Mete and Asa

The impact of genomics on endocrine pathology practice

THE IMPACT OF GENOMICS ON ENDOCRINE PATHOLOGY PRACTICE
Ozgur Mete, M.D
Sylvia L. Asa, M.D., Ph.D.

University Health Network
and University of Toronto
Toronto, Ontario, Canada
ozgur.mete2@uhn.ca
sylvia.asa@uhn.ca

DISCLOSURES

• Sylvia L. Asa is a member of the
  Leica Aperio Medical Advisory Board

• Ozgur Mete has nothing to disclose

LEARNING OBJECTIVES

• Understand diagnostic, predictive, and prognostic
  biomarkers in endocrine tumors
• Discuss genotype-phenotype correlations in
  endocrine tumors
• Establish the importance of clinicopathological
  correlations in endocrine disease
• Clarify the clinical relevance of complete and
  accurate surgical pathology in the management of
  patients with endocrine tumors
CASE 1

- 61 year old woman
- palpable nodule in the left thyroid
- otherwise healthy
- no history of thyroid disease or malignancy in her or her family
- ultrasound 6 cm lesion, solid and uniformly hyperechoic with a well-defined border and no calcification
- Biopsy: abundant thyroid follicular cells and was diagnosed as “suspicious for follicular neoplasm”
- left hemithyroidectomy was performed
The impact of genomics on endocrine pathology practice

PTC

Classical variant with true papillae
### Classification of Thyroid Neoplasia

<table>
<thead>
<tr>
<th>Follicular cell derivation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>• Follicular adenoma</td>
<td></td>
</tr>
<tr>
<td>• Differentiated thyroid carcinoma</td>
<td></td>
</tr>
<tr>
<td>• Poorly differentiated thyroid carcinoma</td>
<td></td>
</tr>
<tr>
<td>• Anaplastic thyroid carcinoma</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C cell derivation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>• Medullary carcinoma</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td></td>
</tr>
</tbody>
</table>

---

**Follicular Adenomas**

- Follicular (microfollicular (fetal), macrofollicular)
- Cytologic variants: oncocytic, clear cell, signet-ring cell, mucinous
- Stromal variants: lipoadenoma
- Architectural variant: papillary hyperplasia ("toxic")
- Atypical*

* Atypical: NO invasion and NO nuclear features of papillary carcinoma

---

**Follicular Adenoma with Papillary Hyperplasia: Toxic Adenoma**

- Hot nodules on scan
- Papillary architecture
- Benign cytology
- Solitary or in MNG (Plummer’s disease)
- Molecular features:
  - monoclonal
  - activating mutations of TSH receptor or GNAS
- Biologically benign

---


The impact of genomics on endocrine pathology practice

TSHR/GNAS SIGNALING

DISTINCT SIGNALING PATHWAYS

RAS MUTATIONS IN FOLLICULAR LESIONS

- Follicular adenoma
- Follicular Variant PTC
- Follicular Carcinoma
CRITERIA FOR THE CLASSIFICATION OF FOLLICULAR LESIONS

• Capsular invasion
• Vascular invasion
• Nuclear atypia

All are subject to interpretation and controversy exists with extensive intra- and inter-observer variability.

CAPSULAR INVASION: FOLLICULAR LESIONS

• Minimally invasive carcinoma
  ≈ 100% 10 year survival
• Widely invasive carcinoma
  25-45% 10 year survival


IS THIS CAPSULAR INVASION?

Mete and Asa, Endocrine Pathology, Cambridge University Press, 2016
WHAT ABOUT UNENCAPSULATED TUMORS?

- Thyroid tumors may NOT have a capsule
- Capsular invasion cannot be evaluated
- Invasion must be assessed as infiltration into surrounding parenchyma, perineural or vascular involvement
ANGIOINVASION IN THYROID TUMORS
• Controversial at best
• If unequivocal, predicts distant metastasis

PSEUDOVASCULAR INVASION
1. Tumor cells bulging into an endothelial-lined lumen
2. Intravascular tumor nests covered with endothelium
3. Tumor casts within vessel lumen

ATYPICAL ADENOMAS, UMP, NIFT-P: CYTOLOGY 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 pseudo-inclusions
**3D RECONSTRUCTION OF PTC NUCLEI**

