Acta Oncologica 28 (1989) Fasc. 3

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DIAGNOSTIC PATHOLOGY OF GASTROINTESTINAL AND PANCREATIC NEUROENDOCRINE TUMOURS

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Abstract

The increased knowledge of the pathobiology of gastrointestinal and pancreatic neuroendocrine tumours and the improved therapeutic possibilities have brought a demand for more precise diagnosis. Although the neuroendocrine tumours can often be tentatively recognized in routinely processed microscopic slides, their more accurate identification requires additional diagnostic procedures. General neuroendocrine markers, such as the argyrophil reaction of Grimelius and immunohistochemistry with application of antibodies against chromogranin A and of neuron-specific enolase are discriminatory staining methods which are used to reveal the neuroendocrine origin of almost all highly differentiated neuroendocrine tumours of the gastrointestinal tract (carcinoids) and pancreas (insulomas). Midgut carcinoids, which predominate among these tumours almost unexceptionally contain serotonin. This biogenic amine can be demonstrated by the argentaffin reaction of Masson, serotonin immunoreactivity or by formalin-induced fluorescence. The characteristic staining pattern of midgut carcinoids is almost invariably preserved in the metastases and can thus be used to reveal a primary midgut carcinoid. The enterochromaffin-like (ECL) cell carcinoids of the body and fundic area of the stomach are argyrophil with Sevier-Munger silver stain. Other neuroendocrine tumours, viz. antral, duodenal and rectal carcinoids and insulomas, should be studied by a battery of relevant peptide hormone antisera for adequate diagnosis. About 50% of all insulin-producing insulomas are endowed with stromal amyloid deposits, which chemically are composed of a peptide designated islet amyloid polypeptide. This molecule has been observed by electron microscopical immunocytochemistry to occur exclusively in the β-cells and is co-stored with insulin in the β-cell granules.

Key words: Neuroendocrine tumours, gastrointestinal tract, pancreas, histopathology, tumour markers.

During the last decade the knowledge of the pathobiology of gastrointestinal and pancreatic neuroendocrine tumours has increased considerably. Nowadays, most of these tumours can be fairly well characterized with regard to their hormone content and biological behaviour. For several reasons, but not least due to the fact that the different types of tumours can be treated successfully with various antitumour agents, a precise diagnosis of as many tumours as possible should be the goal. In this chapter accurate basic methods for histopathological diagnosis of neuroendocrine tumours of the gastrointestinal tract (carcinoids) and pancreas (insulomas) are presented. Mostly the methods employed can be applied to routinely formalin fixed and paraffin embedded specimens.

Routine histology

Often neuroendocrine tumours are rather easily identified in routinely stained histological sections, as they are mostly highly differentiated, built up of regular tumour cells with monomorphous nuclei, and exhibit characteristic regular growth patterns. Sometimes, however, they may be difficult to discriminate from other highly differentiated tumours of ecto-, endo- or mesodermal origin. Moreover, neuroendocrine tumours may—though rather infrequently—display a low degree of differentiation and thus be difficult to distinguish from poorly differentiated and undifferentiated carcinomas (1–4). In fact, some poorly differentiated tumours can be arbitrarily diagnosed as neuroendocrine carcinomas or as undifferentiated carcinomas with neuroendocrine differentiation. Although such tumours may occur at any location, they appear to be more frequenct in the stomach and pancreas than in other areas of the digestive system.

It must also be kept in mind that ordinary adenocarcinomas may contain a subpopulation of tumour cells with neuroendocrine differentiation (5–9). Generally such features are quite common but they are of no diagnostic

Presented at the Kättilö meeting, June 12–15, 1988.

