1018-17 Limited Tissue Samples in the Era of Personalized Medicine: Diagnostic Challenges, Molecular Analysis and Controversies

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DISCLOSURE

In the past 12 months, I have not had any significant financial interest or other relationship with the manufacturers of the products or providers of the services that will be discussed in my presentation.

Overview: The New Era for Head and Neck

- AJCC 8th edition
- WHO 2017
- Milan System for Reporting of Salivary Gland Cytology
AJCC 8th edition changes

- New (or newly separate) chapters
  - HPV mediated oropharynx
  - Nasopharynx
  - HPV negative oropharynx and hypopharynx
  - Unknown primary
  - Cutaneous squamous cell carcinoma of head and neck

- Key staging modifications
  - Oral cavity and skin – depth of invasion is part of T stage
    - Extrinsic tongue muscle no longer part of pT4
    - Advocacy for a specimen drive margin assessment
    - Extranodal extension is part of N stage (except nasopharynx and HPV mediated oropharynx)

Depth of Invasion and T stage – Oral Cavity

<table>
<thead>
<tr>
<th>Size</th>
<th>Depth ≤ 5 mm</th>
<th>Depth &gt;5 mm but ≤ 10 mm</th>
<th>Depth &gt;10 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 2 cm</td>
<td>pT1</td>
<td>pT2</td>
<td>pT3</td>
</tr>
<tr>
<td>&gt; 2 cm but ≤ 4 cm</td>
<td>pT2</td>
<td>pT2</td>
<td>pT3</td>
</tr>
<tr>
<td>&gt; 4 cm</td>
<td>pT3</td>
<td>pT3</td>
<td>pT3</td>
</tr>
</tbody>
</table>

“Plumb line” method (good for flat lesions, less so for ulcerated and exophytic lesions)
A thing of the past???

WHO 3rd edition vs 4th edition

<table>
<thead>
<tr>
<th>3rd edition</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Nasal cavity and paranasal sinuses</td>
</tr>
<tr>
<td>• Nasopharynx</td>
</tr>
<tr>
<td>• Hypopharynx, larynx and trachea</td>
</tr>
<tr>
<td>• Oral cavity and oropharynx</td>
</tr>
<tr>
<td>• Salivary glands</td>
</tr>
<tr>
<td>• Ear</td>
</tr>
<tr>
<td>• Paraganglionic system</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>4th edition</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Nasal cavity, paranasal sinuses, and skull base</td>
</tr>
<tr>
<td>• Nasopharynx</td>
</tr>
<tr>
<td>• Hypopharynx, larynx, trachea and parapharyngeal space</td>
</tr>
<tr>
<td>• Oral cavity and oropharynx</td>
</tr>
<tr>
<td>• Salivary glands</td>
</tr>
<tr>
<td>• Ear</td>
</tr>
<tr>
<td>• Paraganglionic system</td>
</tr>
</tbody>
</table>

The Milan System for Reporting Salivary Gland Cytopathology

• Salivary cytology confounded by rarity and diversity of salivary gland lesions

• International effort to standardize reporting for salivary gland fine needle aspiration

• Diagnostic categories with ascending risk of malignancy to guide management
1016-17 A Brave New World in Head and Neck Pathology: Updates from the New WHO, AJCC and Milan system

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Outline

- AJCC 8th ed. - Changes to head and neck cancer staging (pN, extranodal extension)
- The ideal margins: specimen margins
- WHO: new salivary entities & names
Changes To Head And Neck Cancer Staging (pN, extranodal extension)

- New chapter/staging for HPV-associated oropharyngeal carcinomas
  - HPV-negative oropharyngeal carcinomas remain with hypopharynx
- pN: introduction of extranodal extension: ENE(+) or ENE(-)

P16 Testing is Mandatory for All Oropharyngeal SCC: If Not Performed, Stage as p16(-)!

Positive, as a surrogate HPV marker

Negative, as a surrogate HPV marker

Oropharyngeal p16(+) SCC: Terminology (No Grading!)

- Non-keratinizing: “poorly differentiated” should be avoided
  - non-keratinizing morphology closely mimics the specialized oropharyngeal epithelium; most non-keratinizing oropharyngeal SCCs are highly radiosensitive and have excellent outcome.
- Basaloid – specific type of SCC
pN: HPV(+) Oropharyngeal Carcinomas Vs. HPV (-) Oropharyngeal (& Extra-oropharyngeal) Carcinomas

<table>
<thead>
<tr>
<th>Criterion</th>
<th>HPV(+) Oropharyngeal Carcinoma</th>
<th>HPV(-) Oropharyngeal &amp; Extra-Oropharyngeal Carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of involved lymph nodes</td>
<td>Yes: ≤4 (pN1) or &gt; 4 (pN2)</td>
<td>Yes: 1 or &gt;1</td>
</tr>
<tr>
<td>Size of the metastasis</td>
<td>No</td>
<td>Yes, with cut offs of 3 cm &amp; 6 cm</td>
</tr>
<tr>
<td>NEW: ENE</td>
<td>No</td>
<td>Yes (e.g., pN1 = ENE1); ENE(+) = pN2a - single ipsilateral &lt;3 cm; pN3b - for all other LN</td>
</tr>
<tr>
<td>Laterality</td>
<td>No</td>
<td>Yes: contralateral node leads to higher pN</td>
</tr>
</tbody>
</table>

