Recent Updates on Neuroendocrine Tumors From the Gastrointestinal and Pancreatobiliary Tracts

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Context.—Gastrointestinal (GI) and pancreatobiliary tracts contain a variety of neuroendocrine cells that constitute a diffuse endocrine system. Neuroendocrine tumors (NETs) from these organs are heterogeneous tumors with diverse clinical behaviors. Recent improvements in the understanding of NETs from the GI and pancreatobiliary tracts have led to more-refined definitions of the clinicopathologic characteristics of these tumors. Under the 2010 World Health Organization classification scheme, NETs are classified as grade (G) 1 NETs, G2 NETs, neuroendocrine carcinomas, and mixed adenoneuroendocrine carcinomas. Histologic grades are dependent on mitotic counts and the Ki-67 labeling index. Several new issues arose after implementation of the 2010 World Health Organization classification scheme, such as issues with well-differentiated NETs with G3 Ki-67 labeling index and the evaluation of mitotic counts and Ki-67 labeling.

Objective.—To understand clinicopathologic characteristics of NETs from the GI and pancreatobiliary tracts.

Data Sources.—PubMed (US National Library of Medicine) reports were reviewed.

Conclusions.—In this review, we briefly summarize recent developments and issues related to NETs of the GI and pancreatobiliary tracts.


Neuroendocrine tumors (NETs) from the gastrointestinal (GI) and pancreatobiliary tracts are heterogeneous tumors with diverse biologic and clinical behaviors that vary according to the primary tumor origin, type of neuroendocrine cell, and pathologic features. The distribution patterns of NETs in the GI tract seem to be different between Eastern and Western populations. The most common location of NETs in the GI tract among patients in the United States is due to increased detection of gastric and rectal NETs. In contrast, the rectum (48%) is the most frequent location of NETs in the GI tract of patients in Korea, followed by the stomach (15%), pancreas (9%), colon (8%), small intestine (8%), liver (7%), appendix (3%), and biliary tract (2%). The incidence of NETs in the GI tract and pancreas has increased in recent years, mainly because of a marked increased detection of rectal NETs, whereas the incidences of NETs in other parts of the GI tract are unchanged in the Korean population.

DISTRIBUTIONS OF NORMAL ENDOCRINE CELLS AND THEIR PRODUCTION

The GI and pancreatobiliary tracts contain a variety of neuroendocrine cells that constitute a diffuse endocrine system. Endocrine cells in the GI tract consist of less than 1% of the mucosa; are normally distributed at the surface or base of glandular epithelial cells, such as in the gastric pits of the stomach and the crypts of the small intestine and colorectum; and contain secretory granules that release various peptide hormones. Endocrine cells comprise 1% to 2% of the volume of the adult pancreas and most form well-circumscribed nests called islets of Langerhans; a few scattered endocrine cells are also present in the main pancreatic and larger interlobular ducts but are not observed in the smaller ducts. Endocrine cells in the pancreas produce several peptide hormones, including insulin, glucagon, somatostatin, pancreatic polypeptide (PP), and vasoactive intestinal peptide. The most common cells are insulin-producing β cells, which account for 60% to 80% of all islet cells and are centrally located in the islets, whereas glucagon-producing α cells are located at the periphery of
islets and constitute 15% to 20% of the islet volume. Somatostatin-producing δ cells and PP-producing cells constitute the remaining portions. Extrapancreatic biliary epithelia also contain scattered endocrine cells in the intrapancreatic portion of the common bile duct.

Understanding the normal distribution of endocrine cells in the GI and pancreatobiliary tracts is important because there is a correlation between the distributions of specific types of endocrine cells and the preferential primary sites of specific hormone-producing NETs in the GI and pancreatobiliary tracts. However, there are some exceptions, such as no occurrence of cholecystokinin-, gastrin-, motilin-, or secretin-producing tumors in the small intestine.

Similarly, the predominance of enterochromaffin (EC) cell serotonin-producing NETs in the ileum and appendix and δ cell somatostatin-producing NETs in the duodenum and ampulla is abnormal despite the even distribution of EC and δ cells throughout the GI and pancreatobiliary tracts. Aberrant gastrin-producing tumors (gastrinomas) in the pancreas also cannot be explained by the normal distribution of endocrine cells in the GI and pancreatobiliary tracts. We summarize the normal distributions of various types of endocrine cells in the GI and pancreatobiliary tracts in Table 1.

**NEUROENDOCRINE MARKERS**

Endocrine cells in the GI and pancreatobiliary tracts and NETs are labeled by neuroendocrine markers, including synaptophysin, chromogranin A, CD56/NCAM1, Leu7/3B3GAT1, protein gene product 9.5 (PGP9.5), and neuron-specific enolase. Synaptophysin is considered the most sensitive neuroendocrine marker, whereas chromogranin A is the most specific. Therefore, only synaptophysin and chromogranin A are recommended for use in routine practice, and other neuroendocrine markers, such as CD56/NCAM1, Leu7, and neuron-specific enolase, are not recommended because of their low specificity.

