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

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Chapter 9

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Summary main observations

Clinical behaviour of gastroenteropancreatic neuroendocrine tumours (GEP-NETs) varies strikingly, both in terms of symptoms and outcome[1-3]. An understanding of the basic biology unique to GEP-NETs is necessary for optimal management of patients with these complex tumours. Although markers for GEP-NETs exist, sensitive and specific markers that predict tumour growth and behaviour are lacking[4]. The general purpose of the studies described in this thesis was to investigate the epidemiology, diagnosis and pathogenesis of GEP-NETs in The Netherlands, to reveal insights in the underlying mechanisms contributing to the development and progression of these tumours. The major findings reported in this thesis are highlighted in Figure 1.

Epidemiology of GEP-NETs in The Netherlands

Although GEP-NETs were considered as rare tumours, incidence rates have been reported to increase substantially in recent years[5-7]. Furthermore, a relatively high number of incidental findings of clinically silent NETs by autopsy studies was suggested in literature[8,9]. We calculated the current incidence of gastrointestinal carcinoids and duodeno-pancreatic NETs in The Netherlands, by the use of the PALGA database (**Chapter 2** and **Chapter 3**)[10]. Interestingly, the incidence of non-functional pancreatic and duodenal NETs showed a significant increase from 1991 till 2009, whereas the incidence of gastrointestinal carcinoids increased significantly over 2000 to 2009 as well. Although this increase in incidence of GEP-NETs is likely to be the result of improved diagnostics rather than an actual increase in occurrence of these tumours, non-functional tumours are still detected at a relatively late stage illustrated by the larger size and a diagnosis at an older age than in those patients affected by a functional neuroendocrine tumour. In **Chapter 2**, we provided an overview on recent developments in the diagnosis of GEP-NETs, to increase the intelligibility and awareness of these tumours among clinicians and pathologists, in order to facilitate earlier detection and to prevent morbidity of GEP-NETs.

Gastrinomas

Gastrinomas, after insulinomas, are the most common type of functional neuroendocrine tumours. They are frequently located in the pancreas and duodenum[11]. However, gastrinomas can also occur at ectopic sites[12-14]. In **Chapter 4** we described a unique case of recurring hepatic gastrinomas in a patient suffering from the Zollinger-Ellison syndrome (ZES), in whom no other (primary) tumour was detected, even though with a follow-up of almost 20 years. In this context, we reviewed the literature on primary liver gastrinomas, and found 16 studies in which gastrinomas in the liver were defined as primary. However, we believe that the interpretation of hepatic gastrinomas as primary lesions can still be questioned. Nonetheless, our study showed that a gastrin-producing tumour in the liver can recur. As most cases lack an investigational and well-documented follow-up, we recommend a long-lasting follow-up including frequent serum gastrin measurements and repeated imaging investigations in case of a suspected hepatic gastrinoma.

Gastrinomas produce and secrete gastrin, a hormone normally produced by G-cells in the stomach to stimulate the acid secretion. Patients with gastrinomas therefore suffer from symptoms related to hyperacidity, such as acid reflux, abdominal pain, recurrent ulcers, and diarrhoea. Together these symptoms are called the Zollinger-Ellison syndrome (ZES)[15]. Usually, ZES is suspected in case of increased fasting serum gastrin levels and/or the presence of symptoms. However, the increased use of proton pump inhibitors or other acid reducing medications often masks symptoms, contributing to a delay in diagnosis in these patients[16]. Furthermore, serum gastrin levels can be non-conclusive. The secretin stimulation test has widely been used to diagnose ZES[17]. In the literature, however, the dosage of secretin and the criteria for a positive test have been disputed[18-22]. We discussed the diagnostic efficacy of the secretin stimulation test in patients with ZES by comparison of two different doses of secretin and selecting the most optimal criteria for a positive secretin test (**Chapter 5**). We found a gastrin increase of >100 ng/L to be the most sensitive and specific criterion for a positive secretin stimulation test. When this criterion was applied to both our

study and confirmation group, we found that a higher dose of secretin (0.78 µg/kg) did not contribute to a more valuable secretin stimulation test in diagnosing ZES. Therefore, we recommend the use of the low dose of secretin (0.26 µg/kg) in combination with a gastrin increase >100 ng/L as the optimal criterion for a positive secretin stimulation test to diagnose ZES.

