GEP-NET : rare tumour connections. Pathophysiological aspects of gastroenteropancreatic neuroendocrine tumours

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Chapter 1

General Introduction

and

Outline of the Thesis

Gastroenteropancreatic neuroendocrine tumours

Gastroenteropancreatic neuroendocrine tumours (GEP-NETs) comprise a heterogeneous group of uncommon neoplasms, including the pancreatic neuroendocrine tumours (PNETs) and gastrointestinal (GI) neuroendocrine tumours[1] (GI-NETs, Table 1).

Table 1. Neuroendocrine tumours
Carcinoids *(gastrointestinal neuroendocrine tumours)*
Non-carcinoid gastroenteropancreatic tumours *(pancreatic, duodenal and gastrointestinal neuroendocrine tumours)*
Catecholamine-secreting tumours *(phaeochromocytomas, paragangliomas, ganglioneuromas, ganglioneuroblastomas, sympathoblastoma, neuroblastoma)*
Medullary carcinomas of the thyroid
Chromophobe pituitary tumours
Small cell lung cancer
Merkel cell tumours

Table 1. All tumours which are classified and defined as 'neuroendocrine tumour'.

The total incidence is estimated at 2-5 patients per 100.000 persons per year, although recent epidemiological studies have shown that their incidence is increasing remarkably[2-5]. Nevertheless, they only comprise approximately 2% of all malignant tumours of the gastrointestinal tract.

GEP-NETs are considered to originate from the cells from the diffuse neuroendocrine system. There are at least 15 neuroendocrine cell types, scattered along the entire length of the gastroenteropancreatic tract. These cells are called neuroendocrine because their many similarities to neural cells. Not only do they have several histological similarities such as secretory granules and the expression of neuroendocrine cell markers, they also produce bioactive substances that have transmitter function. GEP-NETs are characterized by their ability to synthesize, store and secrete biogenic amines and neuropeptides. Although various neuroendocrine cell markers have been identified, the presence of chromogranin

A is nowadays widely used to identify GEP-NETs (Table 2). GEP-NETs occur mainly in the gastrointestinal tract and pancreas (2/3rd), the pulmonary system being the next most frequent location[1,6,7].

Table 2. Neuroendocrine cell markers	
General markers	
Chromogranin A, B	
Pancreatic polypeptide	
Neuron-specific enolase	
Human chorionic gonadotrophin alpha/beta subunits	
Specific markers	
Insulin	(insulinoma)
Gastrin	(gastrinoma)
Glucagon	(glucagonoma)
Somatostatin	(somatostatinoma)
VIP	(VIPoma)
ACTH	(ACTHoma)
GrH	(GrHoma)
Serotonin	(carcinoid)
Calcitonin	(calcitoninoma)

Table 2. Overview of general and specific neuroendocrine cell markers in GEP-NETs.

The clinical presentation of GEP-NETs depends on the location of the primary tumour, the presence of metastases, and the peptide(s) secreted. The diagnosis of GEP-NETs is frequently delayed, and metastases are often present when the tumour is detected. The diagnosis of GEP-NETs is based on clinical presentation, hormone assays, and pathological examination of the tumour. The detection of some biochemical markers in plasma or serum of patients with GEP-NETs raises the suspicion of a specific tumour, whereas other markers are common to several types of GEP-NETs[2] (Table 2). Commonly used imaging modalities include CT, MRI, transabdominal ultrasonography, gastrointestinal endoscopy, selective angiography, nuclear imaging such as somatostatin-receptor scintigraphy, endoscopic ultrasonography[8]. Frequently, primary tumours can not be localized, because of their small size and occult localization[2].

As GEP-NETs show a large variation in tumour behaviour and a wide spectrum of clinical manifestions, treatment of these tumours should be individualized per patient, based on the tumour type and presence of symptoms. Surgery is the treatment of choice in a large percentage of GEP-NETs, especially in patients with limited disease[2]. For patients with advanced or unresectable disease, surgery can be palliative, and even reduce morbidity and mortality. Furthermore, recent studies to medical treatment of GEP-NETs using somatostatin analogues show promising results. The prognosis of GEP-NETs varies strikingly, and is mainly dependent on the size and localization of the primary tumour, and metastatic involvement. However, GEP-NETs show less aggressive behaviour than the more common gastrointestinal carcinomas and pancreatic adenocarcinomas.

The majority of GEP-NETs are sporadic, although they can be multiple and occur as part of a hereditary syndrome, such as Multiple Endocrine Neoplasia type 1, von Hippel-Lindau disease, or neurofibromatosis type 1[9]. The model of neuroendocrine tumour development resembles that from colorectal cancer[1] (Figure 1).