Need for Reproducible Histologic Criteria of ENE: “Yes” versus “No”

- Widely variable incidence of ENE
- Low inter- and intraobserver agreement
- No consensus on the extent of lymph node sampling
- Few ENE studies account for HPV status:
  - HPV+ metastases are
    - Larger
    - More cystic
    - Lack desmoplasia

Challenges of Evaluating Extranodal Extension. When in Doubt – assign lower category! p. 57

Note:
Lymph node capsule is incomplete in the hilum, hence the preference for “ENE” over “extracapsular” term.
Example of Unequivocal ENE

When In Doubt – Assign Lower Category!

Intracapsular Tumor Deposits

When In Doubt – Assign Lower Category!

Post-FNA Change: ENE?
If so, <2 mm?

For oral cavity SCC, only ENE >2 mm define pENE?!
(AJCC manual, pp. 85, 127, 153)
(p)ENE in Matted Lymph Nodes, Soft Tissue Deposits – Not Addressed Directly…
Definition of Clinical ENE, Prior Literature May Help

Intranodal fibrous Septae, thick-walled vessels, large nerves, adipose tissue entrapped within the tumor mass.

The Ideal Manner of Margin Assessment – Specimen Driven Approach, p. 85

In ENT pathology, mph = margins per hour

The Ideal Manner of Margin Assessment – Specimen Driven Approach, p. 85

Tumor bed margin, taken from the patient, by surgeon? No
Specimen margin, taken by pathologist? Yes
Margin Sampling: Technical Aspects and Examples (Partial Glossectomy, Video)

<table>
<thead>
<tr>
<th>Margin Type</th>
<th>Radial</th>
<th>Shave</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allows to measure distance to margin</td>
<td>No distance; 2-tier reporting: benign vs malignant</td>
<td></td>
</tr>
<tr>
<td>Fewer frozen vs permanent sampling issues</td>
<td>3-5% chance of frozen vs permanent discrepancy</td>
<td></td>
</tr>
<tr>
<td>Easier to interpret – smaller (inked) area to scrutinize</td>
<td>Greater margin area is examined microscopically</td>
<td></td>
</tr>
<tr>
<td>May yield additional histologic findings - PNI</td>
<td>Under-estimates margin clearance in irregular or curved specimens</td>
<td></td>
</tr>
</tbody>
</table>

Irregular or Curved Specimen – Shave
Margins Under-estimate Distance to Margin

Tumor Bed Margin and Resection Specimen (Base of Tongue)
Margin Revision: Orienting Tumor Bed Margins – Where is the New True Margin Surface?

Orientering Tumor Bed Margins – Ink On New True Surface!

Note: Need this orientation to resolve potential frozen vs permanent discrepancies

WHO Update: Salivary Tumors

- Polymorphous Low Grade Adenocarcinoma
- Low Grade Cribriform Cystadenocarcinoma = Intraductal Carcinoma
- Apocrine nature of salivary duct carcinoma
- Mammary analogue secretory carcinoma
WHO Update: Salivary Tumors

- Polymorphous Low-Grade Adenocarcinoma (PAC)
  - High grade cases do exist
  - Updated diagnostic line: "Polymorphous adenocarcinoma, low grade"

- Cribriform adenocarcinoma of tongue (CAT)/minor salivary glands (CAMSG) is a synonym/variant (not a distinct entity)

High Grade Polymorphous Adenocarcinoma

High Grade Polymorphous Adenocarcinoma
Immunoprofile of Selected Salivary Carcinomas

<table>
<thead>
<tr>
<th></th>
<th>S100</th>
<th>SOX-10</th>
<th>P63/P40</th>
<th>Androgen Receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymorphous adenocarcinoma</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mammary analogue secretory carcinoma</td>
<td>+</td>
<td>+</td>
<td></td>
<td>Minor in situ component</td>
</tr>
<tr>
<td>Acinic cell carcinoma</td>
<td>Mostly ++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Low grade cribriform cystadenocarcinoma</td>
<td>+</td>
<td>+</td>
<td></td>
<td>Extensive intraductal component</td>
</tr>
<tr>
<td>Salivary duct carcinoma</td>
<td>-</td>
<td>-</td>
<td>occasional in situ focus</td>
<td>+</td>
</tr>
<tr>
<td>Adenoid cystic carcinoma</td>
<td>-</td>
<td>+</td>
<td></td>
<td>Highlights biphasic nature (+ in basal cells)</td>
</tr>
</tbody>
</table>

**Highlights**
- Biphasic nature (+ in basal cells)

Polymorphous Adenocarcinoma – Typical Immunoprofile

- Extensive intraductal component distinguishes LGCCA from mammary analogue secretory carcinoma
Low Grade Cribriform Cystadenocarcinoma (LGCCA; Intraductal Carcinoma)

