

Oberndorfer and his successors: from carcinoid to neuroendocrine carcinoma

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In 1907 Oberndorfer reported on little carcinomas of the small intestine [13]. He described seven cases and emphasized the benign nature of these lesions. In retrospect it is clear that similar tumors had been reported before 1907. In 1838 Merling reported a tumor of the appendix that could have been a carcinoid [12]. In 1867 Langhans saw a polypous tumor in the ileum [8] and in 1882 Beger described an adenocarcinoma of the appendix [1]. In 1888, Lubarsch gave a classical description of multiple carcinoids in the ileum in two patients and he called these tumors little carcinomata [9].

When Oberndorfer demonstrated his results on the carcinoids before the German Pathology Society in Dresden, his suggestion that the lesions represented a special cancer were heavily debated [14]. A number of renowned pathologists considered them malformations, adenomyomas, or a tumorous change of a heterotopic pancreas anlage [18]. However, as more and more small tumors were detected and described in the intestine, the neoplastic nature of the lesion was generally accepted.

In 1910, Hübschmann compared the tumor cells with the cells that had been described by Kultschitzky in the crypts of Lieberkühn [6]. These cells corresponded to those that had already been found and described by Heidenhain in the stomach in 1870 [5]. Soon after Hübschmann, Masson developed his argentaffinity reaction and demonstrated that the granules in the Kultschitzky cells and the cells of the carcinoids both stained with his silver technique [10]. Consequently, Masson termed the intestinal carcinoids argentaffinoma [11]. Although many other histogenetic pathways were discussed over the years, the origin from the so-called enterochromaffin cells of the intestinal mucosa was finally accepted [4].

The endocrine cell system that gives rise to carcinoids was further expanded by the work of Feyrter on the diffuse endocrine system that was composed of argentaffin-positive and argyrophilic clear cells [3]. A further step forward in characterizing these endocrine cells was made by Anthony Pearse in 1969 [16], who called the cells APUD (amine precursor uptake

MOLECULAR EVENTS IN GASTROINTESTINAL AND PANCREATIC NEUROENDOCRINE TUMORS

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BACKGROUND

GI and pancreatic NETs originate from the cells of the diffuse endocrine system derived from the embryonic neural crest, neuroectoderm and endoderm. NETs can be functional (release hormones) or non-functional (1-3).

It is important to recognize that molecular studies of GI and pancreatic NETs to date have been affected by several factors:

- 1) Heterogeneity of tumors;
- 2) Difficulty in predicting tumor behavior and prognosis;
- 3) Rarity of tumors and relatively small study sample size;
- 4) Use of different GI-NET classifications by investigators for tumor selection;
- 5) Differences in genetic composition of GI-NETs vs. epithelial tumors;
- 6) Tendency to study known epithelial genes/oncogenes first.

GI-NET Classifications Used in Molecular Studies (1, 4-6)

GI CARCINOIDS are traditionally considered serotonin (5-hydroxytryptamine)-secreting and argentaffin positive:

Foregut: stomach, first part of duodenum and pancreas

Midgut: small intestine (second portion of duodenum, jejunum, ileum) and large intestine (appendix, ascending colon)

Hindgut: transverse colon, descending colon and rectum

PANCREATIC AND DUODENAL NETS (functional) are commonly classified according to the predominant hormone secreted: gastrinoma, insulinoma, VIPoma, glucagonoma, somatostatinoma. Clinically silent NETs are called non-functional (2).

WHO CLASSIFICATION OF ENDOCRINE TUMORS (4)

Well-differentiated endocrine tumors (benign or uncertain behavior)

Well-differentiated endocrine carcinomas (low-grade malignant behavior)

Poorly differentiated endocrine carcinomas (high-grade malignant behavior)

MOLECULAR GENETICS OF GI- AND PANCREATIC NETs

The hope is that the finding of specific genetic alterations that are characteristic of GI-PNETs will lead to improved diagnosis and characterization of these tumors and will enable a modification of current morphologic classifications.

Because the enteroendocrine cells are epithelial cells derived from the same stem cell as the other cell lineages, the investigation of the same mechanisms of neoplastic progression leading to adenocarcinoma were applied to many studies of gut endocrine tumors. However, the results of such studies led to conclusions that GI-PNETs do not share the same mechanism of development and neoplastic progression with GI epithelial tumors (2, 3).

Epithelial Carcinoma Genes Are Not Involved in GI-PNET Tumorigenesis (2, 3)

Despite *APC* (adenomatous polyposis coli; familial adenomatous polyposis) gene's ubiquitous expression and crucial role in cellular homeostasis and neoplastic progression in colon and small bowel adenocarcinomas, it does not seem to have an impact on neoplastic progression in enteroendocrine cells. *DCC* (deleted in colon cancer gene) gene is not involved. DNA mismatch repair (*MSH2* and *MLH1*) genes, (Lynch syndrome) are not involved.

MOLECULAR EVENTS IN TUMORS OF FOREGUT

PANCREAS

Pancreatic NETs occur in three hereditary syndromes such as Multiple Endocrine Neoplasia type 1 (MEN1), von Hippel-Lindau disease (VHL) and von Recklinghausen's disease (Neurofibromatosis 1) (2). The syndromes are caused by defects in three known tumor suppressor genes, respectively: *MEN1* on chromosome 11q13 (610-amino acid protein, *MENIN*); *VHL* on chromosome 3p25.5 (213-amino acid protein, *VHL*) and the gene on chromosome 17q11.2 (2485 -amino acid protein, *neurofibromin*). Pancreatic tumors are multiple but their type, location and incidence are different in each syndrome (2). MEN1 pancreatic NETs are located in the pancreas and duodenum with the incidence of 80-100% (non-functioning pancreatic tumors>gastrinomas>insulinomas). Pancreatic NETs in VHL disease occur only in the pancreas, are non-functioning and are seen in 12-17% of patients. Pancreatic somatostatinomas occur in 6% of patients with Neurofibromatosis 1.

Pancreatic and GI NETs in Hereditary Syndromes		
Syndrome	Gene location (product)	NET Frequency
Multiple Endocrine Neoplasia type 1	11q13 (610-amino acid protein, <i>MENIN</i>)	80-100% pancreas+duodenum (NF>gastrinoma>insulinoma Gastric carcinoids)
Von Hippel-Lindau disease	3p25.5 (213-amino acid protein, <i>VHL</i>)	12-17% pancreas all non-functioning
Von Recklinghausen's disease	17q11.2 (2485 -amino acid protein, <i>neurofibromin</i>)	6% pancreatic somatostatinoma

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MEN1 patients have multiple endocrine tumors of the parathyroid glands, pituitary, pancreas, duodenum as well as gastric, lung and thymic carcinoids (7). Like in

hereditary tumors in patients with MEN1, *MEN1* gene alteration is an important initiating event in about 1/3 of sporadic non-functioning pancreatic NETs, insulinomas, and gastrinomas and is documented in tumors regardless of metastases (2, 8-9). Genetic analysis of the counterpart sporadic pancreatic NETs demonstrated somatic *MEN1* gene mutations accompanied by loss of the wild-type allele in 10-27% of insulinomas and 39-45% of gastrinomas (8, 10). The mutation rate in non-functioning NETs in different reports is 15-26% (10-11). Because the rate of 11q13 LOH in sporadic pancreatic NETs is on average 46%, and LOH is not always accompanied by somatic mutation, other mechanisms of *MEN1* gene inactivation or other genes may play a role in sporadic tumor development.

Thus LOH analysis for more telomeric markers on 11q showed that allelic loss consistently and continuously spanned to 11qter (12). These findings support the hypothesis that additional onco/suppressor gene(s) may reside at 11q distal to the *MEN1* gene and may play a role in the pathogenesis of pancreatic NETs (13-15).

VHL disease patients develop CNS and retinal hemangiomas, renal cysts and carcinomas, pancreatic and epididymal cystadenomas and pheochromocytomas. Pancreatic non-functioning NETs are seen in 12-17% of VHL patients (16-17). Loss of heterozygosity at 3p25.5 gene locus is documented in only 30% of sporadic pancreatic NETs and is usually not accompanied by somatic *VHL* gene mutation (18-20). These data indicate that *VHL* gene is not a factor in sporadic pancreatic NET development and another gene telomeric to the *VHL* 3p locus may be involved.

Variations in the genetic makeup of functioning versus non-functioning pancreatic NETs have been demonstrated in small tumors (<2 cm in diameter) by CGH (21). 9q gains with a common region of involvement at 9q34 were observed in 46.4% of functioning tumors, of which in 50% of insulinomas. However, diffuse genetic instability with multiple chromosomal aberrations per tumor was observed, making it difficult to evaluate the significance of specific findings.

In sporadic gastrinomas, either homozygous deletion or hypermethylation at the 5' region of the *p16/MTS1* or *p16^{INK4a}* tumor suppressor gene on chromosome 9p21 was demonstrated (22-23). Such an abnormality was not observed in 17 insulinomas (24) and in 41 pancreatic NETs of different types (25), despite the presence of 9p LOH in 30% of cases. CGH and high-resolution allelotype analysis confirmed the involvement of 9p defects in 20–30% of cases investigated (26-28). Interestingly, *p16/MTS1* gene mutation was observed in only one out of a total of 68 tumors investigated in different reports. Overall, these findings indicate that other potential tumor suppressor genes on chromosome 9p are involved in the genesis of pancreatic NETs and suggest that *p16/MTS1* or *p16^{INK4a}* defect is restricted to gastrin-producing tumors.

No defect of the retinoblastoma *Rb* gene on chromosome 13q was observed in any type of pancreatic NET investigated to date (29-30).

High LOH rates for markers on chromosome 22q (93%) were observed in both benign and malignant insulinomas (31). An overexpression of cyclin D1 was demonstrated by both immunohistochemistry and Northern analysis in 43% of PET, although there was no correlation with any specific tumor phenotype (32). Although possibly important in initiation of tumorigenesis in both benign and malignant pancreatic NETs these changes were not accompanied by gene mutations and, therefore, require further investigation.

The promoter region CpG island methylation of 12 genes potentially involved in endocrine tumor development such as *p14*, *p16*, the estrogen receptor (*ER*), retinoic acid receptor-beta 2 (*RAR-β*), *O*⁶-methyl-guanine-methyltransferase (*O*⁶-*MGMT*), *MEN1* and cyclooxygenase 2 (*COX2*), recently proved to affect especially the *ER* gene in 9 out of 11 pancreatic NETs (2 gastrinomas and 1 insulinoma) (33). A recent similar investigation for 11 candidate tumor suppressor genes in 48 well-differentiated tumors (of which three were functional) proved a high frequency of methylation for the Ras-associated domain gene family 1A (*RASSF1A*) (75% of

cases), *p16* (40%), *O⁶-MGMT* (40%), *RAR-β* (25%) and *hHMLH1* (23%) (34). In addition, a recent investigation of pancreatic cancer showed *RASSF1A* promoter methylation in 10 out of 12 pancreatic NETs with a similar incidence in both malignant (*n*=6) and benign lesions (35). Since *RASSF1A* gene mutation is very rarely observed in human cancer (36) these findings strongly support the methylation mechanism for multiple gene inactivation in PETs and suggest that the *ras* pathway is involved via *RASSF1A* methylation.