Table 1

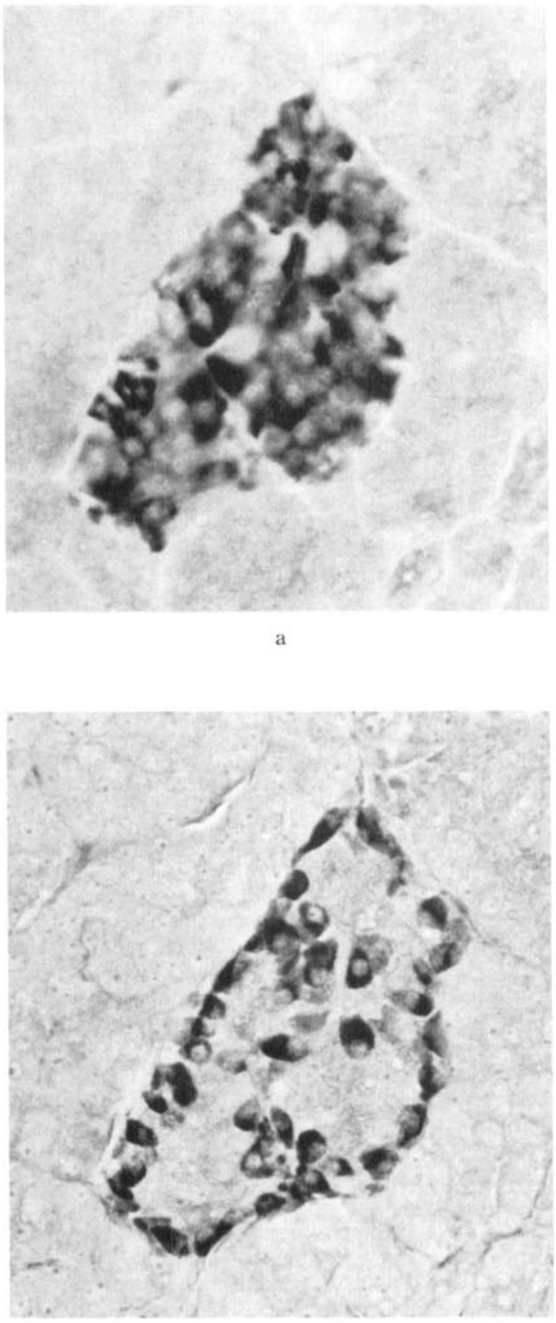
Interrelationship between neuroendocrine and non-neuroendocrine differentiated epithelial tumours

Mixed tumours (goblet cell carcinoid, muco-carcinoid, adeno-carcinoid)	←	*(Adeno-) Carcinoma with scattered neuroendocrine cells*
↓		↑
Highly differentiated neuroendocrine tumour carcinoid, islet cell tumour)		*Highly differentiated (adeno-) carcinoma*
↓		↑
Neuroendocrine carcinoma	→	*Undifferentiated carcinoma*

significance unless the tumour is of the rare type of mixed tumour with obvious components displaying both adenocarcinomatous and neuroendocrine features (see Table 1). The goblet-cell carcinoid of the appendix is perhaps the most distinct example of such a tumour.

Among the gastrointestinal and pancreatic neuroendocrine tumours, the midgut carcinoids (in jejunum, ileum and proximal colon) possess the most typical morphology. These tumours, which are also called 'classical' carcinoids, are built up of insular-like formations of regular tumour cells, surrounded by an often relatively pronounced fibrotic stroma. Most foregut carcinoids (stomach and duodenum) show a mixed growth pattern with solid, ribbon-like, trabecular, rosette-like or acinar structures. Hindgut carcinoids (distal colon and rectum) are often trabecular or solid (10). A variable growth pattern is seen in the insuloma, a tumour type which must be considered when an epithelial neoplasm in the pancreas is identified without the typical appearance of an ordinary adenocarcinoma.

About 50% of all insulin-producing tumours contain amyloid in the tumour stroma, of varying amounts (11). This material can be identified by staining with alkaline Congo Red, with which the amyloid displays a green birefringence on examination in polarized light. Amyloid also occurs in a majority of medullary carcinomas of the thyroid gland, but is not present in gastrointestinal carcinoids or in noninsulin-producing islet cell tumours. The amyloid in insulinomas is identical with the amyloid deposits found in the islet tissue of patients with type II diabetes. Chemical characterization of the islet amyloid has disclosed a novel peptide of 37 amino acids, which shows approximately 50% homology with calcitonin gene-related peptide (12–14). The amyloid peptide has been designated IAPP (islet amyloid polypeptide). Immunohistochemistry with anti-IAPP antiserum has revealed that IAPP is a component of the islet β-cells, while the other endocrine cell types remain unstained (Fig. 1). Electron microscopic examination with the immunogold technique has shown that IAPP is co-stored with insulin in the β-cell secretory granules and thus may possess a hormonal function (15).



