Emerging Trends in Neuroendocrine Tumor Management

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Objectives

1) Diagnostic dilemmas:
   - Understand the features distinguishing slow-growing tumors from the aggressive endocrine carcinomas

2) Therapeutic conundrums:
   - Can novel effective therapy be based on underlying pathogenetic/progression factors?
Aims:

- Advances in classification of NETs
- Clinical Features
- Biomarkers
- Molecular imaging
- Today’s therapeutic landscape
Myth #1

- Neuroendocrine tumors (NETs) are extremely rare
Increasing Incidence of neuroendocrine tumors in the western world

5.0/100,000 inhabitants

GEP-NET ~ 75%

Lung
Small intestine
Rectum

Surveillance, Epidemiology and End Results (SEER), US population 1974-2005

Modlin et al., Lancet Oncol. 2008
Myth #2

- Neuroendocrine tumors (NETs) are not really cancers
Survival in patients with pancreatic endocrine tumors
2004 WHO grading

Ekeblad et al
Myth #3:

- Neuroendocrine tumors are gut tumors resulting largely in flushing and diarrhea
## Cellular Classification of GEP Endocrine Tumors

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Cell</th>
<th>Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrin</td>
<td>G</td>
<td>ZE syndrome</td>
</tr>
<tr>
<td>Glucagon/GLP</td>
<td>A/L</td>
<td>DM/rash</td>
</tr>
<tr>
<td>Insulin</td>
<td>β</td>
<td>hypoglycemia</td>
</tr>
<tr>
<td>Secretin</td>
<td>S</td>
<td>WDHA</td>
</tr>
<tr>
<td>Serotonin</td>
<td>ED</td>
<td>“carcinoid”</td>
</tr>
<tr>
<td>Somatostatin</td>
<td>D</td>
<td>DM/gallstone</td>
</tr>
<tr>
<td>VIP</td>
<td>?</td>
<td>VM syndrome</td>
</tr>
</tbody>
</table>
## Classification of GEP Endocrine Tumor Cells

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Cell</th>
<th>Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholecystokinin</td>
<td>I</td>
<td>?</td>
</tr>
<tr>
<td>Pancreatic polypeptide</td>
<td>PP</td>
<td>?</td>
</tr>
</tbody>
</table>
GEP-Neuroendocrine tumours

- Carcinoid 50%
- Gastinomas 20%
- Insulinoma 15%
- VIPoma 7%
- Glucagonoma 5%
- Other 3%
Anatomic Distribution of GEP tumours

- appendix 35%
- ileum 20%
- stomach 10%
- rectum 10%
- bronchus 10%
- colon 5%
- duodunum 5%
- other 5%
“Carcinoid” or “Endocrine Carcinoma”

- well differentiated neuroendocrine tumors
- clinical “carcinoid syndrome”
- prognosis
Histologic Diagnosis of Endocrine Carcinoma

- Difficulties:
  - primary not obvious
  - malignancy not obvious
Immunohistochemical Diagnosis of Neuro-Endocrine Carcinomas

- General:
  - Neuron specific enolase (NSE)
  - Synaptophysin
  - Chromogranin
  - Thyroid transcription factor (TTF1)

Immunohistochemical Diagnosis of Neuro-Endocrine Carcinomas

- **Specific:**
  - **Hormones**
    - **Eutopic**
      - gastrin, insulin, glucagon, VIP, etc
    - **Ectopic**
      - GHRH, CRH, etc.
Criteria of Malignancy in GEP Tumors

- Cytologic features
- DNA Ploidy
- Proliferation markers:
  - *Ki 67 or MiB1 labeling index
- Invasion
  - capsular, adjacent tissues, perineural, vascular
### WHO 2004 Grading of Well-Differentiated Neuroendocrine Tumors