Activating mutations in the *ras* family of proto-oncogenes, *K-ras*, *H-ras*, *N-ras*, are absent or exceedingly rare in large series of pancreatic NETs investigated (20, 37-39) although it was reported as a frequent event in a single study of malignant insulinomas(40). Overall, the *ras* oncogene seems not play a direct role in the development of most pancreatic NETs, with the possible exception of malignant insulinomas. However, the *ras* pathway may be involved in pancreatic endocrine tumorigenesis via promoter methylation of the *RASSF1A* gene (see above) (33, 35).

Several studies have tried to address the relationship between genetic defects and tumor progression or malignancy. Most relevant data, however, require further confirmation. The deletion of either arm of chromosome 1 was found in 10 out of 17 metastatic pancreatic NETs, with no tumor type prevalence (41). This finding was not confirmed by CGH and genome-wide allelotype studies (26-27). LOH at 3p25, centromeric to the locus for VHL disease, was shown to be associated with malignancy (18). This observation was further supported by CGH data on 44 tumors of different types (26) and by a large study of 99 pancreatic NETs (42). The frequent occurrence of allelic loss at 6q was documented in pancreatic tumors, although its association with malignancy and tumor progression was observed by one group (26, 43) and not confirmed by another (27).

Well differentiated NETs only rarely display *p53* mutations (20, 44-45). LOH of *p53* gene chromosomal markers on 17p13 was reported in 25% of investigated cases and found to be associated with malignancy (38). The absence of relevant *p53* mutation

suggested that an additional tumor suppressor gene might occur on 17p telomeric to *p53*. Poorly differentiated NE carcinomas of any site show high chromosomal instability and frequent *p53* changes (46). It is likely that *p53* alteration is not involved in pancreatic NET initiation but that it is a late progression event in a poorly differentiated endocrine carcinoma of the pancreas

Allelic loss for markers on chromosome X has been frequently demonstrated in malignant compared with benign endocrine tumors (47). The analysis was extended to chromosome Y in male patients, resulting in a significant association with short survival, the presence of metastases, local invasion and a high Ki-67 proliferation rate (48).

Published data suggest that multiple genetic defects may accumulate, resulting in tumor progression and malignancy. LOH for markers of seven different oncosuppressor genes was significantly more frequent in malignant (40%) than in benign (17%) tumors (38). A paired CGH study of primary tumors and their metastases with a control group of non-metastatic tumors displayed more frequent genomic aberrations in metastases than in the corresponding primary tumors when compared with non-metastatic cases (49). In non-functioning pancreatic tumors, a high frequency of chromosomal markers loss (fractional allelic loss) correlates with aneuploid status and a poorer clinical outcome (27). Finally, in an investigation of multiple chromosomal markers, higher percentages of allelic imbalances were reported in pancreatic NETs, suggesting chromosomal instability as basis for malignant progression (50).

STOMACH AND DUODENUM

NETs of stomach and duodenum display frequent LOH for the *MEN1* locus at 11q13 in both familial and sporadic cases (15, 51-52). LOH at the *MEN1* locus occurred in 75% of gastric (ECL) carcinoids in 23 familial cases and 41% in 46 sporadic cases (10). Four out of five poorly differentiated tumors of the stomach also showed allelic loss of the *MEN1* gene (15, 51, 53). A similar frequency of 11q13 LOH was observed

in both familiar and sporadic duodenal gastrinomas (28% and 25% respectively), whereas mutation for the *MEN1* gene was found only in 22 out of 67 sporadic cases (33%) (10). The findings support the initiating role of the *MEN1* gene in the development of many gastric carcinoids and duodenal gastrinomas.

Other data are scattered through small studies on various GI-NETs and are therefore fragmented. The promoter methylation of several genes, including *p14*, *p16*, *COX2* and *ER*, was frequently observed in two gastric and two duodenal well-differentiated tumours(33). Larger series are needed to confirm such observations.

An investigation of multiple chromosomal markers in nine gastric NETs, six of which were poorly differentiated, demonstrated frequent and diffuse allelic imbalances mostly in aggressive carcinomas (50). This finding was further demonstrated in 19 cases of aggressive gastric endocrine carcinoma, 10 of which were poorly differentiated, by an independent study (47). Both studies showed frequent LOH for markers at the *MEN1* and *p53* gene loci and demonstrated high chromosomal instability. In addition, a loss of *Rb* expression was frequently observed in 6 out of 9 well-differentiated gastric endocrine carcinomas and in 7 out of 10 poorly differentiated endocrine carcinomas (54). Finally, extensive losses of X chromosomal markers were present in four malignant tumors (two of which were poorly differentiated) but virtually absent in 29 benign neoplasms investigated (55).

MOLECULAR EVENTS IN TUMOURS OF MIDGUT

Midgut NETs rarely display *MEN1* gene alterations. A recent study of 16 ileal, 6 appendicular and 3 rectal well differentiated NETs showed an overall LOH rate of 9% for informative microsatellites at the *MEN1* locus on 11q13 (12). This was recently confirmed in five further cases (50). *MEN1* gene mutation was found in only 1 of 12 midgut endocrine tumors studied (56-57). These data do not support a role for the *MEN1* gene in the development of midgut NETs.

Accumulating evidence suggests the role of genes located on chromosome 18 in the induction of well-differentiated midgut NETs. An imbalance of chromosome 18, especially the loss of 18q markers, is the most frequent abnormality detected by different techniques in these tumors and appears to be typical of midgut carcinoids (58- 61). A combined CGH/LOH study of 18 classical midgut EC cell tumors losses at 18q22-qter were seen in 67% of cases (60), whereas a genome-wide LOH screening of 8 tumors showed 18q21 losses to be very frequent (88% of cases) and highlighted specific alterations in these neoplasms (61). These alterations were telomeric to the loci of the genes *SMAD2*, *SMAD4* and *DCC*, largely involved in colorectal cancer.

The promoter methylation of different genes is reported in 6 out of 7 well-differentiated ileal tumors, indicating that this mechanism of gene inactivation plays a role in such neoplasms and suggesting that different genes are involved compared with pancreatic NETs (33). However, larger studies are necessary to confirm such observations.

Seventeen midgut NETs showed a low frequency of LOH for X chromosome markers in malignant tumors (15% of informative markers investigated) and no losses in benign tumors (55). A CGH study of 13 primary and 5 metastatic classical midgut carcinoids showed that losses at 16q21-qter and gains at 4p14-pter were rare or absent in primary tumors and frequent in most metastatic tissues investigated (61). As for tumors of foregut derivatives, *p53* mutations are only rarely seen in NETs of the small intestine (62).

MOLECULAR EVENTS IN TUMORS OF HINDGUT

Only a few data are available for this area. Of 15 NETs of large intestine (7 in the appendix), 7 showed LOH for *MEN1* gene markers, whereas only 1 of 10 studied for mutation had one (52, 63). A mutation of *p53* was observed in 1 out of 9 ‘carcinoids’ of the colorectum and in 0 of 6 cases in the appendix (44). Similar to conventional adenocarcinoma, poorly differentiated (small-cell) endocrine carcinomas of the large

intestine display frequent LOH for *p53*, *DCC* and the adenomatous polyposis coli (*APC*) tumour suppressor loci (64). In the same report, no abnormality of the *DCC* and *APC* gene chromosomal loci was observed in four well-differentiated tumors, 'carcinoids' (2 from foregut, 1 from the midgut and 1 from the hindgut). Such findings suggest that common genetic events lie at the basis of colon cancer and poorly differentiated endocrine carcinoma.

Interestingly, poorly differentiated endocrine carcinomas may be synchronous with conventional adenocarcinomas or develop within adenomas. An investigation of poorly differentiated carcinomas arising within in situ/early invasive adenocarcinomas suggested a potential clonal divergence with different oncogenic pathways (65). A similar conclusion was drawn by LOH analysis for multiple chromosomal markers in a rare case of small-cell carcinoma mixed with adenocarcinoma of the appendix (66). Indeed, the investigation of 9 small-cell carcinomas of the colorectum compared with 12 adenocarcinomas revealed higher chromosomal instability and different genetic abnormalities (54). All studies on colonic endocrine small-cell carcinomas reported the high frequency of *p53* gene abnormality with nuclear protein hyperexpression/accumulation.

CONCLUSIONS

- 1) The molecular genetic mechanism of tumor development of GI and pancreatic NETs is complex and largely unknown.
- 2) Multiple genes appear to be involved with significant differences for tumors of different embryological derivatives.
- 3) The *MEN1* gene is involved in initiation of 33% of foregut NETs.
- 4) 18q defects are present almost exclusively in mid/hindgut NETs.
- 5) X-chromosome markers are associated with malignant behavior in foregut tumors only.

6) Poorly differentiated NE carcinomas of any site show high chromosomal instability and frequent *p53* alterations.

Future Studies

- What classification to use when selecting tumors for analysis?
- Need molecular genetic analysis of LARGE tumor series of EACH SPECIFIC tumor type .

REFERENCES

1. Rindi G and Bordi C. Endocrine tumours of the gastrointestinal tract: aetiology, molecular pathogenesis and genetics. *Best Pract Res Clin Gastroenterol* 2005, 19:519-534.
2. Lubensky IA. Endocrine Pancreas. In "Endocrine Pathology". Ed by LiVolsi VA and Asa S; *W. B. Saunders Co.*, Philadelphia, London, Toronto, 2002, 205-235.
3. Furth EE. Gastrointestinal Tract. In "Endocrine Pathology". Ed by LiVolsi VA and Asa S; *W. B. Saunders Co.*, Philadelphia, London, Toronto, 2002, 2237-2260.
4. Rindi G, Klöppel G, Alhman H, Caplin M, Couvelard A, de Herder WW, Eriksson B, Falchetti A, Falconi M, Komminoth P, Körner M, Lopes JM, McNicol A-M, Nilsson O, Perren A, Scarpa A, Scoazec J-Y, Wiedenmann B, and all other Frascati Consensus Conference participants. TNM staging of foregut (neuro) endocrine tumors: a consensus proposal including a grading system. *Virchows Arch* 2006, 449:395-401.
5. Kidd M, Modlin IM, Mane SM, Camp RL, Eick GN, Latich I and Zikusoka MN Utility of molecular genetic signatures in the delineation of gastric neoplasia. *Cancer* 2006, 106:1480-1488.
6. Zikusoka MN, Kidd M, Latich, and Modlin IM. The molecular genetics of gastroenteropancreatic neuroendocrine tumors. *Cancer* 2005, 104:2292-309.
7. Chandrasekharappa, SC, Guru SC, Manickam P Olufemi SE, Collins FS, Emmert-Buck MR, Debelenko LV, Zhuang Z, Lubensky IA, Liotta LA, Crabtree JS, Wang Y, Roe BA, Weisemann J, Boguski MS, Agarwal SK, Kester MB, Kim YS, Heppner C, Dong Q, Spiegel AM, Burns AL and Marx SJ. Positional cloning of the gene for multiple endocrine neoplasia-type 1, *Science* 1997, 276:404-407.
8. Zhuang Z, Vortmeyer AO, Pack S, Huang S, Pham T, Wang C, Park WS, Agarwal S, Debelenko LV, Kester MB, Guru S, Manickam P, Olufemi S-E, Yu F, Heppner C, Crabtree J, Skarulis M, Venzon DJ, Emmert-Buck MR, Spiegel AM,