a

b

Fig. 1. Light micrographs of identical sections of human pancreatic islet. The islet tissue was a) immunostained with islet amyloid polypeptide antiserum, destained, and b) restained with the argyrophil stain of Grimelius. It is seen that the IAPP-immunoreactive cells correspond to the non-argyrophil cells of the pancreatic islets, ×360.

General tumour markers

As mentioned above, most neuroendocrine tumours are tentatively identified at routine histology. However, to verify their neuroendocrine origin and differentiation, various markers are recommended. In this presentation the

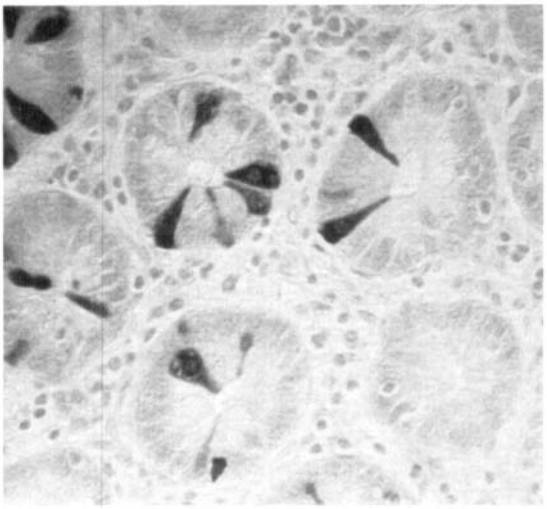


Fig. 2. Light micrograph of human intestinal mucosa after immunostaining with monoclonal chromograin A antibodies. Scattered flask-shaped neuroendocrine cells are seen, ×360.

argyrophil reaction of Grimelius, chromogranin A immunoreactivity and neuron-specific enolase (NSE) immunoreactivity, all of which have now been extensively studied, are described. Recently a number of additional neuroendocrine markers have become available, e.g. synaptophysin. However, antibodies of this compound give uncertain results in ordinary formalin fixed preparations.

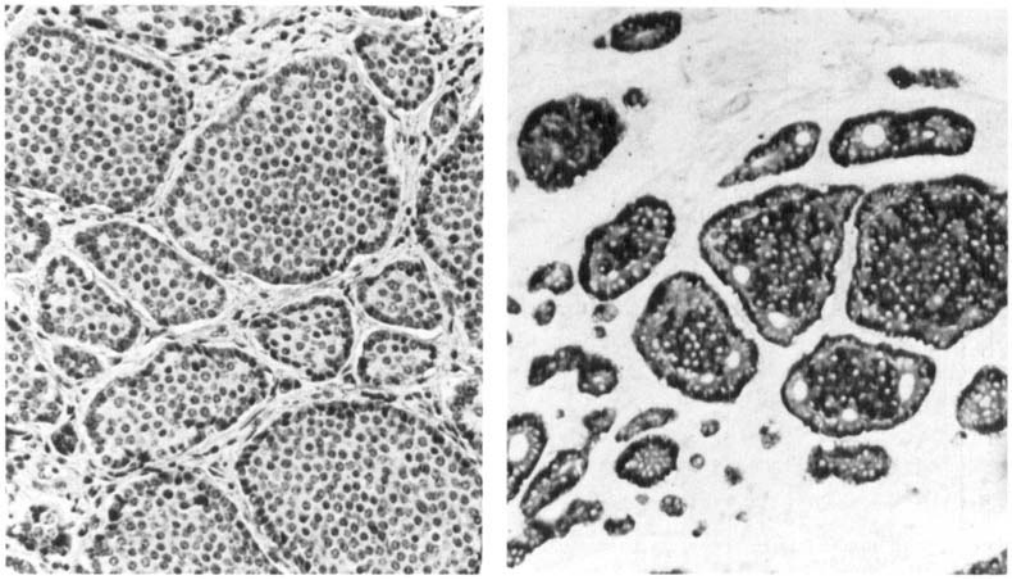
The argyrophil reaction (Grimelius). This method has been used for almost two decades to identify normal neuroendocrine cells and corresponding tumours in various human tissues. It stains the A (glucagon) cells and PP (pancreatic polypeptide) cells of the pancreatic islets and enterochromaffin (EC) cells, and most but not all of the other hormone peptide-producing cells of the gastrointestinal canal. Practically all foregut and midgut carcinoids display an argyrophil reaction in the majority of the tumour cells, while hindgut (rectal) carcinoids are either argyrophil or unreactive (16–19). About 90% of insulomas possess obvious argyrophil properties. A negative reaction is especially seen in pure B (insulin) cell tumours (20).

