Molecular pathology of ameloblastoma: towards targeted therapy

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• 1. Discuss genomic differences between odontogenic neoplasia
• 2. Understand driver genomic changes in odontogenic neoplasia
• 3. Consider targeted therapeutic options
• 4. Appreciate various genomic techniques applied to archival tissue.
Genetic analysis of ameloblastoma

- Whole transcriptome sequencing
- Targeted DNA sequencing
- V600E mutant BRAF immunohistochemistry
BRAF in other odontogenic tumors

- Ameloblastic fibro-odontomas 33%
- Ameloblastic fibroma 40%
- Ameloblastic carcinoma 25% (1/4)
- Dentigerous cysts 0%

Calcifying cystic odontogenic

- CTNNB1 mutation in Calcifying cystic odontogenic tumors
- Removing the phosphorylation sites Asp32, Ser33, or Ser37,
- Similar to mutations in pilomatrixoma and craniopharyngioma
Ameloblastoma mutation discovery

- rRNA-depleted total RNA from FFPE of 2 cases
- Custom analytical pipeline identified
  - high-confidence single-nucleotide variations (SNVs)
  - no gene fusions.

- Candidate mutations were validated in an independent cohort consisting of 26 cases from 4 institutions
  - targeted-capture deep sequencing
  - PCR with Sanger sequencing.
Mutations are oncogenic

- **BRAF, KRAS, and FGFR2 mutations**
  - activating mutations present in other cancers
- **SMO(W535L)**
  - frequent activating mutation in sporadic basal cell carcinomas.
- **SMO(L412F)**
  - recently reported to be a recurrent mutation in a subset of meningiomas

FGFR/MAPK (BRAF) pathway
Hedgehog pathway activation of wildtype or mutant forms of SMO

Gli-luciferase reporter assay in Smo-/- mouse embryonic fibroblasts

- Sensitive to arsenic trioxide and cyclopamine
- Inhibitory effect was not observed for vismodegib

SMO L412F mutant shows constitutive activity

Hedgehog pathway activation of wildtype or mutant forms of SMO
Gli-luciferase reporter assay in Smo-/- mouse embryonic fibroblasts

Sweeney et al, Nat Gen 2014
SMO L412F mutant shows constitutive activity

- Gli-luciferase reporter assay in Smo-/- mouse embryonic fibroblasts
- Hedgehog pathway activation of wildtype or mutant forms of SMO

Crystal structure of human SMO bound to the LY2940680 inhibitor

Sweeney et al, Nat Gen 2014
Effect of treatment with Hedgehog-pathway inhibitors

Sweeney et al, Nat Gen 2014
SMO Leu412Phe enhances ameloblast-lineage cell proliferation

Sweeney et al, Nat Gen 2014
AM-1 cells are sensitive to the BRAF inhibitor vemurafenib

Sweeney et al, Nat Gen 2014
Ameloblasts in tooth development

Early bell stage

Tucker et al, Nature Genetics Reviews, 2004
Ameloblasts and ameloblastoma
Embryology: the epidermal placode

- Epidermal placodes: mini-organs that generate both teeth and hair

Interaction between epithelium and mesenchyme
Embryology: the epidermal placode

- Epidermal placodes: mini-organs that generate both teeth and hair

- Hedgehog (SMO) and the FGFR/MAPK (BRAF) pathways are essential

- Expression patterns are quite similar in teeth and hair development
Tooth development

- Loss of Hedgehog pathway leads to stunted growth and morphogenesis — does not prevent differentiation

Dassule et al, Development 2000
Gorlin syndrome

- Gorlin syndrome (aka nevoid basal cell carcinoma syndrome) is defined by germline inactivating PTCH1 mutations.
- Characterized by multiple developmental abnormalities including a predisposition to neoplasia:
  - including basal cell carcinomas
  - keratocystic odontogenic tumors
- Highlights the relationship between ontogenesis and oncogenesis.
Hedgehog pathway

Amakye et al, Nat Med 2013
Going to the bedside: BRAF targeted therapy

- BRAF V600E mutation–positive metastatic melanoma
  - 50% response rate
- Treatment with the MEK and BRAF inhibitor combination was statistically superior to the BRAF inhibitor alone

Long et al, NEJM 2014 and Larkin et al, NEJM 2014
Case report 1 – metastatic ameloblastoma

- 40-year-old African American male
- 30 year history of ameloblastoma
- Now with unresectable locally recurrent ameloblastoma and multiple pulmonary metastases
- BRAF V600E detected in cancer panel screen
BRAF mutation testing

• DNA sequencing
  – Single gene PCR sequencing
  – Cancer panel approach

• Immunohistochemistry (antibody specific for BRAF V600E protein)
BRAF IHC in ameloblastoma
Case report 1 – metastatic ameloblastoma

• Dabrafenib at 150 mg twice daily and trametinib (Selective MEK-1/2 Inhibitor) at 2 mg once daily

• 8 weeks later, repeat PET CT scan
PET CT scans 8 weeks of dual inhibitor therapy

- Disappearance of FDG activity in ameloblastoma in the lungs
- Reduction of ameloblastoma mass in the face, jaw, and neck.

Kaye et al, JNCI 2014
Follow up

Patient without evidence of disease after 4 years
Case report 2 – primary ameloblastoma

- 85-year-old Caucasian male
- S/p enucleation 1 year ago
- Gnathic ameloblastoma, follicular and plexiform patterns
- 4.5 cm tumor
- BRAF mutation V600E
- 73 days of dabrafenib (BRAF inhibitor) 150 mg PO q12h
Timeline of treatment response

Week 0 | Treatment
---|---
Week 10 | CT imaging
Week 16 | Surgery
Case report 2 – primary ameloblastoma

• A month and a half later, the patient underwent a left mandible composite resection of the tumor with titanium plate placement and pectoralis major skin paddle
Timeline of treatment response

- Treatment
- CT imaging
- Surgery

Week 0 → Week 10 → Week 16
Cavity lining
Sub centimeter nodules
Subluminal tumor
Intramandibular tumor
Conclusions from the bedside

- BRAF mutant targeted therapy results in a significant clinical response
- BRAF targeted therapy might be useful in certain clinical settings of primary ameloblastoma
  - tumors of advanced local stage where a neoadjuvant reduction might alter the extent of surgery
  - instances of local recurrence where surgical options are limited
  - Mandibular vs maxillary?
- BRAF targeted therapy is likely to be useful in clinical settings of metastatic ameloblastoma
  - Ameloblastic carcinoma
- Testing for BRAF mutation is essential
  - BRAF negative cases exist (SMO mutants and BRAF/SMO -/-)
Conclusions from the bedside

• BRAF mutant targeted therapy results in a significant clinical response
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Phase 2 “basket” study of vemurafenib in BRAF V600 cancers

- 122 patients with BRAF V600 cancer
- Response rate varied from 3-43% depending on diagnosis

- The pathologic diagnosis is an important determinant of response in BRAF V600–mutated cancers
- Ameloblastomas are relatively genetically simple and therefore likely to respond well

Hyman et al, NEJM 2015
Technical challenges

- Quality of RNA and DNA in archival sample:
  - RNA integrity number
SMART-3SEQ template switching
Reverse genomics
(microfluidic library preparation)

Microfluidic sorting $\rightarrow$ Expression profiling

In situ organization

Sample organization

Forward genomics
(microdissection library preparation)

Microdissection $\rightarrow$ Expression profiling

Inferred cell types

Observed cell types
Future directions

• SMO mutation targeting (maxillary ameloblastomas)
• Biomarker for response
• Molecular classification of odontogenic tumors
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