Gastroenteropancreatic neuroendocrine tumors: classification, diagnostic pathology and molecular advances

Jean-Yves SCOAZEC

Service central d’Anatomie et Cytologie Pathologiques, Hôpital Edouard Herriot, Hospices Civils de Lyon
Lyon Cancer Research Center, INSERM U1052/CNRS U5286

Tumeurs neuroendocrines du tractus gastro-intestinal et du pancréas Classification et difficultés diagnostiques

Jean-François Fléjou
Service d’Anatomie Pathologique
Hôpital Saint-Antoine, AP-HP
Faculté de Médecine Pierre et Marie Curie, Paris

en collaboration avec Jean-Yves Scoazec
(Hôpital Edouard Herriot, Lyon)
What is a «neuroendocrine» tumor?

- A tumor made of neoplastic cells of epithelial derivation, characterized by structural, phenotypic and/or functional properties recalling those of normal peptide- or amine-producing endocrine cells

Distribution of NETs

<table>
<thead>
<tr>
<th>Type</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digestive NETs</td>
<td>67.5%</td>
</tr>
<tr>
<td>Bronchial NETs</td>
<td>25.3%</td>
</tr>
<tr>
<td>Genito-urinary NETs</td>
<td>1%</td>
</tr>
<tr>
<td>Other sites</td>
<td>6.2%</td>
</tr>
<tr>
<td>Thymus, skin, breast, ear</td>
<td></td>
</tr>
</tbody>
</table>

Modlin et al. Cancer 2003;97:934
The contributions of the pathologist to NET diagnosis and … to patient care

- To identify the neuroendocrine nature of a tumor
- To evaluate its risk of malignancy and rate of progression
- To bring up additional informations useful for prognosis evaluation, treatment and follow-up

Diagnosis and classification

Diagnosis: how to identify a tumor as a «neuroendocrine» tumor?
The diagnosis is easy when the tumor is well differentiated

The immunophenotype of peptide (or amine)-producing endocrine cells

- Chromogranin A in the endocrine pancreas
- Endocrine markers: chromogranins A et B, other granins
- Large dense core granules containing mature hormones and their precursors
- Small synaptic vesicles containing neuropeptides
- Neuro-endocrine markers: synaptophysin, ...
- Neuro-endocrine markers: NSE, PGP9.5
- Synaptophysin expression by intestinal endocrine cells
What is required for the pathological diagnosis?

- One peptidergic endocrine marker
  - Chromogranin A

  and

- One or several neuro-endocrine markers:
  - Synaptophysin
  - NCAM
  - Leu 7 (CD57)
  - PGP9.5

The diagnosis may be difficult when the tumor is poorly differentiated

- «Small cell» morphology (usually)
- «Large cell» morphology (rarely)
- High mitotic activity
- Frequent necrosis
**Immunohistochemistry is mandatory for a correct diagnosis**

- To demonstrate the neuroendocrine differentiation of tumor cells
- To rule out an undifferentiated tumor of other origin
- To confirm the poorly differentiated nature of the tumor in difficult cases

<table>
<thead>
<tr>
<th>Endocrine markers</th>
<th>Chromogranin A</th>
<th>Frequently negative</th>
<th>Sometimes positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuroendocrine markers</td>
<td>Synaptophysin</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leu7, PGP9.5, NCAM, NSE</td>
<td>Frequently positive</td>
<td></td>
</tr>
<tr>
<td>Other markers</td>
<td>Keratins</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EMA Carcino-embryonic antigen</td>
<td>Frequently positive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p53</td>
<td>Strongly positive</td>
<td></td>
</tr>
</tbody>
</table>
Malignancy and site

- Incidence of malignancy according to the site of the primary:
  - Pituitary, parathyroids: exceptional
  - Bronchi: 20%
  - Digestive tract and pancreas: 60%

Three related questions

- How to identify an already malignant tumor?
- How to identify a tumor which may eventually behave as a malignant tumor?
- For malignant tumors, how to predict the rate of evolution?
After the diagnosis ...

- Classification
- Grading
- Staging

Classifications

- Integration of morphological, biological and molecular informations
- in order to define categories of prognostic significance and clinical relevance (follow-up, therapeutic strategy)
- based on simple, robust, validated and reproducible criteria
After the diagnosis ...

- Classification
- Grading
- Staging

- WHO
- ENETS/UICC
- TNM

All three informations are complementary and mandatory

Recent classifications

- The modern era starts with a common effort

- Rapidly, classifications diverged

Thoracic NETs, 2004
GEP-NETs, 2000 - 2004 - 2010
Digestive NETs: too much?

