Molecular pathology of thyroid cancers

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Thyroid nodules

- 4-7% of population with palpable nodules
- More frequent in women
- Incidence increasing with age
- 5-10% of solitary cold nodules are malignant

The question is which one will become malignant?
Thyroid cancer

- 1.5-2.1% of all newly diagnosed cancers
- Increasing in number during the last 25 years:
  - From 4.8 to 8.0 persons per 100,000
  - 11.7 females, 4.2 males /100,000
Worldwide Incidence and Mortality by Cancer Type

Most Frequent Cancers Worldwide (2008): Both Sexes

- Incidence
- Mortality

New thyroid cases diagnosed in 2012: 298,000 (2.1%)

Classification of thyroid cancers

Of follicular origin:

- Differentiated
  - Papillary 80%
  - Follicular 10%
  - Hürthle cell 3-5%

- Non-differentiated
  - Anaplastic 1-2%

Of parafollicular origin:

- Medullary 5%
Thyroid cancer and US

- hypodensity
- microcalcification
- hypervascularization
- solitary
- irregular borders
- lack of halo sign

The more of the above signs present, the more likely the nodule is malignant.
FNAB

- FNAB is the most reliable in determining dignity
- 4 result categories:
  - non-diagnostic
  - benign
  - uncertain: AUS/FLUS; FN/SFN; SMC
  - malignant
- Limitations of FNAB:
  - No differentiation between benign follicular or Hürthle cell adenoma or malignant versions
  - False negative: < 5%
  - 10-40%!
Genetic tests

- Genetic alterations are causative factors
- Genetic alterations are consequences of cancer

Is genetic testing a good marker for malignancy?
Main transduction pathways in thyroid cancer
General scheme of thyroid tumorigenesis

Differentiated thyroid cancers

- PTC and FTC comprise 90% of all thyroid malignancies.
- PTC is the most common histological type of all thyroid malignancies (60-80%). Somatic mutations are found in more than 40-70% of papillary carcinoma cases.
- FTC is the second most common histological type with a frequency is 10-15%. Mutation is present in 30-50%.

Genetic alterations in PTC

PTC:
- BRAF (v-raf murine sarcoma viral oncogene homolog B1)
- RET/PTC (RET tyrosine-kinase protooncogene / papillary thyroid carcinoma)
- RAS (rat sarcoma viral oncogene homolog) mutációk

BRAF mutation is thought to correlate with tumor aggressivity:
- extrathyroidal growth
- lymphnode involvement
- radioiodine resistance
- tumor reoccurrence
Genetic alterations in FTC

FTC:
- RAS
Risk of Cancer in nodules positive for RAS mutations

**Table 1. Molecular Abnormalities Detected in AUS/FLUS FNA Samples and Associated Cancer Risk**

<table>
<thead>
<tr>
<th>Mutation Type</th>
<th>Number of mutation-positive samples</th>
<th>Nodules with surgical outcome</th>
<th>Cancer on surgery (cancer risk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRAS</td>
<td>7</td>
<td>5</td>
<td>3 (60%)</td>
</tr>
<tr>
<td>HRAS</td>
<td>7</td>
<td>6</td>
<td>5 (83%)</td>
</tr>
<tr>
<td>KRAS</td>
<td>3</td>
<td>3</td>
<td>3 (100%)</td>
</tr>
<tr>
<td>PTEN</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>EIF1AX</td>
<td>2</td>
<td>2</td>
<td>1 (50%)</td>
</tr>
<tr>
<td>BRAF V600E</td>
<td>1</td>
<td>1</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>BRAF K601E</td>
<td>1</td>
<td>1</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>TSHR</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gene fusions:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>THADA</td>
<td>3</td>
<td>3</td>
<td>2 (67%)</td>
</tr>
<tr>
<td>PPARG</td>
<td>1</td>
<td>1</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>NTRK3</td>
<td>1</td>
<td>1</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>NTRK1</td>
<td>1</td>
<td>1</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>ALK</td>
<td>1</td>
<td>1</td>
<td>1 (100%)</td>
</tr>
</tbody>
</table>

**79%**

RAS mutation-positive „benign” nodules

Evidence for clonal neoplasm and early transformation to cancer

Nikiforov et al. Thyroid 2015. Sept 10. [Epub]
Genetic alterations in FTC

FTC:
- RAS
- RET/PTC
- PAX8/PPAR-gamma mutations

RAS family influencing 3 signaling pathways:
- MAP-kinase
- phosphatidylinositol-3-kinase/protein-kinase-B (PI3K/AKT)
- adhesion and migration

Mutated RAS protein elicits GTP-ase effect and a consequent constitutive activation of follicular cell proliferation (genomic instability, increased growth potential, tumor development).
Other genetic alterations in thyroid cancer

A RET proto-oncogene encodes a thyrosine-kinase transmembrane receptor.