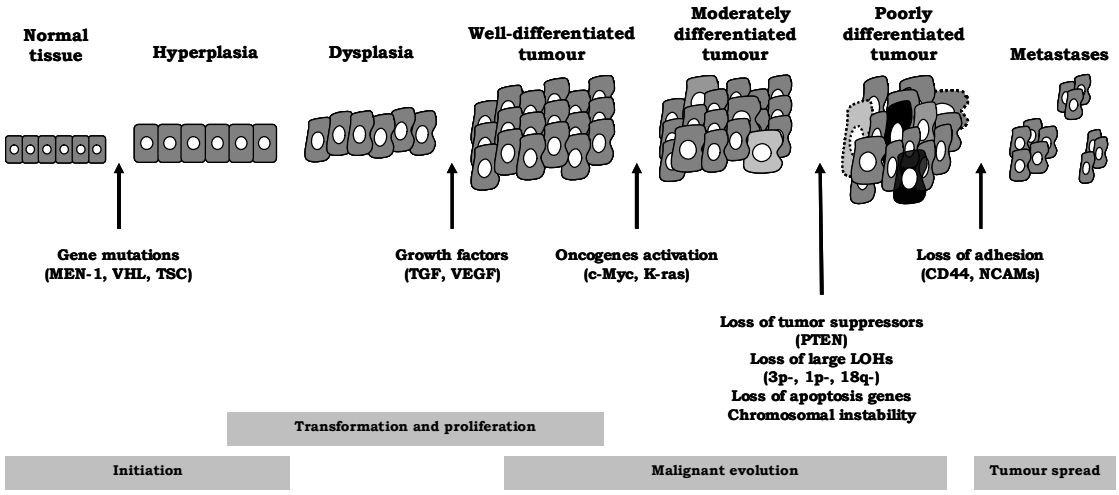

Figure 1. The neuroendocrine tumourigenesis, from normal tissue to the formation of metastases, is shown. The first step in the development of neuroendocrine tumours is the transformation of normal neuroendocrine cells into hyperplastic and/or dysplastic tissue, as a result of gene mutations. Next, the tumour differentiates into a well-, moderately or poorly differentiated tumour, in which growth factors, oncogenes and tumour suppressor genes play an important role. Eventually, tumours spread into the circulation and form metastases. Figure based on Barakat *et al*[1].

The classification of the World Health Organization (WHO) for GEP-NETs is widely used to categorize these tumours. This classification is mainly based on

histopathology and biological behaviour of tumours, divided per tumour localization, i.e., stomach, duodenum and the upper part of the jejunum, appendix, small bowel, including the second part of the jejunum, colon and rectum, and pancreas. Finally they are divided into three classifications, based on differentiation and malignant behaviour, characterized by the presence of angioinvasion and/or metastases[10] (Table 3).

Table 3. World Health Organization Classification for GEP-NETs
1a. Well-differentiated neuro-endocrine tumour with benign or uncertain behaviour
1b. Well-differentiated neuro-endocrine carcinoma with low-grade malignant behaviour
2. Poorly differentiated neuro-endocrine carcinoma with high-grade malignant behaviour

Table 3. Classification of the World Health Organization for GEP-NETs, introduced in 2000.

Pancreatic neuroendocrine tumours

Pancreatic neuroendocrine tumours (PNETs) are often referred to as pancreatic endocrine tumours (PETs), pancreatic islet cell tumours or pancreatic islet cell carcinomas. They comprise less than 2% of all pancreatic cancers, and must be distinguished from the more common pancreatic adenocarcinomas, which have a poorer prognosis[11,12]. PNETs can secrete several hormones, dependent on the cell type of origin, and are therefore divided into functional and non-functional tumours. Tumours are referred to as functional in case of the presence of a clinical syndrome resulting from hormone production, e.g., gastrin, insulin, glucagon, vasoactive intestinal peptide (VIP) or somatostatin, by the tumour. In contrast, non-functional tumours can remain clinically silent for a relatively long time and are only detected when morbidity is caused by tumour mass leading to biliary duct obstruction, bowel obstruction, and development of metastases or invasion into adjacent organs[2,12]. Although PNETs have a relatively slow growing rate, the majority of tumours are malignant. Treatment of PNETs is directed to both the tumour and the associated clinical symptoms. Medical therapies like proton pump inhibitors and somatostatin analogues can control hormonal symptoms, whereas antitumoural treatment is necessary to improve and prolong survival, and

includes chemotherapy, hepatic artery or chemo-embolisation, radioablative therapy, and surgical resection[2,13].

