Molecular Diagnostics in Thyroid Tumors

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Outline

• Overview of genetic alterations in thyroid cancer
• Diagnostic mutational markers in thyroid nodules
• Prognostic mutational markers for thyroid cancer
Well Differentiated Cancer
Poorly Differentiated/AC

PI3K/AKT pathway

MAPK pathway

RTK

TSHR

Adenylate cyclase

cAMP

PKA

α

β

γ

PI3K/AKT

β

γ

Adenylate cyclase

cAMP

PKA

α

β

γ

PI3K/AKT

β

γ

Adenylate cyclase

cAMP

PKA

α

β

γ

PI3K/AKT

β

γ

Adenylate cyclase

cAMP

PKA

α

β

γ

PI3K/AKT

β

γ

Adenylate cyclase

cAMP

PKA

α

β

γ

PI3K/AKT

β

γ

Adenylate cyclase

cAMP

PKA

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β

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PI3K/AKT

β

γ

Adenylate cyclase

cAMP

PKA

α

β

γ

PI3K/AKT

β

γ

Adenylate cyclase

cAMP

PKA

α

β

γ

PI3K/AKT

β

γ

Adenylate cyclase

cAMP

PKA

α

β

γ

PI3K/AKT

β

γ

Adenylate cyclase

cAMP

PKA

α

β

γ

PI3K/AKT

β

γ

Adenylate cyclase

cAMP

PKA

α

β

γ

PI3K/AKT

β

γ

Adenylate cyclase

cAMP

PKA

α

β

γ

PI3K/AKT

β

γ
Somatic Mutations in Thyroid Cancer

- NRAS
- PIK3CA
- TP53
- NTRK1
- PPARγ
- BRAF
- HRAS
- KRAS
- PTEN
- RET
- BRAF

Intrachromosomal rearrangement
Interchromosomal rearrangement
Point mutation
Frequency of Mutations in Thyroid Cancer

Papillary Carcinoma

- None
- RAS 15%
- RET/PTC 15%
- BRAF 45%

75% of tumors with known mutations

Follicular Carcinoma

- None
- RAS 40%
- PAX8/PPARY 30%

70% of tumors with known mutations

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BRAF Mutations

- **K601E**
- **VK600-1E**
- **V599Ins**
- **V600D- FGLAT601-605Ins**
- **T599I-VKSR600-3del**

**AKAP9/BRAF**

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Prevalence: 40-45% in PTC and 20-30% in PDC/AC
RET/PTC Rearrangement

RET receptor

Chromosome 10

Frequency of RET/PTC types

RET/PTC1 70%
RET/PTC2 <3%
RET/PTC3 30%

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Prevalence and Specificity of RET/PTC Rearrangement

**Clonal RET/PTC:** present in >1% of tumor cells

- Standard Sensitivity RT-PCR
- FISH with appropriate cut-off levels
- Southern Blot

**Non-clonal RET/PTC:** present in <1% of tumor cells

- FISH with appropriate cut-off levels
- High Sensitivity RT-PCR

Zhu et al. *JCEM* 2006, 91:3603-10
Prevalence and Specificity of RET/PTC Detection

- Prevalence of **clonal** RET/PTC:
  - Adults: 10-20% (reported range 0-80%)
  - Children, sporadic: 40-50%
  - Children, h/o radiation: 50-80%

- Specificity:
  - **Clonal** rearrangement specific for papillary CA
  - Non-clonal rearrangement found in other lesions
RAS Mutations

- Point mutations
- Most common hotspots in thyroid: NRAS codon 61
  HRAS codon 61
  KRAS codons 12/13

<table>
<thead>
<tr>
<th></th>
<th>Goiter</th>
<th>Follicular adenoma</th>
<th>Follicular Carcinoma</th>
<th>Papillary Carcinoma</th>
<th>PDCA and AC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>0-20%</td>
<td>20-45%</td>
<td>30-50%</td>
<td>0-20%*</td>
<td>20-90%</td>
</tr>
<tr>
<td>Average</td>
<td>5%</td>
<td>30%</td>
<td>40%</td>
<td>15%</td>
<td>30%</td>
</tr>
</tbody>
</table>

*Almost all are follicular variants of PTC

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Meta-analysis of literature 2003-2009
• t(2;3)(q13;p25)
• Fusion involves PAX8 and PPARγ genes

Meta-analysis of literature 2003-2009

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I. Single mutation:
   - BRAF
   - RET/PTC
   - RAS