- Classification
  - WHO
    - 2000 ... 2004
    - 2010
- Grading
- Staging
  - ENETS
    - ENETS 2006
    - ENETS/UICC 2010
  - TNM
    - ENETS (2006-2007)
    - UICC (2010)

WHO classification, 2000

- Well differentiated endocrine tumors
  - Benign behavior
  - Uncertain behavior
- Well differentiated endocrine carcinomas
  - Low grade of malignancy
- Poorly differentiated endocrine carcinomas
  - High grade of malignancy
First criteria: morphological differentiation

<table>
<thead>
<tr>
<th>Category</th>
<th>Characteristics</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poorly differentiated</td>
<td>Always malignant</td>
<td>5-10%</td>
</tr>
<tr>
<td></td>
<td>High grade</td>
<td></td>
</tr>
<tr>
<td>Well differentiated</td>
<td>Benign or malignant?</td>
<td>90-95%</td>
</tr>
<tr>
<td></td>
<td>Not predictive of the behavior</td>
<td></td>
</tr>
</tbody>
</table>

Second criteria: Evidence of objective signs of malignancy

- Metastatic dissemination
- Local invasion

If yes, diagnosis:
Well differentiated endocrine carcinoma (low grade of malignancy)

If not, diagnosis:
Well differentiated endocrine tumor
**Third criteria:**
Evidence of *predictive* signs of malignancy

- Size (< or > threshold)
- Mitotic index or proliferation index (< or > threshold)
- Angioinvasion
- Perineural invasion
- Functioning syndrome (except insulinoma)

**If no at all the criteria:**
Well differentiated endocrine tumor, benign behavior

**If yes at least one criteria:**
Well differentiated endocrine tumor, uncertain behavior

**A complex assemblage of criteria, adapted to each anatomical site**

<table>
<thead>
<tr>
<th>WHO 2000</th>
<th>Well differentiated endocrine tumor</th>
<th>Well differentiated endocrine carcinoma</th>
<th>Poorly differentiated endocrine carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Benign behavior</td>
<td>Uncertain behavior</td>
<td>Uncertain behavior</td>
</tr>
<tr>
<td><strong>Histological differentiation</strong></td>
<td>Well differentiated</td>
<td>Well differentiated</td>
<td>Well differentiated</td>
</tr>
<tr>
<td><strong>Metastasis</strong></td>
<td>Absent</td>
<td>Absent</td>
<td>Possible</td>
</tr>
<tr>
<td><strong>Size</strong></td>
<td>Stomach, small bowel: (\leq 1) cm</td>
<td>Stomach, small bowel: &gt;1 cm</td>
<td>Stomach, small bowel: usually &gt;1 cm</td>
</tr>
<tr>
<td></td>
<td>Appendix, colon, rectum, pancreas: ≤2 cm</td>
<td>Appendix, colon, rectum, pancreas: &gt;2 cm</td>
<td>Appendix, colon, rectum, pancreas: Usually &gt;2 cm</td>
</tr>
<tr>
<td><strong>Angioinvasion Perineural invasion</strong></td>
<td>Absent</td>
<td>Present *</td>
<td>Possible</td>
</tr>
<tr>
<td><strong>Mitotic index</strong></td>
<td>&lt;2</td>
<td>&gt;2</td>
<td>Usually &gt;2</td>
</tr>
<tr>
<td><strong>Ki 67 index</strong></td>
<td>≤2%</td>
<td>&gt;2%</td>
<td>Usually &gt;2%</td>
</tr>
<tr>
<td><strong>Functional syndrome (clinical)</strong></td>
<td>No (except insulinoma)</td>
<td>Yes (except insulinoma)</td>
<td></td>
</tr>
</tbody>
</table>

* except for appendiceal tumors
Clinical validation

Problems

- Terminology
- Complexity
  - Combination of histological and clinical criteria
  - Combination of classification and staging
- Inadequacies
  - Not applicable to biopsies and cytological examinations
  - Lack of relevance of the category « well differentiated endocrine tumors of uncertain behavior »
  - Lack of accurate description and typing of poorly differentiated tumors
  - Lack of accurate prognostic evaluation of well differentiated carcinomas
Histological grading
(ENETS, 2006)

**Grading system (ENETS, 2006)**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Mitotic count (10 HPF)</th>
<th>Ki-67 index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>&lt;2</td>
<td>≤2</td>
</tr>
<tr>
<td>G2</td>
<td>2–20</td>
<td>3–20</td>
</tr>
<tr>
<td>G3</td>
<td>&gt;20</td>
<td>&gt;20</td>
</tr>
</tbody>
</table>

*10 HPF: high power field=2 mm², at least 40 fields (40x magnification) evaluated in area of highest mitotic density