PTC:
- RET/PTC1 fusion protein results in reduced malignancy
- RET/PTC2 rare
- RET/PTC3 fusion protein results in enhanced malignancy

A PAX8 (paired box 8) gene encodes a transcriptional factor that has a role in the tissue differentiation.
The PAX8/PPAR-gamma-1 gene rearrangements has been observed in FTC.
TERT point mutations in thyroid cancer

- 469 patients with FCDTC
- Mean follow-up 7.8 ± 5.8 y

Melo M et al. JCEM 2014:99(5):E754-65
Identification of driving ALK fusion genes and genomic landscape of medullary thyroid cancer

Typical genotype-phenotype relationships in differentiated thyroid cancer

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Histological Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ret/PTC Family</td>
<td>Classic PTC</td>
</tr>
<tr>
<td>Ret/PTC 1</td>
<td>Classic PTC</td>
</tr>
<tr>
<td></td>
<td>Often radiation related</td>
</tr>
<tr>
<td>Ret/PTC 3</td>
<td>Solid variant PTC</td>
</tr>
<tr>
<td></td>
<td>Often radiation related</td>
</tr>
<tr>
<td>TRK</td>
<td>PTC</td>
</tr>
<tr>
<td></td>
<td>Often radiation related</td>
</tr>
<tr>
<td>ALK Fusions</td>
<td>Poorly differentiated thyroid cancer</td>
</tr>
<tr>
<td></td>
<td>Anaplastic thyroid cancer</td>
</tr>
<tr>
<td>PAX8-PPARγ</td>
<td>Follicular thyroid cancer</td>
</tr>
<tr>
<td></td>
<td>Follicular variant, papillary thyroid cancer</td>
</tr>
<tr>
<td></td>
<td>Follicular adenoma</td>
</tr>
<tr>
<td>RAS</td>
<td>Follicular thyroid cancer</td>
</tr>
<tr>
<td></td>
<td>Follicular variant, papillary thyroid cancer</td>
</tr>
<tr>
<td></td>
<td>Poorly differentiated thyroid cancer</td>
</tr>
<tr>
<td></td>
<td>Anaplastic thyroid cancer</td>
</tr>
<tr>
<td></td>
<td>Follicular adenoma</td>
</tr>
<tr>
<td>BRAF V600E</td>
<td>Tall cell variant PTC</td>
</tr>
<tr>
<td></td>
<td>Classic PTC</td>
</tr>
<tr>
<td>PI3K/AKT</td>
<td>Follicular thyroid cancer</td>
</tr>
<tr>
<td></td>
<td>Poorly differentiated thyroid cancer</td>
</tr>
<tr>
<td></td>
<td>Anaplastic thyroid cancer</td>
</tr>
<tr>
<td>PTEN</td>
<td>Poorly differentiated thyroid cancer</td>
</tr>
<tr>
<td></td>
<td>Anaplastic thyroid cancer</td>
</tr>
<tr>
<td>TERT</td>
<td>PTC</td>
</tr>
<tr>
<td></td>
<td>Poorly differentiated thyroid cancer</td>
</tr>
<tr>
<td></td>
<td>Anaplastic thyroid cancer</td>
</tr>
<tr>
<td></td>
<td>Hürthle cell carcinoma</td>
</tr>
</tbody>
</table>

# Genetic alterations in thyroid cancers in Hungary

**Age (year)**

<table>
<thead>
<tr>
<th></th>
<th>n=177</th>
<th>men (n=52)</th>
<th>52.9 ± 15.9</th>
<th>women (n=125)</th>
<th>50.3 ± 14.7</th>
</tr>
</thead>
</table>