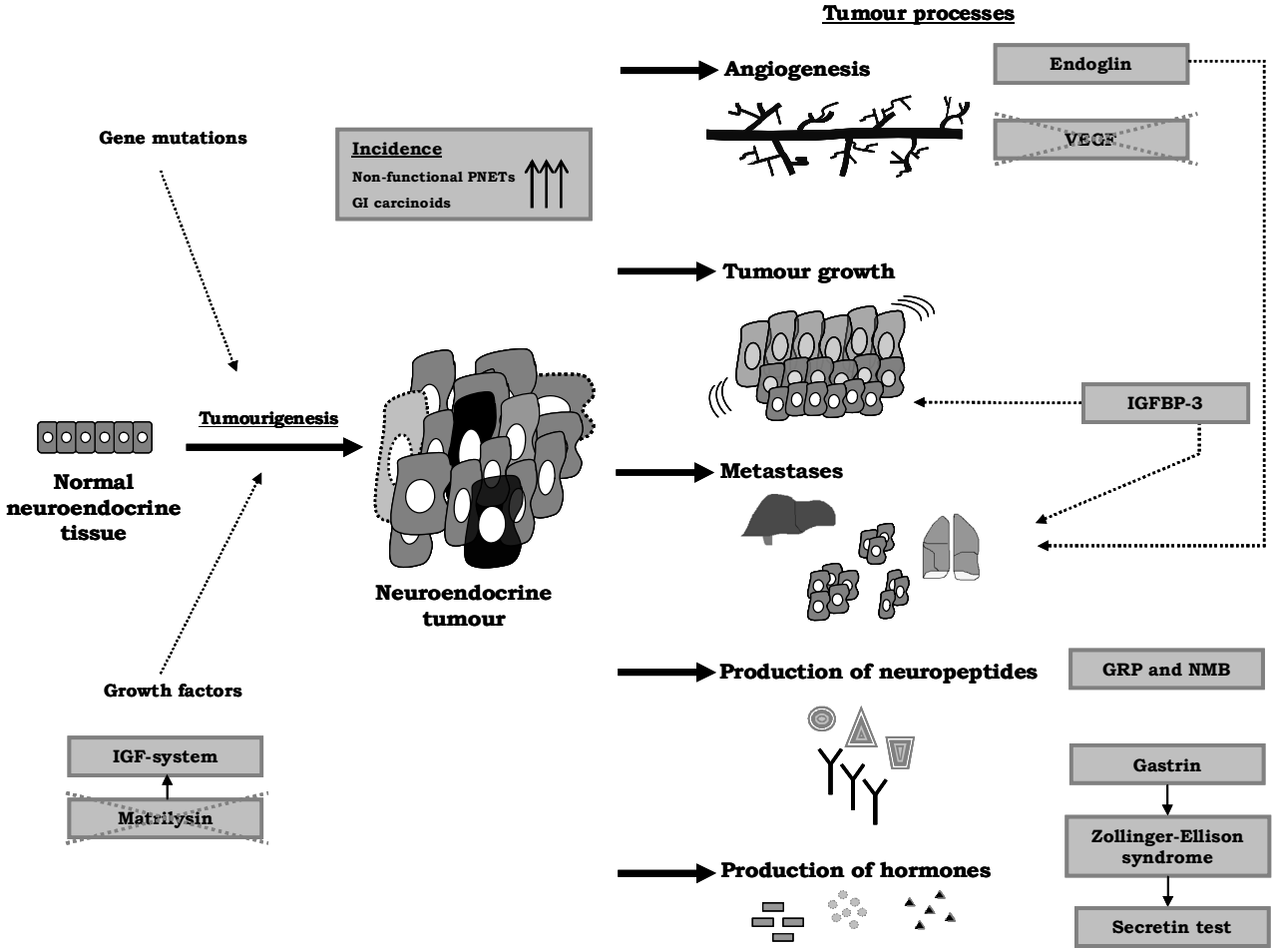

Figure 1. Summary of the results obtained in the studies as described in this thesis.

Heterogeneity and tumour markers in GEP-NETs

Neuroendocrine tumours of the gastroenteropancreatic tract are a group of diverse, heterogeneous tumours. Although gastrointestinal carcinoids and pancreatic neuroendocrine tumours share their common origin of neuroendocrine cells of the digestive tract, these tumours show variable histopathological characteristics and behaviour, making it hard to predict outcomes and prognosis on basis of these features[23]. Therefore, we aimed to identify tumour parameters which might have clinical implications in these tumours.

Carcinoids are predominantly found in the gastrointestinal (2/3rd) or pulmonary system (1/3rd). These tumours are able to secrete bioactive peptides, such as the bombesin-like peptides (BLPs) gastrin releasing peptide (GRP) and neuromedin B (NMB). In addition to stimulating a variety of physiological responses in the human body, BLPs are involved in the development and progression of several human cancers. By binding to their membrane-bound receptors on tumour cells, BLPs are able to activate autocrine loops, leading to growth of the tumour[24]. In small cell and non-small cell lung cancer, an autocrine loop involving BLPs has been suggested, whereas in colorectal cancer BLPs have been observed to act both as morphogens and mitogens[25-27]. We investigated the quantity and localization of bombesin receptors in gastrointestinal and pulmonary carcinoids, and revealed whether bombesin-like peptides and their receptors are of any value in distinguishing pulmonary carcinoids from carcinoids of intestinal origin (**Chapter 6**). Based on our results, we conclude that in both pulmonary and intestinal carcinoids, all three bombesin receptors are present, although the quantity and ligand binding affinities are diverse on carcinoids of different origin; apparently on pulmonary carcinoids, bombesin receptors have a low binding affinity for bombesin, while intestinal carcinoids possess predominantly receptors with a high ligand binding affinity. Therefore, overall bombesin receptor expression seems not to be a very useful marker to distinguish carcinoids based on tumour origin. The combined presence of bombesin and its receptors might suggest the presence of a paracrine or autocrine growth loop in carcinoids, although further research is required to confirm this hypothesis.