Most GI NETs express CDX2, whereas some pancreatic NETs also express CDX2. Several transcription factor proteins, such as pancreatic and duodenal homeobox 1 (PDX1), islet 1 (ISL-1), and PAX6, have been reported to be pancreas specific. In the setting of metastatic NETs with an unknown primary site, use of a panel of immunohistochemical staining with CDX2, ISL-1 (or PDX1), and thyroid transcription factor 1 (TTF-1) can help to identify the primary origin of the metastatic NETs, although some studies reported that these markers can also be expressed in NETs from other locations.

BCL2 overexpression, loss of RB expression, and abnormal p53 expression (either total loss or overexpression) were more commonly seen in poorly differentiated neuroendocrine carcinomas (NECs), whereas expression of those proteins was reported in a few well-differentiated NETs. Therefore, BCL2, RB, and p53 immunohistochemical staining can be useful in some settings for discriminating well-differentiated NETs from poorly differentiated NECs.

**WORLD HEALTH ORGANIZATION CLASSIFICATIONS OF NEUROENDOCRINE TUMORS**

The term carcinoid has been used for several decades to describe most GI NETs after it was proposed by the World Health Organization (WHO) in 1980. The term is not used for several other tumors, such as pancreatic islet cell tumors and small cell carcinomas.

The WHO 2000 classification divided NETs from the GI and pancreatobiliary tracts into well-differentiated endocrine tumors, well-differentiated endocrine carcinomas, and poorly differentiated endocrine carcinomas, based on the degree of differentiation. Well-differentiated endocrine tumors were further classified into benign tumors and low-grade malignant tumors, based on the tumor size, mitotic rate, Ki-67 labeling index, lymphovascular invasion, and symptoms, in association with hormonal oversecretion, whereas poorly differentiated endocrine carcinomas usually indicate small cell and large cell carcinomas. Well-differentiated NECs have been regarded as low-grade malignancies, and poorly differentiated NECs were considered high-grade malignant tumors. Both well-differentiated and poorly differentiated endocrine carcinomas are invasive cancers with the ability to metastasize to distant organs. The term neuroendocrine neoplasm has been accepted as general nomenclature instead of carcinoid because carcinoid does not convey the malignant nature of the tumors and can be confused with carcinoid syndrome.

The recent WHO 2010 classification categorized all NETs from the GI and pancreatobiliary tracts as malignant tumors, except for gangliocytic parangangioma and pancreatic neuroendocrine microadenomas, which are classified as...
benign tumors, and L-cell–type (glucagon-like peptide [GLP] and peptide YY [PYY]-producing) NETs and tubular carcinoids, which are classified as uncertain malignancies. We summarize the NETs from the GI and pancreatobiliary tracts under the current WHO 2010 scheme in Table 2. In general, well-differentiated NETs are well-circumscribed, cellular tumors with sheets of uniform tumor cells. Variable growth patterns, including nests, trabecular, glandular, gyriform, acinar, and solid patterns, have been observed for NETs from the GI and pancreatobiliary tracts. The nuclei are round to oval and stippled, and the chromatin shows the typical “salt-and-pepper” pattern. The 2010 WHO classification divides NETs of the digestive tracts into NET grade (G) 1, NET G2, and NECs, based on mitotic counts and the Ki-67 proliferation index, regardless of tumor size, extent, or location (Table 3). In contrast, mixed adenoneuroendocrine carcinomas contain both malignant glandular and NEC components, and each component should be more than one-third of the tumor volume (Figure 1, A).

Grade 1 is a NET with a mitotic count of less than 2 per 10 high-power fields (HPFs) and/or less than 3% Ki-67 labeling index; G2 is a NET with a mitotic count of 2 to 20 per 10 HPFs and/or a 3% to 20% Ki-67 labeling index; and NEC is a small cell carcinoma or large cell carcinoma with a mitotic rate of more than 20 per 10 HPFs and/or greater than a 20% Ki-67 labeling index. For precise evaluation of the grading, a minimum of 50 HPFs for the mitotic count and at least 500 cells for the Ki-67 labeling index should be counted from hot spots. In about one-third of the NET cases, a discrepancy between the grades of the mitotic count and Ki-67 labeling index are observed and, on these occasions, the higher grade, either that of the mitotic count or the Ki-67 labeling index, should be used. An increased mitotic activity and proliferation index have been associated with an aggressive clinical behavior and poor prognosis.

### ISSUES WITH GRADING

#### Issues With the Mitotic Count

The mitotic count should be calculated from the most active areas (or hot spots), which are recognized by scanning the sample under intermediate magnification. As described above, a minimum of 50 HPFs should be carefully evaluated to precisely determine the mitotic count, a task that requires a minimum of about 3 minutes. In general, 10 HPFs with a ×40 objective lens on a light microscope are equivalent to an area of 2 mm². However, the exact area depends on the eyepiece field, which varies among light microscope manufacturers and models. It is extremely difficult to discriminate true mitosis from mitosis mimics, including pyknosis, apoptotic bodies, or shrunken nuclei, but such discrimination is important because, otherwise, the area is not counted as mitosis. These issues lead to poor reproducibility of mitotic counts between observers. Recently, a mitosis-specific marker, phosphohistone H3 (PHH3), was introduced for the assessment of mitotic counts in NETs. Mitotic counts determined by PHH3 staining and hematoxylin-eosin staining showed a high concordance rate, but their results need to be validated using many cases.