Insulinomas

Insulinomas are the most frequent occurring functional PNETs, and are primarily considered to be benign. They originate from the pancreatic beta-cells and are characterized by overproduction of the hormone insulin, leading to hypoglycemia-associated symptoms, like dizziness, lethargia and palpitations. The diagnosis of insulinoma can be established by determination of plasma insulin, proinsulin, C-peptide and glucose levels. Alternatively, a 48-72 hours fasting test can be performed to diagnose or exclude an insulin-secreting tumour[2,14,15]. About 5-10% of the insulinomas are part of the hereditary MEN-1 syndrome, while the remaining part occurs sporadically. Females seem to be slightly more affected. Most insulinomas are located in the pancreas, with an equal distribution over the pancreatic head, body and tail. The prognosis for patients with insulinomas is relatively good, showing an overall 5-year survival around 97%[16].

Gastrinomas

Gastrinomas are malignant gastrin-producing tumours, arising from the G-cells of the pancreas. Symptoms as dyspepsia, heart burn, diarrhea and peptic ulcers are the result of an increased gastrin production by the tumour, and are collectively named as the Zollinger-Ellison syndrome (ZES)[15]. ZES is seen more commonly in males than in females (ratio 3:2)[16]. Frequently, patients present with a long mean delay in diagnosis. With the widespread use of the proton pump inhibitors (PPIs) and other acid-suppressing medications, delays in presentation are even increasing. The diagnosis of ZES is suspected in case of increased fasting serum gastrin levels (hypergastrinemia), which have been reported to occur in 97% to 99% of the patients[17]. However, in a large percentage of patients the fasting serum gastrin levels alone are not sufficient to diagnose ZES, and therefore additional testing is needed. The secretin stimulation test is considered as the most sensitive and reliable diagnostic tool in gastrinoma patients[18].

Although the majority of gastrinomas is located in the so-called gastrinoma triangle, the anatomical area comprising the pancreatic head, superior and descending portions of the duodenum and the nearby lying lymph nodes, other primary sites of gastrinomas that have been identified are stomach, jejunum, bilitary tract, kidneys, ovaries and liver[19,20] (Figure 2). Gastrinomas occur mainly sporadic, although 30% of the tumours are part of the MEN-1 syndrome[21]. The peak incidence of gastrinomas lies between 40 and 50 years of age[17]. As gastrinomas have a relatively slow growth rate, 5- and 10-year survival rates are estimated to be 65% and 51%, respectively[16]. Even in case of metastatic disease, patients with gastrinomas have a relatively good chance of survival (5-year survival about 40% to 50%). However, patients with pancreatic gastrinomas show a worse prognosis than those with a gastrinoma located in the duodenum[22].

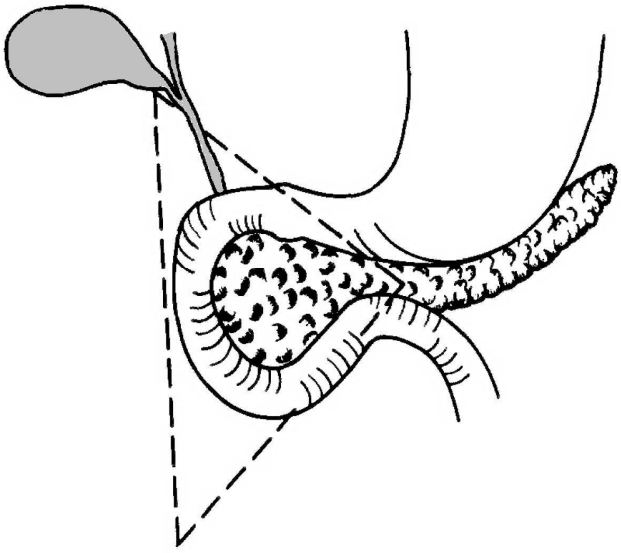

Figure 2. Gastrinoma triangle, which angles are formed by the cystic and common bile ducts, the junction of the neck and body of the pancreas, and the junction of the second and third portion of the duodenum. Figure adapted from Stabile *et al.*[19]

Glucagonomas

Glucagon-producing tumours, or glucagonomas, arise from the alpha-cells of the pancreas. Associated clinical symptoms are hyperglycemia, weight loss, anemia, venous thromboses and a typical skin rash called 'necrolytic migratory erythema' (NME)[15]. Glucagonomas are most frequently found in the pancreatic tail. Extrapancreatic glucagonomas are extremely rare[16]. Glucagonomas usually present with a delay in diagnosis, and are often large at first presentation (>6 cm).

At time of diagnosis, metastases are found in approximately 60% to 70% of the patients[16]. Determination of glucagon serum levels contribute to the diagnosis of a glucagonoma (>500 – 1000 pg/mL)[17].