Intraductal Carcinoma (Low Grade Cribriform Cystadenocarcinoma): S100 +, SOX-10 +, AR -. No Relationship to Salivary Duct Carcinoma

p63 IHC in LGCCA and MASC

Low grade cribriform cystadenocarcinoma: smaller cystic spaces, almost entirely surrounded by basal cells

MASC: 30% of cases show preserved basal cells. Tumor lobules are larger & <10-20% of the circumference is surrounded by basal cells
Apocrine Nature of Salivary Duct Carcinoma, or, Practical Utility of Androgen Receptor Positivity

- 98% of Salivary Duct Carcinomas are AR"+"

WHO: approximately 70% of SDC are AR"+

Mimics of AR "-" SDC:

Other types of salivary carcinomas with high grade transformation:
- Comedo-necrosis ≠ SDC
- Search for better differentiated areas

Squamous cell carcinomas metastatic to intraparotid lymph nodes (p63 "+")

SDC: An Apocrine High Grade Adenocarcinoma

Apocrine Phenotype

AR Expression
Mimics of SDC: Adenoid Cystic Carcinoma with High Grade Transformation (HGT)

- AR-negative;
- Pulmonary metastasis with unequivocal morphology of adenoid cystic carcinoma;
- No MYB/NFIB translocation by FISH.

Mimics of SDC: MASC with High grade Transformation

Conventional MASC
Take Home Messages

• ENE and P16 drive new AJCC staging of head and neck carcinomas and terminology (oropharynx!)
• Relevant margins come from resection specimens
• Salivary tumors: new names, entities... made easier with S100, SOX-10, p63/p40, and AR

2017 WHO Classification of Head & Neck Tumours: What's New in the Classification of Odontogenic Pathology?

Elizabeth Bilodeau DMD, MD, MSEd
University of Pittsburgh

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WHO Blue Books

• 1971 Histologic Typing of Oral & Oropharyngeal Tumours
• 1992 Histologic Typing of Odontogenic Tumours
• 2005 Pathology & Genetics Head & Neck Tumours
• 2017 WHO Classification of Head & Neck Tumours

Overview of Changes in Odontogenic Classification

• Simplified
• Reclassifications
  – Keratocystic odontogenic tumor
  – Calcifying cystic odontogenic tumor
• Additions
  – 50% more entities
  – Cysts now (re)introduced
  – New entities
    • Sclerosing odontogenic carcinoma
    • Odontogenic carcinosarcoma
    • Primordial odontogenic tumor
  – Molecular pathogenesis
• Deletions
  – Ameloblastic fibro-odontoma
  – Ameloblastic fibro-dentinoma
  – Odontoma-meloblastoma

Malignant Tumours

<table>
<thead>
<tr>
<th>Odontogenic carcinomas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metastasizing (malignant) ameloblastoma</td>
</tr>
<tr>
<td>Ameloblastic carcinoma- Primary-type</td>
</tr>
<tr>
<td>Ameloblastic carcinoma- Secondary-type</td>
</tr>
<tr>
<td>Primary intraosseous SCC- solid</td>
</tr>
<tr>
<td>Primary intraosseous SCC- From KGCT</td>
</tr>
<tr>
<td>Primary intraosseous SCC- From odontogenic cysts</td>
</tr>
<tr>
<td>Clear cell odontogenic carcinoma</td>
</tr>
<tr>
<td>Ghid cell odontogenic carcinoma</td>
</tr>
<tr>
<td>Sclerosing odontogenic carcinoma</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Odontogenic Sarcomas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ameloblastic fibrosarcoma</td>
</tr>
<tr>
<td>Ameloblastic fibro-odontosarcoma</td>
</tr>
<tr>
<td>Odontogenic Carinosarcoma</td>
</tr>
</tbody>
</table>
Malignant Odontogenic Tumours

2005 WHO
- Odontogenic carcinomas
  - Metastasizing (malignant) ameloblastoma
  - Ameloblastic carcinoma: Primary type
  - Ameloblastic carcinoma: Secondary type
  - Odontogenic sarcomas: Intraosseous and peripheral
  - Primary intraosseous SCC: Solid
  - Primary intraosseous SCC: From KCOT
  - Primary intraosseous SCC: From odontogenic cysts
  - Clear cell odontogenic carcinoma
  - Ghost cell odontogenic carcinoma

2017 WHO
- Odontogenic carcinomas
  - Ameloblastic carcinoma
  - Primary intraosseous carcinoma, NOS
  - Sclerosing odontogenic CA
  - Clear cell odontogenic carcinoma
  - Ghost cell odontogenic carcinoma

Odontogenic Carcinosarcoma

Odontogenic Sarcomas

Clear Cell Odontogenic Carcinoma

- Clear cell odontogenic carcinoma (CCOC) is an uncommon intraosseous neoplasm seen in the jaws
- There is a predilection for the mandible & females
- Commonly presents in the 5th decade of life with swelling, +/- pain
- 1/3 of cases exhibit local/regional recurrence
- CCOCs have considerable histologic and immunophenotypic overlap with clear cell carcinoma (CCC)
CCC vs CCOC
Any distinguishing features?
Clear Cell Carcinoma and Clear Cell Odontogenic Carcinoma: a Comparative Clinicopathologic and Immunohistochemical Study