#### Issues With Ki-67 Quantification

Ki-67 is expressed in cells during all active phases of the cell cycle, except for the resting (G0) phase. As the time from clamping of vessels and surgical resection of NET to tissue fixation increases, the mitotic counts in surgically resected specimens tend to decrease abruptly. Therefore, grading by the Ki-67 labeling index is always higher than grading by mitosis. Evaluation of the Ki-67 labeling index may be influenced by several factors, such as the use of different clones of the Ki-67 antibody, use of different Ki-67 staining protocols among laboratories, different thicknesses of the section used for Ki-67 staining, and the density of the

### Table 2. Distribution and International Classification of Diseases for Oncology, 3rd Ed. (ICD-O-3) Codes of Neuroendocrine Tumors (NETs) in the Gastrointestinal and Pancreatobiliary Tracts in the 2010 World Health Organization Classification

<table>
<thead>
<tr>
<th>NET Classification</th>
<th>Location</th>
<th>ICD-O-3 Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>NET G1</td>
<td>All organs</td>
<td>8240/3</td>
</tr>
<tr>
<td>NET G2</td>
<td>All organs</td>
<td>8249/3</td>
</tr>
<tr>
<td>Neuroendocrine carcinoma</td>
<td>All organs</td>
<td>8246/3</td>
</tr>
<tr>
<td>Large cell NEC</td>
<td>All organs</td>
<td>8013/3</td>
</tr>
<tr>
<td>Small cell NEC</td>
<td>All organs</td>
<td>8041/3</td>
</tr>
<tr>
<td>EC cell serotonin-producing NET</td>
<td>All organs</td>
<td>8241/3</td>
</tr>
<tr>
<td>Gastrin-producing NET (gastroinoma)</td>
<td>Stomach, ampulla, small intestine, pancreas</td>
<td>8153/3</td>
</tr>
<tr>
<td>Glucagon-producing NET (glucagonoma)</td>
<td>Pancreas</td>
<td>8152/3</td>
</tr>
<tr>
<td>Somatostatin-producing NET (somatostatinoma)</td>
<td>Ampulla, small intestine</td>
<td>8156/3</td>
</tr>
<tr>
<td>Insulin-producing NET (insulinoma)</td>
<td>Pancreas</td>
<td>8151/3</td>
</tr>
<tr>
<td>VIPoma</td>
<td>Pancreas</td>
<td>8155/3</td>
</tr>
<tr>
<td>L cell, Glucagon-like peptide and PP/PYY-producing NETs</td>
<td>Small intestine, appendix, colorectum</td>
<td>8152/1</td>
</tr>
<tr>
<td>Goblet cell carcinoid</td>
<td>Appendix, extrahepatic bile duct</td>
<td>8241/3</td>
</tr>
<tr>
<td>Tubular carcinoid</td>
<td>Appendix, extrahepatic bile duct</td>
<td>8245/1</td>
</tr>
<tr>
<td>Mixed adenoneuroendocrine carcinoma (MANEC)</td>
<td>All organs</td>
<td>8244/3</td>
</tr>
<tr>
<td>Neuroendocrine microadenoma</td>
<td>Pancreas</td>
<td>8150/0</td>
</tr>
</tbody>
</table>

Abbreviations: EC, enterochromaffin; G, grade; NEC, neuroendocrine carcinoma; NET, neuroendocrine tumor; PP, pancreatic polypeptide; PYY, peptide YY; VIP, vasoactive intestinal peptide.

### Table 3. World Health Organization 2010 Classification of Neuroendocrine Tumors (NETs) in the Gastrointestinal and Pancreatobiliary Tracts

<table>
<thead>
<tr>
<th>Grade</th>
<th>Mitotic Count/10 HPFs</th>
<th>Ki-67 Labeling Index, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>NET, grade 1</td>
<td>&lt;2</td>
<td>&lt;3</td>
</tr>
<tr>
<td>NET, grade 2</td>
<td>2–20</td>
<td>3–20</td>
</tr>
<tr>
<td>NEC, grade 3</td>
<td>&gt;20</td>
<td>&gt;20</td>
</tr>
</tbody>
</table>

Abbreviations: HPF, high-power field; NEC, neuroendocrine carcinoma.
tumor cells. For Ki-67 counting, strong, dark-brown nuclear staining is recommended for counting, whereas cytoplasmic staining or weak nuclear labeling should not be counted. In routine pathology practice, the most commonly used method for the evaluation of Ki-67 labeling is a quick count under microscopic examination, the so-called eyeball estimation. In addition, several other methods are used to assess Ki-67 labeling, including manual counting and automated digital image analysis. Although there are still controversies about the agreement of Ki-67 labeling with the eyeball estimation (good versus poor inter-observer agreement rate), use of the eyeball estimation is discouraged in routine practice because of its inaccuracy, especially at the G1 to G2 boundary. On the other hand, manual counting of camera-captured or printed images is considered to be the most practical, reproducible, and cost-effective method of calculating the Ki-67 labeling index.