- Chandrasekharappa SC, Collins FS, Burns AL, Marx SJ, Jensen RT, Liotta LA and Lubensky IA. Somatic mutations of the MEN1 tumor suppressor gene in sporadic gastrinomas and insulinomas. *Cancer Res* 1997, 57:4682-4686.
9. Goebel SU, Heppner C, Burns L, Marx SJ, Spiegel AM, Zhuang Z, Lubensky IA, Gibril F, Jensen RT, Serrano J: Genotype/phenotype correlation of MEN1 gene mutations in sporadic gastrinomas. *J Clin Endocr Metab* 2000, 85:116-123.
10. G. Rindi, V. Villanacci, A. Ubiali and A. Scarpa. Endocrine tumors of the digestive tract and pancreas: histogenesis, diagnosis and molecular basis. *Expert Rev Mol Diagn* , 2001, 1:323–333.
11. Moore PS, Missiaglia E, Antonello D, Zamò A, Zamboni G, Corleto V, Falconi M and Scarpa A. Role of disease-causing genes in sporadic pancreatic endocrine tumors: MEN1 and VHL. *Genes Chromosomes Cancer* 2001, 32:177–181.
12. D'Adda T, Pizzi S, Azzoni C, Bottarelli L, Crafa P, Pasquali C, Davoli C, Corleto VD, Delle Fave G and Bordi C. Different patterns of 11q allelic losses in digestive endocrine tumors. *Hum Pathol* 2002, 33:322–329.
13. Eubanks PJ, Sawicki MP, Samara GJ, Gatti R, Nakamura Y, Tsao D, Johnson C, Hurwitz M, Wan Y-JY and Passaro E, Jr. Putative tumor-suppressor gene on chromosome 11 is important in sporadic endocrine tumor formation. *Am J Surg* 1994, 167:180–185.
14. Chakrabarti R, Srivatsan ES, Wood TF, Eubanks PJ, Ebrahimi SA, Gatti RA, Passaro E, Jr and Sawicki MP. Deletion mapping of endocrine tumors localizes a second tumor suppressor gene on chromosome band 11q13. *Genes Chromosomes Cancer* 1998, 22:130–137.
15. D'Adda T, Keller G, Bordi C and Hoffler H. Loss of heterozygosity at 11q13-14 regions in gastric neuroendocrine tumors not associated with multiple endocrine neoplasia type 1 syndrome. *Lab Invest* 1999, 79:671–677.
16. Libutti SK, Choyke PL, Alexander HR, Glenn G, Bartlett DL, Zbar B, Lubensky I, McKee SA, Maher ER, Linehan WM and Walther MM. Clinical and genetic analysis of patients with pancreatic neuroendocrine tumors associated with von Hippel-Lindau disease. *Surgery* 2000, 128:1022-1028.
17. Lubensky IA, Pack S, Ault D, Vortmeyer A, Libutti S, Choyke P, Walther M, Linehan WM and Zhuang Z. Multiple neuroendocrine tumors of the pancreas in von Hippel-Lindau disease patients: histopathological and molecular genetic analysis. *Am J Pathol* 1998, 153:223-231.

18. Chung DC, Smith AP, Louis DN, Graeme-Cook F, Warshaw AL and Arnold A. A novel pancreatic endocrine tumor suppressor gene locus on chromosome 3p with clinical prognostic implications. *J Clin Invest* 1997, 100:404–410.
19. Chung DC, Brown SB, Graeme-Cook F, Tillotson LG, Warshaw AL, Jensen RT and Arnold A. Localization of putative tumor suppressor loci by genome-wide allelotyping in human pancreatic endocrine tumors. *Cancer Res* 1998, 58:3706–3711.
20. Moore PS, Orlandini S, Zamboni G, Capelli P, Rigaud G, Falconi M, Bassi C, Lemoine NR and Scarpa A. Pancreatic tumours: molecular pathways implicated in ductal cancer are involved in ampullary but not in exocrine nonductal or endocrine tumorigenesis. *Br J Cancer* 2001, 84:253–262.
21. Speel EJM, Scheidweiler AF, Zhao JM, Matter C, Saremaslani P, Roth J, Heitz PU and Komminoth P. Genetic evidence for early divergence of small functioning and nonfunctioning endocrine pancreatic tumors: gain of 9Q34 is an early event in insulinomas. *Cancer Res* 2001, 61:5186–5192.
22. Muscarella P, Melvin WS, Fisher WE, Foor J, Ellison EC, Herman JG, Schirmer WJ, Hitchcock CL, DeYoung BR and Weghorst CM. Genetic alterations in gastrinomas and nonfunctioning pancreatic neuroendocrine tumors: an analysis of p16/MTS1 tumor suppressor gene inactivation. *Cancer Res* 1998, 58: 237–240.
23. Serrano J, Goebel S, Peghini P, Lubensky IA, Gibril F, Jensen RT. Alterations of the p16^{INK4a}/CDKN2A tumor suppressor gene in gastrinomas. *J Clin Endocrinol Metab* 2000, 85:4146-4156.
24. Bartsch DK, Kersting M and Wild A. Low frequency of p16^{INK4a} alterations in insulinomas. *Digestion* 2000, 62:171–177.
25. Moore PS, Orlandini S, Zamboni G, Corleto V, Falconi M and Scarpa A. Pancreatic tumours: molecular pathways implicated in ductal cancer are involved in ampullary but not in exocrine nonductal or endocrine tumorigenesis. *Br J Cancer* 2001, 84:253–262.
26. Speel EJM, Richter J, Moch H, Egenter C, Saremaslani P, Rutimann K, Zhao J, Barghorn A, Roth J, Heitz PU and Komminoth P. Genetic differences in endocrine pancreatic tumor subtypes detected by comparative genomic hybridization. *Am J Pathol* 1999, 155:1787–1794.
27. Rigaud G, Missiaglia E, Moore PS, Zamboni G, Falconi M, Talamini G, Pesci A, Baron A, Lissandrini D, Rindi G, Grigolato P, Pederzoli P and Scarpa A. High resolution allelotype of nonfunctional pancreatic endocrine tumors: identification of two molecular subgroups with clinical implications. *Cancer Res* 2001, 61: 285–292.

28. Terris B, Meddeb M, Marchio M, Danglot G, Fléjou JF, Belghiti J, Ruzniewski P, Bernheim A. Comparative genomic hybridization analysis of sporadic neuroendocrine tumors of the digestive system. *Genes Chromosomes Cancer* 1998, 22:50–56.
29. Pearce SH, Trump D, Wooding C, Sheppard MN, Clayton RN and Thakker RV. Loss of heterozygosity studies at the retinoblastoma and breast cancer susceptibility (BRCA2) loci in pituitary, parathyroid, pancreatic and carcinoid tumours. *Clin Endocrinol (Oxf)* 1996, 45:195–200.
30. Chung DC, Smith AP, Louis DN, Graeme-Cook F, Warshaw AL and Arnold A. Analysis of the retinoblastoma tumour suppressor gene in pancreatic endocrine tumours. *Clin Endocrinol (Oxf)* 1997, 47:523–528.
31. Wild A, Langer P, Ramaswamy A, Chaloupka B and Bartsch DK. A novel insulinoma tumor suppressor gene locus on chromosome 22q with potential prognostic implications. *J Clin Endocrinol Metab* 2001, 86:5782–5787.
32. Chung DC, Brown SB, Graeme-Cook F, Seto M, Warshaw AL, Jensen RT and Arnold A. Overexpression of cyclin D1 occurs frequently in human pancreatic endocrine tumors. *J Clin Endocrinol Metab* 2000, 85:4373–4378.
33. Chan AO, Kim SG, Bedeir A, Issa J-P, Hamilton SR and Rashid A. CpG island methylation in carcinoid and pancreatic endocrine tumors. *Oncogene* 2003, 22:924–934.
34. M.G. House, J.G. Herman and M.Z. Guo *et al.*, Aberrant hypermethylation of tumor suppressor genes in pancreatic endocrine neoplasms. *Ann Surg* 2003, 238:423–431 (discussion 431–432).
35. Dammann R, Schagdarsurengin U, Liu L, Otto N, Gimm O, Dralle H, Boehm BO, Pfeifer GP and Hoang-Vu C. Frequent RASSF1A promoter hypermethylation and K-ras mutations in pancreatic carcinoma. *Oncogene* 2003, 22:3806–3812.
36. Dammann R, Schagdarsurengin U, Strunnikova M, Rastetter M, Seidel C, Liu L, Tommasi S and Pfeifer GP. Epigenetic inactivation of the Ras-association domain family 1 (RASSF1A) gene and its function in human carcinogenesis. *Histol Histopathol* 2003, 18:665–677.
37. Pellegata NS, Sessa F, Renault B, Bonato M, Leone BE, Solcia E and Ranzani GN. K-ras and p53 gene mutations in pancreatic cancer: ductal and nonductal tumors progress through different genetic lesions. *Cancer Res* 1994, 54:1556–1560.

38. Beghelli S, Pelosi G, Zamboni G, Falconi M, Iacono C, Bordi Cand Scarpa, A. Pancreatic endocrine tumours: evidence for a tumour suppressor pathogenesis and for a tumour suppressor gene on chromosome 17p. *J Pathol* 1998, 186:41–50.
39. Yashiro T, Fulton N, Hara H *et al.*. Comparison of mutations of ras oncogene in human pancreatic exocrine and endocrine tumors. *Surgery* 1993, 114:758–763 (discussion 763–764).
40. Pavelic K, Hrascan R, Kapitanovic S, Vranes Z, Belicza M, Kruslin B and Cabrijan T. Multiple genetic alterations in malignant metastatic insulinomas. *J Pathol* 1995, 177: 395–400.
41. Ebrahimi SA, Wang EH, Wu A, Schreck RR, Passaro E, Jr. and Sawicki MP. Deletion of chromosome 1 predicts prognosis in pancreatic endocrine tumors. *Cancer Res* 1999, 59:S311–315.
42. Barghorn A, Komminoth P, Bachmann D, Rütimann K, Saremaslani P, Muletta-Feurer S, Perren A, Roth J, Heitz PU and Speel EJM. Deletion at 3p25.3-p23 is frequently encountered in endocrine pancreatic tumours and is associated with metastatic progression. *J Pathol* 2001, 194:451–458.
43. Barghorn A, Speel EJM, Farspour B, Saremaslani P, Schmid S, Perren A, Roth J, Heitz PU and Komminoth P. Putative tumor suppressor loci at 6q22 and 6q23-q24 are involved in the malignant progression of sporadic endocrine pancreatic tumors. *Am J Pathol* 2001, 158:1903–1911.
44. Lohmann DR, Funk A, Niedermeyer HP, Haupel S and Hofler H. Identification of p53-gene mutations in gastrointestinal and pancreatic carcinoids by nonradioisotopic SSCA. *Virchows Arch [B]* 1993, 64: 293–296.
45. Pellegata NS, Sessa F, Renault B, Bonato M, Leone BE, Solcia E and Ranzani GN. K-ras and p53 gene mutations in pancreatic cancer: ductal and nonductal tumors progress through different genetic lesions. *Cancer Res* 1994, 54:1556–1560.
46. La Rosa S, Sessa F, Capella C, Riva C, Leone BE, Klersy C, Rindi G and Solcia E. Prognostic criteria in nonfunctioning pancreatic endocrine tumours. *Virchows Arch* 1996, 429: 323–333.
47. Pizzi S, D'Adda T, Azzoni C, Rindi G, Grigolato P, Pasquali C, Corleto VD, Delle Fave G and Bordi C. Malignancy-associated allelic losses on the X-chromosome in foregut but not in midgut endocrine tumours. *J Pathol* 2002, 196:401–407.