Somatostatinomas

Somatostatinomas originate from the pancreatic delta-cells, and produce the hormone somatostatin. Although slow-growing, these tumours do show malignant behaviour. They occur mainly in the duodenum or pancreas, of which only tumours in the latter usually lead to a clinical syndrome[17]. Characterizing symptoms for the so-called somatostatinoma-syndrome are steatorrea, cholelithiasis, diabetes mellitus type-2 and hypochlorhydria. Somatostatinomas in the duodenum are often part of a genetic syndrome, such as the MEN-1 or neurofibromatosis (NF-1) syndrome[15]. No specific tests to establish the diagnosis of a somatostatinoma are available. Only pancreatic somatostatinomas are associated with elevated levels of somatostatin in plasma. Frequently, somatostatinomas are found by incidence, during gastrointestinal imaging studies for cholecystectomy or abdominal pain. The overall 5-years survival is about 75% or 60% in case of metastatic disease[16].

VIPomas

VIPomas secrete vasoactive intestinal peptide (VIP), leading to the Verner-Morrison syndrome or watery diarrhea hypokalemia achlorhydria (WDHA) syndrome. Symptoms characterized by WDHA are mainly the result of the severe secretory diarrhea, caused by the secretion of VIP, and are typically dehydration, hypokalemia and achlorhydria. Approximately 80% of VIPomas occur in the pancreas[15], in particular the pancreatic tail[47]. Females are affected more frequently than males[16]. Increased serum levels of VIP (>500 pg/mL) in combination with severe diarrhea are highly suggestive for VIPomas[17]. The 5-year survival rates for patients with VIPomas with or without metastases are estimated to be 60% and 95%, respectively[16].

Other functional pancreatic neuroendocrine tumours

Other functional PNETs include ACTHomas and GRFomas, which are both extremely uncommon[16]. ACTHomas secrete adrenocorticotrophic hormone (ACTH), leading to the Cushing's syndrome. GRFomas produce growth-hormone releasing factor (GRF), and are characterized by acromegaly. Furthermore, PNETs can secrete calcitonin, enteroglucagon, cholecystokinin (CKK), gastric inhibitory peptide, gastrin-releasing peptide (GRP) and ghrelin, although rare[16,17].

Non-functional pancreatic neuroendocrine tumours

Non-functional pancreatic neuroendocrine tumours comprise about 70% of all PNETs. These tumours are not related to any clinical syndrome caused by hormonal overproduction. However, they may show immunohistochemical positivity for hormones or neuropeptides, and frequently increased serum/plasma levels of chromogranin A or PP are found[15,23]. Whereas functional tumours cause symptoms relating to hormone production, non-functional tumours often cause tumour mass related complaints[1]. Furthermore, symptoms can be vague and aspecific, i.e., abdominal pain, anorexia, nausea and weight loss. Frequently, this leads to a delayed detection and the presence of local invasion and/or distant metastases at time of diagnosis. A small percentage of non-functional PNETs are found incidentally at surgery or autopsy[16]. The majority of non-functional PNETs can be classified as well-differentiated neuroendocrine carcinomas[23]. It is important to distinguish these tumours from the more common and aggressive pancreatic adenocarcinomas. Most non-functional PNETs are located in the head of the pancreas. Non-functional PNETs can occur as part of the MEN-1 syndrome or may be associated with Von-Hippel Lindau disease (VHL). These tumours show a more aggressive course than their functional counterparts, although 5-year survival has been reported to lie around 65%[16].

Duodenal neuroendocrine tumours

Duodenal NETs can generally be classified into five tumour types; gastrinomas, somatostatinomas, non-functional NETs, gangliocytic paragangliomas, and poorly

differentiated neuroendocrine carcinomas. The majority of these tumours occur in the first or second part of the duodenum. Duodenal NETs are usually small, i.e., <2cm in diameter. Although they are often limited to the (sub)mucosa, regional lymph node metastases can be found in about 40% to 60% of the patients. Liver metastases are seen less frequently (<10%). Duodenal NETs are usually single lesions. When multiple tumours are detected, the MEN-1 syndrome should be suspected. Functional syndromes are rare in these tumours, comprising mainly ZES or the carcinoid syndrome when they do occur[24,25].