Elizabeth A. Blodow, DMD, MD* Haim Weinreb, MD† Cristina R. Amescua, MD‡
Lei Zhuang, PhD‖ Sarah Bual, MS‖ Simon Mulley, PhD‖ Breck Warner, PhD‡ and Anne B. Sevadjian, MD§

Am J Surg Pathol • Volume 37, Number 7, July 2013

Clear Cell Odontogenic Carcinomas
Show EWSR1 Rearrangements
A Novel Finding and a Biological Link to Salivary Clear Cell Carcinomas

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Positive</th>
<th>Negative</th>
<th>Fail</th>
<th>Overall, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCC</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>12/13 (92.3)</td>
</tr>
<tr>
<td>CCOC</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>5/16 (31.3)*</td>
</tr>
<tr>
<td>SRCCA</td>
<td>—</td>
<td>1</td>
<td>—</td>
<td>0/1 (0)</td>
</tr>
<tr>
<td>CC CECT</td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>0/3 (0)</td>
</tr>
<tr>
<td>CC SCC</td>
<td>—</td>
<td>1</td>
<td>—</td>
<td>0/1 (0)</td>
</tr>
</tbody>
</table>

*The rate of CCOC EWSR1 rearrangement was 31.3% (5/16) after 2 cases were reclassified as CC CECT.
Sclerosing Odontogenic Carcinoma

Case courtesy of Dr. Raja Seethala

Odontogenic Carcinoma w/ Dentinoid


Clear Cell Odontogenic Carcinoma 2017 WHO Updates

• “More than 80% of cases show rearrangement of EWSR1… ATF1 was confirmed as the fusion partner”

• “This is the same translocation found in CCC, and given their morphologic similarity, it has been theorized that these are related tumours”

• “Dentinoid has been reported in 7% of cases…occasional cases have shown extensive dentinoid and may be a separated entity”
There have been significant publications describing mutations in the MAPK & Hedgehog pathway in ameloblastomas

BRAF V600E in Ameloblastomas

- Present in 64% (110/172) of ameloblastomas reported
  - Higher frequency in mandibular ameloblastomas
- Predicts recurrence free survival
- These patients are frequently younger
- BRAF & RAS family mutations (KRAS, HRAS, NRAS, FGFR2) are mutually exclusive
- BRAF & SMO usually mutually exclusive
- 100% concordance reported with IHC & molecular testing

Mutations in Ameloblastoma
Significance of \textit{BRAF} V600E mutation in ameloblastoma

Anatomic Distribution of Mutations

\textbf{BRAF} V600E in other odontogenic tumors?

\begin{tabular}{|c|c|c|c|c|}
\hline
Tumor & Positivity & Brown et al. & Brunner et al. & Diniz et al. \\
\hline
AFO/AFDO & 42\% (9/21) & 3/3 & 6/18 & - \\
AF & 40\% (2/5) & - & 2/5 & - \\
AC & 31\% (4/13) & 0/1 & 1/4 & 3/8 \\
CCOC & 29\% (2/7) & 0/5 & 1/1 & 1/1 \\
\hline
\end{tabular}


WHO Classification, 2017 Odontogenic Tumours

Benign Tumors

- Odontogenic epithelium, mature fibrous stroma, w/o ectomesenchyme
  - Ameloblastoma
  - SOTs
  - CEOTs
  - AOTs
  - KCOTs

- Odontogenic epithelium, (ecto)mesenchyme w/ or w/o hard tissue
  - Ameloblastic fibroma
  - Ameloblastic fibro-odontoma
  - Ameloblastic fibrodentinoma
  - Odontoma
  - CCOT
  - Dentinogenic ghost cell tumor
  - Odontoameloblastoma
  - Primordial odontogenic tumor

Benign mixed epithelial & mesenchymal odontogenic tumors

- (Ecto)mesenchyme w/ or w/o odontogenic epithelium
  - Odontogenic fibroma
  - Odontogenic myxoma
  - Cementoblastoma
  - Cemento-ossifying fibroma

Previously in Bone-related tumors

- Cyst Cyst Hamartoma

Eliminated

- Odontoma
Benign Odontogenic Tumours

2005 WHO
- Odontogenic epithelium with mature, fibrous stroma w/o odontogenic ectomesenchyme
- Odontogenic epithelium w/ odontogenic ectomesenchyme w/ or w/o hard tissue formation
- Mesenchyme &/or odontogenic ectomesenchyme w/ or w/o odontogenic epithelium

2017 WHO
- Benign epithelial odontogenic tumors
- Benign mixed epithelial & mesenchymal odontogenic tumors
- Benign mesenchymal odontogenic tumors

