Changing Concepts Thyroid Pathology

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Cancers on the Rise:
Trends in SEER Incidence Rates by Primary Cancer Site 1992-2002

Underlying rates are per 100,000 and age-adjusted to the 2000 U.S. standard population.

* The APC is significantly different from zero (p<0.5). Graph excludes deaths from cancers on the decline.


Thyroid Cancer Mortality in the United States

- It is estimated that 1,530 americans (880 women, 650 men) will die of thyroid cancer in 2007.
- Thyroid cancer is the fastest-rising cause of cancer-related death in men.
- ¾ of annual deaths are from well-differentiated thyroid cancers.

Thyroid Histology: The “Gold Standard”

Hyperplasia vs Neoplasia
Benign vs Malignant
Indolent vs Aggressive Malignancy

- Observer-dependent
- Inconsistent
- Lack scientific criteria

**Question:** 1. Follicular Adenoma or 2. Papillary Carcinoma?

- 75%
- 25%

**The Answer: 5 Years Later**

- Do we overcall many to catch this one?
- Do we undercall many and miss this one?
- Do we find scientific markers to predict behavior?

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**Sporadic Nodular Goiter**

- Multinodular “colloid” goiter
- Occasionally associated with hyperthyroidism
  - “Plummer’s disease”
- Etiology and pathogenesis NOT understood

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**Clonality Studies of Sporadic Nodular Goiter**

- Dominant nodules often monoclonal
- Nodules may show LOH or aberrant methylation
- Multiple nodules from a single goiter exhibit activation of the same allele

**Diagnostic criteria**

**Follicular Adenomas with Papillary Architecture**

- “Papillary adenomas”
- Monoclonal benign neoplasms
- Activating mutations of TSH-receptor or Gsα
- Plummer’s disease


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**Follicular Adenoma & Carcinoma**

- Encapsulated expansile growth
- Malignant by capsular or vascular invasion
- Hematogenous spread

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**Definitions: Capsular Invasion**

1. Nests, cords or cells in capsule
2. Islands in capsule associated with perpendicular rupture of collagen
3. In capsule beyond bulk of lesion
4. Total thickness into adjacent parenchyma

*Artefactual trapping postFNA*

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**What If There Is NO Tumor Capsule?**

- Capsular invasion cannot be evaluated
- Invasion must be assessed as infiltration into surrounding parenchyma, perineural or vascular involvement
**Classification of Follicular Carcinoma**

- Minimally invasive carcinoma
  - up to 100%
  - 10 year survival

- Widely invasive carcinoma
  - 25-45%
  - 10 year survival

- Angioinvasive carcinoma
  - controversial

**Vascular Invasion by Endocrine Neoplasms**

1. Tumor cells bulging into an endothelial-lined lumen
2. Intravascular tumor nests covered with endothelium
3. Tumor casts within vessel lumen
4. Thrombus adherent to invasive tumor

**Identification of Vascular Invasion by Follicular Neoplasms**

- Rigid criteria predict high likelihood of metastasis
- EVEN in differentiated thyroid carcinoma

Mete and Asa, submitted

**Papillary Carcinoma: A Cytologic Diagnosis**

- Architecture irrelevant
  - Papillary, Follicular, Mixed, Solid, Cystic
  - Diffuse sclerosis variant is hard to recognize

- Invasion not a criterion
  - Encapsulated variant

- Nuclear features predict behavior
Papillary Carcinoma
- Often multifocal
- Locally infiltrative
- Lymphatic spread

Follicular Variant of Papillary Ca
- Encapsulated expansile growth
- Malignant by nuclear features
- Often multifocal
- Lymphatic spread

Cytologic Features of Papillary Carcinoma
1. Enlarged, overlapping nuclei
2. Pale vacuolated nucleoplasm with peripheral margination of chromatin
3. Irregular nuclear membrane
4. Nuclear grooves
5. Nuclear pseudoinclusions

Emerin Identifies Nuclear Features
**Markers of Thyroid Malignancy: HBME-1**
- Monoclonal antibody
- Unknown epitope
- Unknown significance
- Identified in 60% of thyroid malignancies, not in normal or benign lesions

**Markers of Thyroid Malignancy: Galectin-3**
- 31kD β-galactoside-binding lectin
- High percentage of malignant thyroid tumors, not in normal or benign lesions

**Markers of Papillary Carcinoma: CK19**
- One of many keratins
- Identified diffusely in 60% of papillary carcinomas
- Also seen in reactive nontumorous thyroid