*Ki-67 antibody, % of 2,000 tumor cells in areas of highest nuclear labeling

---

Histological grading

Histologically well differentiated carcinomas

Histologically moderately differentiated carcinomas

Histologically poorly differentiated carcinomas

---

Histological grading

Histologically well differentiated carcinomas

Histologically moderately differentiated carcinomas

Histologically poorly differentiated carcinomas
Histological grading

Well differentiated carcinomas

<table>
<thead>
<tr>
<th>Grade</th>
<th>Microm vasc (10 HPF)</th>
<th>Ki-67 index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI</td>
<td>&lt;2</td>
<td>≤2</td>
</tr>
<tr>
<td>G2</td>
<td>2-20</td>
<td>3-20</td>
</tr>
<tr>
<td>G3</td>
<td>&gt;20</td>
<td>&gt;20</td>
</tr>
</tbody>
</table>

(10 HPF: high power field ×2 mm², at least 40 fields at 40x)

Clinical validation

Neuroendocrine Tumors of Midgut and Hindgut Origin: Tumor-Node-Metastasis Classification Determines Clinical Outcome

Prognostic Validity of a Novel American Joint Committee on Cancer Staging Classification for Pancreatic Neuroendocrine Tumors

Journal of Clinical Oncology
Histological grading: issues and perspectives

- Is the determination of the proliferation index simple, robust and reproducible?
- How accurate are the cut-offs currently in use?

Staging:
TNM classification
Integration between prognostic evaluation and clinical staging: ENETS proposals (2006-2007)

• for all digestive NETs:
  – «benign» and «malignant»
  – well and poorly differentiated
• based on criteria adapted to the anatomical location

Clinical validation

Cancer 2008;113:256  Hôpital Edouard Herriot

- Specific classification:
  - Well differentiated NETs of the digestive tract
- Classification identical to that of carcinomas of the same anatomical location:
  - Poorly differentiated NETs of the digestive tract
  - Pancreatic NETs (well and poorly differentiated)
  - Lung NETs (well and poorly differentiated)

Which are the differences between ENETS proposals and UICC/AJCC classification?

- ENETS=UICC for:
  - Well differentiated neuroendocrine tumors of stomach, small bowel, colon and rectum
- ENETS≠UICC for:
  - Well differentiated neuroendocrine tumors of the appendix and pancreas
  - Poorly differentiated neuroendocrine tumors
WHO Classification of Tumours of the Digestive System
WHO classification, 2010

- Neuroendocrine tumor/neoplasm, G1
- Neuroendocrine tumor/neoplasm, G2
- Neuroendocrine carcinoma
  - Small cell type
  - Large cell type
- Mixed adeno-neuroendocrine carcinoma

WHO classification, 2010

- Neuroendocrine neoplasm, G1
  - Well differentiated morphology
  - MI <2 and Ki67 index ≤2%
- Neuroendocrine neoplasm, G2
  - Well differentiated morphology
  - MI: 2-20 and Ki67 index: 3-20%
- Neuroendocrine carcinoma
  - Small cell type
  - Large cell type
- Mixed adeno-neuroendocrine carcinoma
WHO classification, 2010

Changes in terminology and classification mean changes in principles and concepts

- Changes in scope
  - Full separation between typing (WHO) and staging (TNM)
- Changes in basic concepts
  - All NET are potentially malignant
  - Proliferation fraction, and not only morphological differentiation, is a key parameter

Table 4: Grading proposal for foregut (neuroendocrine) tumors

<table>
<thead>
<tr>
<th>Grade</th>
<th>Mitotic count (10 HPF)</th>
<th>Ki-67 index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>&lt;2</td>
<td>2</td>
</tr>
<tr>
<td>G2</td>
<td>2-20</td>
<td>3-20</td>
</tr>
<tr>
<td>G3</td>
<td>&gt;20</td>
<td>&gt;20</td>
</tr>
</tbody>
</table>

*10 HPF: high power field=2 mm², at least 40 fields at 40x magnification evaluated in areas of highest mitotic density

**Ki67 antibody, % of 2,000 tumor cells in areas of highest nuclear labeling

Homologies ...

<table>
<thead>
<tr>
<th>Thoracic NETs WHO 2004</th>
<th>Typical carcinoid</th>
<th>Atypical carcinoid</th>
<th>Large cell neuroendocrine carcinoma</th>
<th>Small cell neuroendocrine carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>GEP-NETs WHO 2010</td>
<td>Neuroendocrine tumor G1 (or carcinoid)</td>
<td>Neuroendocrine tumor G2</td>
<td>Neuroendocrine carcinoma, large cell type</td>
<td>Neuroendocrine carcinoma, small cell type</td>
</tr>
</tbody>
</table>

mitoses

A progress ?

