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Molecular Signaling in Benign Odontogenic Neoplasia Pathogenesis

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Abstract

Several molecular pathways have been shown to play critical roles in the pathogenesis of odontogenic tumors. These neoplasms arise from the epithelial or mesenchymal cells of the dental apparatus in the jaw or oral mucosa. Next generation genomic sequencing has identified gene mutations or single nucleotide polymorphisms associated with many of these tumors. In this review, we focus on two of the most common odontogenic tumor subtypes: ameloblastoma and keratocystic odontogenic tumors. We highlight gene expression and protein immunohistological findings and known genetic alterations in the hedgehog, BRAF/Ras/MAPK, epidermal growth factor receptor, Wnt and Akt signaling pathways relevant to these tumors. These various pathways are explored to potentially target odontogenic tumors cells and prevent growth and recurrence of disease. Through an understanding of these signaling pathways and their crosstalk, molecular diagnostics may emerge as well as the ability to exploit identified molecular differences to develop novel molecular therapeutics for the treatment of odontogenic tumors.

Keywords

odontogenic tumors; ameloblastoma; hedgehog; BRAF; epidermal growth factor receptor

Introduction

Odontogenic neoplasms are a heterogeneous group of tumors which arise in the jaw from epithelial or mesenchymal cells of the dental apparatus. The prevalence of the different types of odontogenic tumors varies among reports, but ameloblastomas, keratocystic odontogenic tumors (KCOTs), and odontomas are the most frequently diagnosed. This review focuses on two of these tumors, ameloblastomas and KCOT, which are both locally invasive, epithelial lesions of the jaw. These neoplasms demonstrate locally aggressive and destructive local behavior and are mostly intraosseous, primarily within the posterior mandible [1]. There is wide variability in the surgical treatment used for these tumors including surgical removal

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Compliance with Ethics Guidelines

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(resection), decompression, marsupilization, and enucleation (with or without Carnoy's solution) [2]. This variation is thought to lead to the overall recurrence rates of 24–29% for ameloblastomas and 22–26% for KCOT [3–7]. Ameloblastomas can occur at any age, but are mostly diagnosed in adults aged 40–50 years and are more common in men than women (Table 1). In contrast, KCOTs are more likely to occur at any age and most commonly diagnosed in the second to third decades of life, with syndromic cases being diagnosed at a significantly lower mean age, mostly in adolescence [12, 86, 88]. KCOTs are also more prevalent in males. To decrease or prevent recurrence and aid in the treatment of odontogenic tumors, many scientists and physicians have suggested the use of targeted therapies, which are commonly used to treat various types of cancer. Here, we review the current literature regarding the therapeutic targets that may be useful for the treatment of these odontogenic neoplasms.

In the literature, the majority of research on odontogenic neoplasms has been restricted to immunohistochemical studies of tumors tissues and the surrounding stroma. These studies have identified potential markers, dysregulation of gene expression, and characterization of the tumor tissue of origin. More recent studies have utilized various sequencing approaches to identify gene mutations or single nucleotide polymorphisms associated odontogenic neoplasms. During the last decade, the isolation and cultivation of cell lines and cell populations from odontogenic tumors have started to improve our understanding of the molecular characteristics of these tumors and have provided models to explore alternative and complimentary treatments. In this review, we describe some of the molecules and signaling pathways which are thought to be related to the molecular pathogenesis of these diseases and the progress currently being made toward their targeting these pathways for therapeutic purposes.

Hedgehog (HH) Signaling

Aberrant hedgehog signaling plays a well-known role in the development and tumorigenesis of KCOT, also known as odontogenic keratocysts. KCOTs are highly associated with Nevroid basal cell carcinoma syndrome (NBCCS), also known as Gorlin's syndrome (OMIM 109400), occurring in 66 to 92% of these patients [8–11]. NBCCS is an autosomal dominant genetic disease characterized by heterogeneous mutations in three genes: most often Patched 1 on 9q22.32 (*PTCH1*; OMIM 601309), to a lesser extent Patched 2 on 1p34.1 (*PTCH2*; OMIM 603673), or the Suppressor of Fused on 10q24.32 (*SUFU*; OMIM 607035). Mutations in *PTCH1*, a transmembrane protein in HH pathway, are also found in sporadic cases of KCOT, leading to increased activity of HH signaling within the tumor that is associated with increased proliferation and neoplastic growth [12–14]. *PTCH1/2* are a cell surface receptors that represses HH pathway signaling. *PTCH* binds HH ligands (sonic, indian, and desert HH). In the absence of the ligand, *PTCH* inhibits the smoothened (SMO) receptor that activates the HH pathway and downstream glioma-associated oncogene (Gli) transcription factors (*GLI1/2*) [15] (Figure 1). Beside its association with NBCCS and KCOT, HH activation possibly as a result of *PTCH* mutations has been reported in ovarian, colon, and pancreatic cancer [16]. Further supporting the role of HH in NBCCS and KCOT, patched heterozygous knockout mice (*Ptc* +/-) have a phenotype typical of NBCCS, including the incidence of medullablastomas, basal cell carcinoma (BCC)-like lesions, and

