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From Basic to Clinical Research in Gastroenteropancreatic Neuroendocrine Tumor Disease – The Clinician-Scientist Perspective

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Abstract
Patients with rare tumors represent a diagnostic and therapeutic challenge for non-specialized physicians, surgeons and other medical doctors. Whereas several specialized centers have gathered data for an improved diagnosis and therapy of neuroendocrine tumor disease, numerous clinical issues have not been resolved on an evidence-based medicine level. Furthermore, the evaluation of new treatment options has been overshadowed by the low incidence of the disease. In this article, a major medical challenge for the diagnosis and therapy of neuroendocrine tumor disease is addressed. As well, new therapeutic treatment options translated from current findings in the fields of molecular and tumor biology are discussed.

Neuroendocrine tumors (NETs) originate in different organs and sites [1, 2]. Based on their diverse primary tumor localizations, NETs of the gastroenteropancreatic (GEP) system encompass a family of distinct or even individual tumors, which have to be considered as distinct as adenocarcinomas of the stomach, rectum and pancreas [3]. NET cells also exhibit, in relation to their primary origin, distinct cell biological features, such as distinct secretory as well as growth and differentiation properties [4].

For example, NETs located in the rectum (also called rectal carcinoids) practically never secrete hormones or biogenic amines to cause hypersecretion-related symptoms and syndromes. They usually grow slowly and metastasize late, i.e. only once a tumor exceeds a diameter of 1–2 cm [5, 6].

By contrast, NETs of the colon are usually dedifferentiated and metastasize early [7]. On the other hand, they are similar to NETs of the rectum in that they are nonfunctional, i.e. no secretion of hormones and biogenic amines is observed which can cause hypersecretion-related syndromes and symptoms. Despite this, however, functionally inactive polypeptides such as chromogranin A can be detected in patients with metastatic disease in the bloodstream [8].

By contrast, NETs of the pancreas often secrete hormones (e.g. insulin, gastrin, glucagon and VIP) but very

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rarely biogenic amines (e.g. serotonin and catecholamines) [5, 9].

This clinical and tumor biological phenomenon is contrasted by NETs of the ileum, which often secrete biogenic amines but rarely hormones (the only exception being tachykinins). Similar for both NETs of the pancreas and the ileum is their usually low proliferation index as determined by Ki67 (<10%) [2, 5].

Based on these tumor biological and clinical facts, NETs have been diagnosed and treated as separate diseases, i.e. according to their primary location, state of differentiation and stage [10, 11].

NET patients often represent a difficult diagnostic challenge at their first doctor's visit. This holds especially true for patients who only exhibit discrete functionality such as mild phases of impaired consciousness (e.g. insulinoma), epigastric pain (e.g. gastrinoma) or intermittent, nocturnal diarrhea (e.g. carcinoid syndrome).

This also holds true for patients with MEN-1, which are known to be genetically affected by a menin mutation [12, 13]; however, in the early tumor stage, only provocation tests can detect small or even minimal disease. Clearly, laboratory diagnosis without provocation tests will practically always be negative in these very early tumor stages. Aside from provocation tests, the bona fide tumor markers, chromogranin A and 5-HIAA, will only be raised once metastases have formed. Furthermore, these markers have to be considered with care, since synthesis of these marker molecules depends on the primary tumor location as well as the state of tumor differentiation [14].

Histological diagnosis, in a preoperative setting, requires the imaging of NET lesions, be they liver metastases, gastric or rectal polyps or pancreatic lesions. In some cases, functionality as well as a positive laboratory test may be present but no lesion can be detected [4, 15]. Only during the course of the disease can the tumor lesion be detected. Therefore, more sensitive diagnostic procedures are required allowing the consistent earlier detection of tumor lesions smaller than 5 or even 2 mm. This would also imply the hope that tumors consisting of less than, for example, 1 million tumor cells (corresponding to a diameter of approximately 3 mm) should be detectable in living tissue. Clearly, in contrast to the given limited in vivo conditions, immunohistology can allow the detection of a single tumor cell, for example in lymph nodes or in bone marrow. Thus, improvement of our current in vivo imaging standards is required to come close to the well-established in vitro imaging conditions.