<table>
<thead>
<tr>
<th>Behavior</th>
<th>BENIGN</th>
<th>UNCERTAIN</th>
<th>LOW GRADE CARCINOMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extra-pancreatic extension</td>
<td>no</td>
<td>no</td>
<td>grossly visible (and/or)</td>
</tr>
<tr>
<td>Metastasis</td>
<td>no</td>
<td>no</td>
<td>yes (and/or)</td>
</tr>
<tr>
<td>Angioinvasion</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Perineural invasion</td>
<td>no</td>
<td>yes</td>
<td>±</td>
</tr>
<tr>
<td>Size</td>
<td>&lt;2 cm</td>
<td>&gt;/= 2cm</td>
<td>any</td>
</tr>
<tr>
<td>Mitosis/10HPF</td>
<td>&lt;/= 2</td>
<td>&gt;2</td>
<td>2-9</td>
</tr>
<tr>
<td>Mib1 (Ki-67)</td>
<td>&lt;/= 2%</td>
<td>&gt; 2%</td>
<td>2-10%</td>
</tr>
<tr>
<td>Hormones</td>
<td>non-functioning or insulin</td>
<td>gastrin, insulin, VIP, glucagon, somatostatin or ectopic ACTH, GH or PTHrP or non-functioning</td>
<td>gastrin, insulin, VIP, glucagon, somatostatin or ectopic ACTH, GH or PTHrP, or non-functioning tumors.</td>
</tr>
</tbody>
</table>
Translations of 7th edition planned
Carcinoids and Neuroendocrine Tumours

Staging

GI tract:
- Carcinoid: separate staging by site
- Small cell/large cell: stage as carcinoma

Pancreas: stage as carcinoma

Lung: stage as carcinoma

Skin: separate classification for Merkel cell carcinoma
Is it all in the genetic information?

Gain or loss of genetic information

Diagram showing various processes:
- Inherited or acquired “first hit”
- Acquired “second hit”
  - Terminal deletion
  - Mitotic recombination
  - Loss of reduplication
  - Loss without reduplication
  - Point mutation
  - Interstitial deletion
  - Disruptive translocation
  - Gene conversion
  - Epigenetic silencing

Legend:
- Black dot: Mutation
- Red: Gene conversion
- Blue: Epigenetic silencing

Note: No “second hit” required in this case.
Cancer Susceptibility Genes

[Graph showing relative risk vs. minor allele frequency for various genes related to cancer susceptibility, with highlights for TP53, BRCA1, BRCA2, PTEN, CDH1, STK11, BRIPI, ATM, PALB2, CHEK2, FGFR2, TOX3, MAP3K1, AKAP9, LSP1, and CASP8.]
Identifying Genetic Signatures in Cancers
DNA Microarray Analysis: GI Carcinoids and PNETs Cluster independently

Duerr et al. Endocr Rel Cancer. 2008
Benign and malignant PNETs cluster independently

- 112 genes differentially expressed with $P < 0.05$

- “benign” cluster
  - 3/3 WDETs – benign
  - 8/9 WDETs – LGM
  - 1/7 WDEC

- “malignant” cluster
  - 6/7 WDECs
  - 1/9 WDETs - LGM

Distinction between pNET and PDAC
### Table 1

Comparison of commonly mutated genes in PanNETs and PDAC

<table>
<thead>
<tr>
<th>Genes</th>
<th>PanNET</th>
<th>PDAC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MEN1</strong></td>
<td>44%</td>
<td>0%</td>
</tr>
<tr>
<td><strong>DAXX, ATRX</strong></td>
<td>43%</td>
<td>0%</td>
</tr>
<tr>
<td>Genes in mTOR pathway</td>
<td>15%</td>
<td>0.80%</td>
</tr>
<tr>
<td><strong>TP53</strong></td>
<td>3%</td>
<td>85%</td>
</tr>
<tr>
<td><strong>KRAS</strong></td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>CDKN2A</strong></td>
<td>0%</td>
<td>25%</td>
</tr>
<tr>
<td><strong>TGFBR1, SMAD3, SMAD4</strong></td>
<td>0%</td>
<td>38%</td>
</tr>
</tbody>
</table>

**a** Includes point mutations and indels.

**b** Data from Joneset *et al.*, *Science* 321, 1801 (2008).

**c** Based on 68 PanNETs and 114 PDACs.
Altered Telomers in DAXX/ATRX + pNETs

Science. 2011 Jul 22;333(6041):425
The Elusive Small Bowel NET Gene

Recurrent somatic mutations and deletions in CDKN1B, the cyclin-dependent kinase inhibitor gene, which encodes p27. Frameshift mutations of CDKN1B in 14 of 180 SI-NETs, and hemizygous deletions encompassing CDKN1B in 7 out of 50 SI-NETs: nominating p27 as a tumor suppressor and implicating cell cycle dysregulation in the etiology of SI-NETs.