48. Missiaglia E, Moore PS, Williamson J, Lemoine NR, Falconi M, Zamboni G and Scarpa A. Sex chromosome anomalies in pancreatic endocrine tumors. *Int J Cancer* 2002, 98:532–538.
49. Zhao JM, Moch H, Scheidweiler AF, Baer A, Schäffer AA, Speel EJM, Roth J, Heitz PU and Komminoth P. Genomic imbalances in the progression of endocrine pancreatic tumors. *Genes Chromosomes Cancer* 2001, 32:364–372.
50. Furlan D, Cerutti R, Uccella S, La Rosa S, Rigoli E, Genasetti A and Capella C. Different molecular profiles characterize well-differentiated endocrine tumors and poorly differentiated endocrine carcinomas of the gastroenteropancreatic tract. *Clin Cancer Res* 2004, 10:947–957.
51. Debelenko LV, Emmert-Buck MR, Zhuang Z, Epshteyn E, Moskaluk CA, Jensen RT, Liotta LA and Lubensky IA. The multiple endocrine neoplasia type I gene locus is involved in the pathogenesis of type II gastric carcinoids. *Gastroenterology* 1997, 113:773–781.
52. Debelenko LV, Zhuang Z, Emmert-Buck MR, Chandrasekharappa SC, Manickam P, Guru SC, Marx SJ, Skarulis MC, Spiegel AM, Collins FS, Jensen RT, Liotta LA and Lubensky IA. Allelic deletions on chromosome 11q13 in multiple endocrine neoplasia type I-associated and sporadic gastrinomas and pancreatic endocrine tumors. *Cancer Res* 1997, 57:2238–2243.
53. Bordi C, Falchetti A, Azzoni C, D'Adda T, Canavese G, Guariglia A, Santini D, Tomassetti P and Brandi, ML. Aggressive forms of gastric neuroendocrine tumors in multiple endocrine neoplasia type I. *Am J Surg Pathol* 1997, 21:1075–1082.
54. Pizzi S, Azzoni C, Bassi D, Bottarelli L, Milione M and Bordi C. Genetic alterations in poorly differentiated endocrine carcinomas of the gastrointestinal tract. *Cancer* 2003, 98: 1273–1282.
55. D'Adda T, Candidus S, Bordi C and Hoffler H. Gastric neuroendocrine neoplasms: tumor clonality and malignancy associated large X-chromosomal deletions. *J Pathol* 1999, 189:394–401.
56. Toliat MR, Berger W, Ropers HH, Neuhaus P and Wiedenmann B. Mutations in the MEN I gene in sporadic neuroendocrine tumours of gastroenteropancreatic system. *Lancet* 1997, 350:1223.
57. Görtz B, Roth J, Krähenmann A, De Krijger RR, Muletta-Feurer S, Rutimann K, Saremaslani P, Speel EJM, Heitz PU and Komminoth P. Mutations and allelic deletions of the MEN1 gene are associated with a subset of sporadic endocrine pancreatic and neuroendocrine tumors and not restricted to foregut neoplasms. *Am J Pathol* 1999, 154:429–436.

58. Zhao JM, de Krijger RR, Meier D, Speel EJM, Saremaslani P, Muletta-Feurer S, Matter C, Roth J, Heitz PU and Komminoth P. Genomic alterations in well-differentiated gastrointestinal and bronchial neuroendocrine tumors (carcinoids)-marked differences indicating diversity in molecular pathogenesis. *Am J Pathol* 2000, 157:1431–1438.
59. Tonnies H, Toliat MR, Ramel M, Pape UF, Neitzel H, Berger W and Wiedenmann B. Analysis of sporadic neuroendocrine tumours of the enteropancreatic system by comparative genomic hybridization. *Gut* 2001, 48: 536–541.
60. Kytola S, Hoog A, Nord B, Cedermark B, Frisk T, Larsson C and Kjellman M. Comparative genomic hybridization identifies loss of 18q22-qter as an early and specific event in tumorigenesis of midgut carcinoids. *Am J Pathol* 2001, 158:1803–1808.
61. Löllgen RM, Hessman O, Szabo E, Westin G and Akerström G. Chromosome 18 deletions are common events in classical midgut carcinoid tumors. *Int J Cancer* 2001, 92:812–815
62. Lohmann D, Putz B, Reich U, Bohm J, Prauer H and Hofler H. Mutational spectrum of the p53 gene in human small-cell lung cancer and relationship to clinicopathological data. *Am J Pathol* 1993, 142:907–915.
63. Jakobovitz O, Nass D, De Marco L, Barbosa AJA, Simoni FB, Rechavi G and Friedman E. Carcinoid tumors frequently display genetic abnormalities involving chromosome 11. *J Clin Endocrinol Metab* 1996, 81:3164–3167.
64. Vortmeyer AO, Lubensky IA, Merino M, Wang C, Pham T, Furth EE, Zhuang Z. Concordance of genetic alterations in poorly differentiated colorectal neuroendocrine carcinomas and associated adenocarcinomas. *J Natl Cancer Inst* 1997, 89:1448-1453.
65. Ubiali A, Benetti A, Papotti M, Villanacci V and Rindi G. Genetic alterations in poorly differentiated endocrine colon carcinomas developing in tubulo-villous adenomas: a report of two cases. *Virchows Arch* 2001, 439:776–781.
66. Rossi G, Bertolini F, Sartori G, Bigiani N, Cavazza A, Foroni M, Valli R, Rindi G, De Gaetani C and Luppi G. Primary mixed adenocarcinoma and small cell carcinoma of the appendix: a clinicopathologic, immunohistochemical, and molecular study of a hitherto unreported tumor. *Am J Surg Pathol* 2004, 28:1233–1239.

Precursor lesions of gastroenteropancreatic neuroendocrine tumors

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Introduction

For diagnosis and therapy as well as for the understanding of the tumorigenesis of malignant tumors the identification of lesions that precede neoplastic growth and may represent a sequence of cellular changes from hyperplasia to neoplasia is of great interest.

There are some endocrine tumors that develop on the basis of hyperplastic changes, i.e. medullary thyroid carcinoma or pheochromocytoma. Furthermore, it is likely that the adenomatous changes of the parathyroid in multiple endocrine neoplasia type 1 (MEN1) patients originate from hyperplastic changes. All such conditions, except for the ECL-cell hyperplasia in chronic atrophic gastritis, are associated with an inherited endocrine disorder [1-3].

This lecture focuses on endocrine precursor lesions and microadenomatosis in the duodenum and pancreas of patients with MEN1. Molecular concepts and the potential clinical significance are discussed. In addition, two new disease entities are presented. Both are characterized by non-MEN1-associated microadenomatosis of the pancreas, but one shows glucagon-producing tumors, while the other displays multiple insulinomas.

Duodenum

Gastrin-producing tumors are the most common type of neuroendocrine tumor (NET) in the duodenum [4-6]. Most of them are associated with a Zollinger–

Ellison syndrome (ZES) characterized by elevated fasting gastrin serum levels, a positive gastrin secretin stimulation test and clinical symptoms such as recurrent peptic ulcer disease, gastroesophageal reflux disease and, occasionally, diarrhea [7]. Though duodenal gastrinomas are often small in size (diameter less than 1 cm), they tend to metastasize early to regional lymph nodes and the liver [8,9]. Surgical treatment of sporadic gastrinomas by local excision and lymphadenectomy is in many cases curative, while the same procedure usually does not cure the patient with MEN1-associated duodenal gastrinomas [8].

In 1990 it was noticed that many duodenal gastrinomas arising in the setting of MEN1 are multiple, in contrast to sporadic gastrinomas [10]. Recently, it has been shown that in addition to gastrinomas, somatostatin-producing tumors can also arise in the duodenum of patients with MEN1. These multicentric gastrin- and somatostatin-producing NETs were found to be associated with gastrin and somatostatin cell precursor lesions within the nontumorous duodenal mucosa [9], whose spectrum of proliferative changes was similar to that described for ECL cells in chronic atrophic fundus gastritis [11]. Therefore, an analogous classification has been proposed (Table 1) that distinguishes between diffuse, linear and micronodular hyperplasia of gastrin cells associated either with the crypts or with Brunner's glands. Lesions more than 300 μm in size, which were encountered less frequently than the hyperplastic changes, were classified as microtumors. The proliferative nature of these lesions was confirmed by enhanced Ki-67 expression, contrasting with the lack of Ki-67 expression in nonhyperplastic gastrin cells. The

hyperproliferative and hyperplastic lesions were found in all patients with MEN1 but were absent in patients with sporadic (non-MEN1-associated) duodenal gastrinomas and ZES. Because of the smooth transition from hyperplastic to early neoplastic gastrin cell lesions these alterations were considered to be precursor lesions of the MEN1-associated duodenal gastrinomas. The fact that such gastrin cell hyperplasia occurred at various sites in the duodenal mucosa explains the multifocality of MEN1 gastrinomas and the failure to cure patients with MEN1-associated ZES by simple tumor excision.

Tumor precursor lesions are assumed to show a sequence of genetic changes that lead to overt neoplasia. In MEN1 patients all somatic cells harbor a germline mutation of the MEN1 gene, which is considered to be a tumor suppressor gene [3,12,13]. Recently, a loss of heterozygosity (LOH) of the *MEN1* gene and/or the centromere 11 was demonstrated in approximately 50% of MEN1-associated duodenal NETs [14]. Allelic loss was detected in tumors as small as 300 μm (gastrin) and 400 μm (somatostatin) in diameter. In contrast to tumors, the hyperplastic gastrin and somatostatin cells consistently lacked LOH on chromosome 11q13. This finding suggested that though the hyperplastic cells were hyperproliferative and carried the *MEN1* germline mutation, they had not yet assumed the neoplastic genotype characterized by the allelic loss of 11q13. We do not know what mechanisms enhance the proliferation of gastrin cells and produce hyperplasia, but they could be related to an increased responsiveness of the gastrin cell bearing the germ cell MEN1 mutation to certain growth factors.

In conclusion, allelic deletion of the second *MEN1* gene seems to be a pivotal event in the development of multifocal gastrin and somatostatin cell neoplasms in the duodenum of MEN1 patients. The observation of distinct deletion patterns in the synchronous MEN1 tumors supports the concept that each gastrin-producing tumor in an individual MEN1 patient arises from an independent cell clone.

Pancreas

Most pancreatic NETs are sporadic tumors. Approximately 10% of pancreatic NETs arise in patients with a hereditary background of MEN1 [3,13]. The presence of multiple small endocrine tumors in the pancreas (i.e. up to 5 mm in diameter) has been referred to as microadenomatosis and has been described in association with the MEN1 syndrome [15]. MEN1-associated pancreatic microadenomatosis is usually accompanied by one or more macrotumors (diameter >5 mm), some of which may be functionally active [13,15,16].

Although the identification of the MEN1 gene on 11q13 has led to extensive studies of the genetic and clinical features of the MEN1 syndrome, our knowledge of the development and pathology of pancreatic endocrine neoplasms in MEN1 is mainly based on reports of individual cases. Only few studies on a series of MEN1 patients have been published [15,16]. In a recent study by our group we analyzed pancreatic specimens from 28 MEN1 patients [17]. Previous studies showed that microadenomatosis in MEN1 patients is

highly variable as far as the number of tumors and their hormonal profiles are concerned [15,16,18]. We confirmed these observations and established a high penetrance for pancreatic microadenomatosis (> 80%) in MEN1 patients. The hormone-positive microadenomas were usually multihormonal, with glucagon as the predominant hormone. Although many patients had numerous microadenomas they did not appear to be associated with a hormonal syndrome. If symptoms of inappropriate hormone secretion, such as persistent hyperinsulinemic hypoglycemia (PHH), were present, they could be related to one or more insulin-producing macrotumors.