**Number of DNA samples**

<table>
<thead>
<tr>
<th>Type of Cancer</th>
<th>Number of Samples</th>
<th>BRAF</th>
<th>HRAS</th>
<th>KRAS</th>
<th>NRAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>papillary cc.</td>
<td>154</td>
<td>59 (38.3%)</td>
<td>3 (1.95%)</td>
<td>1 (0.9%)</td>
<td>2 (1.3%)</td>
</tr>
<tr>
<td>follicular cc.</td>
<td>16</td>
<td>2 (16.7%)</td>
<td>1 (8.3%)</td>
<td>0</td>
<td>4 (25.0%)</td>
</tr>
<tr>
<td>other cc.</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (14.3%)</td>
</tr>
<tr>
<td>normal tissue</td>
<td>163</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>total</strong></td>
<td><strong>340</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Number of RNA samples**

<table>
<thead>
<tr>
<th>Type of Cancer</th>
<th>Number of Samples</th>
<th>RET/PTC1</th>
<th>RET/PTC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>papillary cc.</td>
<td>97</td>
<td>7 (7.2%)</td>
<td>2 (2.1%)</td>
</tr>
<tr>
<td>follicular cc.</td>
<td>16</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>other cc.</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>normal tissue</td>
<td>120</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>total</strong></td>
<td><strong>240</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**No PAX8/PPARγ!**

Tobias B et al, Pathol Oncol Res. 2015 Aug 11. Epub ahead of print
CYP24A1 gene expression in PTC

- CYP24A1 is a calcitriol-neutralizing enzyme
- Increased expression in colon and liver cc

CYP24A1 gene expression in PTC compared to normal thyroid tissues

CYP24A1 gene expression in PTC compared to normal thyroid tissues

CYP24A1 expressional activity is highly proportional to tumor aggressivity!

Follow-up study

- FNAB samples collected
- 779 samples collected (cytologically benign)
- Genetic alteration examined
- At least 3 yrs of follow-up planned
- Frequency of genetic alterations, incidence of malignancies recorded
- Can genetic alterations predict malignancy?
Follow-up of 779 FNAB samples

Genetic alterations | Frequency
--- | ---
BRAF | 39
NRAS | 23
HRAS | 9
KRAS | 1
RET/PTC3 | 73

Specificity: 93.3%, sensitivity 46.2%, negative predictive value is 96%
Follow-up of 504 FNAB samples

<table>
<thead>
<tr>
<th>Genetic alterations</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF</td>
<td>12</td>
</tr>
<tr>
<td>NRAS</td>
<td>5</td>
</tr>
<tr>
<td>HRAS</td>
<td>7</td>
</tr>
<tr>
<td>KRAS</td>
<td>1</td>
</tr>
<tr>
<td>RET/PTC3</td>
<td>1</td>
</tr>
</tbody>
</table>

Specificity: 96.4%, sensitivity 30% negative predictive value is 95.6%
Follow-up of 250 FNAB samples

<table>
<thead>
<tr>
<th>Genetic alterations</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF</td>
<td>4</td>
</tr>
<tr>
<td>NRAS</td>
<td>1</td>
</tr>
<tr>
<td>HRAS</td>
<td>7</td>
</tr>
<tr>
<td>KRAS</td>
<td>1</td>
</tr>
<tr>
<td>RET/PTC3</td>
<td>1</td>
</tr>
</tbody>
</table>

Specificity: 96.2%, sensitivity 38.5%, negative predictive value is 96.6%
**Single gene/limited gene mutation panel**
**conventional sequencing**

**BRAF only**

- **Gene mutations (DNA):**
  - BRAF
  - NRAS
  - HRAS
  - KRAS

**Sensitivity 45-50%; Specificity >99%**

Meta-analysis of 16170 pts/9924 FNA samples

**7-8 gene panels**

- **Gene mutations (DNA):**
  - RET/PTC1
  - RET/PTC3
  - PAX8/PPA
  - RG

**Sensitivity 60-70%; Specificity 60-95%**

Nikiforov et al. JCEM 2009;94:2092
Cantara et al. JCEM 2010;95:1365
Nikiforov et al. JCEM 2011;96:3390
Giordano et al. Hum. Pathol. 2014;45:1339
Molecular testing from FNAB

- Cleveland Clinic TSHR mRNA assay
- Veracyte Afirma Gene Classifier (167-gene panel)
- Asuragen miRInform (7-gene panel)
- ThyroSeq v.2 (56-gene panel)
- PentaCore Thyreon (7-gene panel)
**ThyroSeq v.2 (56-gene panel)**