Similarly, endoscopic ultrasound, the continuously improving MRI technology as well as somatostatin receptor scintigraphy represent moves in the right direction in order to come closer to the ideal in the possible detection of the first and only tumor cell [16, 17].

As far as new therapeutic options in NET disease are concerned, surgical methods have been improved by new minimally invasive procedures, for example in laparoscopic ileocecal resection. However, it remains to be determined if this approach will substitute for the conventional 'open' approach. Considering, for example, that we can evaluate the lymph node status in ileal NET just as well by laparoscopy as by 'open surgery' is very promising.

Furthermore, local ablative procedures have increasingly been used by now in NET disease. Although promising in terms of control of symptoms, no data have been obtained so far in a prospective, randomized, multicentric setting demonstrating a prolonged survival in NET patients.

Similarly, peptide-guided radioreceptor therapy has been used in several trials and shown to be quite promising for both control of hypersecretion-related symptoms as well as control of tumor growth [17–19]. So far, however, only one prospective multicenter trial with radiolabeled octreotate coupled to yttrium 90 via a chemical DOTATOC bridge (Octreother) has been performed. Final results of this trial are not yet ready.

Furthermore, chemotherapy has only partially been effective in two NET groups: in pancreatic as well as in undifferentiated NETs. In the first group, streptozotocin-based regimens combined with 5-FU or doxorubicin are of some value [20]. For undifferentiated, anaplastic NETs, cis-platinum plus VP16/etoposide can lead to some responses [21]. However, these responses last only a few months. Based on these limited effects of presently used chemotherapeutic agents, new chemotherapeutics may be worth testing such as oxaliplatin- or taxol-based regimens in anaplastic NETs [22].

Clearly, based on the above-given limited clinical knowledge in the field of diagnostics, as well as therapeutics in NET disease, a substantial number of clinical questions have to be answered in prospective pan-European or even global trials.

The major issues and questions to be answered are: (1) development of a staging, grading and subsequent TNM classification as an objective measure for prognosis in NET patients; (2) evaluation of conventional enteroclysma as compared to the newly developed MR-Sellink procedure; (3) determination of the cost-effectiveness of somatostatin receptor scintigraphy in comparison to other imaging procedures in a prospective multicentric setting; (4) performance of a randomized prospective study

Table 1. Overexpression of growth factors and their cognate receptors in GEP NETs

Growth factor	Receptor	Reference
PDGF	PDGF-α-R	25
bFGF	FGF-RI, FGF-RII	26
TGF-α	EGF-R	27, 28, 29
TGF-β	TGF-β-RI, TGF-β-RII	24
HGF	HGF-R	29
IGF	IGF-R	30, 31
VEGF	KDR, Flt-1	23

on surgical debulking vs. medical therapy in patients with noncuratively resectable cancer; (5) evaluation of local ablative procedures (radiofrequency thermal ablation, laser-induced thermotherapy and others) in comparison with medical therapy under the aspects of both, control of symptoms as well as tumor growth; (6) evaluation of embolization vs. chemoembolization in a prospective setting; (7) determination of the antiproliferative effect of biotherapeutics in relation to primary localization, tumor differentiation and drug bioavailability; (8) evaluation of the antiproliferative effect of 'cold' vs. 'hot' somatostatin analogues; (9) evaluation of newly developed biotherapeutics (see below); (10) evaluation of the effect of peritoneal carcinosis on gastrointestinal motility; (11) evaluation of the prognostic value of micrometastasis in lymph nodes, liver and blood, and (12) evaluation of certain tumor biological phenomena such as anoikis, angiogenesis and cell cycle activity in differentiated and undifferentiated NET cells in vitro. In addition to new chemotherapeutic agents, biotherapy or targeted therapy should be helpful in the expansion of our current therapeutic armamentarium (see below).

Clearly, on a cellular level, we will have to learn more about the key molecular players involved in the tumor biology of NET disease. Furthermore, as far as NET cell crosstalk is concerned, aside from NET cells, we have very little knowledge concerning the interaction of immunocytes, endothelial cells, non-NET epithelial cells and neurons with NET cells. Furthermore, we do not know if clonal development of NET cells varies in relation to a given specific cellular compartment.