The silver grains in the argyrophil reaction are concentrated to the neurohormonal secretory granules in the cells, and a light microscopically observed silver reaction thus reveals the presence of such granules, which otherwise can only be identified at electron microscopy. However, all granule-containing tumours do not display an argyrophil reaction, for example some insulin-producing insulomas and rectal carcinoids are not argyrophil and a negative reaction does not absolutely rule out the existence of a neuroendocrine tumour. Furthermore, in poorly differentiated neuroendocrine tumours with a low concentration of cytoplasmic hormone granules the silver stain may be either weak or absent.

Chromogranin A. The chromogranins are a family of acidic polypeptides constituting the major part of the soluble proteins in the hormonal secretory granules of many, but not all, neuroendocrine cell types. Originally the peptides were isolated from the chromaffin granules of the adrenal medulla (21–24). One of the major constituents of the peptide family is chromogranin A, a molecule of 431 amino acids with the gene code on chromosome 14 (25–28). The physiological role of the chromogranins is unclear, but the concentration of chromogranin A has been found to be elevated in the plasma of patients with a great variety of tumours of the neuroendocrine type. For that reason chromogranin A is presumed to be secreted from neuroendocrine cells and possibly to possess a hormonal function (29).

Polyclonal and monoclonal antibodies against chromogranin A are now available and can be studied in neuroendocrine tissues with immunohistochemical techniques (Fig. 2) (30, 31). The chromogranin A-immunoreactive neuroendocrine cells and tumours correspond to the argyrophil cells with the method of Grimelius. Thus the peripheral A (glucagon) cells of the pancreatic islet, the EC cells and several other hormone peptide-producing cells of the gastrointestinal mucosa are chromogranin A-immunoreactive. Further, foregut and midgut carcinoids are chromogranin A-immunoreactive, while rectal carcinoids are either reactive or unstained (Fig. 3). The almost total correspondence between neuroendocrine cells and tumours showing chromogranin A-immunoreactivity and an argyrophil reaction has led to the proposal that chromogranin A has an affinity for silver ions and is one component responsible for the tissue argyrophilia. In accordance with this view, pure chromogranin A has been found to respond to an argyrophil reaction in vitro (Wilander, unpublished data), while other hormonal substances such as insulin, glucagon, calcitonin and pentagastrin were unreactive (Fig. 4) (31). Generally chromogranin A-immunoreactivity is more specific than the argyrophil reaction since in addition the latter identifies several other intracellular components, for instance melamin.

Neuron-specific enolase (NSE). NSE is a glycolytic enzyme, which originally was thought to be present only in neurons of the central nervous system. However, further studies showed that NSE also occurred in the peripheral neuroendocrine cell system and could be used as a marker for neuroendocrine tumours. NSE is not a component of the secretory granules and the presence of a positive immunoreaction is not related to the amount of secretory granules in the cytoplasm of the cells studied (32–38). NSE immunoreactivity occurs in most insulomas and carcinoids (20, 39). The marker is useful in rectal carcinoids, which may fail to stain with the argyrophil reaction and with chromogranin A antibodies. Furthermore, it is often possible to obtain NSE immunoreactivity in neuroendocrine tumours with a low granular content, mostly poorly differentiated ones, e.g. small cell carcinoma of the lung and adrenal neuroblastoma. However, caution should be observed when interpreting an NSE



a b

Fig. 3. 'Classical' midgut carcinoid a) routinely stained with haematoxylin-eosin, and b) after chromogranin A immunostaining. The insular-like tumour aggregates are chromogranin A-immunoreactive, ×210.

immunoreaction as positive, as tumours that are considered not to be of neuroendocrine origin, including breast carcinomas, may be found to contain considerable amounts of NSE both on immunohistochemical examination and in analyses of tumour extracts. However, mostly the amounts of NSE in neuroendocrine tumours definitely exceed those observed in ordinary carcinomas (40).