*Raphael et al, Mod Pathol. 1995;8(8):870-2*
**Follicular Adenomas with Papillary Architecture**
- “Papillary adenomas”
- Monoclonal benign neoplasms
- Activating mutations of TSH-receptor or Gsα
- Plummer’s disease


**BRAF Mutations**
- Most common genetic event in thyroid cancer
- Diagnostic marker of PTC
- Genotype-phenotype correlations
  - BRAF\(^{V600E}\) in classical variant PTC (common)
  - BRAF\(^{K601E}\) in FVPTC (rare)
  - VK600-1E deletion (BRAF\(^{VK600-1E}\)) in solid variant (single case)
- Prognostic significance controversial

**Ret/PTC Rearrangements**
- Chromosomal rearrangement involving chromosome 10 ret
- Fusion of the ret tyrosine kinase to:
  - CCDC6 (H4) = ret/PTC1*
  - R1α = ret/PTC2
  - NcoA4 (ele) = ret/PTC3*
- Chromosome 10 inversions most common
- At least 15 identified to date
**Ret/PTC Rearrangements**

These rearrangements result in cytoplasmic protein; antibodies against ret identify the C terminus that is conserved.

Different promoters drive transcript levels that modulate oncogenicity of RET/PTC oncoproteins.


**Methods of Ret/PTC Analysis**

- **DNA**
  - PCR analysis difficult due to variable break-point sites leading to heterogeneous tumor profiles
- **RNA**
  - RT-PCR for ret/PTC mRNA is the "gold standard"
  - Variability of expression; not "all or none"
- **Protein**
  - Immunohistochemistry using antisera to C terminus
- **FISH**
  - Not widely available but promising

Rhoden et al, JCEM 2006

**RAS Mutations Characterize Follicular Lesions**

- Follicular Variant PTC
- Follicular Adenoma
- Follicular Carcinoma
- Poorly Differentiated Carcinoma

**Pax 8-PPARγ1 Fusion Oncogene**

- Identified in angioinvasive follicular carcinoma
- Diagnostically applicable by FISH and IHC for PPARγ
- Also found in PTC
**CTNNB1 Mutations are Found in Poorly Differentiated (Insular) Thyroid Carcinoma**

- Reduced membrane stain for \( \beta \)-Catenin correlates with dedifferentiation
- Nuclear translocation due to exon 3 mutation in 25% of insular carcinomas and 65% of anaplastic carcinomas

Garcia-Rostan et al, Am J Pathol 2001;158:987

**PIK3CA Mutations Predict Aggressive Behavior**

- Identified in anaplastic carcinoma
  - Wang et al, JCEM 2007;92:2387-90
- Accompanies other mutations in aggressive papillary carcinoma and metastases
  - Costa et al, Clin Endocrinol 2008;68:618-34
  - Ricate-Filho et al, Cancer Res 2009;69:4885-93

**p53 Alterations in Thyroid Carcinoma**

Mutations are common in Anaplastic carcinoma

Immunolocalization correlates with extent of disease, extrathyroidal involvement, recurrence and poor outcome in differentiated carcinoma

Hosal et al, Endocr Pathol 1997, 8:21-28

**Molecular Studies: Progression in Thyroid Cancer**

- TSH-R
- Gs
- Ras
- PPARγ
- BRAF
- RET/PTC
- TRK
- \( \beta \)-catenin
- PIK3CA
- p53

Hyperplasia

Follicular Adenoma

Functioning Follicular Adenoma

Follicular Carcinoma

Metastatic Papillary Carcinoma

Tall Cell Papillary Carcinoma

Thyroid Follicular Cell

Insular Carcinoma

Anaplastic Carcinoma
What is the Clinical Significance of Papillary Microcarcinoma?

1. Potentially metastasizing
2. Metastatic focus of papillary carcinoma
3. Clinically insignificant

ret/PTC in Multifocal Papillary Carcinoma

- ret/PTC expression is highly prevalent in multifocal micropapillary thyroid cancer
- Identical ret/PTC rearrangements are found in 32% of patients
  - possible spread of a single tumor
- Discordant ret/PTC patterns in 68%
  - discrete primary tumors

Sugg et al, J Clin Endocrinol Metab 83:4116-4122, 1998
- Identical data using X-chromosome inactivation

Implications of ret/PTC Data in Multifocal Papillary Carcinoma

- One major rationale for completion thyroidectomy in patients with “low risk” papillary carcinoma is unjustified