ISSUES WITH G3 WELL-DIFFERENTIATED NEUROENDOCRINE TUMORS

In the 2010 WHO classification scheme, NECs are defined as poorly differentiated tumors with a mitotic rate of more than 20 per 10 HPFs and/or a greater than 20% Ki-67 labeling index. The NECs are subclassified as small cell carcinomas or large cell carcinomas. However, a recent study of pancreatic NETs showed that the survival time of patients with well-differentiated G2 discordant (mitotic count G2 and Ki-67 index G3) NETs was better than that of patients with poorly differentiated NECs and worse than that of patients with well-differentiated G2 concordant (both mitotic count and Ki-67 index G2) NETs. Well-differentiated tumors with a Ki-67 index less than 55% do not respond as well to a platinum-based chemotherapy regimen as do poorly differentiated tumors with a higher Ki-67 index. Based on these results, G3 tumors, according to the 2010 WHO classification scheme, can be further classified by tumor differentiation as well as proliferation status.

STAGING OF GI AND PANCREATOBILIARY TRACT NEUROENDOCRINE TUMORS

The American Joint Committee on Cancer (AJCC) and the European Neuroendocrine Tumor Society (ENETS) proposed special staging systems for GI tract and pancreas.
NETs according to the tumor size and extension in each organ. In the staging system of the AJCC, NETs of the stomach, small intestine, colorectum, and appendix have specially designated staging systems for NETs distinct from their cancer staging, whereas pancreatic NETs share a single staging system with exocrine pancreatic carcinomas.43 The AJCC and ENETS staging for almost all GI tract NETs, including NETs of the stomach, duodenum, ampulla, jejunum, ileum, and colorectum, are identical, whereas some differences exist in the classification schemes of the pancreatic and appendiceal NETs between AJCC and ENETS staging.42,43 There is no suggested staging system for biliary tract NETs in either the AJCC or the ENETS staging system. Several studies have compared T-classification schemes of the AJCC and ENETS staging systems of pancreas NETs and reported different results.40–41 One study40 demonstrated the superiority of the ENETS staging system, whereas another study41 reported superiority of the AJCC staging system for pancreas NETs.

### GASTRIC NETS

Most of the GI tract NETs are solid masses, whereas multiple NETs are usually associated with multiple endocrine neoplasia type 1 (MEN1) or Zollinger-Ellison syndrome, especially in the stomach or duodenum. These NETs are well-circumscribed masses located in the mucosal or submucosal layer of the GI tract. The most common gastric NETs are enterochromaffin-like (ECL)-cell (histamine-producing) tumors. Interestingly, the International Classification of Diseases for Oncology (ICD-O) code for ECL-cell–producing NETs is not present in the gastric NET section in the 2010 WHO “Blue Book.”42 Other hormone-producing NETs, including EC-cell (serotonin-producing) NETs or G-cell gastrin-producing tumors (also known as gastrinomas), are very rare.4 Immunohistochemical detection of ECL cells is specifically performed not by histamine but by vesicular monoamine transporter 2 because of the difficulty in detecting histamine immunohistochemical staining.42,43

The ECL-cell NETs are categorized into 3 subtypes, based on the histology of the adjacent mucosa, antral G-cell hyperplasia, hypergastrinemia, and accompanying clinical condition.33 The subtypes of gastric ECL-cell NETs are summarized in Table 4. Briefly, type I ECL-cell NETs are the most common subtypes and are associated with autoimmune chronic atrophic gastritis. Usually, multiple small-sized tumors (0.5–1 cm) are observed in the body or fundus. Hypergastrinemia and antral G-cell hyperplasia are commonly observed. Based on the 2010 WHO scheme, all type-I ECL-cell NETs are categorized as G1 NETs.43

Type-II ECL-cell NETs occur in patients with MEN1 or Zollinger-Ellison syndrome. MEN1 is a tumor suppressor gene on band 11q13 that encodes the menin protein.45–49 Biallelic inactivation through a mutation in 1 allele of MEN1, coupled with the loss of the remaining wild-type allele, is identified in almost 90% of gastric NETs. Multiple tumors of less than 2 cm are noted in the body or fundus, and the adjacent mucosa is hypertrophic.43 Type-II ECL-cell NETs are categorized as G1 and, rarely, as G2 NETs.41 Lymph node metastasis is more commonly observed in type-II tumors than in type-I tumors.