II. Panel of mutations:
    BRAF, RET/PTC, RAS, PAX8/PPARg
Management of Patients with Thyroid Nodules

1. Identification of Thyroid Nodule
   - Amenable to FNA?

2. Assess TSH
   - TSH Suppressed
   - TSH Unsuppressed

3. Ultrasound Guided Ultrasound Guided FNA
   - FNA cytology

4. RAI Scan
   - Cold Nodule Present?

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Thyroid Nodules and FNA Cytology

Bethesda Classification

<table>
<thead>
<tr>
<th>Diagnostic Category</th>
<th>Risk of malignancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>&lt;3%</td>
</tr>
<tr>
<td>Malignant</td>
<td>&gt;98%</td>
</tr>
</tbody>
</table>

**Indeterminate cytology**

- Follicular lesion of undetermined significance (FLUS) 5-10%
- Follicular neoplasm/lesion 20-30%
- Suspicious for malignancy 50-75%

- Nondiagnostic

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BRAF V600E is Highly Specific for Malignancy

### Specificity of BRAF in Thyroid FNAs

<table>
<thead>
<tr>
<th>Reported Studies</th>
<th>Samples (n)</th>
<th>BRAF-positive (n)</th>
<th>Final Surgical Pathology Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 FNA Prospective</td>
<td>1814</td>
<td>159</td>
<td>PTC: 159 (100%)</td>
</tr>
<tr>
<td>7 FNA retrospective</td>
<td>685</td>
<td>291</td>
<td>PTC: 291 (100%)</td>
</tr>
<tr>
<td>2 Research FNAs</td>
<td>267</td>
<td>131</td>
<td>PTC: 130 (99.2%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HN: 1 (0.8%)</td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td><strong>2766</strong></td>
<td><strong>581</strong></td>
<td>PTC: 580 (99.8%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HN: 1 (0.2%)</td>
</tr>
</tbody>
</table>
Testing for a Panel of Mutations in Thyroid FNA Samples

**BRAF**
- V600E
- K601E

**RAS**
- NRAS codon 61
- HRAS codon 61
- KRAS codons 12/13

**RET/PTC**
- RET/PTC1
- RET/PTC3

**PAX8/PPARγ**

**Controls:**
- Quality and quantity of DNA and RNA
- Quantity of epithelial cells

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# Performance of Mutational Panel in Thyroid FNA Samples

<table>
<thead>
<tr>
<th>Prospective FNA Study</th>
<th>Location</th>
<th>Number of FNA Samples/ Sample Type</th>
<th>Number of Mutations Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nikiforov et al, JCEM 2009</td>
<td>Cincinnati, Denver, USA</td>
<td>470 FNAs consecutive, all types</td>
<td>32 (7%)</td>
</tr>
<tr>
<td>Cantara et al, JCEM 2010</td>
<td>Siena, Italy</td>
<td>285 FNAs from patients with surgery</td>
<td>67 (29%)</td>
</tr>
<tr>
<td>Nikiforov et al, (submitted)</td>
<td>Pittsburgh, USA</td>
<td>1149 FNAs consecutive, indeterminate and positive cytology</td>
<td>126 (11%)</td>
</tr>
</tbody>
</table>
# Performance of Mutational Panel in Thyroid FNA Samples

<table>
<thead>
<tr>
<th>Propective FNA Studies</th>
<th>BRAF (n=123)</th>
<th>RAS (n=79)</th>
<th>RET/PTC (n=20)</th>
<th>PAX8/PPARγ (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nikiforov et al, JCEM 2009</strong></td>
<td>100%</td>
<td>87%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Cantara et al, JCEM 2010</strong></td>
<td>100%</td>
<td>74%</td>
<td>100%</td>
<td>-</td>
</tr>
<tr>
<td><strong>Nikiforov et al, (submitted)</strong></td>
<td>100%</td>
<td>84%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>100%</strong></td>
<td><strong>83%</strong></td>
<td><strong>100%</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

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RAS Mutations

Molecular
- RAS+ (71)
- Surgery (67)

Cytology
- Indeterminate (64)
  - FLUS (19)
  - FN/FL (25)
  - Suspicious (10)
  - Negative (3)

Histology
- PTC (52)
- FTC (4)
- FA (11)

84%
16%

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RAS Mutations in Follicular Variant PTC
Molecular Testing in FLUS Cytology

513 samples

124 (24%) FLUS

117 (94%) acceptable for molecular analysis

12/12 (100%)

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Positive 12 (10%)

RAS - 8
BRAF - 3
PAX8/PPARg - 1

Negative 105 (90%)

8/105 (7.6%)

Revised Management Guidelines for Patients with Thyroid Nodules and Differentiated Thyroid Cancer

*American Thyroid Association, 2009*

[A12] Indeterminate cytology (follicular or Hürthle cell neoplasm follicular lesion of undetermined significance, atypia).