**EMERIN IDENTIFIES NUCLEAR FEATURES OF PTC**

**THE SOLUTION TO CONTROVERSY? WORKING GROUP MARCH 2015**

Tumor name:
- "Non-invasive follicular thyroid neoplasm with papillary-like nuclear features"
- Note: This is a neoplasm of very low malignant potential. Studies indicate that no further surgery after complete excision or RAI therapy is required for majority of these lesions based on prior studies of non-invasive encapsulated follicular variant of papillary carcinoma.
NOMENCLATURE REVISION FOR ENCAPSULATED FOLLICULAR VARIANT PTC

1. Encapsulation or clear demarcation
2. Follicular growth pattern
   <1% papillae (virtually no papillae!)
   no psammoma bodies
   <30% solid/trabecular/insular growth
3. Nuclear Features of PTC (Score 2 or 3)
4. No capsular or vascular invasion
5. No tumor necrosis
6. No mitotic activity


PAPILLARY CARCINOMA

- Complex papillary architecture
- Marked nuclear atypia
- Psammoma bodies
- Lymph node mets
- BRAF V600E mutation
  - Includes tall cell and hobnail variants

Kimura et al, Cancer Res 2003
Cohen et al, JNO 2003
Soares et al, Oncogene 2003
**RET/PTC REARRANGEMENTS**

- Chromosomal rearrangements involving RET on chromosome 10
  - Greeco et al., Cell. 1990;60:557-63
- Fusion of the RET tyrosine kinase:
  - CCDC6 (H4) = RET/PTC1*: Classical variant
  - R1a = RET/PTC2
  - NcoA4 (ELE) = RET/PTC3*: Solid growth

*Chromosome 10 inversions most common
At least 17 identified to date

---

**PAX 8-PPARγ FUSION ONCOGENE**

- Identified in angioinvasive follicular carcinoma
  - Kroll TG et al., Science, 2000; 289:135
- Diagnostically applicable by FISH and IHC for PPARγ
- Also found in PTC
  - Valiuvou et al, AJSP2002;26(8):1016-23

---

**OTHER UNUSUAL EVENTS**

- TRK rearrangements
  - Classical variant
- BRAF K601E mutation
  - Follicular variant
- ALK rearrangements
  - Diffuse sclerosing variant
  - Class A (ALK/CCND1) or B (BCR/ALK)
- APC mutation (germline or somatic)
  - Cribriform morular variant
- Oncocytic tumors
  - Mutations in non-neoplastic & neoplastic oncocytic cells
  - Associated with BRAF (V600E/PTC1)
  - mDNA somatic events
  - GRIM19 (19p13.2) somatic and germline events

Maximo et al, Virchows Arch 2000
Mattina et al, Virchons Arch 2006
The impact of genomics on endocrine pathology practice

THE BRAF V600E-RAS SCORE

THYROID DIFFERENTIATION SCORES

GENETICS OF THYROID TUMORS
EPIGENETIC CONTROL: DNA METHYLATION


CYCLIN D1 AND P27 PREDICT METASTASIS IN PTC

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

PROTEOMIC BIOMARKERS IN PTC

410 PTCs with morphologic and clinical data
BRAF status known
TMA analysis of correlates of ETE, LN+, VI:
• 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

TCGA: FACTORS DETERMINING OUTCOME

- **AGE**
  - Mutation density correlated with age, recurrence & MACIS score
  - Association of risk with age-corrected mutation density remained, whereas MACIS scores did not
  - Suggests that age should be used as a continuous variable in risk stratification, instead of a threshold of 45 years used in many staging systems
- **Mutation densities were NOT associated with genotype or radiation exposure**
- **MORPHOLOGY**
  - Tall Cell Variants and other histological features of aggressivity correlated with highest mutation density
TCGA THYROID
- TCGA results propose a reclassification of thyroid cancers into molecular subtypes that better reflect their underlying signaling and differentiation properties, which has the potential to improve their pathological classification and better inform the management of the disease.

FVPTC VS FOLLICULAR CARCINOMA
- Both are differentiated thyroid malignancy
- Both have follicular architecture
- Similar biologic behavior
- Similar genetic profiles (RAS)
- Are they really the same thing?
- Are the criteria wrong?
- Does the distinction matter?

IS IT TIME TO SIMPLIFY??