Type-III tumors are sporadic tumors and usually present as a single mass. Type-III ECL-cell NETs occur sporadically in the absence of ECL-cell hyperplasia or dysplasia and are not associated with hypergastrinemia, chronic atrophic gastritis, MEN1, or Zollinger-Ellison syndrome.44 Usually, single large tumor (>2 cm) can be observed in any part of the stomach. Type-III tumors occasionally display more aggressive behaviors than do type-I and type-II tumors, and type-III ECL-cell NETs are categorized as G1 to G3 NETs.43

### Duodenal and Ampullary NETs

G-cell (gastrin-producing) NETs are the most common duodenal NETs, followed by somatostatin-producing NETs and gangliocytic paragangliomas. G-cell (gastrin-producing) NETs occur sporadically or in association with MEN1.4 G-cell (gastrin-producing) NETs associated with Zollinger-Ellison syndrome are frequently metastatic and are usually deeply infiltrating tumors with unfavorable clinical outcomes.20 Somatostatin-producing NETs are associated with neurofibromatosis type 1 and have a typical glandular pattern containing psammoma bodies.50–52 Gangliocytic paragangliomas occur predominantly in the second portion of the duodenum and ampulla and show characteristically triphasic cellular components, including epithelioid neuroendocrine cells, schwannian cells, and ganglion cell components (Figure 1, B). The neuroendocrine cells have an eosinophilic cytoplasm with ovoid nuclei arranged in a pseudoglandular pattern or solid nests. Epithelioid cells are immunoreactive for progesterone receptor and PP, schwannian cells stain positive for S100 protein, and ganglion cells are immunopositive for synaptophysin.51,53,54

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**Table 4. Classification of Gastric Enterochromaffin-Like Cell Histamine-Producing Neuroendocrine Tumors**

<table>
<thead>
<tr>
<th>Classification</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence, %</td>
<td>55–88</td>
<td>8–13</td>
<td>12–23</td>
</tr>
<tr>
<td>Multilocality</td>
<td>Multiple</td>
<td>Multiple</td>
<td>Single</td>
</tr>
<tr>
<td>Peritumoral oxyntic mucosa</td>
<td>Atrophic</td>
<td>Hypertrophic</td>
<td>Normal</td>
</tr>
<tr>
<td>Size, cm</td>
<td>0.5–1</td>
<td>&lt;2</td>
<td>&gt;2</td>
</tr>
<tr>
<td>Location</td>
<td>Corpus</td>
<td>Corpus</td>
<td>Any</td>
</tr>
<tr>
<td>Sex</td>
<td>M &lt; F</td>
<td>M = F</td>
<td>M &gt; F</td>
</tr>
<tr>
<td>Hypergastrinemia</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Antral G-cell hyperplasia</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Associated disease</td>
<td>Chronic atrophic gastritis</td>
<td>Multiple endocrine neoplasia</td>
<td>Zollinger–Ellison syndrome</td>
</tr>
<tr>
<td>Precursor lesion</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>WHO 2010 classification</td>
<td>Grade 1</td>
<td>Grades 1 or 2</td>
<td>Grades 1–3</td>
</tr>
<tr>
<td>Lymph node metastasis, %</td>
<td>5</td>
<td>30</td>
<td>70</td>
</tr>
</tbody>
</table>


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paragangliomas usually have a benign clinical course, but larger tumors (>2 cm) can metastasize to the regional lymph node with mainly neuroendocrine components. \(^{55,56}\)

**Jejunal and Ileal NETs**

Jejunal and ileal NETs comprise about half of small intestinal NETs and are predominantly located in the terminal ileum. \(^{57,58}\) Jejunal and ileal NETs are multiple tumors in up to one-third of the cases. \(^{59-61}\) Ileal NETs are composed of EC-cell serotonin-producing tumors with insular growth patterns. Many cases show invasion to the proper muscle layer or beyond and/or metastases at the time of diagnosis. One-third of patients with ileal NETs have metastasis, and patients with liver metastasis show signs of carcinoid syndrome, which manifests with symptoms of flushing and diarrhea from bypassing the hepatic clearance of serotonin from the portal circulation. \(^{60}\)

**Appendiceal NETs**

Appendiceal NETs can be classified as classic NETs, including EC-cell (serotonin-producing) NETs, L-cell-type NETs, and tubular and goblet cell carcinoids, which are mixed adenoendocrine carcinomas with a more-aggressive clinical behavior. \(^{62}\) Tubular carcinoids are arranged in small, discrete tubules, occasionally with inspissated mucin. Thus, this neoplasm can be misdiagnosed as a metastatic adenocarcinoma.

Goblet cell carcinoids are composed of tumor cells of partial neuroendocrine differentiation, mixed with nests of signet ringlike cells, with mild-to-moderate dysplasia (Figure 1, F). \(^{60}\) Tumor cells are focally positive for staining with synaptophysin, chromogranin A, and CD56 and are diffusely positive for cytokeratin 20 and MUC2. \(^{64-66}\) Goblet cell carcinoids are more aggressive than other appendiceal carcinoids, and 20% of cases have metastasis at the time of diagnosis. \(^{67-69}\) Goblet cell carcinoids are subclassified according to history and mucin immunophenotype as typical goblet cell carcinoids, signet ring cell type adenocarcinomas, and poorly differentiated adenocarcinomas, and patient survival depends upon the subtype. \(^{67}\)

