Thyroid cancer: an update on cytology, histology and molecular pathology

Belgium Thyroid Club, April 28, 2012

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Medical Faculty of the University of Porto, Hospital S. João and IPATIMUP (Institute of Molecular Pathology and Immunology of the U.Porto)

The importance of understanding
MOLECULAR PATHOLOGY

MOLECULAR GENETICS

MOLECULAR MEDICINE

MOLECULAR GENETICS - germline DNA alterations

MOLECULAR PATHOLOGY - somatic, non-hereditary genetic alterations

Sobrinho-Simões M, American Board of Pathology, S. Francisco, 2001
The primary occurrence of BRAF(V600E) is a rare clonal event in papillary thyroid carcinoma


• Using a more sensitive approach than Sanger sequencing (pyrosequencing) it was shown that only a fraction of tumor cells (44.7% - 5%) displayed the BRAF V600E mutation

• BRAF mutated tumours are genetically heterogenous

• The BRAF V600E mutation in PTCs occurs as a late clonal event during tumor development.
• Benign VS Malignant VS Borderline (Uncertain malignant potential – UMP)

• Lobectomy VS Lobectomy plus isthmectomy VS Total thyroidectomy

• Radioactive iodine: Yes or No?

• Targeted therapies: When and Which one(s)?

Cytology
Histology
+ Molecular Pathology
THYROID CARCINOMAS

Follicular carcinoma
Papillary carcinoma
(Hürthle cell carcinoma)

Medullary carcinoma
Poorly differentiated ca

Undifferentiated ca

WHO book on Endocrine Tumours, 3rd edition, 2004
Thyroid carcinoma is the most frequent endocrine tumour
Clinically evident papillary carcinoma >80% of thyroid carcinomas
Occult papillary carcinoma (OPC) >99% of thyroid carcinomas
(1/6 of OPC have lymph node metastases)

What's the Fastest Growing Cancer Diagnosis in U.S.?

By Simeon Margolis, M.D., Ph.D

Posted Fri, Sep 05, 2008, 9:54 am PDT
Major problems in thyroid oncology

• Separation of follicular cell from C-cell derived tumours

• Risk stratification in pre-malignant lesions
  Nodular C cell hyperplasia versus micro medullary ca
  Incipient foci of malignancy in an otherwise benign lesion

• Diagnosis of malignancy

• Prognosis

• Therapy selection
<table>
<thead>
<tr>
<th>Follicular cell</th>
<th>Conventional Papillary ca</th>
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<tr>
<td></td>
<td>RET/PTC (mainly RET/PTC1) and TRK rearrangements</td>
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<tr>
<td></td>
<td>BRAF mutations (V600E)</td>
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<tr>
<td></td>
<td>C-MET and EGF-R overexpression</td>
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<td></td>
<td>mTOR overexpression</td>
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<td>TGFβ and STAT 3 alterations</td>
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<td>E-cadherin downregulation</td>
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</table>

- **Irradiation**
- **Other factors**
  - No chromosomal instability
  - Diploidy; Near diploidy
Papillary thyroid carcinoma oncogene (RET/PTC) alters the nuclear envelope and chromatin structure.

Hashimoto thyroiditis

RET/PTC positive
Hashimoto thyroiditis

68% RET/PTC positivity

Rhoden KJ. J Clin Endocrinol Metab 91:2414-23, 2006
RET/PTC rearrangements arising from a small population of papillary thyroid carcinoma cells, possible candidate for passenger mutation

Tadao Nakazawa · Shin-ichi Murata · Tetsuo Kondo · Dongfeng Niu · Kunio Mochizuki · Tomonori Kawasaki · Tetsu Yamane · Nobuki Nakamura · Ryohei Katoh
BRAF mutations in about 70% of melanocytic nevi

BRAF may lead to senescence
Several other pathways: PI3K/Akt, NFκβ, mTOR, STAT3
Two main paradigms for understanding human diseases

1. Core biological processes associated with a disease are driven by responses to changes in a small number of genes.
2. Disease states are considered as emergent properties of molecular networks originating from a very complex interplay between constellations of changes in DNA and a broad range of factors such as diet, age, gender and exposure to environmental toxins.