Specific markers

While the general neuroendocrine markers serve as tools to discriminate between neuroendocrine tumours and neoplasms of other types, the more specific markers are used to characterize the tumours in detail with regard to their synthesis, storage and secretion of specific hormonal products, such as biogenic amines and/or peptide hormones.

Detection of serotonin. Serotonin (5-hydroxytryptamine) can be visualized in neuroendocrine tissues and tumours with mainly three techniques, namely: Formalin-induced fluorescence (FIF), the argentaffin reaction of Masson, and immunohistochemically with poly- or monoclonal antibodies to serotonin (41–45). The different techniques are not absolutely specific, as FIF may derive from other biogenic amines than serotonin and the argentaffin reaction is of low chemical specificity and additionally stains melanin granules and phenols (catecholamines). On examination of the normal mucosal EC cells with the different techniques, relatively good correspondence is found, but more cells display serotonin immunoreactivity than FIF or an argentaffin reaction (46). This may be due to a higher sensitivity of the serotonin antibodies. In the case of carcinoids the discrepancy is more pronounced. 'Classical' midgut carcinoids, which are known to almost invariably store and secrete serotonin, almost constantly show FIF and an argentaffin reaction in the majority of the tumour cells, while many of the same tumours are negative or weakly stained with serotonin antibodies. By contrast, foregut and hindgut carcinoids and islet cell tumours more often contain tumour cells displaying serotonin immunoreactivity than FIF and an argentaffin reaction (47). In routine diagnostic work, the argentaffin stain is a reliable method of identifying primary 'classical' midgut carcinoids and their metastatic deposits and discriminating them from neuroendocrine tumours with other topographical locations.

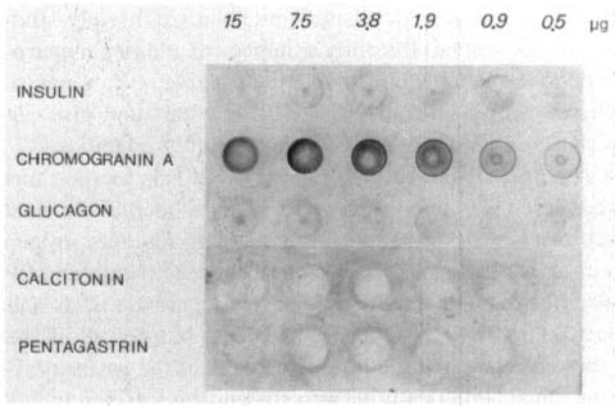


Fig. 4. Pure chromogranin A and various peptide hormones are applied to nitrocellulose. After formalin fixation and staining with the Grimelius' technique, chromogranin A displays a dose-related argyrophil reaction, while the other peptide hormones are unreactive.

Table 2

Characteristic silver staining and immunohistochemical features of carcinoids and pancreatic islet cell tumours. (NSE = neuron-specific enolase. PYY = polypeptide YY. PP = pancreatic polypeptide. VIP = vasoactive intestinal polypeptide)

	Masson stain	Sevier-Munger stain	Grimelius stain	NSE	Chromogranin A	Peptide hormones
Fundic carcinoid	−	+	+	+	+	−
Antral-duodenal carcinoid	−	+/−	+	+	+	Gastrin Somatostatin
Midgut carcinoid	+	+	+	+	+	− *
Hindgut carcinoid	−	+/−	+/−	+	+/−	PYY PP Glucagon Glicentin
Pancreatic islet-cell tumours	−	−	+	+	+	Insulin Gastrin VIP Glucagon Somatostatin PP

* Midgut carcinoids contain peptides of the tachykinin family. However, they are mostly difficult to demonstrate in routinely formalin fixed specimens.