**Colorectal NETs**

Colorectal NETs are composed of colonic-predominant EC-cell (serotonin-producing) NETs and rectum-predominant L-cell-type (GLP- and PP/PYY-producing) NETs. \(^{20}\) In 2010 WHO classification, L-cell-type NETs are classified as tumors with uncertain malignant potential (ICD-O code, M8152/1) although no specific diagnostic criteria of L-cell-type NETs exist. \(^{20}\) Therefore, pathologists are confused about assigning behavior coding for rectal NETs as NET G1 (M8240/3) or L-cell-type NETs (M8152/1). In general, rectal NETs usually manifest as a single, smooth submucosal nodule or polyp with normal-appearing mucosa. \(^{70}\) About 80% of rectal NETs are 1 cm or smaller. \(^{71}\) The L-cell NETs are detected in 50% to 80% of rectal NETs with various combinations of L-cell markers, including GLP1, GLP2, PYY, and PPY. \(^{71-74}\) Histologically, L-cell-type NETs have predominantly trabecular patterns (Figure 1, D). L-cell-type NETs are tumors with uncertain malignant potential in the 2010 WHO scheme. \(^{25}\) Biologic behaviors of rectal NETs depend on the L-cell immune phenotype, tumor size (<1 cm), tumor grade, extension, and lymph node metastasis. \(^{75,76}\) Small (<1 cm) rectal NETs tend to have no recurrence, even with incomplete resection. \(^{76}\) On the other hand, large rectal NETs (>1 cm) and non-L-cell phenotype tumors have an aggressive clinical behavior and worse prognosis. \(^{71}\)

**Pancreatic NETs**

Pancreatic NETs account for about 3% of pancreatic neoplasms. \(^{77,78}\) The tumors show an expansile growth pattern with pushing borders and have a yellow, fish-flesh, or tan-to-brown color (Figures 2, A and B). \(^{79}\) Some tumors show peliosis or hemorrhage. Approximately 10% of pancreatic NETs are unilocular cystic tumors surrounded by fibrous rims and contain straw-colored cystic fluid (Figure 2, C). \(^{80}\) Microscopically, cystic NETs are lined by thin fibrous bands. \(^{80,81}\) Most sporadic pancreatic NETs are solitary, whereas some pancreatic NETs from patients with hereditary syndrome tend to have multiple tumors (Figure 2, D). \(^{82}\)

Well-differentiated pancreatic NETs can be classified into functioning and nonfunctioning tumors based on the clinical symptoms induced by hormonal hypersecretion. About one-half of pancreatic NETs are functioning tumors, and insulinomas are the most common, followed, in order of frequency, by glucagonomas, gastrinomas, and somatostatinomas. Occasional stromal or intracellular amyloid deposition is noted in many cases of insulinomas. \(^{79}\) For functioning tumors, insulinomas have an indolent clinical behavior, whereas gastrinomas, glucagonomas, and somatostatinomas are associated with a high malignant potential. \(^{83,84}\) Similarly, patients with insulin-immuno–labeled NETs have better survival, whereas those with gastrin–immuno–labeled NETs have worse survival, regardless of clinical symptoms. \(^{85}\) In addition to the typical features of NETs, some pancreatic NETs show morphologic variations, including clear cell, oncocytic, and pleomorphic types. Clear cell NETs will be discussed in the section on von Hippel-Lindau syndrome (Figure 3, A). Oncocytic pancreatic NETs contain large polypoid lesions with eosinophilic granular cytoplasm and prominent nucleoli (Figure 3, B). Some studies reported that oncocytic tumors have a malignant clinical behavior. \(^{86,87}\) In the setting of liver metastasis of oncocytic pancreatic NETs, immunohistochemical staining with neuroendocrine markers can help to differentiate hepatocellular carcinomas. Pleomorphic NETs contain more than 20% of tumor cells with marked nuclear pleomorphism (Figure 3, C). A bizarre, pleomorphic nuclear appearance does not affect clinical behavior and patient survival. \(^{88}\) Pleomorphic NETs can be misdiagnosed as ductal adenocarcinoma, and immunohistochemical staining is helpful for differential diagnosis. Serotonin-producing tumors account for about one-quarter of pancreatic NETs; the tumor cells show a predominantly trabecular pattern with stromal fibrosis and uniquely involve main pancreatic ducts (Figure 3, D). \(^{89}\)

Poorly differentiated NECs are further classified as small cell carcinomas and large cell carcinomas. Abundant apoptotic bodies, mitosis, and extensive necrosis are commonly observed in poorly differentiated NECs. Small cell carcinomas are composed of sheets or nests of tumor cells with a high nuclear to cytoplasmic ratio, hyperchromatic nuclei, inconspicuous nucleoli, and nuclear molding (Figure 3, E). Large cell carcinomas consist of large polygonal cells with large nuclei and prominent nucleoli. Their tumor cells form solid or nested growth patterns (Figure 3, F). In the pancreas, large cell carcinomas are more common than small cell carcinomas. \(^{90}\)
Extrahepatic Biliary Tract NETs