Schadt EE, Nature, September 10, 2009
Cancer genome landscapes

Driver genes & Passenger genes

Weaknesses of the concept of oncogene addiction

- Multiple genetic and epigenetic abnormalities (genomic instability)
- Tumour heterogeneity
- Differences among stem cells and their progeny (differences in their intracellular circuitry)
- Differences in the host microenvironment

Felsher DW, Oncogene addiction versus oncogene amnesia: Perhaps more than just a bad habit? Cancer Res 68:3081, 2008
### CANCER GENES

<table>
<thead>
<tr>
<th>Oncogenes</th>
<th>Driver genes</th>
<th>Gatekeepers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour-suppressor genes</td>
<td>Passenger genes (Different qualities of passengers...)</td>
<td>Caretakers</td>
</tr>
<tr>
<td>“Secondary” genetic alterations</td>
<td></td>
<td>Landscapers</td>
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<td></td>
<td>Forerunners</td>
<td>Late bloomers</td>
</tr>
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</table>

PLUS THE ROLE PLAYED BY THE STROMA AND THE IMMUNE CELLS THAT CONTRIBUTE TO (IN)ACTIVATE GENES IN A CONTEXT DEPENDENT MANNER
mTOR pathway overactivation in BRAF mutated papillary thyroid carcinoma


STAT3 Negatively Regulates Thyroid Cancer Growth In vivo (Xenografts and Transgenic Models)

...We observed that 58% of 59 human primary papillary thyroid carcinoma (PTC) cases expressed nuclear pSTAT3 in tumor cells, preferentially in association with the tumor stroma. ...STAT3 knockdown by shRNA in representative thyroid cancer cell lines (8505C, TPC-1, HTH7 and SW1736) that express high levels of pSTAT3 had no effect on in vitro growth. However, xenografted shSTAT3 cells generated larger tumors than shControl cells. Similarly, STAT3 deficiency in a murine model of BRAFV600E-induced PTC led to thyroid tumors that were more proliferative and larger than those expressing STAT3wt.

Couto JP et al. (submitted)
Chromosomal, epigenetic and microRNA-mediated inactivation of LRP1B, a modulator of the extracellular environment of thyroid cancer cells.


Oncogene 30:1302-17, 2011
TGF-beta/Smad pathway and BRAF mutation play different roles in circumscribed and infiltrative papillary thyroid carcinoma


• Transforming growth factor beta (TGF-beta)/Smad dependent pathway activity at the periphery of infiltrative PTCs is associated with epithelial-to-mesenchymal transition (EMT) and local invasion, as well as to nodal metastization
240 cases (1978-2003) with nodal and/or distant metastases

NOT A SINGLE CASE OF:

Follicular tumour of uncertain malignant potential

Well differentiated tumour of uncertain malignant potential

Minimally invasive follicular carcinoma without vascular invasion

Encapsulated follicular variant of PTC without invasion

Consortium IPO-IPATIMUP, 2011 (unpublished results)
Major problems in thyroid oncology

• Separation of follicular cell from C-cell derived tumours

• Risk stratification in pre-malignant lesions

• Diagnosis of malignancy

• Prognosis

• Therapy selection
At variance with PTC, fine needle aspiration cytology and frozen section rarely give useful informations in FTC
Impact of mutational testing on the diagnosis and management of patients with cytologically indeterminate thyroid nodules: A prospective analysis of 1056 FNA samples


• Adequate material in 967 samples with indeterminate cytology: atypia of undeterminate significance, follicular neoplasm/suspicious for a follicular neoplasm and suspicious for malignant cells

• Results: 87 mutations – 19 BRAF, 62 RAS, 1 RET/PTC and 5 PAX8/PPARY

• The detection of any mutation conferred higher risk of malignancy
The PAX8-PPARγ translocation is found in follicular tumours of uncertain malignant potential, follicular thyroid carcinoma and poorly differentiated carcinoma and is not associated with angioinvasion, capsular penetration or prognosis.