Follicular carcinoma
- A well differentiated malignancy of thyroid follicular epithelial cells that exhibits either nuclear atypia or invasive behavior or both

Asa et al: Implications of the TCGA Genomic Characterization of Papillary Thyroid Carcinoma for Thyroid Pathology: Does Follicular Variant Papillary Thyroid Carcinoma Exist? Thyroid 2015 Jan;25(1):1-2
**THYROID DIFFERENTIATION**

- Papillary Carcinoma
- Follicular Carcinoma
- Poorly Differentiated Carcinoma
- Anaplastic Carcinoma

- Follicular Carcinoma
- Papillary Carcinoma
- Poorly Differentiated Carcinoma
- Anaplastic Carcinoma

**THYROID CANCER RISK STRATIFICATION**

- High Risk
- Intermediate Risk
- Low Risk

Haugen et al. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer, Thyroid 2016

**CASE 1**

- Follicular variant papillary carcinoma (RAS-like) with focal transformation to classical variant (BRAF-like) papillary carcinoma
CASE 2

• Previously healthy 51-year-old woman
• Referred to us for pathology review
• Multiple pulmonary nodules ranging from 0.1 cm to 1.2 cm, in the right lower lobe
• No comprehensive endocrine workup

Right lower wedge resection
Could this be a pulmonary neuroendocrine tumor?

The impact of genomics on endocrine pathology practice

Could this be a pulmonary neuroendocrine tumor?

THE ROLE OF IMMUNOHISTOCHEMISTRY IN THE DIAGNOSIS OF NEUROENDOCRINE TUMORS

1. Confirm neuroendocrine differentiation
2. Confirm epithelial differentiation
3. Hormones
   - Determine the peptide profile
   - Define precursor lesions
   - Help to determine certain cell types
4. Determine the cellular origin (transcription factors)
5. Determine the proliferative rate
6. Prognosticators

Chromogranin-A
Synaptophysin
CAM5.2, AE1/AE3

Negative for CDX-2, serotonin, alpha-subunit, p63
The impact of genomics on endocrine pathology practice

What is your diagnosis?
No necrosis
Mitotic activity <1/10 HPF
MIB-1 labeling index 1.5%

What is your diagnosis?
A) Multifocal Typical carcinoid tumor
B) Multifocal Atypical carcinoid tumor
C) Metastatic neuroendocrine tumor
D) Metastatic paraganglioma
E) Multicentric pulmonary paraganglioma

PULMONARY NEUROENDOCRINE NEOPLASMS

1. Pulmonary NETs
   - Well differentiated neuroendocrine tumors (Carcinoid Tumors)
     Typical carcinoid tumor (low grade)
     Atypical carcinoid tumor (intermediate grade)
   - Poorly differentiated neuroendocrine carcinoma
     small cell, large cell
   - Mixed endocrine and exocrine carcinoma
     e.g. Mixed adenoneuroendocrine carcinoma-MANEC

2. Paragangliomas

3. Metastatic Neuroendocrine Tumor/Carcinoma
PARAGANGLIOMA SHOULD BE EXCLUDED IN A KERATIN- AND TRANSCRIPTION FACTOR-NEGATIVE NEUROENDOCRINE TUMOR

Pulmonary NET ("carcinoid" tumor)
orParaganglioma

- Clinical management is different for paragangliomas
- Unlike other NETs, paragangliomas are MIBG-avid
- Criteria of malignancy in paragangliomas

S100 POSITIVE-SUSTENTACULAR NETWORK IS NOT SPECIFIC TO PARAGANGLIOMAS


S100 protein
PARAGANGLIOMAS CAN SECRETE OTHER PEPTIDES
- ACTH, CRH, IGF-1
- VIP, GHRH
- β-endorphin, Serotonin
- Galanin, Adrenomedullin
- Somatostatin, ADH
- gastrin-like peptide
- peptide YY, neuropeptide Y
- Calcitonin
- Calcitonin gene-related peptide

Back to our case…
Tyrosine Hydroxylase (-)  GATA-3 (+)

What is your diagnosis?
D) Metastatic paraganglioma
E) Multicentric pulmonary paraganglioma
MULTIFOCAL PARAGANGLIOMA IN THE LUNG

PRIMARY? vs METASTASIS?
GENETIC PREDISPOSITION?

FAMILIAL PARAGANGLIOMA

- Often multicentric synchronous and asynchronous presentations
- Up to 40% of paragangliomas are associated with genetic susceptibility
  - SDHA, SDHB, SDHC, SDHAF2, SDHD genes
  - RET, NF-1, VHL, TMEM127, MAX, KIF1B-β, HIF2a (EPAS1), PHD2/EGLN1, PHD1, FH, BAP1, MDH2 genes mutations
  - Somatic H/K/N-RAS, IDH, ATRX, HIF-1α (ANR3T), HIF2a, RET, VHL, NF-1, CSDE1, and a few additional mutations and gene fusions including MAML3 fusions
- At least 50% of SDHB gene mutations are associated with malignancy
- Use an antibody against SDHB
  - Sporadic tumors and normal tissue elements are positive
  - SDHx-related tumors show loss of SDHB expression
  - Genetic testing is required when SDHB stain is negative