Gastrointestinal neuroendocrine tumours

Gastrointestinal neuroendocrine tumours (GI-NETs) are heterogeneous regarding histological differentiation, hormone production and biology. Frequently, GI-NETs are referred to as carcinoids[26]. They derive from cells of the diffuse neuroendocrine system, and can be divided into serotonin-producing enterochromaffin (EC) or Kulchitsky's cells, and the gastric histamine-secreting enterochromaffin-like (ECL) cells. Carcinoids are able to produce vasoactive substances like amines (serotonin, catecholamines, and histamine) and prostaglandins[26,27]. About only 10% of the carcinoid patients actually suffer from the classical carcinoid syndrome, characterized by symptoms as flushing, hypotension, diarrhea, wheezing, and heart disease, as a consequence of the serotonin secretion. GI-NETs occur predominantly in the gastrointestinal system (70%) or pulmonary tract (25%). Other known, but rare sites of GI-NETs are the ovaries, breast, larynx, thymus and gall bladder[1]. Among the gastrointestinal system, the small intestine and appendix are most commonly affected[27-30].

Dependent on their localization, GI-NETs can remain indolent for a long time. Frequently, symptoms arise when metastases have developed[31].

Besides the determination of chromogranin A levels, 5-HIAA measurements can aid in diagnosing serotonin-producing carcinoids. Although the specificity of the 5-HIAA test is about 100%, sensitivity is only 35%. Treatment options for patients with GI-NETs include somatostatin analogues, alpha-interferon, radiation,

chemotherapy, and surgery. The decision for a medical or surgical approach is based on the location of the primary tumour, and the presence of metastases[27-29].

Multiple endocrine neoplasia type 1 syndrome (MEN-1 syndrome)

The multiple endocrine neoplasia type 1 syndrome (MEN-1 syndrome) is an autosomal dominant inherited disorder, caused by mutations in the MEN-1 gene, located on chromosome 11q13. This syndrome is characterized by tumours in the parathyroid, pancreas, and anterior pituitary. Familial MEN-1 is defined as one patient with MEN-1 and one first-degree relative are affected with at least one tumour in one of the three key organs[9].

In 30% to 75% of the patients with MEN-1 pancreatic tumours are seen[15]. In particular gastrinomas are associated with this hereditary syndrome (20% to 60%), followed by insulinomas (30%) and VIPomas (5%). Non-functional PNETs occur in approximately 50% of the patients with MEN-1. MEN-1 related tumours occur at a relatively earlier age, and have a better prognosis compared to sporadic tumours. They may be multiple and vary in size from small microadenomas to large tumours[23]. Other hereditary syndromes which are associated with pancreatic or gastrointestinal neuroendocrine tumours are VHL-disease and tuberous sclerosis[9].

Neuropeptides

GEP-NETs express a variety of peptide hormones and bioactive amines, including serotonin, chromogranin A, calcitonin, corticotrophin, neuron specific enolase, substance P, gastrin and bombesin-like peptides[28,32]. Bombesin was initially isolated from amphibian skin, and received its unusual name after the genus of the frog, i.e., Bombina bombina. Gastrin releasing peptide (GRP) and neuromedin B (NMB) are the mammalian analogs of bombesin, and belong to the family of bombesin-like peptides (BLPs)[33]. In humans, they are distributed in neural and endocrine cells, especially throughout the gastrointestinal tract. In addition to stimulating a variety of physiological responses in the human body, BLPs are involved in development and progression of several human cancers. For example, it has been shown that these peptides can stimulate the growth of lung, CNS,

breast, cervix and prostate cancer cell lines, both in vivo and in vitro[34,35]. BLPs mediate their biological actions through binding to the G-protein coupled gastrin-releasing peptide receptor (GRPR, BB2R), neuromedin B receptor (NMBR, BB1R), bombesin receptor subtype 3 (BRS3, BB3R) and bombesin receptor subtype 4 (BRS-4, BB4R). Activation of various bombesin receptor subtypes has growth effects in both normal and neoplastic tissues, and several studies have reported an upregulation of bombesin receptors in tumour samples compared to associated normal tissue[36-38].

Angiogenesis

Angiogenesis, the formation of new blood vessels from the existing vascular bed, is a physiological process involved in several events like wound healing and embryonic development[39,40]. Furthermore, it is a critical process for tumourigenesis, as tumours need the development of new blood vessels for their growth and further expansion[41-44]. Tumour cells stimulate mature blood vessels nearby to sprout new microvessels towards the tumour by production of angiogenic factors like transforming growth factor-beta (TGF-β), fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF). Obviously, angiogenesis provides the tumours with an efficient route of exit for tumour cells to leave the primary tumour, enter the blood or lymph stream and form metastases[40] (Figure 3). In various cancers, increased vascular density has been shown to be related to an increased amount of metastases and decreased survival[46].