<table>
<thead>
<tr>
<th>Gene mutations (DNA)</th>
<th>Gene Fusions (RNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF</td>
<td>RET</td>
</tr>
<tr>
<td>NRAS</td>
<td>TSHR</td>
</tr>
<tr>
<td>HRAS</td>
<td>AKT1</td>
</tr>
<tr>
<td>KRAS</td>
<td>TP53</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>GNAS</td>
</tr>
<tr>
<td>PTEN</td>
<td>CTNNB1</td>
</tr>
<tr>
<td>TERT</td>
<td>EIF1AX</td>
</tr>
</tbody>
</table>

- RET
- PPARG
- NTRK1
- NTRK3
- BRAF
- ALK
- Other

<table>
<thead>
<tr>
<th>Gene expression (RNS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGK1</td>
</tr>
<tr>
<td>KRT7</td>
</tr>
<tr>
<td>TG</td>
</tr>
<tr>
<td>TTF1</td>
</tr>
<tr>
<td>NIS</td>
</tr>
<tr>
<td>Calcitonin</td>
</tr>
<tr>
<td>PTH</td>
</tr>
<tr>
<td>KRT20</td>
</tr>
<tr>
<td>Other</td>
</tr>
</tbody>
</table>

- **14 genes for mutations, > 1000 hotspots**
- **42 fusion types**
- **16 genes for expression**

Nikiforov et al. Thyroid 2015. Sept 10. [Epub]
ThyroSeq v2 performance in AUS/FLUS cytology nodules

143 consecutive FN/SFN nodules with surgery

Retrospective group
- Mutation NEGATIVE n=64
  - CANCER n=2
  - BENIGN n=62
  - Sensitivity 92%
  - Specificity 94%
  - PPV 85%
  - NPV 97%
  - Accuracy 93%
- Mutation POSITIVE n=27
  - CANCER n=23
  - BENIGN n=4

Prospective group
- Mutation NEGATIVE n=37
  - CANCER n=2
  - BENIGN n=35
  - Sensitivity 86%
  - Specificity 92%
  - PPV 80%
  - NPV 95%
  - Accuracy 90%
- Mutation POSITIVE n=15
  - CANCER n=12
  - BENIGN n=3

Overall test performance
- Sensitivity 90% (CI: 80-99%)
- Specificity 93% (CI: 88-98%)
- PPV 83% (CI: 72-95%)
- NPV 96% (CI: 92-95%)
- Accuracy 92% (CI: 88-97%)

Nikiforov et al. Cancer 2014; 120:3627-34
Citology

FNA

FLUS (BC III)

Cancer risk
10-15%

FN/SFN (BC IV)

20-30%

SUSP (BC V)

50-70%

ThyroSeq v2

- 

- 

+ 

+ 

Modified cancer risk

3% 

75-99% 

4% 

75-99% 

20% 

90-99%

Recommended management

Observation

Therapeutic surgery

Observation

Therapeutic surgery

Lobectomy

Therapeutic surgery
<table>
<thead>
<tr>
<th></th>
<th>ThyroSec v2</th>
<th>PentaCore Thyreon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>90</td>
<td>46</td>
</tr>
<tr>
<td>Specificity</td>
<td>93</td>
<td>96</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cancers missed by indeterminate cytology category</th>
<th>ThyroSec v2</th>
<th>PentaCore Thyreon</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUS/FLUS</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>FN/SFN</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>SMC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gene classifier (%)</th>
<th>Molecular panel (%)</th>
<th>TSHR mRNA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>92</td>
<td>60</td>
</tr>
<tr>
<td>Specificity</td>
<td>52</td>
<td>98</td>
</tr>
</tbody>
</table>

Sensitivity and specificity are reported for each method. The percentage of cancers missed based on indeterminate cytology category is shown.

Sources: All data from Refs. 4, 7, and 11.

TSHR mRNA, thyrotropin receptor messenger RNA; AUS/FLUS, atypia/follicular lesion of undetermined significance; FN/SFN, follicular or Hürthle cell neoplasm/suspicious for follicular neoplasm; SMC, suspicious for malignant cells; n/a, not applicable.
Cancer risk stratification based on molecular profiling

High
- Multiple driver mutations (e.g. BRAF and PIK3CA)
- TP53
- TERT

Intermediate
- ALK fusions
- NTRK1 fusions
- NTRK3 fusions
- BRAF V600E
- RET/PTC
- RAS
- PTEN
- BRAF K601E
- PAX8/PPARG

Low
Contribution of molecular information to thyroid diagnostics and therapy

- Additional information to FNAB or histology
- Prediction of malignancy in thyroid nodules
- Individual treatment options (e.g. thyrosine kinase inhibition)
Thank you for your attention!