• 226 thyroid tumours studied by FISH
• PAX8-PPARγ is not a reliable marker for follicular thyroid carcinoma and does not correlate with invasiveness nor with prognosis.
Conventional papillary carcinoma

Nuclear features

Malignancy in papillary carcinoma

Follicular variant of PTC
Malignancy in follicular patterned thyroid tumours (follicular variant of papillary carcinoma and follicular carcinoma)

**Capsular and/or VASCULAR INVASION**

<table>
<thead>
<tr>
<th>Pattern of growth</th>
<th>Solid, insular, trabecular</th>
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<tbody>
<tr>
<td></td>
<td>Embryonal, fetal</td>
</tr>
<tr>
<td></td>
<td>Normofollicular</td>
</tr>
<tr>
<td></td>
<td>Macrofollicular</td>
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| Nuclear features       | PTC NUCLEI                  |
What is the best way to diagnose parenchymatous/vascular invasion?

<table>
<thead>
<tr>
<th>Method</th>
<th>Result</th>
</tr>
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<tbody>
<tr>
<td>Cytopathology</td>
<td>No</td>
</tr>
<tr>
<td>Histopathology</td>
<td>Yes</td>
</tr>
<tr>
<td>Detection of biomarkers in the plasma/blood</td>
<td>May be</td>
</tr>
<tr>
<td>Conventional molecular pathology and high throughput approaches</td>
<td>No</td>
</tr>
</tbody>
</table>
DIAGNOSTIC HINTS

Capsular or, more importantly, vascular invasion
Nuclear features

WHAT ABOUT QUESTIONABLE CASES?

DOES IMMUNOHISTOCHEMISTRY OR MOLECULAR BIOLOGY HELP? NO
What about high throughput results?

Expression profiling of human tumours: The end of surgical pathology?

Kadanyi et al, J Mol Diagn 3:92, 2001

The answer is No: Microarrays, genome-wide, miRNAs,… did not provide so far anything really positive.
WHAT ABOUT FOLLICULAR-CELL TUMOURS WITH EQUIVOCAL CAPSULAR INVASION?
Benign vs Malignant

- Follicular adenoma
- Follicular tumour of uncertain malignant potential
- Follicular carcinoma

WHAT ABOUT FOLLICULAR TUMOURS WITH INTERMEDIATE NUCLEI?
• Well differentiated tumour of uncertain malignant potential

• Well differentiated carcinoma, NOS (If there is invasion)

WHO book on Endocrine Tumours, 3rd edition, 2004
Malignancy in Hürthle cell tumours

Diagnostic hints

Capsular/vascular invasion

Nuclear features
Follicular carcinoma

Minimally invasive

? 

Widely invasive
Follicular carcinoma

Minimally invasive
Angioinvasive
Widely invasive

Rosai et al, 2004
WHO book on Endocrine Tumours, 2004
Major problems in thyroid oncology

• Separation of follicular cell from C-cell derived tumours
• Risk stratification in pre-malignant lesions
• Diagnosis of malignancy
• Prognosis
• Therapy selection
PROGNOSTIC FACTORS IN PAPILLARY AND FOLLICULAR THYROID CARCINOMA

Completeness of surgery and responsiveness to radioactive iodine
A – Age
M – Distant metastases
E – Extrathyroid extension
S – Size of the tumours

Vascular invasion

Still debatable: aneuploidy (D...AMES) and molecular features (MIB1, p53, BRAF)
Most studies did not confirm the worse prognosis of BRAF mutated tumours provided major clinico-pathological features are controlled.
The preeminence of growth pattern and invasiveness and the limited influence of *BRAF* and *RAS* mutations in the occurrence of papillary thyroid carcinoma lymph node metastases

Catarina Eloy · Joana Santos · Paula Soares · Manuel Sobrinho-Simões

*BRAF* V600E mutation did not play a significant role

Eloy et al, Virchows Arch, 459:595, 2011
Few studies have reported the BRAF status of well differentiated, distantly metastatic papillary thyroid carcinomas


Only 4 out 20 (20%) PTCs had the BRAF V600E mutation

**Conclusion:**

**BRAF V600E mutation should not be considered, per se, a negative prognostic marker in PTC.**
What about BRAF mutation in papillary microcarcinoma?
### BRAF and Aggressiveness of Papillary MicroCA