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Gene (Chromosome)</th>
<th>Tumor Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEN2</td>
<td>RET (10q11.2)</td>
<td>+ + +</td>
</tr>
<tr>
<td>VHL</td>
<td>VHL (3p25-26)</td>
<td>+ + +</td>
</tr>
<tr>
<td>NF1</td>
<td>NF1 (17q11)</td>
<td>+ + +</td>
</tr>
<tr>
<td>FHL1</td>
<td>SDH1 (11q23)</td>
<td>+ + +</td>
</tr>
<tr>
<td>PGL2</td>
<td>SDHA (11q12.2)</td>
<td>+ + +</td>
</tr>
<tr>
<td>PGL3</td>
<td>SDHC (1p33)</td>
<td>+ + +</td>
</tr>
<tr>
<td>PGL4</td>
<td>SDHB (1p13.13)</td>
<td>+ + +</td>
</tr>
<tr>
<td>PGL6</td>
<td>SDHx (5q13)</td>
<td>+ + +</td>
</tr>
<tr>
<td>TMEM127</td>
<td>+ + +</td>
<td></td>
</tr>
<tr>
<td>MAX</td>
<td>(14q23.3)</td>
<td>+ + +</td>
</tr>
<tr>
<td>HIF2a</td>
<td>(2p21)</td>
<td>+ + +</td>
</tr>
</tbody>
</table>

Cluster 1: Pseudohypoxia Pathway

Cluster 2: Kinase Signaling Pathway

Can we predict inherited disease?

VHL-related paraganglioma/pheochromocytoma?
VHL-RELATED PARAGANGLIOMAS AND PHEOCROMOCYTOMAS

Hereditary Pheochromocytoma:
The Importance of Nontumorous Medulla

• Characteristic of: RET-driven MEN2 syndrome
• Rarely: TMEM127, SDHB mutations


Normal Medullary Hyperplasia

INTACT SDHB CYTOPLASMIC GRANULAR POSITIVITY
Back to our case

SDHB

CRITERIA OF MALIGNANCY
PARAGANGLIOMA-PHEOCHROMOCYTOMA

Malignancy is defined by the presence of metastases to sites where paraganglial tissue is not normally found

Endocrine-Related Cancer (2011) 18 R253–R276
Back to our case
Station 8

Chromogranin-A

SDHB (LYMPH NODE)
What is your diagnosis?
Multicentric SDH-deficient pulmonary paraganglioma with metastatic spread in the lymph node

CLINICAL ALGORITHMS

TAKE HOME MESSAGES
- The 10% rule is no longer valid!
  - Up to 40% familial
  - At least 50% of SDHB-driven paragangliomas are malignant
  - MAX mutations: increased malignancy
  - Somatic MAML3 fusion- and ATRX-related tumors: aggressive
- Genotype-Phenotype correlations
  - Biochemistry-Genotype correlations
  - Clear cells-VHL disease
  - SDHB antibody-any SDH gene mutations
  - Medullary hyperplasia-RET gene mutations (MEN2)
    - rarely TMEM127 and SDHB mutations
- Normal anatomic distribution of paraganglia should be remembered when distinguishing multifocal disease from metastatic disease
CASE 3

- Previously healthy 40-year-old man
- Sudden right upper quadrant pain
- CT scan:
  - 3 pancreatic lesions
  - 3 mm stone in the right urinary system
- Biochemistry:
  - Inappropriately elevated insulin levels

2.8 cm dominant tumor
MORPHOLOGY OF pNETS

- Solid/nesting pattern
- Trabecular/gyriform
- Glandular
- Tubular-acinar
- Cystic
- Papillary
- Angiomatoid
- Mixed patterns

CYTOLOGY OF pNETS

- Small cell
- Oncocytic
- Rhabdoid
- Spindle cell
- Glycogen rich
- Clear cell
- Papillary
- Angiomatoid
- Mixed patterns

IMMUNOHISTOCHEMISTRY OF NETS

**Neuroendocrine differentiation:**
- Cytosol
- NSE, PGP 9.5
- Secretory Granules
- Chromogranin
- Synaptophysin
- Synaptobrevin, Synaptotagmin, Synapsin
- NESP-SS
- Membrane
- CD56, CD57

