Novel Targets for the Treatment of Ameloblastoma

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The Unpredictable Ameloblastoma

Ameloblastomas are classified by the World Health Organization (WHO) into solid/multicystic, peripheral (extraosseous counterpart of the intraosseous solid/multicystic ameloblastoma), desmoplastic, and unicystic types with implications for treatment (Gardner et al. 2005). The solid/multicystic ameloblastoma is by far the most common type. The peak incidence is in the fourth and fifth decades. The vast majority occur in the mandible, with marked predilection for the posterior region. Ameloblastoma grows slowly, is locally invasive, and has a high recurrence rate, especially if not adequately removed at initial surgery. Therefore, the treatment of choice is jaw resection, which often results in significant morbidity. The molecular background of ameloblastoma has been poorly understood, thus hindering the development of noninvasive therapies. Although a benign tumor, ameloblastoma behavior is unpredictable, and from a clinical perspective, the maxillary tumors carry the worst prognosis. Patients with ameloblastoma should be followed up for a lifetime.

Histologically, ameloblastoma is characterized by islands or strands of odontogenic epithelium with mature connective tissue stroma. Molecularly, ameloblastoma exhibits dental identity as seen by the expression of early dental epithelial transcription factors such as *PITX2*, *MSX2*, and *DLX1,2,3,4* (Heikinheimo et al. 2015). However, the exact cellular origin of ameloblastoma has not been clarified. The pathogenesis of ameloblastoma has also remained elusive until recently, when 3 independent research groups largely unraveled the mutation landscape of ameloblastoma (Brown et al. 2014; Kurppa et al. 2014; Sweeney et al. 2014).

Mutated Pathways in Ameloblastoma

We recently reported frequent mutations in the mitogen-activated protein kinase (MAPK) pathway gene *BRAF* in solid/multicystic mandibular ameloblastomas (15/24 samples, 63%) (Figure A and B; Kurppa et al. 2014). In all cases, the mutation led to amino acid substitution V600E.

This mutation renders the BRAF protein constitutively active and is the most common activating mutation in this gene in melanoma and in thyroid and colorectal cancer (Holderfield et al. 2014). These results indicated that MAPK pathway activation is important in the pathogenesis of ameloblastoma. Normally, MAPK pathway activation is initiated when RAS becomes activated, often by a receptor tyrosine kinase (RTK) (Figure C). The activation of RAS leads to activation of a phosphorylation cascade, where subsequent phosphorylations of RAF, MEK, and ERK result in ERK translocation to the nucleus, where it can activate a number of transcription factors (Figure C). The MAPK signaling pathway is a potent mediator of cell proliferation, differentiation, migration, and survival and is commonly targeted by oncogenic mutations in human malignancies (Holderfield et al. 2014).

Subsequently, 2 independent studies also reported high frequency of MAPK pathway mutations in ameloblastoma (Brown et al. 2014; Sweeney et al. 2014). Sweeney et al. (2014) reported mutations in *BRAF* (V600E, 46%), *KRAS* (14%), and *FGFR2* (18%) genes in their series of 29 mandibular and maxillary ameloblastomas (Figure A; Sweeney et al. 2014). In the most recent study by Brown et al. (2014),

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Figure. Mutations associated with ameloblastoma. **(A)** Summary of the reported mutations in ameloblastoma by 3 research groups. **(B)** Immunohistochemical staining of BRAF V600E in ameloblastoma. Immunohistochemistry using a BRAF V600E-specific antibody (VE1) shows positive staining in the tumor epithelium of a mutation-positive ameloblastoma (top), whereas ameloblastoma lacking the mutation remains negative (bottom). **(C)** Mutated pathways in ameloblastoma and currently approved drugs inhibiting these pathways. Proteins encoded by genes found to be mutated in ameloblastoma are indicated in purple. Multikinase inhibitors are broad-spectrum tyrosine kinase inhibitors such as ponatinib and regoratinib that inhibit FGFR2, among other targets. MEK, mitogen-activated protein kinase kinase; ERK, extracellular signal regulated kinase; TF, transcription factor; Hh, hedgehog; PTCH, patched; GLI2, GLI family zinc finger 2.