*Nature Genetics; 2013:45,1483–1486.*
Progression and survival in classical “carcinoid syndrome”

Carcinoid syndrome

- <10% of carcinoid tumours

Features
- Diarrhea 83%
- Flushing 49%
- Dyspnea 20%
- Bronchospasm 6%
- Valvular heart disease

↑ serotonin levels
- 96% with carcinoid syndrome
- 25% without carcinoid syndrome
Diarrhea

- **Secretory diarrhea**
  - Large volume (>500cc/d)
  - Persists with fasting
- **Hypermotility**
- **Partial mechanical obstruction**
- **Mesenteric vascular insufficiency**
Diarrhea

- Differential diagnosis
  - Medullary cancer of the thyroid
  - VIPoma (WDHHA)
  - Gastrinoma (Zollinger-Ellison syndrome)
  - Rectal villous adenoma
  - Laxative ingestion
flushing
Flushing

- Dry – not associated with sweating
- Foregut tumours
  - Intense, protracted duration
  - Purplish
  - Followed by telangiectasia
  - Upper trunk + limbs
  - Acrocyanotic
  - Skin thickens
Flushing

- Midgut tumours
  - Faint pink – red
  - Face and upper truck (to nipple line)
  - Provoked by EtOH, tyramine containing foods (blue cheese, chocolate, sausage, red wine)
  - Last few minutes
  - No permanent discolouration
Flushed

Differential diagnosis

- Physiologic
  - Menopause, hot drinks, emotional

- Drugs
  - EtOH ± chlorpropramide/disulfiram, niacin, diltiazem, bromocriptine, levodopa

- Tumours
  - MTC, mastocytosis, renal cell carcinoma, VIPoma, diencephalic seizures
Carcinoid heart disease

- Valvular disease
  - Dyspnea
  - Fatigue
  - Ascites
  - Anorexia
  - Edema
Laboratory investigations

- Urinary 5-HIAA
- Serotonin
- Chromogranin A (CgA)
- Others
  - Bradykinin
  - Kallikrein
  - Neuropeptide K
  - Substance P
  - Pancreatic Polypeptide
  - Vasoactive intestinal Peptide
Biomarkers

- General marker: Chromogranin A
  - Pancreatic Polypeptide
  - HCG-subunits

- Specific markers: Gastrin
  - Urinary 5 HIAA
  - Insulin
    - Glucagon
    - Vasoactive intestinal Peptide
Urinary 5-HIAA

- 24 hour urine collection (HPLC)

<table>
<thead>
<tr>
<th>Condition</th>
<th>µmol/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10-42</td>
</tr>
<tr>
<td>Malabsorption</td>
<td>Up to 157</td>
</tr>
<tr>
<td>Carcinoid syndrome</td>
<td>&gt;523</td>
</tr>
<tr>
<td>Metastatic carcinoid w/o carcinoid syndrome</td>
<td>262-1360</td>
</tr>
</tbody>
</table>

- Sensitivity 75%
- Specificity 90%
Serootonin metabolism

Carcinoid cell

Tryptophan → Tryptophan hydroxylase → 5-hydroxytryptophan → Aromatic L-amino acid decarboxylase → Serotonin → Serotonin stored in secretory granules

Serotonin in blood → Serotonin uptake and storage in platelets

5-hydroxyindoleacetic acid in urine
Blood serotonin

- If 5-HIAA testing equivocal
- Normal fasting level 0.4-1.8 µmol/L
- Elevated in carcinoid syndrome (4.5-25.5 µmol/L)
Localization