As tiny as the pancreatic microadenomas in MEN1 were (i.e. less than 200 μm), they showed the signs of neoplastic tumors: (1) monohormonality, (2) trabecular growth pattern and (3) a distinct stromal component [15-17]. Furthermore, Vortmeyer et al. [19] and we [20] demonstrated LOH of the MEN1 gene locus in MEN1-associated pancreatic microadenomas.

Opinions vary as to which type of endocrine cell lesion precedes the development of microadenomas in patients with MEN1. Recently, Vortmeyer et al. [19] found 11q13 LOH in duct-associated lesions but not in islets and their conclusion was that there is a “non-islet cell origin of pancreatic islet cell tumors.” In our study using a technique combining fluorescence *in situ*-hybridization of the *MEN1* locus and the centromeric region of chromosome 11q with hormone immunostaining, we found loss of one *MEN1* allele in all microadenomas and in 19 of 20 monohormonal endocrine cell clusters examined. By contrast, LOH on 11q was absent in islets and ductal or acinar structures. Our results indicate that monohormonal endocrine cell clusters

represent a minute form of microadenomas. The frequent presence of single nonneoplastic insulin cells in microadenomas and the occurrence of monohormonal endocrine cell clusters intermingled with islets suggest an islet origin of microadenomas rather than a non-islet origin.

Non-MEN1-related microadenomatosis of the pancreas

Microadenomatosis of the endocrine pancreas has been described in a single case report of a patient with VHL syndrome [21]. In contrast, in a study by Lubensky et al. [22], the smallest tumor encountered in a series of 30 Von-Hippel-Lindau (VHL)-associated PETs was 0.4 cm in diameter, but microadenomatosis was not described in any of their cases. We screened the clinico-pathological records of 425 patients suffering from pancreatic NETs [17] and found only two who fulfilled the criteria of a VHL syndrome. The two patients exhibited solitary functionally inactive clear cell NETs without associated microadenomatosis. These observations do not exclude the possibility that microadenomatosis is a feature of VHL-associated pancreatic NETs, but might suggest that the frequency of this feature is lower than in MEN1.

A special finding was the identification of pancreatic microadenomatosis in 9 patients who had multiple microadenomas but neither clinical nor genetic features of the MEN1 or VHL syndrome. Five of these 9 patients suffered from hyperinsulinemic hypoglycemia and had, in addition insulin-positive microadenomas, insulin-positive macrotumors. Two of the 5 patients with

hyperinsulinemic hypoglycemia had only insulin-positive microadenomas, most of which were less than 1 mm in diameter. Four other patients had multiple small glucagon-expressing neoplasms, three of them with additional macrotumors. None of the four patients with multiple glucagon-producing NETs showed clinical signs of a glucagonoma syndrome. This observation suggests that what we encountered is two new disease entities characterized by pancreatic endocrine microadenomatosis; one causing hyperinsulinemic hypoglycemia, the other without any clinical syndrome but numerous glucagon-producing tumors. The mechanisms that may cause the microadenomatosis in these patients are as yet unknown.

Brief bullet points

- (1) MEN1-associated duodenal and pancreatic endocrine tumors are preceded by proliferative endocrine cell changes and microtumors
- (2) Allelic deletion of the *MEN1* gene in early neoplastic lesions but not in hyperplastic lesions may reflect a pivotal genetic event in the development of multifocal MEN1-associated endocrine neoplasms.
- (3) The observation of distinct deletion patterns in very small synchronous tumors supports the concept that each MEN1-associated tumor in an individual MEN1 patient arises from an independent cell clone.
- (4) Most sporadic (non-MEN1-associated) endocrine tumors lack evidence of precursor lesions.

(5) Two types of pancreatic microadenomatosis without any hereditary background were identified that were characterized either by multiple hormonally silent glucagon-producing tumors or multiple syndromatic insulin-producing tumors.

References

1. Solcia E, Bordi C, Creutzfeldt W et al. Histopathological classification of nonantral gastric endocrine growths in man. *Digestion* 1988; 41:185-200.
2. Studer H, Derwahl M. Mechanisms of nonneoplastic endocrine hyperplasia - a changing concept: a review focused on the thyroid gland. *Endocr Rev* 1995; 16:411-426.
3. Marx SJ, Simonds WF. Hereditary hormone excess: genes, molecular pathways, and syndromes. *Endocr Rev* 2005; 26:615-661.
4. Burke AP, Federspiel BH, Sobin LH, Shekitka KM, Helwig EB. Carcinoids of the duodenum. A histologic and immunohistochemical study of 65 tumors. *Am J Surg Pathol* 1989; 13:828-837.
5. Capella C, Riva C, Rindi G et al. Histopathology, hormone products, and clinicopathological profile of endocrine tumors of the upper small intestine: a study of 44 cases. *Endocr Pathol* 1991; 2:92-110.
6. Anlauf M, Garbrecht N, Henopp T et al. Sporadic versus hereditary gastrinomas of the duodenum and pancreas: distinct clinico-pathological and epidemiological features. *World J Gastroenterol* 2006; 12:5440-5446.
7. Zollinger RM, Ellison EH. Primary peptic ulcerations of the jejunum associated with islet cell tumors of the pancreas. *Ann Surg* 1955; 142:709-723.
8. Norton JA, Fraker DL, Alexander HR et al. Surgery to cure the Zollinger-Ellison syndrome. *N Engl J Med* 1999; 341:635-644.
9. Anlauf M, Perren A, Meyer CL et al. Precursor lesions in patients with multiple endocrine neoplasia type 1-associated duodenal gastrinomas. *Gastroenterology* 2005; 128:1187-1198.
10. Pipeleers-Marichal M, Somers G, Willems G et al. Gastrinomas in the duodenums of patients with multiple endocrine neoplasia type 1 and the Zollinger-Ellison syndrome. *N Engl J Med* 1990; 322:723-727.

11. Solcia E, Capella C, Fiocca R, Cornaggia M, Bosi F. The gastroenteropancreatic endocrine system and related tumors. *Gastroenterol Clin North Am* 1989; 18:671-693.
12. Chandrasekharappa SC, Guru SC, Manickam P et al. Positional cloning of the gene for multiple endocrine neoplasia- type 1. *Science* 1997; 276:404-407.
13. Calender A, Morrison CD, Komminoth P, Scoazec JY, Sweet KM, Teh BT. Multiple endocrine neoplasia type 1. In: DeLellis RA, Lloyd RV, Heitz PU, Eng C, eds. *Pathology and genetics: tumours of endocrine organs. WHO classification of tumors.* Lyon, IARC Press, 2004, pp 218-227.
14. Anlauf, M., Perren, A., Henopp, T. et al. Allelic deletion of the *MEN1* gene in duodenal gastrin and somatostatin cell neoplasms and their precursor lesions. *Gut* . 2006.
Ref Type: In Press
15. Klöppel G, Willemer S, Stamm B, Häcki WH, Heitz PU. Pancreatic lesions and hormonal profile of pancreatic tumors in multiple endocrine neoplasia type I. An immunocytochemical study of nine patients. *Cancer* 1986; 57:1824-1832.
16. Thompson NW, Lloyd RV, Nishiyama RH et al. MEN 1 pancreas: a histological and immunohistochemical study. *World J Surg* 1984; 8:561-574.
17. Anlauf M, Schlenger R, Perren A et al. Microadenomatosis of the endocrine pancreas in patients with and without the multiple endocrine neoplasia type 1 syndrome. *Am J Surg Pathol* 2006; 30:560-574.
18. Le Bodic MF, Heymann MF, Lecomte M et al. Immunohistochemical study of 100 pancreatic tumors in 28 patients with multiple endocrine neoplasia, type I. *Am J Surg Pathol* 1996; 20:1378-1384.
19. Vortmeyer AO, Huang S, Lubensky I, Zhuang Z. Non-islet origin of pancreatic islet cell tumors. *J Clin Endocrinol Metab* 2004; 89:1934-1938.
20. Perren, A., Anlauf, M., Henopp, T. et al. Multiple endocrine neoplasia type 1: loss of one *MEN1* allele in tumors and monohormonal endocrine cell clusters, but not in islet hyperplasia of the pancreas. A combined

FISH and immunofluorescence study. *J.Clin.Endocrinol.Metab.* 2006.

Ref Type: In Press

21. Chetty R, Kennedy M, Ezzat S, Asa SL. Pancreatic endocrine pathology in von Hippel-Lindau disease: an expanding spectrum of lesions. *Endocr Pathol* 2004; 15:141-148.
22. Lubensky IA, Pack S, Ault D et al. Multiple neuroendocrine tumors of the pancreas in von Hippel-Lindau disease patients: histopathological and molecular genetic analysis. *Am J Pathol* 1998; 153:223-231.

Table 1. Classification of proliferative gastrin cell lesions in the duodenum of patients with MEN1

Pattern	Definition
Diffuse hyperplasia	Increase in single gastrin-positive cells (confirmed by morphometric analysis)
Linear hyperplasia	Formation of chains of five or more gastrin-positive cells and ≥ 2 chains per mm
Micronodular hyperplasia	Nodules of gastrin-positive cells with more than 5 gastrin-positive cells within glands or crypt (30 μm to 90 μm)
Enlarged nodule	Nodules of gastrin-positive cells with solid architecture ($\geq 90 \mu\text{m}$ to $< 210 \mu\text{m}$)
Microinvasive lesion	Small clusters of gastrin-positive cells localized in the lamina propria between the glands and surrounded by thickened collagen
Microtumor	Tumorous lesion with trabecular growth pattern and extensive fibrosis (diameter $> 250 \mu\text{m}$)

PROGNOSTIC FACTORS IN GI NETs

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The (neuro)endocrine tumors of the gastrointestinal tract are believed to originate from cells of the diffuse (neuro)endocrine system (DNES). 14 different endocrine cell types are known and characterized as producing a wide range of hormones and bioactive molecules and display a type-specific regional distribution. Endocrine tumor cells are largely similar in phenotype and distribution to their normal counterpart. The endocrine nature of tumor cells is based on the identification of markers of endocrine differentiation, including chromogranin A (in large dense core vesicles) and synaptophysin (in small synaptic like vesicles). Potentially useful antigens are also the cytosol markers neuron-specific enolase (NSE) and the protein gene product 9.5 (PGP9.5), as well as the membrane-bound marker CD56 n-CAM. Specific neuroendocrine markers correspond to the hormonal product of the endocrine cell type. A tumor counterpart exists only for a fraction of the 14 normal DES cells.

The current WHO classifications [3; 4; 10] provide a common definition frame and specific "clinicopathological correlations" according to tumor anatomical location. Two major tumor categories are identified according to histology and tumor cell differentiation: i) well-differentiated neoplasms; ii) poorly differentiated carcinomas.

Well differentiated neoplasms as a rule express the whole set of general markers of endocrine differentiation and display abundant large dense core vesicles with variable hormone content(s). The site distribution of well differentiated endocrine tumors largely corresponds to the normal DES cell counterpart, suggesting retained differentiation programs. This feature reflects the concept of endocrine tumors heterogeneity according to tumor site of origin and necessarily requires specific tumor cell typing. The genetic background of well differentiated endocrine tumors is complex and may vary depending on the site of origin (foregut, midgut and hindgut). Unexpectedly high frequency of genetic defects is reported. Abnormality of the Multiple Endocrine Neoplasia of type 1 (MEN1) gene is restricted to foregut tumors, while 18q chromosomal abnormality is more frequently observed in lower gut tumors.

