NGS and Thyroid FNA: Utility, Implementation and Pitfalls

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Management of Patients with Thyroid Nodules

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Benign FNA

Indeterminate
20-30% risk of malignancy

Malignant

Benign history
Clinical Follow-up

Diagnostic Lobectomy

Malignant histology
Total thyroidectomy

Modified from Nishino M. Cancer Cytopathology 2015
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Modified from Nishino M. Cancer Cytopathology 2015
Next Generation Sequencing

- Sequence DNA/RNA in massively parallel configuration
- Interrogates multiple regions of genome at once
- Rapid and cost-effective
- High sensitivity
- Quantitative
- Used in clinical setting
Next Generation Sequencing Approaches

Whole genome, Exome, Transcriptome

• Discovery tools
• Expensive
• Time consuming
• Requires large amount of DNA/RNA
• Complex BI analysis and results interpretation
Transcriptome Analysis in Thyroid FNA Samples

- 9 thyroid FNA samples, negative for known alterations, 30 ng of RNA
- Novel fusions detected in 7/9 FNAs
Targeted Next Generation Sequencing

• Sequencing of multiple preselected genes or gene regions
• Requires small amount of NA (fewer number of cells)
• Faster TAT (faster sequencing and data analysis)
• Used in clinical setting
## Targeted Next Generation Sequencing

<table>
<thead>
<tr>
<th>DNA/RNA isolation</th>
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<tbody>
<tr>
<td>NGS library</td>
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<tr>
<td>preparation</td>
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<tr>
<td>Sequencing, BI Analysis</td>
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</tbody>
</table>
NGS technology allows for detection of all types of genetic alterations in a single workflow.

NGS

Point mutations
Indels

Copy number alteration

Gene fusions

Gene expression

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Molecular Alterations in Thyroid Cancer: Diagnostic

TCGA study of PTC

![Diagram showing genomic alterations in thyroid carcinoma](image)

- **Point mutations**: 74%
- **Gene fusions**: 15%

**Gene Mutations and Fusions**
- BRAF
- NRAS
- HRAS
- KRAS
- EIF1AX
- TERT
- TP53
- PTEN
- PIK3CA
- RET
- PPARG
- NTRK1
- NTRK3

Giordano T et al, Cell, 2014; 159:679-90
Molecular Alterations in Thyroid Cancer: Prognostics


Song YS et al. Cancer (2016)

Xing M et al. JCO (2014)

Song YS et al. Cancer (2016)
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Multiple High-Risk Mutations Detected in Thyroid FNA Samples are Associated With Aggressive Cancer

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35 (55%) BRAF + Another HR mutation
18 (29%) RAS + Another HR mutation
3 (5%) Other Multiple HR mutations

55 (98%) Thyroid Cancer

51 (93%) Cancers with Aggressive Features:
• Extrathyroidal extension (55%)
• Vascular invasion (53%)
• Lymph node macrometastasis (47%)
• Poorly differentiated/anaplastic carcinoma areas (14%)
• Distant metastasis (8%)

American Thyroid Association Annual Meeting, 2016
Molecular Alterations in Thyroid Cancer: Therapeutics

Utility of Thyroid NGS Test

- **Diagnostic:** to improve accuracy of diagnosis in thyroid nodules with indeterminate cytology, eliminate diagnostic lobectomy

- **Prognostic:** to identify tumors with aggressive behavior

- **Predictive:** to select appropriate targeted therapy

**Personalized Management of Thyroid Cancer**
NGS Panels for Thyroid FNA Samples

**Ion AmpliSeq™**
- *Cancer hot spot panel (ThermoFisher Scientific)*
- 50 genes

**ThyroSeq**
- *Thyroid specific panel (UPMC)*
- 56 genes
NGS Utility for Diagnosis of Medullary Thyroid Carcinoma and Parathyroid Tissue in Thyroid FNA Samples

4,765 FNA samples (Bethesda III-V) analyzed by ThyroSeq® Genomic Classifier

- 21 (0.4%) FNAs showed MTC profile
  - 5 FLUS/AUS
  - 6 FN/SFN
  - 10 SMC
  - Surgery (n=13)
  - 13/13 cases diagnosed as MTC (100% PPV)

- 26 (0.6%) FNAs showed PT lesion profile
  - 20 FLUS/AUS
  - 6 FN/SFN
  - Surgery (n=10)
  - 10/10 cases diagnosed as Parathyroid Lesions (100% PPV)

American Thyroid Association Annual Meeting, 2016

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Implementation of NGS Test

- Custom NGS panel design vs. commercial product
- NGS instruments/sequencing platform/sequencing chemistry
- Analytical validation
  - Analytical sensitivity and specificity
  - Limits of detection
  - Depth of sequencing
- Clinical validation (NPV, PPV)
• Performed according to CLIA, CAP, and NY State Department of Health
• 413 tissue and FNA samples
• Analytical sensitivity >99% (CI: 96-100%)
• Analytical specificity >99% (CI: 98-100%)
• Validated in FNA, FRZ and FFPE tissues, >500x depth of coverage
• Limits of Detection (LOD) is 3-5% for SNVs, 1% for fusions
NGS Test Clinical Validation

Performance in AUS/FLUS (Bethesda III) Cytology Nodules

- Negative Predictive Value (NPV): 96%
- Positive Predictive Value (PPV): 83%

Performance in FN/SFN (Bethesda IV) Cytology Nodules

- Negative Predictive Value (NPV): 96%
- Positive Predictive Value (PPV): 83%

Nikiforov et al. Thyroid 2015

Nikiforov et al. Cancer 2014
NGS Testing Challenges in Clinical Setting

- FNA sample size
- Cell preservation
- Quality control
- Result annotation
- TAT
- Billing/reimbursement issues
Clinical Case: 60 year-old female with incidentally noted 1.5 cm thyroid nodule and another 0.6 cm nodule

Isthmus - Mixed solid and cystic nodule

Cytology:
- Isthmus nodule
  - Benign
- Right lobe nodule
  - Atypia of Undetermined Significance

AUS/FLUS:
- Cancer risk: 5-15%
- Repeat FNA?
- Diagnostic lobectomy?
- Observation?

Right lobe nodule submitted for NGS analysis
Clinical Case: 60 year-old female with incidentally noted 1.5 cm thyroid nodule and another 0.6 cm nodule

NGS Analysis:

<table>
<thead>
<tr>
<th>Gene</th>
<th>cDNA</th>
<th>Protein</th>
<th>Allelic Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF</td>
<td>c.1799T&gt;A</td>
<td>p.V600E</td>
<td>37%</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>c.3140A&gt;G</td>
<td>p.H1047R</td>
<td>21%</td>
</tr>
<tr>
<td>AKT1</td>
<td>c.49G&gt;A</td>
<td>p.E17K</td>
<td>6%</td>
</tr>
<tr>
<td>TERT</td>
<td>c.1-124C&gt;T</td>
<td>-</td>
<td>77%</td>
</tr>
</tbody>
</table>

Co-occurrence of \( \text{BRAF V600E, TERT, PIK3CA, AKT1} \) mutations:
- ~100% probability of cancer
- Increased risk of aggressive disease

Total Thyroidectomy:
- Papillary carcinoma, 0.6 cm
- Extrathyroidal extension
- Positive resection margin

Diagnostic and prognostic application

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THANK YOU