**RECOMMENDATION 8**

(a) The use of molecular markers (e.g., BRAF, RAS, RET/PTC, Pax8-PPARγ, or galectin-3) may be considered for patients with indeterminate cytology on FNA to help guide management. Recommendation rating: C
Prognostic Molecular Markers
BRAF V600E Is Associated with Tumor Recurrence and Mortality

<table>
<thead>
<tr>
<th></th>
<th>BRAF +  (n=107)</th>
<th>BRAF -  (n=112)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extrathyroidal Extension</td>
<td>41%</td>
<td>16%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LN Metastases</td>
<td>54%</td>
<td>21%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tumor Stage III or IV</td>
<td>30%</td>
<td>14%</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Xing M et al. *JCEM* (2005)
BRAF V600E Predisposes to Tumor Dedifferentiation

BRAF V600E Is Associated with ¹³¹I-Refractory Disease

Mutational frequency of BRAF, RET/PTC, NRAS, HRAS, KRAS, AKT1, and PIK3CA in (A) 18 primary ATC, (B) 34 primary PDTC, and (C) 23 RAIR, FDG-PET-positive PDTC.

Should BRAF-positive PTCs be Treated Differently?

- Retrospective review
  - 106 consecutive patients with BRAF+ PTC compared to a cohort of 100 BRAF- patients
- 75 BRAF+ patients had unknown BRAF status preoperatively
  - 12 received lobectomy then required completion
  - 6 required re-operation for persistent disease within 2 years

Yip L et al. *Surgery* 2009;146:1215
Should BRAF-positive PTCs be Treated Differently?

- 18 of 75 patients (24%) would have received different treatment had BRAF status been known pre-operatively.
- BRAF-positivity more than triples the need for re-operation within the first 2 years of follow up.

Yip L et al. Surgery 2009;146:1215
Potential *BRAF* Impact on Patient Management

- Initial surgical management of PTC, including microcarcinoma
  - Total thyroidectomy, possible central compartment l/n dissection
- Initial radioiodine treatment
  - Higher dose of iodine
- Follow-up of patients
  - TSH level in low subnormal range

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Xing M. *Mol Cell Endocrinol* (2005)
Papillary Thyroid Microcarcinoma (PTMC)

**WHO Definition:**
- Papillary carcinoma 1 cm or less in size
- Incidental finding

**Incidence in autopsy series:**
- 0.5-13% in North America
- 19-28% in Japan
- 36% in Finland

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## Some PTMCs are Aggressive

<table>
<thead>
<tr>
<th>Publication</th>
<th>Patient No. (Follow-up)</th>
<th>Lymph Node Metastases</th>
<th>Distant Metastases</th>
<th>Tumor Recurrence</th>
<th>Cancer-Related Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hay et al. (1992)</td>
<td>535 (16 y)</td>
<td>32%</td>
<td>0.4%</td>
<td>5%</td>
<td>0.4%</td>
</tr>
<tr>
<td>Baudin et al. (1999)</td>
<td>281 (7.3 y)</td>
<td>43%</td>
<td>2.8%</td>
<td>3.9%</td>
<td>0</td>
</tr>
<tr>
<td>Sugitani et al. (1999)</td>
<td>178 (10.7 y)</td>
<td>ND</td>
<td>2.2%</td>
<td>ND</td>
<td>2.2%</td>
</tr>
<tr>
<td>Ito et al. (2003)</td>
<td>732 (1.6-4 y)</td>
<td>48%</td>
<td>0</td>
<td>2.6%</td>
<td>0</td>
</tr>
<tr>
<td>Roti et al. (2006)</td>
<td>243 (5.1 y)</td>
<td>13%</td>
<td>1.6%</td>
<td>1.6%</td>
<td>0</td>
</tr>
<tr>
<td><strong>Summary of 11 studies</strong></td>
<td><strong>4432</strong></td>
<td><strong>28% (12-70%)</strong></td>
<td><strong>0.7% (0-2.8%)</strong></td>
<td><strong>5% (1.6-26%)</strong></td>
<td><strong>0.3% (0-2.2%)</strong></td>
</tr>
</tbody>
</table>

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Mazzaferri EL. *Endocr Pract* (2007)
Molecular and Histopathological Features of Aggressive and Non-Aggressive PTMCs

