupted tooth [53]. They are slow growing asymptomatic lesions with the potential for tooth displacement, occasionally causing expansion of the jaw. The radiographic features are usually distinctive, presenting as a radiopacity surrounded by a thin radiolucent line. The compound odontoma is comprised of malformed tooth-like structures, whereas the complex odontoma is an amorphous radiopaque mass. Microscopically, enamel, dentin, cementum, and pulp tissue can be identified with tooth-like structures seen in the compound odontoma and a haphazard arrangement of tissue found in the complex odontoma. Ghost cells are present in 16 % of complex odontomas and 3 % of compound odontomas [54, 55]. When seen in association with another tumor, the most common combination is an odontoma-associated CCOT, seen in approximately 24 % of CCOT [56].

### Molecular Findings

The Wnt signaling pathway regulates multipotent stem cell development and is important for tooth development [57]. *CTNNB1* is a regulatory gene in this pathway that encodes  $\beta$ -catenin, a downstream transcriptional activator of Wnt.  $\beta$ -catenin accumulates in the nucleus and forms complexes with DNA binding proteins such as TCF and LEF-1 [58].  $\beta$ -catenin can thus be considered a transcriptional co-activator that interacts with LEF-1.  $\beta$ -catenin positivity has been reported in CCOTs and odontomas [59, 60].

Patients with familial adenomatous polyposis (FAP) have mutations in the *APC* gene which may be inherited in an autosomal dominant pattern and may present with extraintestinal manifestations including odontomas (as well as jaw osteomas and supernumerary teeth) [61].

### Applications in Practice

$\beta$ -catenin and LEF-1 are not utilized in the diagnosis of CCOT or odontomas with ghost cells. However, there is an association of  $\beta$ -catenin mutations in tumors with ghost cells and the Wnt signaling pathway may be involved in their pathogenesis. In patients with multiple odontomas, genetic testing for FAP should be considered [62].

## Odontogenic Myxoma

### Clinicoradiographic, Histologic, and Immunophenotypic Features

Odontogenic myxoma (OM) is an uncommon benign neoplasm of the jaws derived from the ectomesenchyme of the tooth forming apparatus. It accounts for approximately 3 % of all odontogenic tumors [63]. Any gnathic site can be affected, although OM shows a predilection for the posterior mandible [64]. The lesion most commonly develops in young adults, but can affect a broad age group. A slight female predilection has been observed [64]. Unlike myxomas of other sites, OMs are invariably sporadic and are not a feature of Carney complex or Mazabraud syndrome. On panoramic radiograph, OM characteristically presents as a multilocular radiolucency, with wispy septations imparting a “soap-bubble”-like appearance [65]. Histologically, OMs are composed of abundant, pale-staining, myxoid ground substance with a paucicellular infiltrate of bland-appearing, thin spindle-shaped cells. Small rests of odontogenic epithelium may be observed within the stroma. The microscopic borders of the tumor are typically poorly delineated, with focal infiltration of soft tissue and bone. This finding likely accounts for the approximately 25 % recurrence rate associated with these tumors [4].

### Molecular Findings

One study noted increased expression of anti-apoptotic proteins Bcl-2 and Bcl-XL in OM cells compared to control tissue using standard IHC procedures [66]. In this study, myxomatous dental follicular and pulpal tissues were used as controls. This would suggest that dysregulated apoptosis might play a role in neoplastic growth of OM. The authors also found that 90 % of tumor cells stained positively for a gelatinase-type matrix metalloproteinase, MMP-2, compared to 10 % of cells staining in the control tissue. Production of MMP-2 by tumor cells would facilitate spread of the lesion within the surrounding tissue. The authors noted a Ki-67 proliferative index of less than 1 %.

Mazabraud syndrome (MS) is a condition characterized by the presence of intramuscular myxomas along with fibrous dysplasia of bone [67]. Guanine nucleotide-binding protein,  $\alpha$ -stimulating activity polypeptide 1 (*GNAS1*) mutations have been implicated in the pathogenesis of McCune–Albright syndrome and have been identified in some patients with MS [68]. *GNAS1* mutations have also been reported in sporadic and syndromic intramuscular myxomas [69]. However, based on one study of 7 cases, OM does not appear to be associated with *GNAS1* mutations [70].

Carney complex (CNC) is a familial multiple neoplasia syndrome characterized by skin and mucosal pigmentation,

endocrine abnormalities, as well as cutaneous and cardiac myxomas [71]. Osteochondromyxoma of bone and myxomas at other sites have also been described in association with this condition [72]. Tumorigenesis in patients with CNC has been linked in part to dysregulation of the cAMP signaling pathways via alterations of *protein kinase A (PKA) regulatory subunit type 1A (PRKARIA)*, on chromosome 17q22-24) which encodes the RI $\alpha$  subunit of PKA type I [72]. *PRKARIA* inactivating mutations are encountered in up to one-half of patients with familial CNC [72]. Interestingly, sporadic (non-CNC) cardiac myxomas have not shown an association with *PRKARIA* gene mutations. Investigation into the role of *PRKARIA* in the pathogenesis of OM indicates a possible commonality of at least a subset of OM with CNC-related tumors. Sequencing of *PRKARIA* confirmed heterozygous mutations in 2 cases of OM [73]. Furthermore, LOH in the *PRKARIA* gene is found within tumor cells in patients with CNC and indicate that RI $\alpha$  might act as a tumor suppressor gene in certain tissues [72, 74]. However, the tumorigenesis is likely more complex and the exact mechanism by which mutations in *PRKARIA* lead to tumorigenesis in CNC and OM has not been fully elucidated. Dysregulation of PKA activity appears to play a role. Despite decreased RI $\alpha$  levels, CNC tumors show increased responsiveness to cAMP compared to non-CNC control tumors, and total PKA activity is preserved. This is likely due to a compensatory overexpression of RI $\beta$  and an increase in PKA II activity [72, 74].

### Applications in Practice

Perdigão et al. [73] found significantly decreased immunohistochemical expression of RI $\alpha$  in 9 of 17 OM (with compensatory increased expression of RI $\alpha$  and/or RI $\beta$ ), when compared to expression of the protein in non-tumor cells within the surrounding normal tissue. Sequencing of *PRKARIA* confirmed heterozygous mutations in 2 cases. One tumor showed a missense mutation in exon 6 (c.725C > A) and the other harbored a single-pair deletion (del774C), also in exon 6. Interestingly, these mutations identified in OMs have not been detected in patients with CNC. At the present time, molecular studies are of limited value in the diagnosis of OM.

## Conclusion

In summary, while recent advances have been made in molecular pathology of odontogenic tumors, including the identification of *BRAF* V600E and *SMO* mutations in ameloblastomas and the *EWSR1-ATF1* translocation in clear cell odontogenic carcinomas, much remains unknown. Clinically, molecular testing has the ability to aid in diagnosis of only a

limited subset of tumors and is currently of limited applicability in practice. However, improved understanding of molecular biology may help refine tumor classification and perhaps even allow for targeted therapeutic approaches. Molecular testing will no doubt improve our understanding of odontogenic tumor pathogenesis and someday will likely be an important component of routine clinical practice and its role will only increase in the coming years. With time, we anticipate more widespread integration of molecular pathology into clinical practice as costs decrease, technology advances, and more is known about the clinical significance of these findings.

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