**Keratins/TyH:**
- To exclude/confirm PGL

**Specific NE cells:**
- Transcription factors
- TTF-1 lung, thyroid
- PDX-1, Isl-1, Pax-6/8 pancreas
dox-2 pancreas, small bowel
- PSAP in rectum
- Pit-1, SF-1, Tpit pituitary

**Hormones:**
- Pituitary hormones
- Calcitonin, bombesin, serotonin in lung and thyroid
- Serotonin, insulin, glucagon, somatostatin, gastrin, PP, PYY, etc in GEP

**Novel circulating tumor markers:**
- C-kit, GEP, insulin, glucagon, somatostatin, gastrin, PP, PYY, etc
**GRADING OF NEUROENDOCRINE TUMORS GEP-NETS**

- Tumor grade refers to the degree of biologic aggressiveness
- Tumor differentiation refers to the extent of resemblance to the normal cellular counterpart

<table>
<thead>
<tr>
<th>Grading - GEPNET</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitotic activity</td>
<td>&lt;2/10 HPF</td>
<td>2-20/10 HPF</td>
<td>&gt;20/10 HPF</td>
</tr>
<tr>
<td>Ki-67 index</td>
<td>&lt;3%</td>
<td>3-20%</td>
<td>&gt;20%</td>
</tr>
</tbody>
</table>

- G3 includes high grade NETs and Neuroendocrine carcinoma (NEC)
  - Large cell NEC, Grade 3
  - Small cell NEC, Grade 3

**MITOSES AND KI67 IN NETS**

- Counting vs eyeballing
- Manual counting
- Digital image analysis

- Ki67 LI assessment
  - percent of 500-2000 cells in areas of hot spot nuclear labeling (in resection specimens)
  - tumor heterogeneity

- Mitotic counting
  - at least 50 HPF in areas at highest mitotic density (in resection specimens)

**EVIDENCE OF INHERITED DISEASE IN pNETS**

- Clinical stigmata of familial tumor syndromes
  - MEN1/MEN4, VHL, NF-1, Tuberous sclerosis, Lynch syndrome
- Multifocality
  - Endocrine or other (VHL – cystic lesions)
- Precursor lesions (MEN1, VHL)
  - Islet dysplasia
  - Nesidioblastosis (Ductulo-insular complexes)
  - Peliosis of nontumorous islets
- Clear cell change
  - Often in VHL disease; inhibin positivity suggests VHL disease

CLEAR CELL CHANGE: VHL SYNDROME

**PATHOGENESIS OF CLEAR CELL CHANGE IN VHL**

- VHL absence → activation of HIF-1α → pseudohypoxia
- HIF-1α and inhibin → Lipid and glycogen accumulation
  - HIF-1α promotes LDL and VLDL uptake through regulation of VLDL gene expression
  - HIF-1α induces glycogen synthesis and promotes tumor cell survival
  - Increase in HIF-1α leads to the induction of VEGF, inhibin, and LH
  - Inhibin blocks the suppressive effect of action that inhibits adipogenesis via affecting lipid accumulation, expression of glycerol-3-phosphate dehydrogenase activity, and expression of adipocyte fatty acid-binding protein mRNA.

**SEQUENCING OF SPORADIC pNETS**

DAXX/ATRX, MEN1 and mTOR Pathway Genes are Frequently Altered in Pancreatic Neuroendocrine Tumors

Yuchen Jiao1,*, Chiqian Shai1, Batish H. Gaba1, Rosand F. B. Widdel2, David S. Kilpatrick3, Anil D. Mahdaviani1, Richard G. Schilsky4, Laura H. Teng5, Christopher L. Wolfgang4, Michael B. Sheld1, Victor E. Velculescu1, Luis A. Diaz Jr.1,5,6, Bert Vogelstein1, Kenneth W. Kinzler1, Ralph H. Hruban7,8, and Nickolas Papadopoulos1,#

1. Ludwig Center for Cancer Genetics and Howard Hughes Medical Institutions, Johns Hopkins-Kimmel Cancer Center, Baltimore, MD 21231
2. Department of Pathology, the Sol Goldman Pancreatic Cancer Research Center, the Johns Hopkins Medical Institutions, Baltimore, MD 21231
3. Department of Surgery, the Sol Goldman Pancreatic Cancer Research Center, the Johns Hopkins Medical Institutions, Baltimore, MD 21231
4. Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, NY 10065
5. Departments of Pathology and Oncology, the Sol Goldman Pancreatic Cancer Research Center, the Johns Hopkins Medical Institutions, Baltimore, MD 21231
6. Swim Across America Laboratory at Johns Hopkins, Baltimore, MD 21231

