**Changing Concepts**

**Thyroid Pathology**

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**Cancers on the Rise:**

**Trends in SEER Incidence Rates by Primary Cancer Site 1992-2002**

- **All Races, Males**
  - Thyroid: 3.1%
  - Liver: 3.0%
  - IBD: 2.5%

- **All Races, Females**
  - Thyroid: 3.3%
  - Liver: 2.3%

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**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.
- ⅔ of annual deaths are from well-differentiated thyroid cancers.

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**Thyroid Histology:**

**The “Gold Standard”**

- Hyperplasia vs Neoplasia
- Benign vs Malignant
- Indolent vs Aggressive Malignancy

- Observer-dependent
- Inconsistent
- Lack scientific criteria


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**Follicular Adenoma or Papillary Carcinoma?**

- The Answer:
  - Do we overcall many to catch this one?
  - Do we undercall many and miss this one?
  - Do we find scientific markers to predict behavior?
**Sporadic Nodular Goiter**

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

**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

(Lyons et al, Science 249:535, 1990;
van Sande et al,
J Clin Endocrinol Metab 80:2577, 1995)

**Follicular Adenoma & Carcinoma**

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

**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

**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

What Can Molecular Pathology Teach Us About Thyroid Cancer?

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
###Mechanisms of Thyroid Tumorigenesis

![Diagram of signaling pathways](image)

- TSH signaling
- MAPK signaling

![Diagram of follicular cell](image)

- Cell differentiation
- Cell proliferation

**Kondo, Ezzat and Asa. Nature Reviews Cancer 2006**

###Follicular Adenomas with Papillary Architecture

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

![Image of adenomas and papillary architecture](image)


###BRAF Mutations

- Most common genetic event in thyroid cancer
- Diagnostic marker of PTC
- Genotype-phenotype correlations
  - BRAFV600E in classical variant PTC (common)
  - BRAFK601E in FVPTC (rare)
  - VK600-1E deletion (BRAFVK600-1E) 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

###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**

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.

**RAS Mutations Characterize Follicular Lesions**
- Follicular Variant PTC
- Follicular Adenoma
- Follicular Carcinoma
- Poorly Differentiated Carcinoma

**Pax 8-PPARY1 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 β-Catenin correlates with dedifferentiation
- Nuclear translocation due to exon 3 mutation in 25% of insular carcinomas and 65% of anaplastic carcinomas

**PIK3CA Mutations Predict Aggressive Behavior**
- Identified in anaplastic carcinoma
  » Garcia-Rostan et al, Cancer Res 2005;65:10199-207
  » 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

**Molecular Studies: Progression in Thyroid Cancer**
**What is the Clinical Significance of Papillary Microcarcinoma?**

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

**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

*Chueh et al, J Clin Endocrinol Metab 85: 876-882, 2000*

**mtDNA, GRIM19**

- Altered ATP synthesis
  - Savagner et al, JCEM 2001;86:4920–4925
- mtDNA somatic events
  - Gasparre et al, PNAS 2007; 104: 9001–9006
- Mutations in non-neoplastic and neoplastic oncocytic cells
  - Not specific to neoplastic transformation
  - Associated with BRAF, ret/PTC etc
- GRIM19 (19p13.2) somatic and germline events
  - Matuso et al, Virchows Arch 2000
**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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**BRAF Kinase Inhibition Arrests Thyroid Cancer Growth In Vivo**

However ..........  
Clinical trials have failed to show effectiveness of BRAF inhibitors

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**Epigenetic Control: DNA Methylation**


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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;145:3540-6

**Are There Other Targets of VD?**

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


**Fibronectin is Upregulated in Papillary Thyroid Carcinoma**

- Increased cDNA expression in microarray studies of papillary carcinoma vs 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**


**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

TMA Profiling Shows Divergent Expression of FGFRs in the Thyroid

FGFR2, Normal thyroid
- FGFR2 is expressed exclusively in normal thyroid

FGFR1, PTC
- FGFR1 is expressed in hyperplastic and neoplastic lesions

St Bernard et al, Endocrinology 146:1145-1153, 2005

FGFR2-IIIb Interrupts Signaling Upstream of BRAF/MAPK

Kondo et al, Cancer Res 2007;67: 5461

FGFR2-IIIb Represses MAGE-A3/6


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"
MAGE-A3 Promotes Migration & Invasion


MAGE-A3 Enhances Tumor Growth


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


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

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