BRAF V600E mutations were detected in 62% (31/50) of the ameloblastomas studied (Figure A; Brown et al. 2014). Most of the *BRAF* mutation-positive ameloblastomas were of the intraosseous solid/multicystic type, but *BRAF* V600E mutation was also found in 1 metastatic ameloblastoma, 1 unicystic ameloblastoma of the mural type, and 1 desmoplastic ameloblastoma (Brown et al. 2014). In addition to *BRAF*, mutations were also found in the *RAS* genes (*KRAS*, 8%; *NRAS*, 6%; *HRAS*, 6%) and in *FGFR2* (6%) (Brown et al. 2014). Identified mutations in the *RAS* genes leading to amino acid substitutions in codons 12 or 61 are canonical activating mutations in these genes and lead to constitutive activation of the *RAS* protein products. The *FGFR2* gene encodes fibroblast growth factor receptor 2, an RTK that is a potent activator of the MAPK pathway (Lemmon and Schlessinger 2010). The identified mutations in *FGFR2* (C382R, V395D, N549K) have previously been shown to result in ligand-independent activation of the receptor and/or have been detected in craniosynostosis and endometrial cancer (Li et al. 1997; Chen et al. 2007; Byron et al. 2012).

In addition to MAPK pathway mutations, the studies by Sweeney et al. and Brown et al. also reported a high incidence of recurrent activating mutations in the hedgehog pathway gene *SMO* (39% and 16% of the cases, respectively) (Brown et al. 2014; Sweeney et al. 2014), suggesting that this pathway could also be involved in the pathogenesis of ameloblastoma. The *SMO* gene encodes Smoothened (*SMO*), a transmembrane activator of the hedgehog pathway. In the absence of hedgehog ligand, *SMO* is repressed by the hedgehog receptor Patched 1 (*PTCH1*) (Figure C). Upon ligand binding, the repression of *SMO* by *PTCH1* is relieved, resulting in the activation of the

transcription factor GLI2 by SMO. Activated GLI2 translocates to the nucleus, where it controls the transcription of hedgehog-dependent target genes (Figure C; Kim et al. 2013). Aberrant hedgehog pathway activity has been linked to cancer, most notably to basal cell carcinoma, in which essentially all cases harbor mutations in either the *PTCH1* or the *SMO* genes (Kim et al. 2013). *PTCH1* mutations are also common in keratocystic odontogenic tumors (odontogenic keratocyst), especially in association with the naevoid basal cell carcinoma syndrome (for review, see Li 2011).

While mutations in the MAPK and hedgehog pathways were most frequent in ameloblastoma, rare mutations in other pathways were also identified. The affected genes included *PIK3CA* (phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha; 6%), *SMARCB1* (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1; 6%), and *CTNNB1* (β -catenin; 4%) (Figure A; Brown et al. 2014).

In the ameloblastoma samples analyzed so far, the mutations in the MAPK pathway genes *BRAF*, *KRAS*, *NRAS*, and *HRAS* were mutually exclusive (Figure A; Brown et al. 2014; Sweeney et al. 2014), which is typical for driver mutations of the same pathway. *FGFR2* mutations also tended to occur in different tumors than did *BRAF* and *RAS* mutations, with the exception of 1 sample harboring both *FGFR2* and *BRAF* mutations (Sweeney et al. 2014). The *SMO* mutations were found to co-occur with mutations in the MAPK pathway genes (Brown et al. 2014; Sweeney et al. 2014), suggesting that hedgehog pathway activation is independent and possibly synergistic with the MAPK pathway activation. Taken together, the results from these studies indicate that aberrant activity of the MAPK pathway and the hedgehog pathway is closely associated with the pathogenesis of ameloblastoma and that *BRAF* V600E mutation is the most frequent genetic alteration in this tumor.

Implications for Diagnosis and Prognosis

BRAF V600E mutation was shown to be associated with young age at diagnosis. The mean age of patients with *BRAF* V600E-positive tumors was 34.5 y at diagnosis, compared with 53.6 y of patients with *BRAF* wild-type tumors ($P < 0.0001$; Brown et al. 2014). In addition, the *BRAF* mutation status was shown to be an independent marker for recurrence-free survival, with the *BRAF* wild-type tumors recurring earlier than the *BRAF* mutant tumors ($P < 0.046$; Brown et al. 2014). This implicated a role for *BRAF* V600E mutation as a prognostic marker in ameloblastoma. Interestingly, the *BRAF* wild-type tumors were reported to be more common in the maxilla as opposed to the mandible (Brown et al. 2014; Sweeney et al. 2014), suggesting differences in the pathogenesis of mandibular and maxillary ameloblastomas. Thus, the differences in the clinical behavior of mandibular and maxillary ameloblastomas,

with the latter typically being more aggressive, could at least partly be explained by the genetic differences between the tumors. To conclude, more data on the mutation status and clinicopathological information, including treatment and follow-up, are needed to associate various mutations and the clinical outcome.