Science. 2011 March 4; 331(6021): 1199–1203
PNETS: LOW MUTATION RATE

- Full exomic sequences of ~18,000 protein-coding genes in a Discovery set of 10 non-familial pNETs
  - 157 somatic mutations in 149 genes
  - mutations per tumor ranged from 8 to 23, mean = 16 (60% less than pancreatic ductal adenocarcinoma)
  - 91% were validated by Sanger sequencing

Science. 2011 March 4; 331(6021): 1199–1203

GENES SEQUENCED IN VALIDATION SET N=58

- MEN1: 5 tumors
- DAXX: 3 tumors
- PTEN: 2 tumors
- TSC2: 2 tumors
- ATRX: 1 tumor
  - product forms a heterodimer with DAXX
- PIK3CA:
  - part of mTOR pathway with PTEN and TSC2

Science. 2011 March 4; 331(6021): 1199–1203

DIFFERENT GENES FOR DIFFERENT PANCREATIC TUMORS

Table 1: Comparison of commonly mutated genes in PanNETs and PDAC

<table>
<thead>
<tr>
<th>Gene</th>
<th>PanNET</th>
<th>PDAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEN1</td>
<td>44%</td>
<td>0%</td>
</tr>
<tr>
<td>DAXX</td>
<td>15%</td>
<td>0%</td>
</tr>
<tr>
<td>PTEN</td>
<td>2%</td>
<td>85%</td>
</tr>
<tr>
<td>TSC2</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>ATRX</td>
<td>0%</td>
<td>25%</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>0%</td>
<td>38%</td>
</tr>
</tbody>
</table>

Science. 2011 March 4; 331(6021): 1199–1203
DIFFERENT MECHANISMS OF MUTATION

- Mutations differ in PDACs and pNETs
  - C to T transitions more common in PDACs
  - C to G transversions more common in pNETs
- Implicates different mechanisms
  - Different environmental carcinogens
  - Different DNA repair pathways

Science. 2011 March 4; 331(6021): 1199–1203

CHROMATIN REMODELING IS A TARGET

- Menin: a component of a histone methyltransferase complex implicated in chromatin remodeling
- DAXX (death-domain associated protein): an H3.3-specific histone chaperone
- ATRX (alpha thalassemia/mental retardation syndrome X-linked), a gene with an ADD (ATRX-DNMT3-DNMT3L) domain
- Non-overlapping mutations of DAXX/ATRX account for 43%
  - (25% DAXX and 17.6% ATRX)
- Some tumors had associated LOH
  - Immunohistochemistry identifies loss of expression even in tumors that were not homozygous, suggesting epigenetic silencing of the normal allele in tumors without LOH

Science. 2011 March 4; 331(6021): 1199–1203

DAXX/ATRX TARGET TELOMERES

- DAXX and ATRX interact for H3.3 incorporation at telomeres
- ATRX suppresses telomeric repeat-containing RNA
- The DAXX-ATRX complex targets CpG islands and G-rich tandem repeats that are prominent close to telomeres
- 61% of pNETs display abnormal telomeres that are characteristic of the telomerase-independent telomere maintenance mechanism known as “alternative lengthening of telomeres” (ALT)
- pNETs with ALT have ATRX or DAXX mutations or loss of nuclear ATRX or DAXX protein
  - An alternative telomere maintenance function

Science. 2011 Jul 22;333(6041):425
ALTERNATIVE LENGTHENING OF TELOMERES (ALT) IN DAXX/ATRX + PNETS

CHROMATIN REMODELING PHENOTYPE IS PROGNOSTIC

THE mTOR PATHWAY IN pNETS
- Expression profiling has identified altered expression of genes in the mTOR pathway in most pNETs
- Mutations of PTEN, TSC2, and PIK3CA were found in 7.3%, 8.8% and 1.4% of cases
- Implications for Everolimus treatment
OTHER PROGNOSTIC/PREDICTIVE MARKERS

- CK19
- CD117
- E-cadherin
- beta-catenin
- CEACAM1
- FGFR4 polymorphism

THERAPY FOR NETS

Surgery
- Primary resection
- Liver metastectomy
- Liver transplant

Medical therapy
- Somatostatin
- Interferon + Somatostatin
- Streptozotocin (pancreas)
- Everolimus and Sunitinib (pancreas)
- Temozolamide
- Cytotoxic chemotherapy

Interventional Radiology
- Bland embolization
- Chemoembolization
- Radioembolization
- Thermal embolization (RFA)