mandibular jaw cysts similar to KCOTs [17]. Transgenic mice over-expressing GLI2 in keratinocytes develop KCOTs from the dental root epithelial rests of Malassez, reinforcing the critical role of this transcription factor in KCOT formation [18]. Li (2011) provides a synopsis of known mutations in NBCCS and non-syndromic KCOT patients, which occur in 16 of the 23 PTCH1 exons and include missense mutations, nonsense mutations, frameshifts, duplications, and exon skipping [12]. Calcifying epithelial odontogenic tumors are also reported to have mutations or single nucleotide polymorphisms in *PTCH1* [19, 20]. In contrast, few ameloblastomas tested have PTCH1 alterations, instead these tumors tend to have mutations in *SMO*, a G protein-coupled receptor, which are thought to be activating mutations also leading to increased HH signaling. 24% of ameloblastomas tested have mutations in *SMO*, but Sweeny et al. (2014) reports that 82% of maxillary ameloblastomas have a *SMO* mutation. Thus, it may be location or development dependent as to the association of *SMO* with ameloblastoma formation [21, 22].

In most studies, all KCOT and ameloblastomas are shown to express many components within the HH signaling pathway except the ligand SHH, but as mentioned above, many tumors may have mutations that render active HH signaling ligand-independent. Also, many studies look at expression in tumor tissue only and SHH may be expressed by the tumor-associated stroma, which is known to play a major role in cancer-related HH signaling. One report found *SMO* expressed in 61% of KCOTs [23]. Other reports state that at both the mRNA and protein level, 100% of KCOT and ameloblastomas express PTCH1, *SMO*, and GLI1 [23–30].

The Food and Drug Administration (FDA) has approved two HH inhibitors, vismodegib (GDC-0449) and sonidegib (LDE225), for the treatment of basal cell carcinoma (BCC), often associated with NBCCS. These inhibitors have also shown to have efficacy against sporadic and NBCCS-related BCC and have great potential to benefit patients with oral odontogenic HH-driven tumors, such as KCOT and a subset of ameloblastomas. Preclinical *in vitro* studies have used cyclopamine, another HH inhibitor that acting on *SMO*, to test the efficacy against ameloblastoma and KCOT cells [15, 29]. Kanda et al. (2013) showed AM-1, an immortalized ameloblastoma cell line, expressed SHH, PTCH, GLI1, GLI2, and GLI3. Both a SHH-neutralizing antibody and cyclopamine were shown to decrease the proliferation of AM-1 cells and the SHH-neutralizing antibody prevented GLI1 and GLI2 nuclear translocation and induced apoptosis in tumor cells [29]. In another study, a KCOT-1 primary cell population was also shown to express SHH, PTCH1, *SMO*, GLI1, and GLI2 [15]. Cyclopamine treatment of KCOT-1 cells reduced cell viability and decreased GLI1, a marker of decreased HH activity. Several studies have highlighted the utility of treating KCOTs with HH inhibitors [12, 15, 31–34]. Clinically two reports have successfully used vismodegib in NBCCS patients with KCOTs [31, 35]. Following 12 weeks of daily treatment, the patient had complete regression of the BCCs and after 2-years of therapy nearly complete remissions of the KCOT lesions with no appearance of additional lesions [31]. In a study of 6 patients with pre-existing KCOTs, after 18 months of treatment the tumors in four patients were reduced in size and no new KCOT developed in any patients [35]. Even though it was not the goal of treatment in these cases, this demonstrates a possible clinical utility for HH inhibitors to treat KCOTs. In both of these studies patients

received oral therapy, perhaps with local administration of HH inhibitors at the tumor site a better clinical response may be observed.