Extrahepatic biliary epithelia contain scattered endocrine cells, which are immunolabeled by neuroendocrine markers.\(^9\) Biliary tract NETs are extremely rare, and only one-fifth of these tumors are well-differentiated NETs.\(^{91,92}\) Similarly, high-grade tumors, such as NECs and mixed adenoneuroendocrine carcinomas, are more common than either NET G1 or G2 tumors in the biliary tract.\(^1\) The common locations of biliary NETs include the common hepatic and proximal common bile ducts.\(^91\) The growth patterns of biliary tract NETs are either nodular or polypoid. In contrast to pancreatic NETs, biliary tract NETs do not have functioning tumors. However, a few biliary NETs express gastrin, serotonin, PP, or somatostatin.\(^{92}\) Larger tumors (>2 cm) are associated with aggressive behavior.\(^{92}\)

HEREDITARY SYNDROMES ASSOCIATED WITH GI AND PANCREATOBLIARY TRACT NEUROENDOCRINE TUMORS

Some GI tract and pancreatic NETs are associated with hereditary syndromes, including MEN1, von Hippel-Lindau syndrome, neurofibromatosis 1, and tuberous sclerosis.\(^95\) The genes, clinical manifestations, and tumors involved in the GI and pancreatobiliary tracts and other organs are summarized in Table 5. All 4 hereditary syndromes are inherited in an autosomal-dominant manner.

MEN1 is a multiple-organ–involving endocrine neoplastic disorder with autosomal-dominant inheritance, characterized by multiple neoplasms in the pituitary glands, parathyroid glands, pancreas, adrenal glands, stomach, duodenum, thymus, and lung. Loss of MEN1 heterozygosity is associated with the generation of multiple tumors. Multiple histamine-producing gastric tumors, multiple gastrin-producing duodenal tumors; and multiple insulin- or gastrin-producing pancreatic NETs are associated with MEN1.\(^83,94\) Numerous endocrine precursor lesions, such as islet hyperplasia and dysplasia, and microadenomas are observed in the pancreas of MEN1 patients.\(^90\)

Von Hippel-Lindau syndrome is an autosomal-dominant familial cancer syndrome caused by germline VHL mutation and characterized by clear cell tumors that affect multiple organs, such as hemangioblastomas of the central nervous system and retina, renal cell carcinomas, pheochromocytomas, and adrenal cortical adenomas. The NETs involving the GI tract have not been described,\(^94\) whereas pancreatic lesions are associated with von Hippel-Lindau syndrome,
Figure 3. Representative microscopic images of pancreatic neuroendocrine tumors. A, A clear cell variant of pancreatic neuroendocrine tumor. B, An oncocytic variant of pancreatic neuroendocrine tumor containing abundant eosinophilic granular cytoplasm with prominent nucleoli. C, A pleomorphic variant of pancreatic neuroendocrine tumor showing bizarre pleomorphic nuclei. D, A serotonin-producing neuroendocrine tumor showing a predominantly trabecular pattern with stromal fibrosis and uniquely involving pancreatic ducts. E, A small cell type of poorly differentiated neuroendocrine carcinoma with a high nuclear to cytoplasmic ratio, hyperchromatic nuclei, inconspicuous nucleoli, and nuclear molding. F, A large cell type of poorly differentiated neuroendocrine carcinoma with large, polygonal cytoplasm; large nuclei; and prominent nucleoli (hematoxylin-eosin, original magnifications ×400 [A, B, and D through F] and ×200 [C]).
Table 5. Hereditary Syndromes Associated With Gastrointestinal (GI) and Pancreatobiliary Tract Neuroendocrine Tumors (NETs)\(^a\)

<table>
<thead>
<tr>
<th>Syndrome/Location</th>
<th>Chromosomal Band Location</th>
<th>Gene/Protein Involved</th>
<th>GI and Pancreatobiliary Tract NETs</th>
<th>Other Tumors in GI and Pancreatobiliary Tracts</th>
<th>Clinical Presentation Outside GI and Pancreatobiliary Tracts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple endocrine neoplasia 1 AD</td>
<td>11q13.1</td>
<td>MEN1/menin</td>
<td>Multiple gastric, duodenal, and pancreatic NETs</td>
<td>Esophageal leiomyoma</td>
<td>Pituitary adenoma, parathyroid hyperplasia, bronchial and thymic NETs, adrenal cortical adenoma</td>
</tr>
<tr>
<td>von Hippel-Lindau AD</td>
<td>3p25.3</td>
<td>VHL/ VHL</td>
<td>Pancreatic clear cell NETs</td>
<td>Pancreas serous cyst adenomas</td>
<td>Hemangioblastomas of the CNS and retina, renal clear cell carcinomas, pheochromocytomas, adrenal cortical adenomas</td>
</tr>
<tr>
<td>Neurofibromatosis 1 AD</td>
<td>17q11.2</td>
<td>NF1/neurofibromin</td>
<td>Duodenal and pancreatic somatostatin-producing NETs</td>
<td>GIST, neurofibromas</td>
<td>Neurofibromatosis, café au lait spots, optic nerve gliomas</td>
</tr>
<tr>
<td>Tuberous sclerosis AD</td>
<td>9q34.13, 16p13.3</td>
<td>TSC1/hamartin, TSC2/tuberin</td>
<td>Pancreatic insulin- and somatostatin-producing NETs</td>
<td>Hamartomatous polyp</td>
<td>Multiple organ hamartomas</td>
</tr>
</tbody>
</table>