SOMATOSTATIN RECEPTORS

- SSTRs 2,3 and 5

- Diagnostic Applications
  - Somatostatin Scintigraphy
  - Indium 111 Ocatotide Planar whole-body imaging
  - Ga68 Dotatate PET CT

- Therapeutics
  - somatostatin analogue Rx
PEPTIDE RECEPTOR RADIOThERAPY (PRRT)

- Yttrium$^{90}$ or Lutetium$^{177}$ labeled Octreotide
  - $^{90}$Y has greater tissue penetration and therefore is better for larger tumors (>4 cm)
  - 2cm to 4cm should be treated with $^{177}$Lu

RESPONSE TO $^{177}$LU-OCTREOTATE


IMPACT OF GENETICS IN pNETS

- Prognosis
- Prediction
- Implications for management
  - The patient
    - This lesion
    - Other lesions
  - The family
CASE 4

- **A 29-year-old man** with a nonfunctioning mass in the right adrenal gland
- **Past medical history:**
  - No history of radiation exposure
  - Smoker
- **Radiology:**
  - **CT scan:** well circumscribed 3.8 cm right adrenal cortical neoplasm (2 years ago 2.2 cm)
MARKERS OF ADRENAL CORTICAL DIFFERENTIATION

- Steroidogenic factor-1 (SF-1)
- Synaptophysin
- Melan-A (Clone A103)
- Inhibin
- Calretinin
- Vimentin

- Negative for:
  - CK7, EMA, Pax-8, TTF-1, CDX-2

Back to our case...

Melan-A Calretinin SF-1 (most specific)
ADENOMA? CARCINOMA?
UNCERTAIN MALIGNANT POTENTIAL (UMP)?

Diagnosis of Adrenal Cortical Carcinoma

- **Clinical Findings**
  - Metastasis
    - DHEA-S, virilization-feminization in adults
  - Morphology
    - Atypical mitoses, increased mitotic activity (>5/50HPF), Necrosis
    - Gross (malignant) invasion**
    - Angioinvasion**
  - >100 g
  - **WEISS scoring scheme has a grey zone and reproducibility issues!**

- **Histochemistry**
  - Loss of normal reticulin network (qualitative and quantitative changes)

- **Immunohistochemistry**
  - IGF-2, p53, Ki67, beta-catenin

- **Molecular Biology**
  - IGF-2, p53, beta-catenin, DLG7, BUB1B, PINK1
RETICULIN ALGORITHM IS VERY USEFUL IN THE DISTINCTION OF MALIGNANCY

If an adrenal cortical neoplasm shows a disruption of the reticulin framework, the diagnosis of malignancy is rendered when at least 1 of 3 following parameters are identified:

1. Necrosis
2. High mitotic activity (>5/50 high power fields)
3. Angioinvasion (Venous invasion)

RETICULIN FRAMEWORK IN ADENOMAS
Back to our case...
Qualitative and Quantitative Alterations in Reticulin Framework

Mitotic count: 12/50 HPF  Necrosis

Vascular invasion

Back to our case...

IGF-2

DIAGNOSIS
Angioinvasive low grade adrenal cortical carcinoma confined to the adrenal gland

Ki67 labeling index?
p53?
Beta-catenin?
MMR status?
The impact of genomics on endocrine pathology practice

Mete and Asa

Syndrome-Associated Cancer

Adrenocortical Carcinoma Is a Lynch Syndrome-Associated Cancer

**Abstract**

Adrenocortical carcinoma (ACC) is an endocrine malignancy with poor prognosis. The association of adverse clinical outcomes with familial predisposition to Lynch syndrome suggests that ACC may be related to Lynch syndrome. To explore this possibility, we analyzed the presence of Lynch syndrome in 135 ACC patients and 135 age, sex, and race-matched controls.

**Methods**

A cohort of 135 ACC patients with a histopathologically confirmed diagnosis was included. A control group of 135 age, sex, and race-matched individuals was obtained from a community hospital. Lynch syndrome was diagnosed based on the presence of at least one first-degree relative with Lynch syndrome and a second affected relative or at least two affected relatives with a hereditary cancer syndrome.

**Results**

In the ACC cohort, the presence of Lynch syndrome was found in 36% of patients, compared to 0% in the control group. All patients with ACC and Lynch syndrome had a family history of Lynch syndrome in at least two first-degree relatives. The presence of Lynch syndrome was associated with a higher incidence of metastases and a shorter overall survival.