BRAF/Ras/MAPK

Constitutively expressed or overactive Ras signaling has been documented in many human tumors and cancers, including colorectal, non-small cell lung, and pancreatic cancers. This pathway can be activated by mutations in many of the pathway components, including receptors fibroblast growth factor receptor 2 (*FGFR2*), downstream serine-threonine kinases (*BRAF*), and the Ras genes themselves (*HRAS*, *KRAS*, *NRAS*) [36]. These mutations can lead to constitutive activation of the mitogen-activated protein kinase (MAPK), resulting in the activation of downstream kinases such as MEK1 and ERK and the transcription of targets leading to proliferation and survival of cancer cells.

Investigations focusing on Ras activation in odontogenic tumors were initiated with the observation that mice expressing a Ras oncogene often developed ameloblastomas, odontomas, or other odontogenic tumors [37, 38]. In 2014, three studies identified a *BRAF* mutation (*BRAFV600E*) in human ameloblastomas and an ameloblastoma cell line (AM-1) [21, 22, 39]. *RAS* and *FGFR2* mutations were also identified in additional ameloblastoma samples. Brown and Betz (2015) summarized these studies, and indicated that 78–88% of ameloblastomas have an activating mutation in the Ras pathway, but rarely have mutations in more than one pathway component [36]. More recent studies have examined subtypes of ameloblastoma and other types of odontogenic tumors. Of note, *BRAFV600E* was detected in unicystic and multicystic ameloblastomas (83% and 78%, respectively) [40]. This mutation was also detected in desmoplastic ameloblastomas, ameloblastic fibro-odontomas, ameloblastic fibromas, and ameloblastic carcinomas [40, 41]. Sweeney et al. (2014) reported that ameloblastoma tumors with *BRAFV600E* mutations were commonly those from mandibular tumors; in contrast, maxillary tumors predominantly had SMO mutations [22]. *BRAFV600E* was also found in a case of clear cell odontogenic carcinoma. KCOT (n=20), ghost cell odontogenic carcinomas (n=1), adenomatoid odontogenic tumors (n=2), calcifying cystic odontogenic tumors (n=2) and odontogenic fibromas (n=5) were all found to be negative for the *BRAFV600E* mutation [21, 40, 41]. Brunner et al. (2015) concluded that the *BRAF* mutation was present only in the epithelial tumor tissue, but was absent from the surrounding stromal tissue [41]. These data show that the activation of the Ras pathway by oncogenic mutation may be an important factor that drives the development and/or progression of odontogenic tumors with an ameloblastic component.

The ameloblastoma AM-1 cell population, harboring a heterozygous *BRAF V600E* mutation, was shown to be sensitive to vemurafenib, a B-Raf inhibitor, within the dose range reported for *BRAF* mutant colorectal cancer and melanoma cells [21, 22]. In vitro studies also revealed that vemurafenib dose-dependently inhibited the phosphorylation and activation of ERK and MEK, downstream enzymes of B-Raf, in AM-1 cells [21]. In a recent clinical report, a patient had recurrent and metastatic Stage 4 ameloblastoma with a tumor mass in the face, jaw, and neck and pulmonary nodules plus a mass in the right bronchus [42]. This was the patient's fourth recurrence. This patient had previously received radiation therapy. Cancer gene profiling demonstrated that there was a *BRAFV600E* mutation, so the

patient received an eight-week course of dabrafenib, another B-raf inhibitor, combined with trametinib, a MEK inhibitor (the combination has been shown to prevent resistance). Within eight weeks, there was a reduction in the facial tumor masses and a response was also seen at the metastatic sites. CT scans at 20 weeks after initiating treatment showed a persistent tumor response at all sites with no drug-related toxicity. These studies demonstrate the clinical significance of the *BRAF*V600E mutation in odontogenic tumors and the promise of using B-raf inhibitors for the treatment of ameloblastomas.

Epidermal Growth Factor Receptor (EGFR)

Overexpression or high expression of the EGFR has been reported for many human tumors and cancers (squamous cell carcinoma, non-small cell lung cancer, colorectal cancer, etc.) as well as in odontogenic tumors [43, 44]. The expression of the EGFR in ameloblastomas has been variable in previous reports, but the most recent studies have report expression in all ameloblastoma tumor tissues examined (193 cases total), including solid/multicystic (plexiform and follicular), unicystic, and desmoplastic subtypes [39, 45–49]. KCOT have been documented to be EGFR-positive in 85–100% cases (60 cases total) [39, 50–52]. Additionally, a recent case report showed strong EGFR staining in a calcifying epithelial odontogenic tumor [53].