- **For diagnosis:**
  - + Applicable to small-sized tissue samples (biopsy, endoscopical resections …), even to cytological preparations
  - + A better definition of poorly differentiated gastroenteropancreatic tumors
- **For prognosis:**
  - + Over estimation of grading ?
  - + Under estimation of morphological differentiation ?
Some important issues

- A new terminology ...
  - Tumor/neoplasm
  - Neuroendocrine
  - Carcinoid: once more ...
  - Neuroendocrine carcinoma in 2010: always poorly differentiated!
- Caution: the reclassification of cases during their follow-up ...

Correspondence 2000/2010
Correspondence 2000/2010

<table>
<thead>
<tr>
<th>WHO 2010</th>
<th>WHO 2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuroendocrine neoplasm G1</td>
<td>• Well differentiated endocrine tumor of benign behavior</td>
</tr>
<tr>
<td></td>
<td>• Well differentiated endocrine tumor of uncertain behavior with mitotic index &lt;2 and Ki67 index ≤2%</td>
</tr>
<tr>
<td></td>
<td>• Well differentiated endocrine carcinoma with mitotic index &lt;2 and Ki67 index ≤2%</td>
</tr>
<tr>
<td>Neuroendocrine neoplasm G2</td>
<td>• Well differentiated endocrine tumor of uncertain behavior with mitotic index 2-20 and/or Ki67 index 3-20%</td>
</tr>
<tr>
<td></td>
<td>• Well differentiated endocrine carcinoma with mitotic index 2-20 and/or Ki67 index 3-20%</td>
</tr>
<tr>
<td>Neuroendocrine carcinoma, small cell type</td>
<td>Poorly differentiated endocrine carcinoma, small cell type</td>
</tr>
<tr>
<td>Neuroendocrine carcinoma, large cell type</td>
<td>No corresponding category</td>
</tr>
<tr>
<td>Adeno-neuroendocrine carcinoma</td>
<td>Mixed tumor</td>
</tr>
</tbody>
</table>

Future trends

- **Definition of NET spectrum**
  - Relation between well and poorly differentiated neoplasms: one or several diseases?
- **Improvement of typing**
  - Neoplasms with well differentiated morphology, G3?
- **Refinement of grading**
  - Is Ki67 index a reproducible criteria?
  - Are the current thresholds accurate and relevant?
- **Validation of staging**
- **Integration of molecular informations**
  - Diagnostic and prognostic markers
  - Predictive markers in the era of targeted therapies
Minimal items for the pathological report

- Diagnostic arguments
  - Morphological arguments
  - Immunohistochemical arguments
- Classification
  - WHO 2010 (WHO 2000 in brackets)
- Histological grading
  - Absolute values
- pTNM
  - UICC 2010 (along with ENETS, especially for appendix and pancreas)

The pathologist may provide useful additional informations
Identification of a syndrome of genetic predisposition: MEN-1

- Histological arguments
  - Endocrine hyperplasia in target tissues

- Molecular arguments
  - Loss of expression of target proteins

Identification of factors predictive of the response to treatment

sst2  MGMT  CD34  EGFR  pmTOR
Predictive markers: MGMT and temozolomide

- **MGMT**
  - Interest in NETs and pheochromocytomas
  - Loss of protein expression and/or gene silencing associated with increased response
  - Evaluation:
    - Immunohistochemistry
    - Promoter methylation


MGMT deficiency:
- 51% pancreatic NET
- 0% gut NET

Molecular advances
Molecular mechanisms involved in early tumorigenesis and tumor progression are poorly known

- Tumorigenesis
  - No role for the «conventional» oncogenes and tumor suppressor genes known to be involved in epithelial carcinogenesis
  - Several genes involved in syndromes of familial predisposition to endocrine tumors, behaving as tumor suppressor genes with a role in early tumorigenesis
    - MEN-1, but also NF1, TSC1/2, VHL ...
  - Some candidate genes emerging for sporadic pancreatic NETs
    - MEN-1, DAXX/ATRX, genes of the mTOR pathway

- Tumor progression
  - Limited knowledge about the molecular mechanisms involved in local invasion and metastatic dissemination

Molecular mechanisms

- Several genes involved in predisposition syndromes encode proteins of the mTOR pathway or of the hypoxia response pathway
- Some of these genes are also involved in sporadic tumors

Molecular mechanisms

Table 1. Comparison of commonly mutated genes in PanNETs and PDAC based on 68 PanNETs and 114 PDACs.

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

Prognostic relevance
Prognostic relevance

Pancreatic Endocrine Tumors: Expression Profiling Evidences a Role for AKT-mTOR Pathway

MEN1, DAXX/ATRX TSC1/2, NF1, PTEN VHL

Pancreatic NETs
The diagnosis of endocrine tumors is usually easy: however, be aware of the possible pitfalls!

Their classification is in a state of flux: validation studies are needed!

New predictive factors would be helpful for a better evaluation of the prognosis and for the design of new therapeutic strategies: more basic, translational and clinical research is required!

Importance of networks and collaborative studies