**Conclusion**

Our findings suggest that ACC is a Lynch syndrome-associated cancer. Patients with ACC and a history of Lynch syndrome may benefit from genetic testing and closer surveillance. Further studies are needed to confirm these findings and to evaluate the impact of Lynch syndrome on ACC treatment and outcomes.
**DIAGNOSIS**

Angioinvasive low grade adrenal cortical carcinoma confined to the adrenal gland

**MMR status:** intact (no deficiency)

**Ki67 labeling index:** 11.67%

**p53:** no overexpression

**Beta-catenin:** no nuclear reactivity

**Functionality:** biochemically non-functioning tumor

---

**CURRENT CONCEPTS IN THE PATHOGENESIS OF ADRENAL CORTICAL NEOPLASIA**

- Recent data and observations suggest that carcinoma can arise in the background of adenoma
- Hyperplasia-neoplasia sequence
- Molecular pathways in cortisol and aldosterone-producing tumors
- Good prognosis cortical carcinomas
- Bad prognosis cortical carcinomas

---

**INHERITED TUMOR SYNDROMES WITH CORTICAL PROLIFERATIONS**

**“HYPERPLASIA-NEOPLASIA SEQUENCE”**

- Beckwith-Wiedeman Syndrome (11p15.5; IGF-2)
- Li-Fraumeni Syndrome (p53)
- Carney Complex (*PRKAR1A*)
- McCune Albright Syndrome (*PRKAR1A*)
- MEN 1 Syndrome (*MEN1*)
- MEN 4 Syndrome (p27^kip1)
- FAP Syndrome (*APC*)
- Lynch Syndrome (MMR)
- Congenital Adrenal Hyperplasia Syndrome (*CYP21, CYP11B1, HSD3B1, CYP17A1, CYP11A, POR*)
- Familial hyperaldosteronism (*KCNJ5, CYP11B1/CYP11B2*)
- Familial macronodular adrenal hyperplasia (*ARMC5*)
Mete and Asa

The impact of genomics on endocrine pathology practice

Adrenal cortical carcinoma in the setting of PPNAD


PATHOGENESIS OF CORTISOL-SECRETING ADENOMAS

CAMP/PKA PATHWAY

GNAS
PRKAR1A, PRKACA
PDE11A*, PDE8B*
(*) inactivating


PRIMARY MACRONODULAR ADRENAL HYPERPLASIA

GERMLINE ARMC5 MUTATIONS ~55%

ALDOSTERONOMAS
“CA++-MEDIATED SIGNALING PATHWAY”

- KCNJ5
- ATP1A1
- ATP2B3
- CACNA1D

GENOTYPE AND PHENOTYPE CORRELATIONS IN SPORADIC ALDOSTERONE-PRODUCING ADENOMAS

KCNJ5 Mutations
- large tumor size
- CYP17A1 is higher than in wild type
- predominant clear cells (Z.F-like)

ATP1A1 and CACNA1D Mutations
- small tumor size
- older males
- more compact cells (Z.G-like)

PROPOSED TRANSCRIPTOME-BASED ADRENAL CORTICAL TUMOR CLASSIFICATION

Transcriptome-based tumor classification

<table>
<thead>
<tr>
<th>Gene</th>
<th>Normal (NG)</th>
<th>Adrenal G (AG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GbonA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GbonB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GbonC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Molecular and Cellular Endocrinology 35 (2012) 111–117
The impact of genomics on endocrine pathology practice
**PROGNOSTICATORS**

- Stage
- Functional status
- Angioinvasion
- Mitotic Grading (based on mitotic activity in hot spots)
  - Low grade: <20/50 high power fields
  - High grade: >20/50 high power fields
- p53 (overexpression)
- Beta-catenin (diffuse nuclear)
- Ki-67 labeling index (hot spots)
- Molecular/Transcriptome profile
  - low PINK1 expression, BUB1B-PINK1 and DLG7-PINK1, and others

**TAKE HOME MESSAGE**

- Altered reticulin framework in association with any of the followings vascular invasion, mitotic activity >5/50HPF, or necrosis is diagnostic of adrenal cortical carcinoma
- Regardless of the grade, IGF-2 overexpression occurs in at least 90% of adrenal cortical carcinomas
- Around 3% ACCs are linked to extra-colic manifestations of Lynch syndrome (role of MMR immunohistochemistry)
- Functionality of the tumor, tumor stage, vascular invasion, mitotic grading, p53, beta-catenin, and Ki67 labeling index are predictive-prognostic parameters of adrenal cortical carcinomas