2005 WHO
- Ameloblastoma
  - Peripheral
  - Desmoplastic
  - Unicystic
  - Giant cell
  - SO T
  - CEOT
  - AOT
  - KCO T
- Ameloblastic fibroma
- Ameloblastic fibro-odontoma
- Ameloblastic fibrodentinoma
- Odontoma
  - Complex
  - Compound
- Odontoameloblastoma
- CCOT
- Dentinogenic Ghost Cell Tumor

2017 WHO
- Benign epithelial odontogenic tumors
  - Ameloblastoma
    - Unicystic
    - Peripheral
    - Desmoplastic
    - Giant cell
  - SO T
  - CEOT
  - AOT
- Benign mixed epithelial & mesenchymal odontogenic tumors
  - Primordial odontogenic tumor
  - Odontoma
    - Compound
    - Complex
- Dentinogenic Ghost Cell Tumor
- Benign mesenchymal odontogenic tumors
  - Odontogenic fibroma
  - Odontogenic myxoma/myxofibroma
  - Cementoblastoma
  - Cemento-ossifying fibroma

NEW-WHO Classification, 2017
Cysts

Inflammatory origin
- Radicular cyst
- Periapical cyst
- Paradoxal cyst
- Buccal bifurcation
- Gingival cyst

Odontogenic & non-odontogenic developmental cysts
- Dentigerous cyst
- Odontogenic keratocyst
- Orthokeratinized odontogenic cyst
- Nasopalatine duct cyst
- Odontogenic intraosseous cyst
Keratocystic Odontogenic Tumor (Odontogenic Keratocyst)

- Cystic lesions that present in the 3rd decade of life
- Asymptomatic, or may be associated with pain and swelling
- Predilection for posterior mandible (angle/ramus)
- Grows in an anteroposterior direction
- Associated with nevoid basal cell carcinoma syndrome

Keratocystic Odontogenic Tumor (& Dentigerous cyst)

Keratocytic Odontogenic Tumor
Hedgehog pathway

Nevoid Basal Cell Carcinoma Syndrome (Gorlin syndrome)

- AD with incidence of 1:57,000-164,000
- Nevoid basal cell carcinomas
- Multiple KCOTs/OKCs
- Palmoplantar pitting
- Calcified falx
- Medulloblastoma (desmoplastic)
- Bifid, fused, or markedly splayed ribs
- 1st degree relative with Gorlin syndrome
- Skeletal abnormalities
- Other tumors
  - Medulloblastoma, meningioma, rhabdomyosarcoma, etc.
Nevoid Basal Cell Carcinoma Syndrome (Gorlin syndrome)

PTCH1 gene mutations in KCOT (OKC)

<table>
<thead>
<tr>
<th>First author, Year (Ref. No.)</th>
<th>No. cases</th>
<th>PTCH1 mutations</th>
<th>Mutation frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barner, 2000 [12]</td>
<td>10</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>Vorechovsky, 2002 [14]</td>
<td>10</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>Pan, 2007 [16]</td>
<td>8</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>Pan, 2009 [17]</td>
<td>8</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>Pan, 2010 [18]</td>
<td>20</td>
<td>13</td>
<td>65</td>
</tr>
<tr>
<td>This study</td>
<td>19</td>
<td>16</td>
<td>84</td>
</tr>
</tbody>
</table>

- PTCH1 mutations were previously reported by other groups.
- PTCH1 mutations were previously reported by our group.

• PTCH1 mutation somatically acquired KCOT ~30%
  - Range 25-84%
  - Qu et al. 16/19 (84%) separated lining from the capsule

• PTCH1 mutation on 9q22.3-q31 ~90% syndromic KCOT
  - 1st “hit” inherited as a germline mutation
  - Functions as a tumor suppressor gene
  - Less common PTCH2 1p34.1 or SUFU 10q24.32

### PTCH alterations in other odontogenic cysts & tumors

- LOH studies/IHC
  - Orthokeratinized odontogenic cysts
  - Calcifying epithelial odontogenic tumors
  - Dentigerous cysts


### Hedgehog pathway

**Targeted Therapy**

<table>
<thead>
<tr>
<th>Hedgehog pathway inhibitors</th>
<th>Vismodegib (Erivedge®)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BCCs/KCOTs &amp; Targeted Therapy</strong></td>
<td>Hedgehog pathway inhibitors - Vismodegib</td>
</tr>
</tbody>
</table>