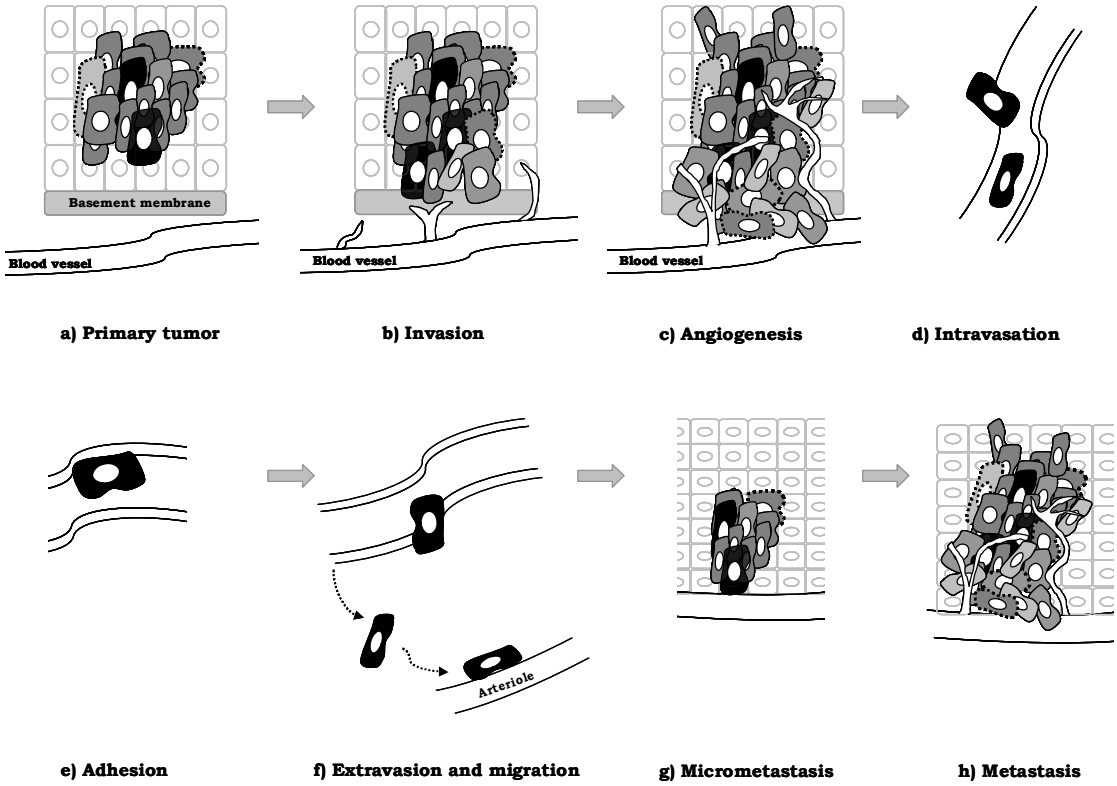

Figure 3. The process of angiogenesis in tumours step-by-step.
a) Primary tumour; b) Tumour cells induce blood vessels to form microvessels in the direction of the primary tumour; c) Angiogenesis, the formation of new blood vessels from existing ones; d) Tumour cells escape from the primary tumour, enter the circulation (intravasation), and e) adhere to other blood vessels; f) Tumour cells leave the circulation (extravasation) and migrate to other places; g+h); where they form (micro)metastases. Figure adapted from Zetter *et al.*[44]

Vascular endothelial growth factor

One of the key factors in angiogenesis is vascular endothelial growth factor (VEGF). VEGF has numerous effects on endothelial cells, including migration and differentiation[47-49]. Its physiological effects are mediated through binding to the VEGF receptor 1 (Flt-1) and VEGF receptor 2 (KDR) [50]. Up-regulation of VEGF in tumours may result from oncogene activation, inhibition of tumour suppression factors, release of growth factors, hypoxia, or necrosis. VEGF primary acts as an endothelial cell mitogen and modulator of changes in vascular permeability, but also mediates the secretion and activation of enzymes involved in the degradation of the extracellular matrix (ECM), thereby further facilitating tumour angiogenesis[51].

Endoglin

Endoglin, or CD105, is a transforming growth factor beta (TGF-β) receptor, which can bind TGF-β1 and TGF-β3 in the presence of the TGF-β receptor type II[52-54]. In the early stages of tumour formation, TGF-β inhibits the proliferation, differentiation and migration of cells, whereas endoglin counteracts these actions, thereby promoting angiogenesis[55]. Endoglin is predominantly expressed on endothelial cells of newly formed (angiogenic) blood vessels[56]. Its expression is up-regulated by hypoxia and TGF-β[57]. In several cancers, increased endoglin levels in tumours are associated with the presence of metastases and a poor survival[58-60].