<table>
<thead>
<tr>
<th></th>
<th>AGGRESSIVE PTMCs N= 29</th>
<th>NON-AGGRESSIVE PTMCs N= 30</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF</td>
<td>20/26 (77%)</td>
<td>8/25 (32%)</td>
<td>0.0016</td>
</tr>
<tr>
<td>ANATOMIC LOCATION</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- LEFT LOBE/ RIGHT LOBE</td>
<td>15 (52%) / 14 (48%)</td>
<td>14 (47%) / 14 (47%)</td>
<td>0.4495</td>
</tr>
<tr>
<td>SUPERFICIAL TUMOR LOCATION</td>
<td>27 (93%)</td>
<td>9 (30%)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>POSITIVE MARGIN</td>
<td>2 (7%)</td>
<td>1 (3%)</td>
<td>0.4876</td>
</tr>
<tr>
<td>COMPLETE CAPSULE</td>
<td>2 (7%)</td>
<td>8 (27%)</td>
<td>0.0469</td>
</tr>
<tr>
<td>INFILTRATIVE BORDER</td>
<td>29 (100%)</td>
<td>24 (80%)</td>
<td>0.0174</td>
</tr>
<tr>
<td>MICROSCOPIC VARIANT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- CLASSIC PAPILLARY</td>
<td>13 (45%)</td>
<td>11 (37%)</td>
<td>0.3546</td>
</tr>
<tr>
<td>- FOLLICULAR VARIANT</td>
<td>8 (28.0%)</td>
<td>18 (60%)</td>
<td>0.0124</td>
</tr>
<tr>
<td>- TALL CELL FEAT/VAR</td>
<td>8 (28%)</td>
<td>1 (3%)</td>
<td>0.0129</td>
</tr>
<tr>
<td>INTRATHYROIDAL SPREAD/MF</td>
<td>26 (90%)</td>
<td>13 (43%)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>TUMOR FIBROSIS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- NO</td>
<td>2 (7%)</td>
<td>9 (30%)</td>
<td>0.026</td>
</tr>
<tr>
<td>- YES, 2-3+</td>
<td>24 (83%)</td>
<td>12 (40%)</td>
<td>0.001</td>
</tr>
<tr>
<td>EXTRATHYROIDAL EXTENSION</td>
<td>19 (66%)</td>
<td>4 (13%)</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

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Niemeyer LA et al. (submitted)
Statistical Analysis: Multivariate Regression Model

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>Std. Error</th>
<th>Z value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BRAF mutation</strong></td>
<td>2.69</td>
<td>1.20</td>
<td>2.24</td>
<td>0.024*</td>
</tr>
<tr>
<td><strong>Superficial tumor location</strong></td>
<td>3.67</td>
<td>1.26</td>
<td>2.91</td>
<td>0.004*</td>
</tr>
<tr>
<td><strong>Intraglandular spread/Multifocality</strong></td>
<td>3.12</td>
<td>1.29</td>
<td>2.41</td>
<td>0.016*</td>
</tr>
<tr>
<td><strong>Fibrosis (2+)</strong></td>
<td>1.69</td>
<td>1.18</td>
<td>1.43</td>
<td>0.15</td>
</tr>
</tbody>
</table>

**MP Score** = 3x **BRAF** + 4x **Superf. location** + 3x **IGS/MF** + 2x **Fibrosis (≥2+)**

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Niemeyer LA et al. (submitted)
Histopathologic Features of Aggressive Papillary Thyroid Microcarcinomas

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Superficial Location

Fibrosis 2+

Niemeyer LA et al. (submitted)
Intraglandular spread/Multifocality

Histopathologic Features of Aggressive Papillary Thyroid Microcarcinomas

Tumor foci in normal thyroid tissue adjacent to main tumor nodule

Isolated psammoma body

Lymphatic invasion

Niemeyer LA et al. (submitted)
Molecular-Pathological Score To Define TPMC Aggressiveness

MP Score

<table>
<thead>
<tr>
<th>BRAF Mutation +</th>
<th>x3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superficial (subcapsular) location</td>
<td>x4</td>
</tr>
<tr>
<td>Intrathyroidal spread/MF</td>
<td>x3</td>
</tr>
<tr>
<td>Tumor fibrosis, ≥ 2+</td>
<td>x2</td>
</tr>
</tbody>
</table>

Validation in independent set of 40 TPMCs

- 70% Aggressive TPMCs
- 25% Not aggressive
- 5% Not aggressive

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Niemeyer LA et al. (submitted)
Conclusions
And Future Directions

Diagnostic use of mutational markers:
• High PPV for cancer (close to 100%, except RAS ~85%)
• Need to define distinct algorithms of patient management based on cytology + molecular analysis

Prognostic mutational markers:
• BRAF V600E tumors - surgical and post-surgical management
• Additional markers of tumor aggressiveness

Future directions:
• Discovery of new mutations in thyroid cancer to completely resolve uncertainty of indeterminate cytology