The role of the EGFR in odontogenic tumors has not been well studied. Rosa et al. (2014) isolated and immortalized cells from a primary ameloblastoma sample to generate the AME-HPV cell population [54]. They demonstrated that both the primary tumor and isolated cells expressed epidermal growth factor (EGF), the ligand for the EGFR, as well as the EGFR, in addition to two potential downstream targets of EGFR signaling, MMP-2 and MMP-9. MMP-2 and MMP-9 are secreted matrix metalloproteinases (MMPs) known to degrade the extracellular matrix surrounding cells and to potentiate the invasion and migration of tumor cells. They are thought to play a role in the locally invasive nature of odontogenic tumors. Many studies have shown the expression of various MMPs in odontogenic tumors, including ameloblastomas and KCOT [48, 51, 55–57]. Treating AME-HPV cells with EGF induced increased expression of MMP-2 and MMP-9, and increased the rates of invasion and migration of these cells [54]. Knocking down EGFR decreased the amount of active MMP-2 and MMP-9, indicating that EGFR signaling mediates the MMP expression in these ameloblastoma cells.

There are currently many targeted therapies available for tumors overexpressing the EGFR or that have overactive EGFR signaling, many of which have been approved by the FDA [43]. Among these are tyrosine kinase inhibitors (erlotinib, gefitinib), which target the intracellular kinase domain responsible for the downstream signaling that stimulates the proliferation and survival of tumor cells. There are also chimeric and humanized antibodies against the EGFR (cetuximab or panitumumab), which are known to induce cytotoxicity and reduce the proliferation of tumor cells. Because a high percentage of ameloblastomas and KCOT apparently express the EGFR, the use of targeted therapies may help to reduce the size of tumors and may prevent tumor recurrence.

Mutations in *EGFR*, particularly within the kinase domain, have been demonstrated to render cancer cells resistant to many tyrosine kinase inhibitors and other therapies targeting the EGFR [58]. However, it has not been shown that odontogenic tumors harbor these mutations or have the capacity to develop them. In addition, some tumors with EGFR mutations still respond to EGFR-targeted therapies. For example, Oikawa et al. (2012) showed, in the 18 cases of ameloblastomas tested, that ameloblastomas lacked these sensitizing mutations with no alterations in *EGFR* exons 19 or 21 [49]. Pereira et al. (2015) examined the localization of the EGFR in ameloblastomas, and reported nuclear localization where the EGFR colocalized with Cyclin D1 in 12 of the 14 cases [59]. Nuclear expression of the EGFR has been suggested to be related to highly proliferative cells and resistance to anti-EGFR therapies. Additionally, the presence of the *BRAF*V600E mutation, which has been documented in ameloblastomas, is known to contribute to resistance to anti-EGFR therapies. Kurppa et al. (2014) established two ameloblastoma cell populations; one showed sensitivity and a dose-dependent response to both anti-EGFR antibodies and EGFR tyrosine kinase inhibitors [39]. However, the other cell population harbored a *BRAF* mutation and was resistant to anti-EGFR therapies. In addition, in studies of non-small cell lung cancer cells, the activation of hedgehog activity (increased SMO, GLI1, and PTCH1 expression) was demonstrated to be important in the acquired resistance to a tyrosine kinase inhibitor, gefitinib [60]. GLI1 expression was also linked to acquired resistance to the anti-EGFR antibody cetuximab in squamous cell carcinoma, but was overcome by combined treatment with cetuximab and a hedgehog inhibitor [61]. Thus, the presence of mutations, nuclear expression of the EGFR, or increased activation of other oncogenic signaling pathways could decrease the utility of these inhibitors for the treatment of odontogenic tumors as it has other human tumors. However, there are currently many treatment modalities being investigated to overcome intrinsic and acquired resistance or that can be used in combination to avoid resistance, such as third-generation tyrosine kinase inhibitors [58]. Using EGFR inhibitors in combination with B-Raf inhibitors, such as vemurafenib, may also have clinical utility. All of these approaches may improve the treatment outcomes for ameloblastoma.