Abbreviations: AD, autosomal dominant; CNS, central nervous system; GIST, gastrointestinal stromal tumor; MEN1, multiple endocrine neoplasia type 1; NF1, neurofibromatosis type 1; TSC1, tuberous sclerosis complex 1; TSC2, tuberous sclerosis complex 2; VHL, von Hippel-Lindau.


including multiple clear cell NETs, serous cystadenomas, and benign serous cysts.\(^{90,94}\) Clear cells in pancreatic NETs show a trabecular, glandular, or solid pattern and have a multivesicular, clear cytoplasm (Figure 3, A). These features are frequently seen not only in von Hippel-Lindau syndrome but also in sporadic pancreatic NETs and in association with MEN1 syndrome.\(^{95}\) Differential diagnosis of clear cell pancreatic NETs includes metastatic renal cell carcinomas, especially in the setting of patients with von Hippel-Lindau syndrome.\(^{90}\)

Neurofibromatosis type 1, also known as von Recklinghausen disease, is an autosomal-dominant disorder characterized by café au lait spots, neurofibromas, optic nerve gliomas, and malignant peripheral nerve sheath tumors.\(^{94}\) Occasional duodenal or ampullary NETs and rare pancreatic somatostatin-producing NETs are noted with characteristic glandular patterns and psammoma bodies.\(^4\)

**PROGNOSTIC MARKERS OF GI AND PANCREATOBILIARY TRACT NEUROENDOCRINE TUMORS**

In addition to their use in the grading and staging of GI and pancreatobiliary tract NETs, several biomarkers were reported to be prognostic factors in GI and pancreatobiliary tract NETs.

In well-differentiated pancreatic NETs, a recent whole-exome sequencing study\(^{96}\) revealed the genomic landscape of pancreatic NETs and the higher frequency of mutations in MEN1, ATRX (alpha thalassemia/mental retardation syndrome X-linked), and DAXX (death-domain associated protein) and a low frequency of mutations in several genes in the mTOR pathways, such as PTEN, TSC2, and PICS3A. The protein expression status of some of these genes affects the survival of patients with pancreatic NETs.\(^{97,98}\) For example, loss of PTEN, ATRX, and DAXX expression has been related to worse survival.\(^{97,98}\) Although cytokeratin 19 was initially proposed as a powerful worse prognostic indicator,\(^{99}\) controversy remains: some studies showed that cytokeratin 19 expression correlated with worse survival,\(^{100,101}\) but others did not find significant prognostic differences according to cytokeratin 19 expression in patients with pancreatic NETs.\(^{102,104}\) Similarly, there have been controversies about the prognostic significance of KIT expression: a few groups showed that KIT-expressing pancreatic NETs had poor survival,\(^{102,105}\) whereas another group observed no survival difference.\(^{100}\) In addition, one study\(^{97}\) showed that progesterone receptor negativity was associated with worse survival and that combined negative progesterone receptor/low PTEN was a worse prognostic indicator in patients with pancreatic NET. Another study\(^{95}\) showed that insulin, GLP1, and increased numbers of peptide hormonal expression were associated with better survival in patients with pancreatic NET, whereas gastrin expression was associated with worse survival. Similarly, expression of cyclooxygenase-2, p21, p18, RB, and thymidylate synthetase and loss of somatostatin receptor 2 expression was related to poorer survival in GI and pancreatobiliary tract NETs.\(^{106-108}\) Loss of p27 was reported to be a negative prognostic indicator in GI and pancreatobiliary tract NETs.\(^{108}\)

**SUMMARY**

The incidence of NETs from the GI and pancreatobiliary tracts is continuously increasing; small intestinal NETs are the most common type among patients in the United States, whereas rectal NETs are the most frequent among patients in Korea. At least 15 types of neuroendocrine cells are distributed in the GI and pancreatobiliary tracts, and there is a correlation between the distributions of specific endocrine cells and the preferential location of specific hormone-
producing NETs in the GI and pancreatic endocrine tracts, although there are some aberrations. The current 2010 WHO classification scheme includes histologic grading based on mitotic counts and the Ki-67 labeling index. Manual counting of camera-captured or printed images is recommended for calculation of Ki-67 labeling. The presence of heterogeneity in G3 NETs—well-differentiated tumors with a high Ki-67 index and poorly differentiated tumors with different histologic and immunohistochemical characteristics and responses to platinum-based treatment—may indicate the need for further classification of these tumor groups. Several prognostic factors have been proposed, but most require further validation studies to stratify the survival of patients with GI and pancreaticobiliary tract NETs.

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References