### The Use of Vismodegib to Shrink Keratocystic Odontogenic Tumors in Patients With Basal Cell Nevus Syndrome

- **Table:** Vismodegib treatment effects and biomarker changes
  - **Patient No.:** Basal Cell Nevoid Syndrome (BCCs/KCOTs) & targeted therapy
  - **Vismodegib:** 200 mg orally daily
  - **Biomarkers:**
    - **Hedgehog pathway:** 
      - **Change in effector activity:**
    - **Cyclooxygenase 2 (COX-2):**
    - **Microphthalmia transcription factor (MITF):**
    - **Vismodegib:**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Vismodegib Treatment</th>
<th>Basal Cell Nevoid Syndrome</th>
<th>Hedgehog pathway</th>
<th>COX-2</th>
<th>MITF</th>
<th>Change in effector activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Before</td>
<td>50</td>
<td>10</td>
<td>55</td>
<td>1.2</td>
<td>0.7</td>
</tr>
<tr>
<td>2</td>
<td>After</td>
<td>45</td>
<td>10</td>
<td>55</td>
<td>1.2</td>
<td>0.7</td>
</tr>
<tr>
<td>3</td>
<td>Before</td>
<td>40</td>
<td>10</td>
<td>55</td>
<td>1.2</td>
<td>0.7</td>
</tr>
<tr>
<td>4</td>
<td>After</td>
<td>35</td>
<td>10</td>
<td>55</td>
<td>1.2</td>
<td>0.7</td>
</tr>
<tr>
<td>5</td>
<td>Before</td>
<td>30</td>
<td>10</td>
<td>55</td>
<td>1.2</td>
<td>0.7</td>
</tr>
<tr>
<td>6</td>
<td>After</td>
<td>25</td>
<td>10</td>
<td>55</td>
<td>1.2</td>
<td>0.7</td>
</tr>
</tbody>
</table>

*Research study on the effects of Vismodegib treatment on Hedgehog pathway inhibitors.*

*Research study on the effects of Vismodegib treatment on COX-2.*

*Research study on the effects of Vismodegib treatment on MITF.*
Questions?

1016-17: A Brave New World In Head & Neck Pathology: The Milan System

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Emory University School of Medicine
Atlanta, Georgia

DISCLOSURE

In the past 12 months, I have not had any significant financial interest or other relationship with the manufacturers of the products or providers of the services that will be discussed in my presentation.
Salivary Gland Tumors

- Salivary gland tumors are uncommon
- Heterogeneous ranging from benign to high grade malignancies
- Most salivary gland tumors are benign neoplasms (~75%)
- Rate of malignancy varies by gland
  - Parotid gland – 20-25%
  - Submandibular gland – 40-50%
  - Minor glands – up to 80%

Utility of Salivary Gland Cytology

- Preoperative triage
- High specificity in differentiating¹...
  - Benign versus malignant (97%)
  - Neoplastic versus non-neoplastic (98%)
- Limit unnecessary surgical excision in some cases²
- Guide extent of surgical excision in combination with frozen section³,⁴

Limitation of Salivary Gland FNA

- Ability to provide specific diagnosis is limited, particularly for malignant tumors⁵,⁶
  - Extensive morphologic overlap with basaloid neoplasms
  - Some malignant tumors have low grade / subtle cytologic features or depend on identification of invasion
    - Adenocarcinoma
    - Low grade mucoepidermoid carcinoma
    - Basal cell adenocarcinoma
- Descriptive terminology is commonly used
  - Lacks uniform diagnostic terminology
  - Limits clinical utility in some cases
  - Limits data collection regarding risk
Recently Proposed Classification Schemes for Salivary Gland Cytopathology

- The Milan System for Reporting Salivary Gland Cytopathology
- Others with traditional cytologic categories
- A pattern based risk stratification scheme
  - Griffith CC et al. AJCP. 2015

The Milan System for Reporting Salivary Gland Cytopathology

- Recently proposed by international group sponsored by ASC & IAC
- Category based using traditional cytologic categories
- Goals:
  - Standardize reporting
  - Provide associated risk of malignancy
  - Provide clinical management algorithm
- Bethesda style atlas expected later this year (2017)

Proposed Milan System Categories

<table>
<thead>
<tr>
<th>Diagnostic Category</th>
<th>Risk of Malignancy*</th>
<th>Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Diagnostic</td>
<td>10-20%</td>
<td>Correlation/repeat FNA</td>
</tr>
<tr>
<td>Non-neoplastic</td>
<td>0-20%</td>
<td>Clinical follow-up</td>
</tr>
<tr>
<td>AUS (atypical)</td>
<td>TBD (28-53%)</td>
<td>Repeat FNA vs surgery</td>
</tr>
<tr>
<td>Neoplastic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benign</td>
<td>5-7%</td>
<td>Conservative surg vs f/u</td>
</tr>
<tr>
<td>Uncertain Malignant Potential</td>
<td>20-40%</td>
<td>Conservative surgery</td>
</tr>
<tr>
<td>Suspicious for malignancy</td>
<td>70-80%</td>
<td>Surgery</td>
</tr>
<tr>
<td>Malignant</td>
<td>85-95%</td>
<td>Surgery (HG vs LG)</td>
</tr>
</tbody>
</table>

* Estimated risks of malignancy based on Milan group literature review
Non-Diagnostic

- Not precisely defined currently
  - “insufficient quantity/quality to make cytologic diagnosis”
- ≤10% of aspirates is goal
- Includes aspirates with:
  - Only benign elements which fail to explain the presence of a tumor (sialoadenosis is one exception)
  - Cyst contents only (non-mucinous)

- Recommendation: repeat aspiration with image guidance versus clinical and radiologic correlation