- Endoscopy/wireless capsule
- CXR
- CT
- MRI
- Angiography
- Octreoscan
- PET
Endoscopy
Abdominal CT

- 87% sensitive for detecting ≥ 1 of:
  - Liver metastases
  - Mesenteric stranding
  - Lymph node enlargement
  - Primary tumour
    - rarely due to submucosal location
Abdominal CT

- Soft tissue mass
- Central calcification
- Desmoplastic response
- Spiculation of adjacent mesenteric fat
Molecular Imaging
Functional techniques

- Octreoscan (somatostatin receptor scintigraphy)

- MIBG-scintigraphy (metaiodobenzylguanidine)

- PET (positron emission tomography) ($^{11}$C-5-HTP, $^{18}$F-DOPA, $^{68}$Ga-Dota-octreotide $^{99}$Tc EDDA-HYNIC-TOC)
Specific isotopes for NETs

- $^{11}$C-5HTP (hydroxytryptophan)
- $^{11}$C-Dopamine
- $^{18}$F-Dopamine
- $^{68}$Ga-Dota Octreotide
- $^{99}$Tc EDDA-HYNIC-octreotide
- [Lys40(Ahx-DTPA-$^{111}$In)NH2]-Exendin-4 (GLP-1)
(A) Computed tomography (CT) scan, (B) somatostatin receptor scintigraphy (SRS), (C) 18F-dihydroxy-phenyl-alanine (18F-DOPA) positron emission tomography (PET), and (D) 11C-5-hydroxy-tryptophan (11C-5-HTP) PET of a 54-year-old male patient with metastatic islet cell tumor.

Advantages:

- No cyclotron required
- More sensitive than Octreoscan
- Possible to use for radioactive or tumor-targeted treatment
- May be possible to quantify somatostatin receptors - tumor-targeted therapy
PET/CT with $^{68}\text{Ga}-\text{DOTA-octreotide}$
Therapeutic Options NETs

- **Surgery**
  - Curative (rarely)
  - Ablative (very often)

- **Debulking**
  - Radiofrequency ablation (RFA)
  - Embolization/chemoembolization/
    radioembolization

- **Irradiation**
  - External (bone, brain-mets)
  - Tumor targeted
  - Radioactive therapy (MIBG, $^{90}$Y-DOTATOC,
    $^{177}$Lu-DOTATATE)

- **Medical therapy**
  - Chemotherapy
  - Biological treatment:
    - Somatostatin analogs
    - $\alpha$-interferon
    - m-TOR inhibitors
    - VEGF R inhibitors
    - Other TKI:s
Factors influencing the therapeutic decision

- Type of NET-tumor
- TNM stage and grade
- Extent of liver involvement
- Functioning v.s. non-functioning tumor
- Patients performance status
- Availability of different therapeutic modalities

*NB!* The treatment of most patients is a combination of surgery, PRRT and medical treatment
Conclusions

- Endocrine carcinomas of the GEP system vary widely in terms of:
  - pathogenetic mechanisms
  - morphologic appearance
  - biologic behavior
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- S. Gallinger
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- C. Moulton

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- R. Tsang
- R. Wong
Management Approaches

Metastatic NET

Surgery (resection, debulking RF, embolization)

WHO 1
Ki-67 <3%

WHO 1-2
Ki-67 3-20%

WHO 3
Ki-67 >20%

Biotherapy
- Somatostatin analogue (SMS)
- α-IFN
- Combination
- SMS + α-IFN
- SMS + Everolimus
- SMS + bevacizumab

Chemotherapy
- STZ + 5FU/Dox
- STZ + RAD001
- Temozolomide + capecitabine
- SMS for symptom control

Chemotherapy:
- Cispl + Etoposide
- Temozolomide
- + capecitabine
- + bevacizumab
- SMS for symptom control

Targeted Radiotherapy
- Lu\textsuperscript{177} DOTA-octreotate, Y\textsuperscript{90} DOTATOC

Experimental Protocols