Poorly differentiated endocrine carcinoma cells usually lack large dense core vesicles and related markers, whereas widely express synaptophysin, NSE and PGP 9.5. Though more often observed in the stomach and large intestine, there is no site-specific distribution. This feature reflects the concept that poorly differentiated endocrine carcinomas are more commonly reputed to derive from endocrine-committed, multipotent cells rather than from differentiated endocrine cells. The genetic background of poorly differentiated endocrine carcinomas consistently display an elevated frequency of genetic defect and is characterized by common p53 gene abnormalities.

While for poorly differentiated carcinomas a poor prognosis is implicit, predicting the behavior of well-differentiated neoplasms is problematic. In well-differentiated neoplasms three categories can be defined by WHO at diagnosis as: i) "benign behavior" tumors, or tumors with potentially benign course, ii) "uncertain behavior" tumors, or tumors with potentially low grade malignant course and iii) "carcinomas", with low grade malignant course.

The following malignancy criteria were adopted by WHO classification for the definition of carcinoma: **a)** evidence of metastasis and **b)** invasion of *muscularis propria*/fat tissue/nearby organs. Notably, for appendiceal endocrine tumors the wall-invasion criterion represents a significant exception. A combination of variables are additionally provided to allocate a specific case to the category of "benign behavior" (low risk) or to the category of "uncertain behavior". Such variables are defined within the frame of the site-specific "clinicopathological correlations" provided by WHO and include: **a)** the clinical variable, i.e. the presence of an hyperfunctional endocrine syndrome (nonfunctioning or functioning tumor); **b)** several variables more pertinent to a staging system like size, angioinvasion and, for appendix only, the wall invasion; and **c)** the proliferation status as assessed by Ki67 index (pancreas only). For numerical variables the cut-offs vary according to tumor anatomical location [10].

The application of the WHO classification following the above criteria proved effective in predicting the behavior of GEP endocrine tumors and useful for the patients' management and treatment [e.g. see 1; 2; 6; 9].

Several other histopathological variables have been investigated especially in foregut endocrine tumors (stomach, duodenum and pancreas) and proved effective predictors of malignancy. For pancreatic endocrine tumors these included: p53 hyperexpression; high index of aneuploidy at flow cytometry; AgNOR percentage >5%; lack of progesterone receptor immunoreactivity; alpha-hCG immunoreactivity; high PCNA index; high Ki67 index; CD10 metalloproteinase expression; low microvessel density; low VEGF expression; increased lymphatic vessel density; size ≥ 3 cm; vascular and perineural invasion; mitotic index ≥ 2 (x10 HPF); nuclear atypia. For midgut tumors, a recent investigation showed that Ki67 $>2.6\%$ proved informative of poor prognosis at univariate analysis, though not informative at multivariate [11]. Genetic markers have been also proposed as informative variables and some of them proved promising or potentially useful. Finally, from a clinical standpoint, the most important predictive factor for poor prognosis appear to be the presence of metastases either to the liver or at distance [6; 11].

Overall, the incidence of histological/genetic variables of poor prognosis appear to follow specific trends along the differentiation spectrum of endocrine tumors of the gut and pancreas (e.g. while, similar to microvessel density, the expression of LDCV markers decreases along this path, by converse the mitotic count, the Ki67 index and the frequency of genetic defects increase).

Previous work suggested the usefulness of a grading system for endocrine tumors too [5; 7; 12]. In the attempt to integrate the WHO classifications and to overcome difficulties in their practical application, a new grading system was recently proposed for foregut tumors [8] based on mitotic count and Ki67 index as follows: G1, mitotic count <2 per 10 high power field (HPF) and/or •2% Ki67 index; G2, mitotic count 2-20 and/or 3-20% Ki67 index; G3, mitotic count >20 per 10 HPF and/or >20% Ki67 index. To allow a practical patients' stratification a TNM proposal was also formulated for foregut endocrine tumors, and separately for tumors of the stomach, duodenum/ampulla/proximal jejunum and pancreas. Following the current TNM format, for tumor (T) sizes, T1 were those defined by the various site-specific WHO "clinicopathological correlations" for "benign behavior" tumors, T2 for tumors of "uncertain behavior" (when available) and T3 and T4 for deeply invasive tumors according to site-specific features. Lymph-node (N) and distant metastasis (M) were defined as absent (N0 or M0) or present (N1 or M1). Accordingly a staging system was defined, with stage I for NET tumors with limited growth, stage II for larger or more invasive tumors though in absence of metastases, stage III for tumors invading the surrounding structures or with loco-regional metastases and stage IV implying distant metastases. Both grading and TNM/staging proposals were formulated, discussed and consensually approved at a dedicated expert meeting involving endocrine pathologists and clinicians from various branches (medicine/endocrinology/gastroenterology; surgery; radiology/radiotherapy). Validation by future clinicopathological work is needed.

REFERENCES

1. Artale S, Giannetta L, Cerea G, Pedrazzoli P, Schiavetto I, Napolitano M, Veronese S, Bramerio E, Gambacorta M, Vanzulli A, Pisconti S, Pugliese R, Siena S (2005) Treatment of metastatic neuroendocrine carcinomas based on WHO classification. *Anticancer Res* 25:4463-9
2. Bajetta E, Catena L, Procopio G, Bichisao E, Ferrari L, Della Torre S, De Dosso S, Iacobelli S, Buzzoni R, Mariani L, Rosai J (2005) Is the new WHO classification of neuroendocrine tumours useful for selecting an appropriate treatment? *Ann Oncol* 16:1374-80
3. DeLellis RA, Lloyd RV, Heitz PU, Eng C (ed) (2004) World Health Organization Classification of Tumours, Pathology and Genetics of Tumours of Endocrine Organs. IARC Press, Lyon
4. Hamilton SR, Aaltonen LA (ed) (2000) World Health Organization Classification of Tumours, Pathology and Genetics of Tumours of the Digestive System. IARC Press, Lyon
5. Hochwald SN, Zee S, Conlon KC, Colleoni R, Louie O, Brennan MF, Klimstra DS (2002) Prognostic factors in pancreatic endocrine neoplasms: an analysis of 136 cases with a proposal for low-grade and intermediate-grade groups. *J Clin Oncol* 20:2633-42
6. Panzuto F, Nasoni S, Falconi M, Corleto VD, Capurso G, Cassetta S, Di Fonzo M, Tornatore V, Milione M, Angeletti S, Cattaruzza MS, Ziparo V, Bordi C, Pederzoli P, Delle Fave G (2005) Prognostic factors and survival in endocrine tumor patients: comparison between gastrointestinal and pancreatic localization. *Endocr Relat Cancer* 12:1083-92
7. Rindi G, Azzoni C, La Rosa S, Klersy C, Paolotti D, Rappel S, Stolte M, Capella C, Bordi C, Solcia E (1999) ECL cell tumor and poorly differentiated endocrine carcinoma of the stomach:

Prognostic evaluation by pathological analysis. *Gastroenterology* 116:532-542

8. Rindi G, Kloppel G, Alhman H, Caplin M, Couvelard A, de Herder WW, Eriksson B, Falchetti A, Falconi M, Komminoth P, Korner M, Lopes JM, McNicol AM, Nilsson O, Perren A, Scarpa A, Scoazec JY, Wiedenmann B (2006) TNM staging of foregut (neuro)endocrine tumors: a consensus proposal including a grading system. *Virchows Arch* 449:395-401

9. Ruzniewski P, Ish-Shalom S, Wymenga M, O'Toole D, Arnold R, Tomassetti P, Bax N, Caplin M, Eriksson B, Glaser B, Ducreux M, Lombard-Bohas C, de Herder WW, Delle Fave G, Reed N, Seitz JF, Van Cutsem E, Grossman A, Rougier P, Schmidt W, Wiedenmann B (2004) Rapid and sustained relief from the symptoms of carcinoid syndrome: results from an open 6-month study of the 28-day prolonged-release formulation of lanreotide. *Neuroendocrinology* 80:244-51

10. Solcia E, Klöppel G, Sobin LH (2000) *Histological typing of endocrine tumours*. Springer-Verlag, New York

11. Tomassetti P, Campana D, Piscitelli L, Casadei R, Nori F, Brocchi E, Santini D, Pezzilli R, Corinaldesi R (2006) Endocrine Tumors of the Ileum: Factors Correlated with Survival. *Neuroendocrinology*

12. Van Eeden S, Quaedvlieg PF, Taal BG, Offerhaus GJ, Lamers CB, Van Velthuysen ML (2002) Classification of low-grade neuroendocrine tumors of midgut and unknown origin. *Hum Pathol* 33:1126-32

GI-NETs - Uniform, But Also Diverse

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Gastrointestinal neuroendocrine tumors (GI NETs), traditionally known as carcinoids, have generated interest far disproportionate to their incidence. When Oberndorfer originally coined the term *Karzinoid* for tumors that resembled adenocarcinomas but did not behave like them, he implied an indolent and benign clinical behavior for these neoplasms⁽¹⁾. When reports of these tumors metastasizing, and of patients dying from them, appeared in the literature, he modified his stance⁽²⁾, and with time the potentially malignant nature of these tumors was gradually established and accepted⁽³⁾.

With the gradual realization that this diversity was not limited to clinical behavior alone, but was also shared by their histologic, histochemical, immunohistochemical and ultrastructural characteristics as well, it became clear that these differences in this seemingly homogeneous family of endocrine tumors were site-related, and led to Williams and Sandler's classification based on the embryologic derivation of their anatomic site-of-origin⁽⁴⁾.

However, inconsistencies gradually became apparent within each subtype as well, when sporadically occurring (Type III) gastric carcinoids were seen to be more aggressive than their broncho-pulmonary counterparts, appendiceal carcinoids to be more indolent than the ileal ones, and rectal carcinoids to be more benign than those arising in the colon.

Further diversities in the clinicopathologic profiles of these tumors became apparent when some tumors were seen to be multicentric, while others were endocrinologically functional and gave rise to a variety of clinical syndromes (acromegaly, Zollinger-Ellison, or Cushing's syndrome etc). Likewise, some tumors were associated with other gastrointestinal and extra-intestinal malignancies^(5,6,7), while yet others were associated with such familial disorders as neurofibromatosis, the MEN-1, von Hippel Lindau, or even the FAP syndromes⁽⁸⁻¹³⁾. Some of these differences, although baffling at first, can now be easily explained by our current knowledge of the various neuroendocrine cell types in the gut, their physiology, their interrelationship with each other and factors that modulate their maturation and their hyperplastic and neoplastic proliferations.

The gastrointestinal mucosa is populated by a constellation of 15 or so morphologically, and functionally distinct neuroendocrine cell types. The distributional pattern and relative numbers of each cell type in any anatomical segment of the gut is dictated by the physiologic needs pertinent to that site. Thus, the histamine-producing ECL cells, that play a pivotal part in gastric acid secretion, are exclusively present in the

oxyntic mucosa, while the gastrin-producing G cells are predominantly located in the antrum where they can fine-tune gastric acid secretion by monitoring the intraluminal pH. Since somatostatin has a much broader sphere of influence, the somatostatin-producing D cells are much more widely dispersed.