Cystic Salivary Gland Lesions

- Benign (neoplastic and non-neoplastic) and malignant lesions may be cystic
- Common non-neoplastic cysts:
  - Ductal retention cyst
  - Branchial cleft cyst
- Neoplasms may also be cystic:
  - Warthin tumor
  - Low grade mucoepidermoid carcinoma
- Aspiration of non-neoplastic cysts may result in resolution of the lesion
- Residual mass lesions should be aspirated

Non-Neoplastic

- Differential diagnosis includes:
  - Inflammatory (sialadenitis)
  - Reactive changes (reactive lymph node)

- Recommendation: Clinical follow-up and radiologic correlation to ensure adequate sampling of lesion
Common Non-Neoplastic Lesions and Morphologic Features

- Acute sialadenitis
  - Neutrophils, fibrin, debris, reactive epithelial cells
- Chronic sialadenitis
  - Hypocellular, cohesive ductal cells, inflammation
- Nodular oncocytic hyperplasia
  - Cohesive clusters of bland oncocyes
  - Overlaps with neoplasia
- Reactive lymph node
  - Polymorphous lymphocytes, macrophages, germinal centers
  - Consider flow cytometry in some cases
- Granulomatous inflammation

Atypia of Undetermined Significance

- Use extremely rarely (<10%)
- Heterogenous group with variable findings:
  - Cases approaching inadequate but with rare, highly atypical cells
  - Specimens compromised by poor quality
  - Mucinous cyst contents without epithelium
- Should NOT be used clearly neoplastic lesions with atypia
- Primary differential considerations:
  - Reactive changes
  - Poorly sampled malignancy

Neoplasm

- Divided into benign neoplasm and salivary gland neoplasm of uncertain malignant potential (SUMP)
- Reflects importance of identifying neoplasia in salivary gland cytology
Benign Neoplasm

- Use in cases of specifically diagnosable benign neoplasms
  - Pleomorphic adenoma
  - Warthin tumor
  - Myoepithelioma
  - Lipoma
- Very low risk of malignancy expected for tumors in this group
- Recommendation: conservative surgery versus clinical follow-up

Salivary Gland Neoplasm of Uncertain Malignant Potential

- Cytologic features are diagnostic of a neoplasm but specific classification not possible
- Basaloid tumors will often be SUMP:
  - Cellular pleomorphic adenoma / myoepithelioma
  - Basal cell adenoma and adenocarcinoma
  - Adenoid cystic carcinoma
- Recommendation: conservative surgery

Suspicious for Malignancy

- Highly suggestive but not diagnostic of malignancy
- Expected to mostly represent poorly sampled high grade malignancies
- Recommendation: surgical excision (frozen section may be useful)
Malignant

- Diagnostic of malignancy
- Primary salivary gland carcinoma should be defined as low grade versus high grade
- Includes other malignancies:
  - Metastasis
  - Lymphoma
  - Sarcoma
- Recommendation: surgical excision

Other Classification Schemes for Salivary Gland Cytology

- Traditional cytologic categories
- A pattern based risk stratification scheme
  - Griffith CC et al. AJCP. 2015

A Pattern Based Approach to Salivary Cytology: A Potential Supplement to Milan

- Identifies common benign neoplasms
  - Pleomorphic adenoma
  - Warthin tumor
- Separates basaloid and oncocytoid neoplasms
  - Divides basaloid neoplasm by stromal features
    - Fibrillary stroma
    - Non-fibrillary stroma
  - Divides oncocytoid neoplasms by cytoplasmic and background features
    - Cystic background
    - Mucinous background
    - Granular or vacuolated cytoplasm
- Identifies overtly malignant high grade tumors
Pattern Based Approach Categories

<table>
<thead>
<tr>
<th>Pattern Based Approach Categories</th>
<th>N (254)</th>
<th>Malignant (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsatisfactory/Non-Diagnostic</td>
<td>83</td>
<td>18.1% (15)</td>
</tr>
<tr>
<td>Cyst Contents Only</td>
<td>31</td>
<td>14.9% (5)</td>
</tr>
<tr>
<td>Non-Neoplastic</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>Pleomorphic Adenoma</td>
<td>49</td>
<td>4.2% (2)</td>
</tr>
<tr>
<td>Monomorphic Cellular Basaloid Neoplasm</td>
<td>30</td>
<td>36.7% (11)</td>
</tr>
<tr>
<td>with Papillary Struma</td>
<td>13</td>
<td>23.4% (3)</td>
</tr>
<tr>
<td>with Myxoid Struma</td>
<td>7</td>
<td>42.9% (3)</td>
</tr>
<tr>
<td>with Myxoid/Otherside</td>
<td>10</td>
<td>40% (4)</td>
</tr>
<tr>
<td>Pleomorphic Basaled Neoplasm</td>
<td>4</td>
<td>100% (4)</td>
</tr>
<tr>
<td>Warthin Tumor</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td>Monomorphic Oncocytoid Neoplasm:</td>
<td>40</td>
<td>90% (12)</td>
</tr>
<tr>
<td>with Cyst Content Background</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>with Other Background</td>
<td>19</td>
<td>21.1% (4)</td>
</tr>
<tr>
<td>with Mucinous Background</td>
<td>10</td>
<td>80% (8)</td>
</tr>
<tr>
<td>Oncocytoid Neoplasm, Granular/Vacuolated</td>
<td>13</td>
<td>84.6% (11)</td>
</tr>
<tr>
<td>Pleomorphic Oncocytoid Neoplasm:</td>
<td>23</td>
<td>100% (21)</td>
</tr>
</tbody>
</table>