WNT

Wingless-type (Wnt) signaling has been shown to induce either oncogenic or tumor suppressor effects in many human cancers. Both canonical signaling involving β -catenin and non-canonical signaling, especially that involving Wnt-5A, have been studied in odontogenic tumors. Canonical Wnt ligands (Wnt-1, 2, 3, 8a, 8b, 10a, and 10b) were reported to be expressed in 100% of ameloblastomas (n=72) representing unicystic, solid/multicystic, and desmoplastic subtypes, and including 10 cases of recurrent ameloblastoma (Siar 2012). The expression of β -catenin, the signal transducer of canonical Wnt signaling, is inconsistently reported, with expression detected by immunohistochemistry in 15–100% of ameloblastomas [62–69]. Overall, 82% of ameloblastomas express β -catenin (n=137). In the studies that included data about the localization of β -catenin, 51% expressed nuclear β -catenin, which would indicate active canonical Wnt signaling (n=71). Two studies of KCOT reported that 100% of tumors expressed β -catenin (n=58) with only 22% expressing nuclear β -catenin (n=40) [70, 71]. Calcifying cystic odontogenic tumors have a high prevalence of nuclear β -catenin expression, as well as frequent mutations in the gene that encodes β -

catenin (*CTNNB1*), which can lead to constitutive activation (94%, n=31) [62, 68, 72, 73]. In contrast, only 8% of ameloblastomas tested had such mutations (n=26) [62, 63]. Bilodeau et al. (2015) reported that 64% of calcifying cystic odontogenic tumor express LEF-1, a transcriptional coactivator of β -catenin [68]. These studies demonstrate that canonical Wnt signaling may be an oncogenic driver in calcifying cystic odontogenic tumors, and possibly also in ameloblastomas and KCOT.

The non-canonical Wnt or β -catenin-independent signaling pathways include planar cell polarity (PCP) and Wnt/ Ca^{++} signaling [74]. Wnt/PCP signaling activates MAPK signaling via Rac1, MAPKK4/7, and Jun kinase (JNK) to promote cytoskeletal remodeling and cell motility. Wnt/ Ca^{++} signaling involves the release of intracellular calcium to activate calmodulin-dependent protein kinase II (CAMKII) and protein kinase C (PKC), which may inhibit canonical Wnt signaling and have tumor-promoting or tumor-suppressing effects based on the cell/cancer type. Non-canonical Wnt ligands (Wnt-4, 5a, 5b, 6, 7a, 7b, and 11) were not expressed in unicystic or solid/multicystic ameloblastoma (n=72) [75]. However, other studies have reported that ameloblastomas and KCOT do express Wnt-5a (n=92 and 11, respectively) [76, 77].

One of these studies examined the function of Wnt-5a in enamel epithelial cells, the precursor cells of odontogenic tumors [76]. The authors determined that 88% of ameloblastoma tumors expressed Wnt-5a (n=52), and it was also expressed in the inner enamel epithelium (IEE) and outer enamel epithelium (OEE) of human molar tooth buds during the cap stage; the IEE, OEE, and presecretory ameloblasts during the early bell stage; and in the enamel knot and cervical loop during the late bell stage. To determine the effects of Wnt-5a expression in the enamel epithelium, LS-8 cells, an immortalized mouse enamel epithelial cell population, were transfected with either a sense or anti-sense Wnt-5A construct to overexpress or knock down Wnt-5a expression. The cells overexpressing Wnt-5a exhibited characteristics of tumor cells, such as a loss of contact inhibition, anchorage-independent growth, increased cell migration, and an enhanced ability to form tumors in athymic mice.

Another study used two immortalized ameloblastoma cell lines (AM-1 and AM-3) to evaluate the expression of Wnt ligands and receptors and the effects of Wnt-3a expression [78]. They showed that AM-1, AM-3, and MOE1b (normal oral epithelium) cells expressed mRNA for both canonical and non-canonical Wnt ligands (Wnt2, 2B, 4, 5A, 5B, 7B, 8B, 9B, and 11) and Wnt receptors (Frizzled1, 2, 3, 4, 5, 6, 7, 8, 9, 10, LRP5, LRP6). MMP-2, a marker of Wnt activity, was shown to be expressed in ameloblastoma cells and oral cancer cells, while MMP-9 (another marker) was only expressed in the ameloblastoma cells. Next, the AM-1 and AM-3 cells were treated with Wnt-3a, which binds to the Frizzled-2 receptor and potentially induces MMP-9 expression. Treating the AM-3 cells with Wnt-3a induced the expression of β -catenin protein and activated MMP-9, indicating activated canonical Wnt signaling, suggesting that Wnt signaling may play a role in the invasive nature of ameloblastoma via the increase in MMPs expression. As in various other human normal and cancer cells, the role of Wnt signaling seems to be complex in odontogenic tumors. The current data suggest that both the canonical and non-canonical Wnt pathways are active, but further studies are needed to understand their roles in odontogenic tumor biology.