Peptide hormone immunohistochemistry. Staining of neuroendocrine tumours with peptide hormone antisera is of diagnostic importance especially for antral, duodenal and rectal carcinoids and pancreatic islet cell tumours. A large battery including antisera against all known peptides occurring in the neuroendocrine cells in the mucosa of the gastrointestinal canal and endocrine pancreas is necessary. Many such antibodies are now commercially available, mostly as polyclonal antisera but a few also as monoclonal antibodies. With these facilities most of the above described neuroendocrine tumours can be properly characterized with regard to their hormonal content. These tumours are often multihormone producers but as a rule one hormonal substance predominates.

Sevier-Munger argyrophil stain. Although the argyrophil reaction of Sevier-Munger is not a specific stain for a single neuroendocrine cell type, it is almost discriminatory when used on carcinoids located in the body and fundic area of the stomach, as the stain characteristically stains the enterochromaffin-like (ECL) cell carcinoids (ECLomas) in that region (48, 49). The ECL cells store an unknown hormone and their identity cannot at present be revealed with any immunohistochemical technique.

Other diagnostic methods

S-100 protein is a highly acidic protein with a molecular weight of 21 000 (50, 51). The substance occurs in glial and Schwann cells of the central nervous system but is also present in several non-neural cell types. S-100 protein antibodies can be used to stain the Schwann cell derived sustentacular cells of the adrenal medulla and the satellite cells of the adenohypophysis and peripheral ganglia (52–55). Long slender cells with cytoplasmic projections displaying S-100 protein immunoreactivity are frequently seen in phaeochromocytomas and paragangliomas. These cells are intermingled with aggregates of neuroendocrine cells. A corresponding picture with buds of neuroendocrine tumour cells surrounded by S-100 protein Schwann-like cells is also seen in most appendiceal carcinoids and in some neuroendocrine tumours of the duodenum (56, 57). It is suggested that tumours with a biphasic pattern and built up of both neuroendocrine and S-100 protein-immunoreactive Schwann-like cells rather represent intestinal paragangliomas and are possibly not closely related to the intestinal carcinoids. While the latter tumours are considered to derive from the mucosal neuroendocrine cells, intestinal paragangliomas possibly arise from subepithelial neuroendocrine cells (possibly related to the peripheral nervous system) located in the intestinal wall (58–61).

General diagnostic principles

On fresh unfixed tumour material from neuroendocrine tumours a number of different histochemical, immunological, cell biological and molecular biological methods can be applied in order to obtain optimal information in each

individual case. However, in routine diagnostic work quite good characterization can be achieved on ordinary paraffin blocks. Moreover, on such material retrospective studies can be performed and it allows pathological laboratories without expensive facilities to study neuroendocrine tumours. The most characteristic features of neuroendocrine tumours and their various locations in the gastrointestinal tract and pancreas are given in Table 2. It is seen that the Grimelius argyrophil technique and chromogranin A-immunoreactivity are discriminatory for carcinoids at all locations except for the rectal type, where NSE immunoreactivity is a more useful diagnostic stain. However, as mentioned above, it must be emphasized that a positive NSE reaction may also occur in non-neuroendocrine tumours.

Most fundic carcinoids are nonargentaffin but stain with the Sevier-Munger technique. In midgut carcinoids the majority of the tumour cell population are almost unexceptionally argentaffin. To identify the hormone production of antral and duodenal carcinoids and islet cell tumours, a battery of relevant peptide hormones must be applied for a proper identification of their secretory products. In rectal carcinoids, also, a more accurate characterization requires the use of peptide hormone antisera. However, in rectal carcinoids identification of the secretory products is of less clinical importance, since so far these products are of no clinical significance. It is clear from Table 2 that there is no need to apply all available staining methods on all tumours, but it is more appropriate to use a few adequate markers chosen with regard to the topographic site of the primary tumour mass.

ACKNOWLEDGEMENT

This work was supported by the Swedish Medical Research Council (Project No. 6817).

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