Despite this, however, new agents developed in the field of targeted therapy will, we hope, allow us to study possible interference/inhibition of various growth factor signalling pathways. Similarly, signalling pathways linked with G-protein-coupled receptors as well as calcium channels represent promising therapeutic targets.

Interference with these pathways will also include interference with angiogenesis and cellular crosstalk (e.g. via integrins). It might also allow an improved therapeutic control of nuclear replication and membrane transport/secretion. This may not only hold true for NET cells but may also include immunocytes and other non-NET cells.

Among the most promising new therapeutic approaches in targeted therapy may be the inhibition of synthesis and/or secretion, as well as receptor binding of growth factors such as vascular endothelial growth factors [23, 24]. Based on their action on endothelial cell activation, followed by a consecutive vascular hyperpermeability and matrix permeation, followed in turn by the induction of endothelial proliferation, migration, lumen formation and stabilization of pericytes, this growth factor family warrants further detailed studies in NETs. This approach is further supported by the fact that NET disease is characterized by hypervascularization within the tumor tissue [25].

Growth factor signalling in GEP NETs has so far been quite extensively studied [23, 24, 26–32]. Biological parameters such as growth, glucose metabolism, survival and mitogenesis have mainly been studied in vitro by the overexpression of growth factors and their cognate receptors in GEP NET cell lines. Details and references on the signalling pathways of PDGF, bFGF, EGF, HGF, IGF and VEGF in GEP NET are shown in table 1. Aside from the biological functions of the various growth factors, the function of somatostatin including its analogues has been extensively studied in NET [33].

As comprehensively discussed by Schmid et al. [34], somatostatin as well its analogues play an important role in the treatment of hypersecretion-related symptoms in NETs. However, in order to improve the potency of these pharmacological agents, more detailed studies are required analyzing the crosstalk of somatostatin analogues with phosphatases and calcium channels. In addition, a variety of mechanisms of interferon-α action on NET cells has been elucidated [35, 36] and justifies this substance as both an antisecretory as well as an antiproliferative agent, although its side effects have to be considered [37, 38].

In this context, it is of note that a recent study by Mergler et al. [39, 40] suggested for the first time that R-type Ca^{2+} channels are expressed in NETs, which in turn can be used as therapeutic targets by interfering with their function such as with SNX-482.

Aside from new drug targets, a number of medical agents are available on the market for other indications.

The antiproliferative action of COX-2/NSAIDs in NET cells has recently been demonstrated in vitro for the first time [41]. Clearly, COX-2 inhibitors represent an interesting new therapeutic approach for NETs based on their low side effects and their possible combination with other orally available agents (e.g. capecitabine).

Similarly, other pharmaceutical agents, characterized by their signalling via growth factor receptors, G-protein-coupled receptors, calcium channels, integrins and nuclear proteins are currently evaluated in numerous clinical, oncological trials for non-NET indications. These include targeted therapeutics such as gefinitib (Iressa®) interfering with EGF receptor signalling; imatinib interfering with PDGF c-kit signalling; SOM 230 interfering with somatostatin signalling; bevacizumab (Avastin®) interfering with VEGF-A signalling; PTK/ZK interfering with VEGFR 1–3, PDGFR c-kit and c-Fms signalling; Medi-522 and cilengitide interfering with integrin-$\alpha_v\beta_3$ signalling; flavopyridol and rapamycin interfering with nuclear replication and membrane transport/secretion.

In summary, numerous questions remain to be answered, both at the level of diagnostics and therapeutics. To answer these primary questions, multicentric, prospective, randomized studies are required in order to generate better evidence-based medicine levels than those that have been obtained so far. Clearly, this also implies the performance of studies evaluating the cost-benefit of currently applied diagnostics and therapeutics. In addition, new therapeutic strategies are required in order to improve current, rather limited treatment options especially in metastatic NET disease. Here, new targeted therapies offer new hope especially in the fields of angiogenesis, nuclear replication, cellular adhesion and signal transduction.

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