GI-NETs arise as a result of one or more genetic and molecular aberrations in cells that are “neuroendocrine-committed” but may or may not be terminally differentiated into a specific cell type. Tumors supposedly arising from terminally differentiated cells generally recapitulate the phenotypic features of a parent cell type that is normally present in the gastrointestinal mucosa, and are therefore commonly referred to as G-, D-, EC-, or ECL cell NETs, as identified by their dominant secretory, immunohistochemical or ultrastructural characteristics. This is also supported by the fact that the distributional pattern of each such tumor closely parallels that of their prototypic progenitor cell type. Thus ECL cell NETs, in keeping with the normal distribution of the histamine-producing ECL cells, arise exclusively in the stomach; while EC cell NETs most often originate in the midgut where the serotonin-producing EC-cells are the dominant cell type. However, there are two notable exceptions to this broad generalization. Although the gastrin-producing G cells are most numerous in the antrum, G-cell NETs are far more common in the duodenum than in the antrum, and even though the somatostatin-producing D cells are dispersed uniformly throughout the gut, D cell tumors in the gut almost exclusively occur in the duodenum. It is possible that since the duodenum is the commonest site for extra-pancreatic G- and D-cell tumors, its local tissue and/or luminal microenvironment may have a role in their histogenesis. These exceptions aside, the concept of GI-NETs arising from “neuroendocrine committed” but variably differentiated cell types also helps explain how certain NETs are more homogeneous (true to type) and have a dominant monohormonal secretory pattern, while others acquire a more variegated phenotype and have a multisecretory profile.

Thus Oberndorfer’s original concept regarding these tumors has gradually evolved into one where despite their sharing a number of generic similarities, they show significant diversity in their morphology, their secretory products, functional characteristics, molecular events involved in their pathogenesis, their association with certain clinical syndromes, familial and non-familial disorders, and most importantly, their biologic behavior and clinical outcome. Some of these aspects are discussed below, and since the others are going to be discussed by the subsequent speakers, they will not be included in this presentation.

Multicentricity:

GI-NETs are often multicentric⁽¹⁴⁾. This tendency for multicentricity appears to be site-related, and is most often seen with gastric and jeuno-ileal NETs^(14,15), less frequently with duodenal tumors^(16,17), and only infrequently with colorectal or appendiceal ones. The high incidence of multicentricity in gastric NETs is easily explained by the fact that the vast majority of such tumors are of the ECL cell type and arise in a background of ECL cell hyperplasia in chronically hypergastrinemic patients⁽¹⁵⁾. Currently, two types of multicentric gastric ECL cell tumors are recognized (type I and type II), and it is of interest that while

they are histologically identical, they differ significantly from each other in their pathogenesis, their biologic aggressiveness and clinical outcome. For their clinical management therefore, they need to be distinguished from each other⁽¹⁵⁾. The sporadically occurring type-I ECL cell tumors arise in patients with pernicious anemia and chronic atrophic gastritis type A, while the type-II tumors are familial and arise in MEN-1 patients with the Z-E syndrome. The salient differences in their clinicopathologic profiles are briefly summarized in Table 1.

Table 1: Comparative Profiles of Gastric NETs Types I & II

Features	Type – I	Type – II
Incidence	++++	+
Age	40 – 80 yrs	20 – 35 yrs
Gender Bias	M:F = 1:2.3	M:F = 1:1
Genetic Bias	No	Yes (MEN-1)
Gastric body mucosa	Atrophic	Hypertrophic
LOH of MEN-1 gene	Occasional	Frequent
Clinical Behavior	Indolent	More Aggressive
Nodal metastases	3 – 8%	~30%
Prognosis	Excellent (++++)	V. Good (+++)

Duodenal gastrinomas can be multicentric too and arise from a similar antropyloric and duodenal G cell hyperplasia, but all such tumors occur in patients with the MEN-1 syndrome and are related to an underlying mutation of the MEN-1 gene^(16,18). It is of interest that while the G cells are most heavily represented in the antrum, these multicentric G-cell tumors are almost exclusively seen in the duodenum, where they occur as tiny mucosal/submucosal tumors that arise in a background of G-cell hyperplasia. Despite their small size, these endocrinologically functional tumors are clinically aggressive and associated with regional nodal metastases in 60-80% of cases at detection⁽¹⁹⁾. In a small subset of these patients, the hypergastrinemia from the concomitant antropyloric and duodenal G-cell hyperplasia induces a secondary ECL cell hyperplasia that often culminates in multicentric ECL cell NETs in these patients⁽²⁰⁾.

No such explanations can be proposed for the multicentricity seen in upto 33% of jejuno-ileal NETs, since these tumors are mostly sporadic, of the EC cell type, and un-associated with any underlying EC cell hyperplasia.

Even though multicentricity may be seen in upto 33% of jejuno-ileal NETs, no such explanations can be proposed for their multicentricity, since these tumors are mostly sporadic, of the EC cell type, and un-associated with any underlying EC cell hyperplasia. Molecular studies indicate that most multicentric jejuno-ileal NETs are independent primaries⁽²¹⁾. Perhaps an unidentified growth factor, a genetic alteration, or a change in the local microenvironment of the affected bowel segment may have a role to play. Be it as it may, multicentric jejuno-ileal NETs tend to occur in younger patients, are more often

associated with a carcinoid syndrome, and have a poorer prognosis⁽²²⁾. Overall therefore, regardless of their tumor stage, multicentric jejuno-ileal NETs tend to behave more aggressively.

Association with Other Tumors:

Pooled data indicates that GI-NETs are associated with other primary intestinal and extra-intestinal tumors in about 17% of cases (range 12-46%), and that this is more often seen with ileal NETs (29-52%), than with appendiceal (13-32%) or colorectal tumors (5-32%). It is of interest that the commonest site for such second primary tumors (SPTs) is the gastrointestinal tract (32-62%), followed by the genitourinary (9-22%) and the lower respiratory tracts (9-13%)⁽²³⁾. The vast majority (59-87%) of such SPTs are synchronous and clearly more malignant than the GI-NETs themselves⁽²³⁾. Since about 87-91% of such patients present with symptoms related to the SPT rather than the GI-NET, this association appears fortuitous, and related to the incidental detection of an asymptomatic NET at surgery or work-up for the SPT⁽²³⁾. This is also supported by the observation that NETs account for nearly 90% of all small bowel tumors found incidentally at autopsy⁽²⁴⁾. Whereas it has been speculated that the secretory products of GI-NETs can themselves influence the initiation and/or promotion of the associated non-endocrine SPTs^(5,25), there is no evidence of any such mechanism. Another likely possibility involves an as yet uncharacterized genetic alteration that predisposes to the development of two independent primaries, one of which happens to be a GI-NET. However, there is no data to indicate that sporadically occurring GI-NETs and their associated SPTs share a common chromosomal abnormality.

Predictors of Malignancy:

Diversity in GI-NETs even extends to such features as predictors of their malignant behavior. While the vast majority of these tumors are well differentiated, some are poorly differentiated neoplasms that behave like conventional carcinomas and have a much poorer prognosis. The recent WHO classification, based on a combination of the site of origin and the clinicopathologic features of these tumors, categorizes them into 1) well differentiated neuroendocrine tumors (WD-NETs) to include tumors that are clearly benign and those that are anticipated to have an uncertain behavior (i.e. a low grade malignant potential); 2) well differentiated endocrine carcinomas (WD-NECAs), and 3) poorly differentiated endocrine carcinomas (PD-NECAs). A clearcut distinction between "benign" (WD-NETs) and "malignant" (WD-NECAs) can be confidently made only in the presence of metastases, or if the tumor is ≥ 2 cm in size, extends deep into the muscularis propria or shows lymphovascular invasion. Since these features are clearly impossible to evaluate in biopsy material, a final judgement should be deferred until the resected specimen has been examined. In the absence of demonstrable metastases, predictions of their aggressive behavior have traditionally relied on a combination of such indicators of malignancy as their anatomic site of origin, their size, histologic grade, extent of their intramural penetration, presence of lymphovascular

invasion, proliferative activity as judged by mitotic count/10 HPF or a high Ki-67/MIB-1 labeling index, and lastly, the functionality of the tumor. The relative significance of each of these features, is again dependent on the site of origin of these tumors.

Tumor Site: The anatomic site of origin of GI-NETs is overall an important predictor of their biologic behavior. Thus only a small proportion of gastric carcinoids (type III NETs) are clearly malignant. Duodenal, jejuno-ileal and colonic carcinoids (even when small) are, on the other hand, often malignant and most have already metastasized by the time they are detected; whereas appendiceal and rectal NETs are usually benign, even though most of them may show deep intramural invasion that is otherwise associated with malignancy.

Tumor Size: Tumor size too is a reliable independent predictor of aggressive behavior in certain sites. In the stomach at least, tumors ≤ 1 cm in size are generally benign, while those ≥ 3 cm in size are invariably malignant⁽²⁶⁾. For duodenal tumors size does not appear to be a reliable predictor of eventual behavior, since even the relatively small tumors tend to be deeply invasive and aggressive. However, for jejuno-ileal tumors, size of turns out to be a significant discriminator since 80% of tumors ≥ 2 cm in size show metastases when first discovered, while less than 2% of those under 1 cm do so⁽²⁷⁾. The aggressive potential of tumors 1-2 cm in size resembles that of the larger lesions. Appendiceal NETs smaller than 2cm, on the other hand, are slow growing indolent tumors and are regarded as benign, while those >2 cm in size are generally regarded as aggressive tumors and treated by a hemicolectomy. However, it should be mentioned that size as a criterion is reliable only for non-functioning tumors, since the functional ones, regardless of whether they are familial or non-familial, are often overtly malignant and metastatic at detection.

Local Invasion: Whereas local tissue invasion by GI-NETs does not in itself imply malignancy, invasion into the muscularis propria is associated with a high incidence of metastatic spread. In one series, 90% of the deeply invasive tumors had already metastasized when first detected, whereas none of the superficially invasive tumors had done so⁽²⁸⁾. The WHO classification too emphasizes tumor invasion of the muscularis propria as an important distinguishing feature of WD-NECAs. But here too appendiceal NETs are an exception. Since even the clearly benign ones often show extension into the muscularis propria, appendiceal NETs are not considered malignant until they extend into the mesoappendix.

Proliferative Index: Though a high Ki-67 index ($\geq 150/10$ HPF) is a reliable independent predictor of malignancy in gastric NETs⁽²⁶⁾, it fails to discriminate between benign and malignant jejuno-ileal or colorectal NETs⁽²⁹⁾. Nonetheless, in keeping with other tumors, a Ki-67 labeling index of $>15\%$ of tumor cell nuclei in gastric NETs, and a similar index of $>10\%$ in jejuno-ileal NETs has been suggested as a reliable marker of aggressive growth⁽³⁰⁾.

Expression of Growth Factors: In addition to their secretory products, GI-NETs are also known to express a variety of growth factors (α -FGF, b-FGF- α & β , IGF and

PDGF etc.) and their receptors. Since the concurrent expression of growth factors and their corresponding receptors is expected to confer an autonomy of growth to the tumor cells by autocrine or paracrine mechanisms⁽³¹⁾, it has been argued that the presence/absence of such expression in GI-NETs might be helpful in separating benign from malignant tumors. However, since such expression appears related to either the site of origin of these tumors or their progenitor cell type, it has not achieved diagnostic utility⁽³²⁾.

In conclusion therefore, it is clear that in the 100 years since Oberndorfer's original description, carcinoids (or GI-NETs as we now refer to them), are thought of as a "family" of tumors that share such generic features as an origin from a "common/closely related" cell lineage (as demonstrated by their reactivity for the common NE markers) and an ability to secrete varying amounts of one or more of a variety of amines/peptides etc. However, they show significant heterogeneity as to their pathogenesis, their ability to give rise to certain clinical syndromes, their association with certain heritable conditions (MEN-1, MEN-2, VRNF-1 and VHL etc.), and last of all their prognostic indicators and biological behavior. Even though significant strides have been made in our understanding of some of these features, it appears that we may be just scratching the surface and that there still remains a lot to be learnt about their "unity in diversity."