Matrilysin

Matrix metalloproteinases (MMPs) are a group of proteolytic enzymes, involved in ECM degradation. In humans, at least 23 different MMPs are known. Based on their structure and their substrate preference, they are classified as gelatinases, collagenases, stromelysins, matrilysins, membrane-type MMPs, and others. MMPs are synthesized as pre-proenzymes. The expression of MMPs is transcriptionally controlled by inflammatory cytokines, growth factors, hormones, cell-cell interactions, and cell-matrix interactions. Next to their main function to degrade and remove ECM molecules from the tissue, MMPs are involved in pathologic processes like angiogenesis, tumour transformation and the development of metastases[61,62].

Matrilysin, or MMP-7, belongs to the subgroup of stromelysins. Matrilysin is secreted as pro-MMP-7, of which proteolytic removal of the 9 kDa prodomain from the N-terminus results in activation of the enzyme. Matrilysin is almost exclusively produced by epithelial tumour cells. Up-regulation of matrilysin in tumours is the consequence of mutations in the Wnt-signaling pathway[63]. Numerous studies have shown that matrilysin is significantly enhanced in several cancers, including breast, prostate, lung, skin, and colorectal cancer, and related to the malignant potential of the tumour[64].

Insulin-like growth factor system

The insulin-like growth factor (IGF) system is crucially involved in growth and development of tissues. Furthermore, by controlling cell cycle progression and preventing apoptosis, it plays an important role in tumourigenesis, tumour cell proliferation and metastatic spread[65]. The IGF-system is composed of two ligands, IGF-1 and IGF-2, three cell-surface receptors, IGF-1 receptor (IGF-1R), IGF-2 receptor (IGF-2R), and the insulin receptor (IR), and a family of six IGF binding proteins (IGFBP-1 to IGFBP-6). IGFBPs are able to regulate the bioavailabity of the IGF ligands in the circulation. IGF-1 is predominantly produced in the liver, and has numerous functions. It acts as a mitogen and an anti-apoptotic survival factor, is involved in the glucose metabolism, and promotes cell migration. The effects of IGF-1 are predominantly mediated via the type I insulin-like growth factor receptor (IGF-1R), which can also bind IGF-2. Recent studies have shown that elevation of serum IGF-1 is associated with an increased risk of tumour development. Furthermore, IGF-1R has emerged as a key regulator of mitogenesis and tumourigenicity, because of its important role in cell transformation, tumour invasion, metastasis and cell survival enhancement[65-67].

Outline of the thesis

Gastroenteropancreatic neuroendocrine tumours (GEP-NETs) are a group of uncommon and heterogeneous neoplasm, which show a large diversity in morphological, histocytopathological and clinical aspects. This thesis describes studies on the epidemiology, diagnosis, and pathogenesis of neuroendocrine tumours of the gastroenteropancreatic tract, in particular the pancreatic neuroendocrine tumours and the gastrointestinal carcinoids. The goal was to elucidate the mechanisms contributing to the diversity of GEP-NETs, and to investigate the role of various factors in the pathogenesis of these tumours (Figure 4).