Fink et al, Modern Pathol 1996; 9: 816-820

Hürthle Cell Tumors

- Hürthle cell adenoma, Hürthle cell carcinoma
  - distinguished by invasive behavior
  - controversial because of unpredictable behavior
- Hürthle cell PTC
  - defined by papillary architecture
**Molecular Basis of Hürthle Cell Papillary Carcinoma**

- ret/PTC identifies Hürthle cell tumors with lymph node mets
  - allows distinction from Hürthle cell adenoma
  - better prognosis than Hürthle cell carcinoma


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**mtDNA, GRIM19**

- Altered ATP synthesis
  - Savagner et al, JCEM 2001; 86:4920–4925
- mtDNA somatic events
  - Bonore et al, Cancer Res 2006; 66:6087–6096
  - Gasparre et al, PNAS 2007; 104, 9001–9006
- Mutations in non-neoplastic and neoplastic oncocyic cells
  - Not specific to neoplastic transformation
  - Associated with BRAF, ret/PTC etc
- GRIM19 (19p13.2) somatic and germline events
  - Maximo et al, Virchows Arch 2000

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**Molecular Diagnosis in Thyroid Aspirates- Papillary Carcinoma**

- ret/PTC
  - Cheung et al, J Clin Endocrinol Metab 2001
- BRAF
  - Salvatore et al, J Clin Endocrinol Metab 2004

Improved diagnosis with combined morphology and molecular testing

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After “The Anatomy Lecture of Dr. Nicolaes Tulp” – Rembrandt, 1632
(Courtesy of Dr. Carlos Gordin, New York, USA)
**BRAF Kinase Inhibition Arrests Thyroid Cancer Growth In Vivo**

However ………

Clinical trials have failed to show effectiveness of BRAF inhibitors


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**Molecular Studies: Progression in Thyroid Cancer**

Epigenetic Dysregulation


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**Molecular Studies: Progression in Thyroid Cancer**
Cyclin D1 and p27 Predict Metastasis in Papillary Carcinoma

Khoo et al. J Clin Endocrinol Metab 2002, 87:1814-8

Vitamin D Targets p27 Degradation in Thyroid Cancer

- VD/EB1089 induce intranuclear p27 accumulation by diminished degradation
- VD/EB1089 hypophosphorylate p27 in a phosphatase dependent process that involves the Akt pathway but may be PTEN independent
  
- In an orthotopic model, in vivo VD administration
  » decreases tumor volume
  » increases p27 accumulation
  » enhances cellular differentiation
  » decreases lung metastases
  
  Dackiw et al. Endocrinology 2004;145:5840-6

Are There Other Targets of VD?

- CITED-1 (L)
- Galectin-3
- Fibronectin (R)
- HGF, MET
- TPO
- COX-2
- CD44V6
- CD57

Prasad et al. Modern Pathology 2005;18:48-57

Fibronectin is Upregulated in Papillary Thyroid Carcinoma

- Increased cDNA expression in microarray studies of papillary carcinoma cf normal
- Diminished FN immunoreactivity reported at invading edge of aggressive thyroid cancers
- Negative in poorly-differentiated and anaplastic carcinomas
- Function unclear
  » Increasing invasion?
  » Reactive upregulation?
### Down-regulation of FN Promotes Tumor Growth and Metastasis

**Graph:**
- X-axis: Days after cancer cell injection
- Y-axis: Tumor volume (mm$^3$)
- Control siRNA: Blue line
- FN siRNA: Red line

<table>
<thead>
<tr>
<th>Mice</th>
<th>n</th>
<th># of mice with mets</th>
<th># of lesions/mouse</th>
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<td>1</td>
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<td>9</td>
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### Fibronectin in Thyroid Cancer

- Fibronectin mediates adhesion in thyroid carcinoma and restrains tumour growth
- VD upregulates fibronectin and restores adhesiveness of thyroid carcinoma
- The PTEN/PI3 Kinase pathway is involved in FN regulation and VD action on FN and adhesion
- The mechanism underlying overexpression in papillary carcinoma is unclear, but appears to be compensatory, and is lost in aggressive and dedifferentiated thyroid cancers


### CEACAM1

**aka biliary glycoprotein (BGP), CD66a, C-CAM1 and pp120**

- A member of the CEA family (Ig superfamily)
- A putative TSG
  - Down-regulated in colon, prostate, liver, endometrial, bladder and breast cancer
  - Reduces proliferation in human prostate cancer cell lines in vitro and in vivo
- Also implicated as an oncogene
  - Over-expressed in gastric cancer, non-small cell lung cancer and malignant melanomas
  - Facilitates metastatic tumor spread
  - Shows angiogenic function as a major target of VEGF