Angiogenesis plays an important role in tumour growth, progression and metastatic development[28]. Vascular endothelial growth factor (VEGF) and endoglin (CD105) are two key factors in angiogenesis. VEGF is a potent angiogenic growth factor stimulating endothelial cell proliferation, whereas endoglin, a TGF-beta co-receptor, is highly expressed on endothelial cells of newly formed blood vessels[29,30]. In **Chapter 7**, a study to evaluate the expression and potential prognostic role of VEGF and endoglin in GEP-NETs is described. Expression of endoglin and VEGF were two to four-fold higher on tumours compared to

associated normal tissue. This increased endoglin tissue expression in tumours was significantly related to the tumour's size, the presence of metastases and a more advanced tumour stage. These findings implicate that endoglin can serve as a marker to detect present and to predict future metastases in GEP-NETs. Assessment of endoglin tumour levels provides information on tumour aggressiveness, which might help to optimize the therapeutic approach in patients with these tumours. As several *in vivo* and *in vitro* studies using anti-endoglin antibodies for anti-cancer treatment show promising results, we suggest that endoglin might provide a new therapeutic vascular target in GEP-NETs as well[31]. Although VEGF tissue levels showed a similar pattern to endoglin, these were not significantly related to any clinicopathological parameter. Therefore, we assume that, although VEGF is most likely to be involved in the process of neoplastic blood vessel formation in GEP-NETs, this key mediator of angiogenesis is not the appropriate prognostic marker in these tumours.

The insulin-like growth factor (IGF) system, composed of the ligands IGF-1 and IGF-2, three receptors and six binding proteins (IGFBPs), plays an important role in cancer[32]. Several studies have shown that the expression of IGF-1 is up-regulated in various tumours, and related to tumour growth and malignant behaviour[33-35]. Matrix metalloproteinases (MMPs) are a family of endopeptidases which act to degrade the extracellular matrix and are essential for tissue remodelling[36].

Matrilysin (MMP-7) is exclusively produced by tumour cells and implicated to be involved in various tumour processes[37]. For example, in pancreatic and colorectal cancer, an increased expression of matrilysin was found to be related to invasion and the presence of metastases[38,39]. Recently, several studies have shown that MMPs, including matrilysin, can regulate the bioavailability of IGFs to tumour cells, thereby participating in IGF-induced growth activation in tumours[40].

We examined the expression of IGF-1, IGFBP-3 and matrilysin in GEP-NETs, in order to investigate their relation to the pathogenetic factors of these tumours (**Chapter 8)**. Tissue levels and expression of IGF-1 and IGFBP-3 were found to be up-regulated in GEP-NETs. In addition, higher IGFBP-3 expression was related to

a larger tumour size and the presence of metastases, which might be indicative for a more malignant tumour. The expression of matrilysin was down-regulated in tumours compared to associated normal tissue, and negatively correlated to the expression of IGFBP-3. These data suggest that IGFBP-3 plays a direct role in the etiopathogenesis of GEP-NETs, whereas matrilysin might indirectly be involved via regulation of this IGFBP-3 expression.

Concluding remarks

GEP-NETs comprise a group of heterogeneous tumours, with a wide and complex spectrum of clinical behaviour. They originate in a great diversity of tissues and are characterized by their ability to produce various hormonal peptides that cause distinct clinical syndromes. As incidence rates of both GI carcinoids and duodenopancreatic NETs are increasing over the past years in the Netherlands, these tumours might not be as uncommon as previously thought. This increasing incidence and large heterogeneity of GEP-NETs underlines the urgent need for better understanding of the underlying pathological mechanisms, in order to facilitate the development of new therapeutic strategies. In this thesis, several studies to reveal new markers in the pathogenesis of GEP-NETs are described. Foremost, we suggest endoglin as a novel marker to indicate the presence and potential development of metastases in GEP-NETs, of potential use in the post-resection approach in the therapy of these tumours. Next, preliminary evidence for a role of two autocrine growth systems, involving the bombesin-like peptides GRP and NMB, and the IGF-system and matrilysin, respectively, in the growth and development of these tumours, is provided. Although further research to reveal the exact mechanism of these autocrine growth systems in GEP-NETs is required, these studies might provide the basis for the development of new anti-cancer therapies in these tumours.

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