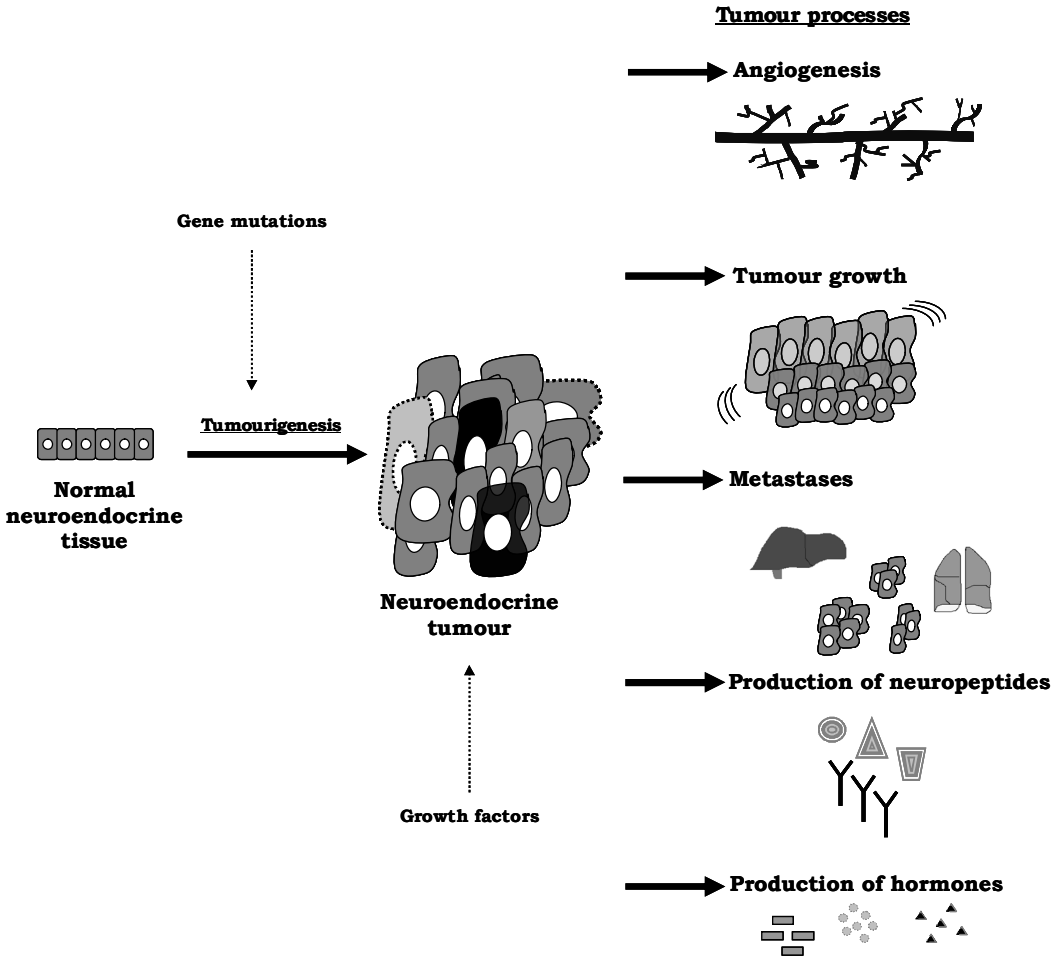

Figure 4. The processes associated with neuroendocrine tumour development, behaviour and progression, as discussed in this thesis, are depicted. As a result of gene mutations and the effects of growth factors produced by tumour cells, normal neuroendocrine tissue cells can proliferate and differentiate into a neuroendocrine tumour. Tumour processes like angiogenesis, tumour growth, metastases and the production of neuropeptides or hormones determine the clinicopathological behaviour and prognosis for the patients.

An overview of the current diagnostic approach of GEP-NETs is given in **Chapter 2**. The need for a standardized diagnostic approach of GEP-NETs is advocated by the rise in incidence of these tumours, as illustrated in **Chapter 3.** This chapter describes an epidemiological study to the incidence of duodeno-pancreatic neuroendocrine tumours from 1991 to 2009 in The Netherlands. Gastrinomas are the most frequent occurring type of malignant functional neuroendocrine tumours, usually located in the pancreatic region. However, **Chapter 4** describes a case report of a patient suffering from the Zollinger-Ellison syndrome with recurrent gastrinomas in the liver, without evidence of any tumour of another primary origin. As the existence of truly *primary* hepatic

gastrinomas is highly questionable, an overview of all liver gastrinomas defined as primary in the literature is given. The diagnosis of a gastrinoma can be established by the use of the secretin stimulation test. Although this test is currently the most used diagnostic tool for gastrinomas, several aspects of this test have been debated. **Chapter 5** describes an intra-individual comparison study using different dosages of secretin in patients and controls to investigate the most optimal criterion and secretin dosage for a positive secretin stimulation test to diagnose the Zollinger-Ellison syndrome.

GEP-NETs are characterized by their ability to secrete neuropeptides, such as gastrin releasing peptide and neuromedin B, the mammalian counterparts of bombesin. A study on the expression of these bombesin-like peptides and their receptors in carcinoids of different origin, i.e., pulmonary and intestinal origin, is reported in **Chapter 6**.

GEP-NETs are highly vascularized tumours. Angiogenesis, the formation of new blood vessels, is a crucial process in tumour development. **Chapter 7** documents an investigation on the expression and role of vascular endothelial growth factor (VEGF) and endoglin (CD105), two key players in angiogenesis, in the tumourigenesis of GEP-NETs.

In order to assess a potential growth activation process of GEP-NETs, the expression of insulin-like growth factor 1 (IGF-1), insulin-like growth factor binding protein 3 (IGFBP-3) and matrilysin (MMP-7) was also investigated. The role of this IGF-matrilysin network in the pathogenesis of GEP-NETs is described in **Chapter 8**.

The aim of the studies described in this thesis was to identify markers with a role in the pathogenesis of GEP-NETs, which contribute to a better understanding of the biology, histopathology and complex heterogeneity of these tumours. Ultimately, these markers might assist in improved histological grading systems and classifications, advanced diagnostics and appropriately targeted treatment for the patients, as summarized and discussed in **Chapter 9**.

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