Akt

Akt is a serine/threonine kinase that phosphorylates a multitude of targets important in cancer progression to activate or inactivate them [79]. The activation of Akt is regulated by phosphatidylinositol-3 kinase (PI3K) and phosphatidylinositol-3,4,5 trisphosphate (PIP3). PIP3 recruits Akt to the plasma membrane, where PIP3 induces a conformational change in Akt allowing it to be phosphorylated and stabilized by 3'-phosphoinositide-dependent kinase 1 (PDK1). PTEN (phosphatase and tensin homologue deleted on chromosome 10) is a negative regulator of Akt activity that functions by dephosphorylating PIP3 to PIP2, thus reducing PI3K and Akt activity. It has been reported that 93% of ameloblastomas express phospho-Ser473 Akt and 65% express phospho-Thr308 Akt (n=60) [80, 81]. It has been thought that phosphorylation at both sites is required for Akt to activate its downstream target, mTOR; however, these studies do not indicate if all of the phospho-Thr308 Akt-positive tumors were also positive for phospho-Ser473 Akt. Chaisuparat et al. (2013) reported that 60% of ameloblastomas were positive for both of these phosphorylated forms of Akt and for phospho-RPS6 (phosphorylated-ribosomal protein S6), a marker of mTOR activity (n=30) [81]. It was reported that 93% of KCOT expressed phospho-Thr308 Akt, 40% expressed phospho-Thr308 Akt, 83% expressed phospho-RPS6, and 30% expressed all three (n=30). Two additional studies reported that 100% of ameloblastomas expressed phosphorylated Akt, but these reports did not indicate which phosphorylation site(s) were examined [67, 82]. However, one study clarified that while all ameloblastomas tested also expressed PTEN, the expression was at a lower level compared to that in normal tooth germ, indicating that there may be increased Akt activity in ameloblastomas (Kumamoto 2007). In addition, Scheper et al. (2008) showed that while 66% of ameloblastomas were positive for PTEN, only 17% expressed phospho-PTEN, the active and stable form [80]. Moreover, increased Akt activity was detected in the ameloblastomas that were negative for PTEN. To further examine mTOR activity, other studies examined the levels of phosphorylated mTOR (p-mTOR) and downstream effectors of mTOR signaling, including phosphorylated ribosomal s6 kinase (pS6K) and phosphorylated translation repressor protein 4EBP1 [80, 83]. These studies found that 75% of ameloblastomas expressed p-mTOR, 62% pS6K, and 65% p-4EBP1; all three were expressed at significantly higher levels in cases of recurrent ameloblastoma (n=33). The authors suggested that the Akt and mTOR activity in ameloblastomas may play a role in the invasive nature of these tumors, and serve as a prognostic indicator.

Signaling Crosstalk

We have provided a concise overview of the literature regarding the molecular pathways involved in the pathogenesis of odontogenic neoplasms. We have focused on the HH, B-Raf/MAPK, EGFR, Wnt, and Akt/mTOR pathways that have presented as separate entities. However, recent studies have pointed out the complex nature of cancer/tumor cells and the crosstalk and overlap between and among signaling pathways. For example, several studies used MMP expression as a marker of pathway activity, but MMPs expression can be induced by each of the pathways described in this review. Many pathways also regulate glycogen synthase kinase-3 (GSK-3), which can act as a repressor of β -catenin, which mediates canonical Wnt signaling [84]. Akt phosphorylates GSK-3, causing its degradation, which

frees β -catenin and activates Wnt signaling. Active GSK-3 negatively regulates the HH pathway as well; it can increase the sequestration of GLI in the cytoplasm to inhibit HH activity. Therefore, deactivation of GSK-3 by Akt can increase HH signaling. ERK is a downstream effector of both the Akt and EGFR-mediated pathways and is expressed in 70% of ameloblastomas [80]. B-Raf signaling through MEK1 may also induce the expression of HH genes, *GLI1* and *PTCH1* [85]. Some EGFR-targeted genes also contain GLI binding sites in their promoters. Therefore, there appears to be overlap, cross-talk and interconnections among these signaling pathways. Inhibiting one pathway with a targeted therapy may therefore inhibit other oncogenic pathway(s). Conversely, when one pathway is inhibited, another may be activated to enhance cell survival. Additional research is needed to determine the full extent of these interactions and relationships and to determine how these can be exploited to obtain the best therapeutic outcome.