Table 1 Analysis of BRAF<sup>V600E</sup> mutation in PTMCs

<table>
<thead>
<tr>
<th></th>
<th>BRAF mutation</th>
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<tbody>
<tr>
<td></td>
<td>Positive (n = 24)</td>
<td>Negative (n = 40)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>49.1 ± 10.4</td>
<td>45.1 ± 11.2</td>
<td>0.533</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male:female ratio</td>
<td>2:22</td>
<td>6:34</td>
<td>0.358</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nodular goiter</td>
<td>6 (25%)</td>
<td>8 (20%)</td>
<td>0.432</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hashimoto thyroiditis</td>
<td>0</td>
<td>8 (20%)</td>
<td>&gt;0.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extrathyroidal extension</td>
<td>12 (50%)</td>
<td>4 (10%)</td>
<td>0.001*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nodes metastasis</td>
<td>12 (50%)</td>
<td>6 (15%)</td>
<td>0.003*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tall cell variant</td>
<td>2 (8.3%)</td>
<td>2 (5%)</td>
<td>0.483</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;:T&lt;sub&gt;3/4&lt;/sub&gt; ratio</td>
<td>12:12</td>
<td>36:4</td>
<td>0.001*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Statistically significant

BRAF mutation in about 20-40% of papillary microcarcinomas
Should they be treated aggressively?

Soares and Sobrinho-Simões, Future Oncol, 2009
There is no solid evidence to support the utilization of the 10mm size as a dividing line between extremely low and low risk papillary thyroid microcarcinomas. (We guess the line might have been drawn at 6 or 12mm in case we had 6 fingers in each hand).

Small papillary thyroid cancers—is BRAF of prognostic value?

Paula Soares and Manuel Sobrinho-Simões

We think the excellent prognosis of microPTC means that it is unrealistic to suggest, as it has been recently advanced,\(^{10}\) that patients with BRAF mutated microPTC should be treated more aggressively just taking into consideration the BRAF status.

Nature Reviews Endocrinology, 2011
3 histopathologic features: superficial tumor location, intraglandular tumor spread/multifocality, and tumor fibrosis cooperate with BRAF mutations.
Completeness of surgery and responsiveness to radioactive iodine
A – Age
M – Distant metastases
E – Extrathyroid extension
S – Size of the tumours

Vascular invasion

Still debatable: aneuploidy (D..AMES) and molecular features (MIB1, p53, BRAF)
- Benign VS Malignant VS Borderline (Uncertain malignant potential – UMP)
- Lobectomy VS Lobectomy plus isthmectomy VS Total thyroidectomy
- Radioactive iodine: Yes or No?
- Targeted therapies: When and Which one(s)?

Cytology
Histology
+ Molecular Pathology
The BRAF$^{V600E}$ Oncogene Induces Transforming Growth Factor β Secretion Leading to Sodium Iodide Symporter Repression and Increased Malignancy in Thyroid Cancer

Garcilaso Riesco-Eizaguirre,¹,² Irene Rodríguez,¹ Antonio De la Vieja,¹,¹ Eugenia Costamagna,¹ Nancy Carrasco,⁵ Manuel Nistal,³ and Pilar Santisteban¹

mTOR pathway overactivation in BRAF mutated papillary thyroid carcinoma

Faustino A,…Soares P. J Clin Endocrinol Metabol, 2012 (in press)

“mTOR overactivation induces repression of Sodium Iodide Symporter (NIS)”
Study of genetic alterations on follicular Hürthle cell tumours

The biology and the genetics of Hürthle cell tumors of the thyroid
Maximo V et al. Endocrine-Related Cancer, 2012 (in press)

“RET/PTC rearrangements are frequently detected in every type of Hürthle cell tumour“
Therapeutic problems

Phenotypic and genotypic heterogeneity and topography

It will be necessary to target the ERK/MAPK (RET, BRAF, RAS,…) and/or the PI3K/AKT/mTOR and/or JAK/STAT3 pathways, as well as mitochondrial and metabolic alterations.