Adapted from Griffith et al. AJCP 2015

Differential Diagnosis by Pattern

<table>
<thead>
<tr>
<th>Pattern Based Approach Categories</th>
<th>Differential Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monomorphic Basaloid Neoplasm:</td>
<td></td>
</tr>
<tr>
<td>with Papillary Struma</td>
<td>Cellular Pleomorphic Adenoma</td>
</tr>
<tr>
<td>with Myxoid/Otherside</td>
<td>Adenoid Cystadenoma</td>
</tr>
<tr>
<td>Pleomorphic Basaloid Neoplasm:</td>
<td>Salivary Gland Carcinoma</td>
</tr>
<tr>
<td>Monomorphic Oncocytoid Neoplasm:</td>
<td>Salivary Duct Carcinoma</td>
</tr>
<tr>
<td>with Cyst Content Background</td>
<td>Ossifying Adenocarcinoma</td>
</tr>
<tr>
<td>with Mucinous Background</td>
<td>Metastatic Breast Carcinoma</td>
</tr>
<tr>
<td>with Other Background</td>
<td>Salivary Duct Carcinoma</td>
</tr>
<tr>
<td>Oncocytoid Neoplasm, Granular/Vacuolated</td>
<td>Mammary Acinic Carcinoma</td>
</tr>
<tr>
<td>Pleomorphic Oncocytoid Neoplasm:</td>
<td>High Grade Mucoepidermoid Carcinoma</td>
</tr>
<tr>
<td>with papillary/Myxoid Struma</td>
<td>Metastatic Breast Carcinoma</td>
</tr>
<tr>
<td>with Cyst Content Background</td>
<td>Mammary Acinic Carcinoma</td>
</tr>
<tr>
<td>with Mucinous Background</td>
<td>Salivary Duct Carcinoma</td>
</tr>
<tr>
<td>with Other Background</td>
<td>Salivary Duct Carcinoma</td>
</tr>
</tbody>
</table>

Adapted from Griffith et al. AJCP 2015

Pattern Based Approach for Salivary FNA: When to Implement

- Primary salivary gland epithelial neoplasms
  - Exclude non-neoplastic aspirates
  - Exclude metastatic lesions when possible
    - Morphology
    - History
    - Immunostains
  - Exclude lymphoproliferative processes and sarcoma
A Pattern Based Approach to Salivary Cytology: A Potential Supplement to Milan

- Can be applied to aspirates that are positive for neoplasm
  - i.e. Positive for neoplasm
    - Oncocytoid neoplasm with mucinous background
- Further improve clinical relevance of salivary FNA
- Limit differential diagnostic considerations and direct ancillary studies
- Provide more granular risks of malignancy and high grade malignancy within a given category

Milan + Pattern Based Cytology

<table>
<thead>
<tr>
<th>Milan Category (est. ROM)</th>
<th>Pattern Based Categories (est. ROM*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Diagnostic (10-20%)</td>
<td>Non-Neoplastic (0-20%)</td>
</tr>
<tr>
<td>Non-Neoplastic (5-7%)</td>
<td></td>
</tr>
<tr>
<td>AUS (TBD)</td>
<td></td>
</tr>
<tr>
<td>Neoplasm</td>
<td></td>
</tr>
<tr>
<td>Benign (8-7%)</td>
<td>Pleomorphic adenoma (4.1%)</td>
</tr>
<tr>
<td></td>
<td>Warthin tumor (0)</td>
</tr>
<tr>
<td></td>
<td>Oncocytoid neoplasm with cystic background (0)</td>
</tr>
<tr>
<td>Uncertain/Malignant Potential (30-40%)</td>
<td>Oncocytoid neoplasm with other background (21.1%)</td>
</tr>
<tr>
<td>Suspicious for Malignancy (70-80%)</td>
<td>Basaloid neoplasm with non-fibrillary stroma (42.9-60%)</td>
</tr>
<tr>
<td>Malignant (85-95%)</td>
<td>Oncocytoid neoplasm with granular/vacuolated cytoplasm (84.6%)</td>
</tr>
<tr>
<td></td>
<td>Pleomorphic basaloid neoplasm (100%)</td>
</tr>
<tr>
<td></td>
<td>Pleomorphic oncocytoid neoplasm (100%)</td>
</tr>
</tbody>
</table>

* Est. ROM from Griffith et al. AJCP 2015

Conclusions

- Aspiration cytology is useful to aid in treatment of salivary gland tumors
- Definitive diagnosis is challenging in some cases
- Classification schemes such as the Milan system offer standardized terminology and treatment options
- A Pattern based approach is also useful to further limit differential diagnostic considerations
- Such schemes even if not implemented completely can provide a framework to improve one’s approach to salivary aspirates
THANK YOU!

References