New Treatment Options for Ameloblastoma

The very high incidence of activating *BRAF* mutations and the additional recurrent, mutually exclusive mutations in the MAPK pathway genes *KRAS*, *NRAS*, and *HRAS* strongly implicate this pathway as the main driver of ameloblastoma growth. In addition, the recurrent mutations in the *FGFR2* gene suggest the role of this RTK as a potent activator of the MAPK pathway in ameloblastoma. Various targeted therapies inhibiting the activity of the MAPK pathway are currently available (Figure C). Selective inhibitors of mutated *BRAF*, vemurafenib and dabrafenib, as well as the MEK inhibitor trametinib, have been approved for the treatment of *BRAF* mutation-positive metastatic melanoma (Menzies and Long 2014). MEK inhibitors also hold promise against mutant *NRAS*-driven tumors (Ascierto et al. 2013). Intriguingly, ameloblastoma cells harboring the *BRAF* V600E mutation were shown to be sensitive to vemurafenib treatment in vitro (Figure C; Brown et al. 2014; Sweeney et al. 2014), suggesting that mutant *BRAF* inhibition could be beneficial in ameloblastoma. Considering the importance of MAPK pathway activation for ameloblastoma, MAPK pathway inhibitors should be evaluated as novel targeted therapy for this disease.

The recurrent *SMO* mutations co-occurring with the MAPK pathway mutations suggest that hedgehog pathway could be a parallel, synergistic pathway contributing to ameloblastoma pathogenesis and a potential therapeutic target. Targeted inhibitors of SMO or downstream effectors of SMO are also available (Figure C). Vismodegib, a specific inhibitor of SMO, has been approved for the treatment of basal cell carcinoma. In addition, Food and Drug Administration (FDA)-approved hedgehog pathway inhibitors, itraconazole and arsenic trioxide (ATO), have been shown to inhibit hedgehog pathway-driven tumors in vivo and in phase II clinical trials (Kim et al. 2013; Kim et al. 2014).

The efficacy of targeted therapies using mutant *BRAF* and *SMO* inhibitors is often faced with drug resistance (Menzies and Long 2014; Kim et al. 2013). In the case of mutant *BRAF*-driven tumors treated with vemurafenib, resistance mechanisms often include compensatory activation of the MAPK kinase pathway (Menzies and Long 2014). In *BRAF* mutation-positive colorectal cancer, the lack of response to targeted mutant *BRAF* inhibition has been shown to be associated with compensatory activation of epidermal growth factor receptor (EGFR) (Prahallad

et al. 2012). This resistance mechanism could also be relevant for targeted treatment of ameloblastoma, as ameloblastomas express high levels of EGFR (Kurppa et al. 2014). Thus, it could be hypothesized that MEK inhibitors could be more suitable for the treatment of ameloblastoma than mutant BRAF inhibitors. In the case of SMO inhibition in hedgehog pathway-driven tumors, resistance mechanisms often involve mutations in the *SMO* gene that inhibit the binding of SMO-targeted drugs (Kim et al. 2013). Accordingly, vismodegib and itraconazole have been shown to be ineffective in inhibiting the activity of ameloblastoma-associated SMO mutants, L412F and W535L (Sweeney et al. 2014). However, ATO and another hedgehog pathway inhibitor, CAAD-cyclopamine, were highly effective against these mutants (Sweeney et al. 2014), suggesting that these agents could be tested for targeted inhibition of the hedgehog pathway in ameloblastoma.

Taken together, the unraveling of the mutation landscape in ameloblastoma has rationalized the development of novel noninvasive treatment options for the management of ameloblastoma. However, the best treatment approaches must be carefully considered before potential future clinical studies.

Author Contributions

K. Heikinheimo, K.J. Kurppa, K. Elenius, contributed to conception, design, and data analysis, drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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