### CEACAM1 Expression Predicts Metastasis in PTC

- CEACAM1 is expressed in a small PTCs with lymph node spread
- CEACAM1 has a novel dual role in thyroid carcinoma: it has a suppressive effect on thyroid cell proliferation and increases adhesion, while promoting invasion and metastasis

* Liu et al, Oncogene 2007; 26:2747-58
**CEACAM1 in Thyroid Cancer**
- CEACAM1 is expressed in a small thyroid malignancies with lymph node spread
- CEACAM1 has a novel dual role in thyroid carcinoma: it suppresses thyroid cell proliferation, while promoting invasion and metastasis
  
  *Liu et al, Oncogene 2007; 26:2747-58*
- VD inhibits CEACAM1 to promote insulin/IGF-I receptor signaling without compromising anti-proliferative action
- CEACAM1 represents a target for VD therapy which may have potential therapeutic applications
  
  *Liu et al, Lab Invest 2011; 91(1):147-56*

**FGFR2-IIIb Interrupts Signaling Upstream of BRAF/MAPK**

**TMA Profiling Shows Divergent Expression of FGFRs in the Thyroid**

**FGFR2-IIIb Represses MAGE-A3/6**

<table>
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<tr>
<th>Gene Symbol</th>
<th>Gene Name</th>
<th>GeneBank Accession</th>
<th>U133Pv2 Probe ID</th>
<th>Ratio 1</th>
<th>Ratio 2</th>
<th>Ratio 3</th>
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<td>2.0</td>
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<td>2.1</td>
</tr>
</tbody>
</table>

**MAGE subgroup I members, MAGE-A, B, C, are expressed in several tumors, but not in normal tissues except testis and placenta**

*[Cancer-testis antigens]*

*Kondo et al, Clin Cancer Res 2007;13(16):4713-20*
**MAGE-A3 Promotes Migration & Invasion**

Liu et al, Cancer Res 2008;68:8104-8112

**MAGE-A3 Promotes Metastasis**

Liu et al, Cancer Res 2008;68:8104-8112

**MAGE in Thyroid Cancer**

- Downregulation or FN or FGFR2 increase tumor growth and metastasis
- Downregulation of FN or FGFR2 induce expression of MAGE-A3 through histone methylation
- MAGE-A3 mediates p21 down-regulation, accelerated cell cycle progression, increased cell migration rate, invasion and metastasis
- MAGE-A3 is a functional integrator of diverse signals in mediating cancer progression

Liu et al, Cancer Res 2008;68:8104-8112

**MAGE-A3 Enhances Tumor Growth**

Liu et al, Cancer Res 2008;68:8104-8112
**MAGE**

- Normal thyroid tissue exhibits weak cytoplasmic and strong nuclear MAGE reactivity.
- Tumors exhibit an increase in cytoplasmic MAGE scores that correlates with clinical behavior
  - larger tumors have higher MAGE scores
  - correlation between MAGE cytoplasmic score and number of lymph node metastases

_Cheng et al. Endocrine Related Cancer 2009;16:455-466_

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**Proteomic Biomarkers in PTC**

- 410 PTCs with morphologic and clinical data
- BRAF status known
- TMA analysis of:
  - Histopathologic biomarkers of malignancy: Galectin-3, CK 19, HBME-1
  - Cell differentiation factors: NIS, CITED-1
  - Nuclear receptors: ERα, ERβ, and PPAR-γ
  - Adhesion molecules: CEACAM-1, Osteopontin, Fibronectin, E-Cadherin
  - Cell cycle regulators: Cyclin-D1, p53, p27, p21

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**BRAF Mutations & Outcome**

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**PTC Proteome**

_ETE:_
- Membraneous CK19, HBME, Gal3, OPN
- Cytoplasmic HBME, CK19
- Nuclear ERβ, Gal3, p53

_LNM:_
- Membranous HBME, CK19, Gal3
- Cytoplasmic FBN and CK19
- Nuclear Gal3, ERβ

_VI:_
- Membranous Gal3
- Cytoplasmic Gal3

**Conclusions**

- The diagnosis of thyroid cancer is evolving as molecular data clarify the significance of morphologic features and behaviors.
- Our data predict the need for targeting epigenetic factors along with intragenic mutations in the control of thyroid cancer progression.

**Thanks To……..**

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