Conclusions

The molecular pathogenesis of odontogenic tumors may involve several cellular signaling pathways. One advantage to targeted treatment of odontogenic tumors is the anatomical accessibility. It may be possible to use targeted therapies locally, directly at the tumor site while avoiding systemic toxicity. Another questions that needs to be explored is whether there are differences between primary and recurrent tumors. These studies have been hindered in the past due to sample availability, but with the development of cellular models of primary and recurrent tumors some of these can be undertaken. Also, the difference between these benign tumors and metastatic cancers is not well understood. As we have described here, many pathways thought to be involved in the pathogenesis of odontogenic neoplasms are those involved in the pathogenesis of human cancers. What is the defining and biological features of these tumors that usually prevents them from metastasizing? The signaling mechanisms presented here which may play a role in the tumorigenic phenotypes of odontogenic neoplasms provides many avenues of study and opportunities to provide a targeted and novel therapeutics to this understudied patient population.

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•• Of major importance

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Fig 1. Histopathology of an ameloblastoma (A) and a keratocystic odontogenic tumor (B). (A) Plexiform pattern of ameloblastoma with cords of epithelium bound by columnar or cuboidal cells surrounded by loose stroma. H&E staining, 10×. (B) Keratocystic odontogenic tumor with a layer of stratified squamous epithelium, basal palisading of nuclei, and luminal parakeratinized layer. H&E staining, 10×

Fig 2. Hedgehog (HH) signaling pathways. The pathway is inactive in the absence of HH ligands or activating mutations. Active HH signaling occurs with ligand binding, *PTCH1/2* mutations (loss of function, red X) or *SMO* mutations (gain of function, yellow X). Activate HH signaling leads to GLI-regulated transcription and in tumors can lead to increased proliferation and tumorigenicity

Fig 3.

Activation and molecular crosstalk amongst signaling pathways. The hedgehog, BRAF/Ras/MAPK, EGFR, Wnt, and Akt signaling pathways each have a canonical signaling pathway. There are also many ways the pathways can interact and regulated the signaling in other pathways. GF=growth factor, EGF=epidermal growth factor, TF=transcription factor

Table 1

Age, Sex, Site, and Associated Genes of Odontogenic Tumors [4, 86–91]

Tumor	Mean Age	Male to Female Ratio	Mandible to Maxilla Ratio	Cellular Origin	Genes Associated with Disease
Ameloblastoma	36	1:1	4:1	Epithelial	<i>BRAF, SMO</i>
Keratocystic Odontogenic Tumor ^a	34.5	2:1	2.4:1	Epithelial	<i>PTCH1, PTCH2, SUFU</i>
Calcifying Epithelial Odontogenic Tumor	40	1:1	2:1	Epithelial	<i>PTCH1</i>
Ameloblastic Carcinoma	33	1:1	3:1	Epithelial	<i>BRAF</i>
Clear Cell Odontogenic Carcinoma	56	1:1	3:1	Epithelial	<i>BRAF, EWSR1-ATF1</i>
Adenomatoid Odontogenic Tumor	20	1:1.9	1:1	Epithelial	
Calcifying Cystic Odontogenic Tumor	30	1:1	1:1	Epithelial	<i>CTNNB1</i>
Ameloblastic Fibro-Odontoma	11	1:1	1:1	Mixed	<i>BRAF</i>
Ameloblastic Fibroma	15	1:1	4:1	Mixed	<i>BRAF</i>
Odontoma	18	1:1	1:1	Mixed	
Odontogenic Fibroma	40	1:2.8	1:1	Mesenchymal	
Odontogenic Myxoma	36	3:1	3:1	Mesenchymal	

^aData represent non-syndromic cases of KCOT. Syndromic cases of KCOT have a lower mean age.