Validation of *BRAF* Mutational Analysis in Thyroid Fine Needle Aspirations: A Morphologic-Molecular Approach

Kerry C. Councilman, MD
Assistant Professor
University of Colorado Denver
Goals: BRAF Mutation Validation Study

• To confirm that Diff Quick stained, direct cytology smears from thyroid can be used for both diagnosis and molecular assays—similar to liquid based samples.

• To show that thyroid cell-specific high-quality DNA can be obtained using pathologist directed microdissection methods for enrichment.

• To compare mutational data from cytology material and surgical pathology sample, in order to validate cytology assay methods.
<table>
<thead>
<tr>
<th>Category</th>
<th>Risk</th>
<th>Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>&lt;1%</td>
<td>Clinical follow-up</td>
</tr>
<tr>
<td>ACUS/FLUS</td>
<td>5-10%</td>
<td>Repeat FNA</td>
</tr>
<tr>
<td>Follicular neoplasm</td>
<td>20-30%</td>
<td>Lobectomy</td>
</tr>
<tr>
<td>Suspicious</td>
<td>50-75%</td>
<td>Thyroidectomy</td>
</tr>
<tr>
<td>Malignant</td>
<td>100%</td>
<td>Thyroidectomy</td>
</tr>
</tbody>
</table>
BRAF Mutation

• Most common genetic alteration in PTC (~45%)
• The most frequent mutation is C.1799T>A (V600E) is basis of assay
• BRAF mutation activates MAPK, promoting cancer formation
**BRAF Mutation Testing of Thyroid Nodules**

- Typically used only for indeterminate cases
- Approximately half of PTC have *BRAF* mutation
- *BRAF* mutation >95% PPV for PTC

### Methodological Approaches

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liquid Based</strong></td>
<td>Ease of transfer</td>
<td>May need extra pass</td>
</tr>
<tr>
<td></td>
<td>Superior DNA</td>
<td>Unknown cell composition</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Must prepare up front</td>
</tr>
<tr>
<td><strong>Cell Block</strong></td>
<td>Validated like surgical</td>
<td>Few cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>May need extra pass</td>
</tr>
<tr>
<td><strong>Direct Smear</strong></td>
<td>Visualize target cells</td>
<td>Slide sacrifice</td>
</tr>
<tr>
<td></td>
<td>Superior DNA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Routinely prepared</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cell specific DNA Enrichment</td>
<td></td>
</tr>
</tbody>
</table>
Processing the DQ Slide
Cytology Cases Tested for *BRAF* Mutation: Validation Study

<table>
<thead>
<tr>
<th>Cytologic Dx</th>
<th>N</th>
<th>Mutation +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>37</td>
<td>5/37</td>
</tr>
<tr>
<td>PTC</td>
<td>9</td>
<td>5/9</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>17</td>
<td>0/17</td>
</tr>
<tr>
<td>Negative</td>
<td>11</td>
<td>0/11</td>
</tr>
</tbody>
</table>
Validation: Concordance Data

- Comparison of FNA cytology and surgical pathology specimen derived BRAF data to validate methodology
  - 100% mutation negative FNA cases were negative (on follow-up surgical when available)
  - 1/9 PTC case was positive in FNA, negative in surgical resection
    - Possible explanations
      - Tumor heterogeneity: Sampled multiple areas of the final resection specimen, some areas (+), other areas (-)
      - Sampling
      - Multiple papillary cell clones may represent separate collision tumors
Summary/Conclusions

• Microdissection of pathologist directed cells from DQ stained direct smear slide is as effective as FFPE sample BRAF mutation analysis.

• Advantages:
  - FNA material can be used to determine if surgery is necessary on indeterminate lesions.
  - Uses routinely prepared and stained cytology smears.
  - Cytopathologist directed cell harvesting.
References

• Nikiforova MN, Nikiforov YE. Molecular diagnostics and predictors in thyroid cancer. *Thyroid.* 2009;19:1351-1361
• Nikiforov YE, et al. Mutational Profiling of Thyroid FNA Samples. *J Clin Endocrinol Metab.* 2011;96:0000
Acknowledgments

• CMOCO (Colorado Molecular Correlates) Laboratory
  – Dara Aisner, MD, PhD
  – Wilbur Franklin, MD
  – Natalie Thomas
  – Justin Bohn
  – Patrick Chesnut
• Department of Pathology
  – Ann Thor, MD
  – Sherif Said, MD, PhD
• Department of Endocrinology
  – Brian Haugen, MD
  – Joshua Klopper, MD
• Photography
  – Lisa Litzenberger
Indeterminates

• Of 17 indeterminate cytology cases tested (all \textit{BRAF}–negative) 8 had surgical follow-up
  – All remained \textit{BRAF} -negative on surgical follow-up
  – Expected as \textit{BRAF} -positive mutation rate is low in indeterminate nodules
      – Suspicious: 19.2%
      – Follicular neoplasm: ~1%
      – ACUS: ~2%
    Our validation study:
      – 1 Suspicious
      – 4 Follicular neoplasm
      – 12 ACUS