References

1. Oberndorfer S: Karzinoide tumoren des Dunndarms, Frankf, Z. Pathol 1, 426-432, 1907.
2. Oberndorfer S: Die Geschwulste des Darms. In. F Henke, O Lubarsch (eds) Handbuch der speziellen pathologischen Anatomie und Histologie, Bd. 4, Teil 3, Verdauungsschlauch. Springer, Berlin, 1929, pp.717-953.
3. Pearson CM, Fitzgerald PJ: Carcinoid tumors. A reemphasis of their malignant nature. Review of 140 cases. Cancer. 2; 1005-1026, 1949.
4. Williams ED, Sandler M: The classification of carcinoid tumors. Lancet, 1, 238, 1963.
5. Zucker KA, Longo WE, Modlin IM et al: Malignant diathesis from jejuno-ileal carcinoids. Am J Gastroenterol 84; 182-186,1989.
6. Gerstle JT, Kauffman GL, Koltun WA: The incidence, management and outcome of patients with gastrointestinal carcinoids and second primary malignancies. J Amer Coll Surg. 180;427-432,1995.
7. Rivadeneira DE, Tuckson WB, Naab T.: Increased incidence of second primary malignancies in patients with carcinoid tumors: case report and literature review. J Natl Med Assoc. 88: 310- 312,1996.
8. Stamm B, Hedinger CE, Saremaslani P: Duodenal and ampullary carcinoid tumors. A report of 12 cases with pathological characteristics, polypeptide content and relation to the MEN-1 syndrome and von Recklinghausen's disease (neurofibromatosis). Virchows Arch. 408, 475-489, 1986.
9. Dayal Y, Tallberg K, Nunnemacher G. et al.: Duodenal carcinoids in patients with and without neurofibromatosis: A comparative study. *Am. J. Surg. Path: 10*, 348-357, 1986.
10. Fendrich V, Ramaswamy A, Slatier EP, et al.: Duodenal somatostatinoma associated with Von Recklinghausen's disease. J. Hepatobiliary Pancreatic Surg. 11;417-421. 2004.
11. Usui M, Matsuda S, Suzuki H, et al: Somatostatinoma of the papilla of Vater with multiple gastrointestinal stromal tumors in a patient with von Recklinghausen's disease. J. Gastroenterol 37; 947-953. 2002.
12. Karasawa Y, Sakaguchi M, Minami S, et al.; Duodenal somatostatinoma and erythrocytosis in a patient with von Hippel-Lindau disease type 2A. Int. Med. 40:38-43, 2001.

13. July LV, Northcott KA, Yoshida EM. et al: Coexisting carcinoid tumors in familial adenomatous polyposis-associated upper intestinal adenomas. *Am J Gastroenterol* 94: 1091-1094, 1999.
14. Martensen M, Nobin A, Sundler F, et al.: Endocrine tumors of the ileum: cytochemical and clinical aspects. *Pathol. Res. Pract.*:180;356,1985.
15. Dayal Y: Recognition and Histopathologic Classification of ECL cell proliferations. *Yale J. Biol. & Med.* 71, 257-272, 1999.
16. Donow C, Pipeleers-Marichal M, Schroder S, et al.: Surgical pathology of gastrinoma: site, size, multicentricity, association with multiple endocrine neoplasia type 1, and malignancy. *Cancer* 68: 1329-1334, 1991.
17. Pipeleers-Marichal M, Somers G, Willems G, et al.: Gastrinomas in the duodenum of patients with multiple endocrine neoplasia type-1 and the Zollinger-Ellison syndrome. *N. Engl. J. Med.* 322.723-727,1990.
18. Anlauf M, Peren A, Meyer CL et al.: Precursor lesions in patients with multiple endocrine neoplasia type 1-associated duodenal gastrinomas. *Gastroenterol* 128: 1187-1198, 2005.
19. Hoffman KM, Furukawa M, Jensen RT.: Duodenal neuroendocrine tumors: Classification, functional syndromes, diagnosis and medical treatment. *Best Pract & Research in Clin Gastroenterol.* 19; #5, 675-697, 2005.
20. Dayal Y.: Neuroendocrine tumors of the gastrointestinal tract. *Pathology Case Reviews* 2007 (in press).
21. Katona TM, Jones TD, Wang M, Abdul-Karim FW, Cummings OW, Cheng L.: Molecular evidence for independent origin of mucosal neuroendocrine tumors of the enteropancreatic axis. *Cancer Res.* 66 (9); 4936-4942, 2006.
22. Yantiss RK, Odze RD, Farraye FA, Rosenberg AE: Solitary versus multiple carcinoid tumors of the ileum: a clinical and pathological review of 68 cases. *Am J Surg Pathol* 27; 811-817, 2003.
23. Habal N, Sims C, Bilchik AJ: Gastrointestinal carcinoid tumors and second primary malignancies. *J Surg Oncol* 75; 301-306, 2000.
24. Berg T, Lindell F.: Carcinoid tumors: frequency in a defined population during a 12-year-period. *Acta Pathol Microbiol Scand.* 84; 322-30,1976.
25. Lauffer JM, Zhang T, Modlin IM: Review article: current status of gastrointestinal carcinoids. *Aliment Pharmacol. Ther.* 13: 271-287, 1999.

26. Rindi G, Azzoni C, La Rosa S, et al: ECL cell tumor and poorly differentiated endocrine carcinoma of the stomach: prognostic evaluation by pathological analysis. *Gastroenterol.* 116; 532-542, 1999.
27. Moertel CG, Sauer G, Dockerty MB, et al.: Life history of the carcinoid tumor of the small intestine. *Cancer* 14: 901-912, 1961.
28. Hajdu S, Winawer SJ, Myers WPL: Carcinoid tumors. A study of 204 cases. *Am J Clin Pathol.* 61: 521-528, 1974.
29. Canavese G, Azzoni C, Pizzi S et al.: p27: a potential main inhibitor of cell proliferation in digestive endocrine tumors but not a marker of benign behavior. *Hum. Pathol.* 32:1094-1101, 2001.
30. Plockinger U, Rindi G., Arnold R et al: Guidelines for the diagnosis and treatment of neuroendocrine gastrointestinal tumors. *Neuroendocrinology* 80; 394-424, 2004.
31. Puztal L, Lewis CE, Lorenzen J, McGee OD.; Growth factors; regulation of normal and neoplastic growth. *J. Pathol.* 169, 191-201, 1993.
32. Rindi G, Bordi C.: Rindi G, Bordi C.: Aetiology, Molecular Pathogenesis and Genetics. *Best Pract. and Res. Clin. Gastroenterol.* 19; 519-534, 2005.

and decarboxylation) cells and postulated that they derived from the neural crest. However, the neural crest origin of the diffuse neuroendocrine system proved to be wrong and has currently been replaced by the concept of the entodermal origin of the neuroendocrine cells of the gastrointestinal tract.

Descriptions of patients suffering from diarrhea, cyanosis, cough and flushing started in 1931 [2, 19]. The first report of a carcinoid syndrome, however, was probably by Ransom [17], who described a 50-year-old woman with severe diarrhea and a metastasizing tumor originating from small nodules in the ileum. It was not until 1953 that the carcinoid syndrome was related to the hypersecretion of serotonin from the carcinoid tumor [7].

Originally Oberndorfer considered carcinoids to be benign, although one of Lubarsch's cases had probably metastasized. As soon as other carcinoids with lymph node metastases were observed, in his contribution to Henke and Lubarsch's textbook on pathological anatomy and histology in 1929 [15] he admitted that there are also metastasizing carcinoids. The discussion on the benign and/or malignant nature of carcinoids continued for a long time until it was generally accepted that all carcinoids have a malignant potential.

In recent years it has become clear that the morphological and biological features of neuroendocrine tumors (NETs), especially those arising from the gastroenteropancreatic system, are heterogeneous. In the last two decades efforts were therefore made to define NET features that discriminate tumors with almost no risk/low risk from low grade malignant well differentiated neuroendocrine tumors and high grade malignant poorly differentiated tumors in the different parts of the digestive system and elsewhere. This resulted in a new WHO classification of the gastroenteropancreatic NETs. Further efforts are still necessary, however, to improve the prognostic assessment of an individual NET.

References

1. Beger A (1882) Ein Fall von Krebs des Wurmfortsatzes. *Klin Wochenschr* (Berlin) 19:616
2. Cassidy MA (1931) Postmortem finding in a case shown on October 10, 1930 as one of abdominal carcinomatosis with probable adrenal involvement. *Proc Roy Soc Med* 24:920
3. Feyrter F (1938) Über diffuse endokrine epitheliale Organe. J.A. Barth, Leipzig
4. Hamperl H (1927) Über die "gelben (chromaffinen)" Zellen im gesunden und kranken Magendarmschlauch. *Virchows Arch path Anat* 266:509-548
5. Heidenhain R (1870) Untersuchungen über den Bau der Labdrüsen. *Arch Mikr Anat* 6:368-460
6. Huebschmann P (1910) Sur le carcinome primitif de l'appendice vermiculaire. *Rev méd Suisse rom* 30:317-332
7. Isler P, Hedinger C (1953) Metastasierendes Dünndarmcarcinoid mit schweren, vorwiegend das rechte Herz betreffenden Klappenfehlern und Pulmonalstenose - ein eigenartiger Symptomkomplex? *Schweiz Med Wochenschr* 83:4-7
8. Langhans T (1887) Ueber einen Drüsenpolyp im Ileum. *Virchows Arch* 38:559-560
9. Lubarsch O (1888) Ueber den primären Krebs des Ileum nebst Bemerkungen über das gleichzeitige Vorkommen von Krebs und Tuberculose. *Virchows Arch* 111:280-317
10. Masson P (1914) La glande endocrine de l'intestin chez l'homme. *C R Acad Sci (Paris)* 138:59-61
11. Masson P (1924) Appendicite neurogène et carcinoides. *Ann Anat Pathol* 1:3-59
12. Merling F (1838) Anatomie pathologique de l'appendice du caecum. *Experience (Paris)* 1:337
13. Oberndorfer S (1907) Karzinoide Tumoren des Dünndarms. *Frankf Z Pathol* 1:425-432

14. Oberndorfer S (1907) Ueber die "kleinen Dünndarmcarcinome". Verh Dtsch Ges Pathol 11:113-116
15. Oberndorfer S (1929) Die Geschwülste des Darms. In: F Henke, O Lubarsch (eds) Handbuch der speziellen pathologischen Anatomie und Histologie, Bd. 4, Teil 3, Verdauungsschlauch. Springer, Berlin, pp 717-953
16. Pearse AG (1969) The cytochemistry and ultrastructure of polypeptide hormone-producing cells of the APUD series and the embryologic, physiologic and pathologic implications of the concept. J Histochem Cytochem 17:303-313
17. Ransom WB (1890) A case of primary carcinoma of the ileum. Lancet ii:1020-1023
18. Saltykow S (1912) Beiträge zur Kenntnis der "karzinoiden Darmtumoren". Verh Dtsch Ges Pathol 15:302-307
19. Scholte AJ (1931) Ein Fall von Angioma teleangiectaticum Cutis mit chronischer Endocarditis und malignem Dünndarmcarcinoid. Beitr Pathol 86:440-443

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