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The Association of Chronic Inflammation and Gastroenteropancreatic Neuroendocrine Tumors (GEP-NETs)

Maja Cigrovski Berković[1], Davorka Herman Mahečić[1], Vedran Tomašić[2], Davor Hrabar[2] and Vanja Zjačić-Rotkvić[1]

[1]*Department of Endocrinology, Diabetes and Metabolism University Hospital Centre "Sestre milosrdnice", Zagreb,*

[2]*Department of Gastroenterology and Hepathology University Hospital Centre "Sestre milosrdnice", Zagreb, Croatia*

1. Introduction

Gastroenteropancreatic neuroendocrine tumors (GEP-NETs) are rare and heterogeneous neoplasms with overall increasing incidence, but not an associated increase in survival rate over the past few decades. Tumors originate from at least 16 different cells of diffuse endocrine system (DES), scattered through mucosa of gastrointestinal tract. They are mainly sporadic, but sometimes exhibit familial inheritance. Tumors often preserve the ability to synthesize, store and secrete numerous hormones and biogenic amines which sometimes lead to distinct hypersecretory and clinically recognizable syndromes (such as carcinoid, Zollinger-Ellison, WDHA etc.).[1] The resulting clinical symptoms are generally well controlled by somatostatin analogs and/or interferon-α.[2]

More often, GEP-NETs remain clinically silent until late, when they present with mass effect, and have unfortunately already locally or distantly spread. In the later case tumor growth and spread are not always well controlled by either biotherapy or chemotherapy. Although many biochemical and tissue markers for GEP-NETs already exist, sensitive and specific markers that predict tumor growth and behavior are lacking.[3]

According to our unpublished data chromogranin A (CgA) and 5-hydroxyindolacetic acid (5-HIAA), currently used as standard biochemical markers of neuroendocrine tumors were only positive in 76.84% and 20.79% of GEP-NET cases respectively. Tumor markers were analyzed in 101 patients (61.2% with localized and 38.8% with metastatic disease) diagnosed with GEP-NETs. According to same investigation, CgA levels were much higher when tumors were part of MEN1 syndrome, while 5-HIAA levels were higher in case of metastatic disease, especially when hepatic metastases were present. When 5-HIAA values were compared among patients with different tumor localizations, the highest values were detected in patients with functional midgut tumors. This is consistent with data of other authors on biochemical diagnostics of gastrointestinal neuroendocrine tumors.[4]

Unfortunately, the correct diagnose of GEP-NETs is delayed for 7-10 years, additionally adding burden to anyhow complex and challenging tumor management.[3] So, in clinical practice, more reliable serum markers as well as precise tumor localization of small, initial lesions together with incorporation of a histological grading system with implemented prognostic implications would help in optimal treatment of patients. The mentioned needs to be supported by better understanding of tumor cell biology and mechanistic regulation of underlying growth processes.[5]

In general, majority of GEP-NETs are represented by well-differentiated cells, and one would expect low proliferating rate, but unfortunately, tumors often present metastatic at the time of diagnosis. This is one of the most intriguing characteristics, and has triggered scientific research aiming to demonstrate specific molecular features that could explain mechanisms underneath the ability of tumor cells to detach from primary malignancy and gain excess to the surrounding structures.[6]

Although development of GEP-NETs is still unclear, significant breakthrough has been made in elucidating molecular genetics of neuroendocrine tumors exhibiting a hereditary background. Those rare tumor types (5-10% of all GEP-NETs) are often caused by mutations in tumor suppressor genes MEN1, VHL, NF-1, TSC1, and TSC2 which in turn lead to development of NETs as a part of multiple endocrine neoplasia type 1, von Hippel Lindau disease, neurofibromatosis type 1 and tuberous sclerosis complex respectively.[7] Besides tumor suppression genes, studies have also demonstrated involvement of oncogenes, each of which may be associated with several different abnormalities that include point mutations, gene deletions, DNA methylation, chromosomal losses and chromosomal gains (Figure 1).[3,8,9]

Perhaps the best characterized is the genetic background of the MEN1 syndrome, which in addition to neuroendocrine tumors of duodenum and pancreas includes adenomas/ hyperplasia of other endocrine glands (parathyroid hyperplasia/hyperparathyroidism, pituitary adenomas and adrenal cortical adenomas). It involves mutations of the MEN-1 tumor suppressor gene. This chromosome 11q13 gene encodes protein menin which interacts with a number of proteins involved in the transcriptional regulation and genome stability, so it has been proposed to be a key player in regulating NET cell proliferation.[8]

The *MEN-1* gene, although conferring a high disease risk in MEN-1 patients where it represents a putative tumor suppressor gene accounts for less than 40 percent of sporadic GEP-NET cases.[10] Thus, the genes involved in neuroendocrine tumorigenesis and the cellular roles of their proteins on proliferation and/or apoptotic pathways remain largely unknown. Studies of comparative genomic hybridization and allelic loss analysis have detected a large number of genomic regions with loss or gain of genetic material, further elucidating genetic differences between GEP-NETs of various primary localizations, and proving the heterogeneity of the tumors.[11]

In general, foregut GEP-NETs often show loss of 11q, while tumors of midgut origin frequently show losses on chromosome 18q. The genetic abnormalities in hindgut NETs have not been well characterized, but it was noticed that larger tumors tend to express transforming growth factor-alpha (TGF-α) more frequently, while epidermal growth factor receptor (EGFR) was expressed in all lesions.[12]

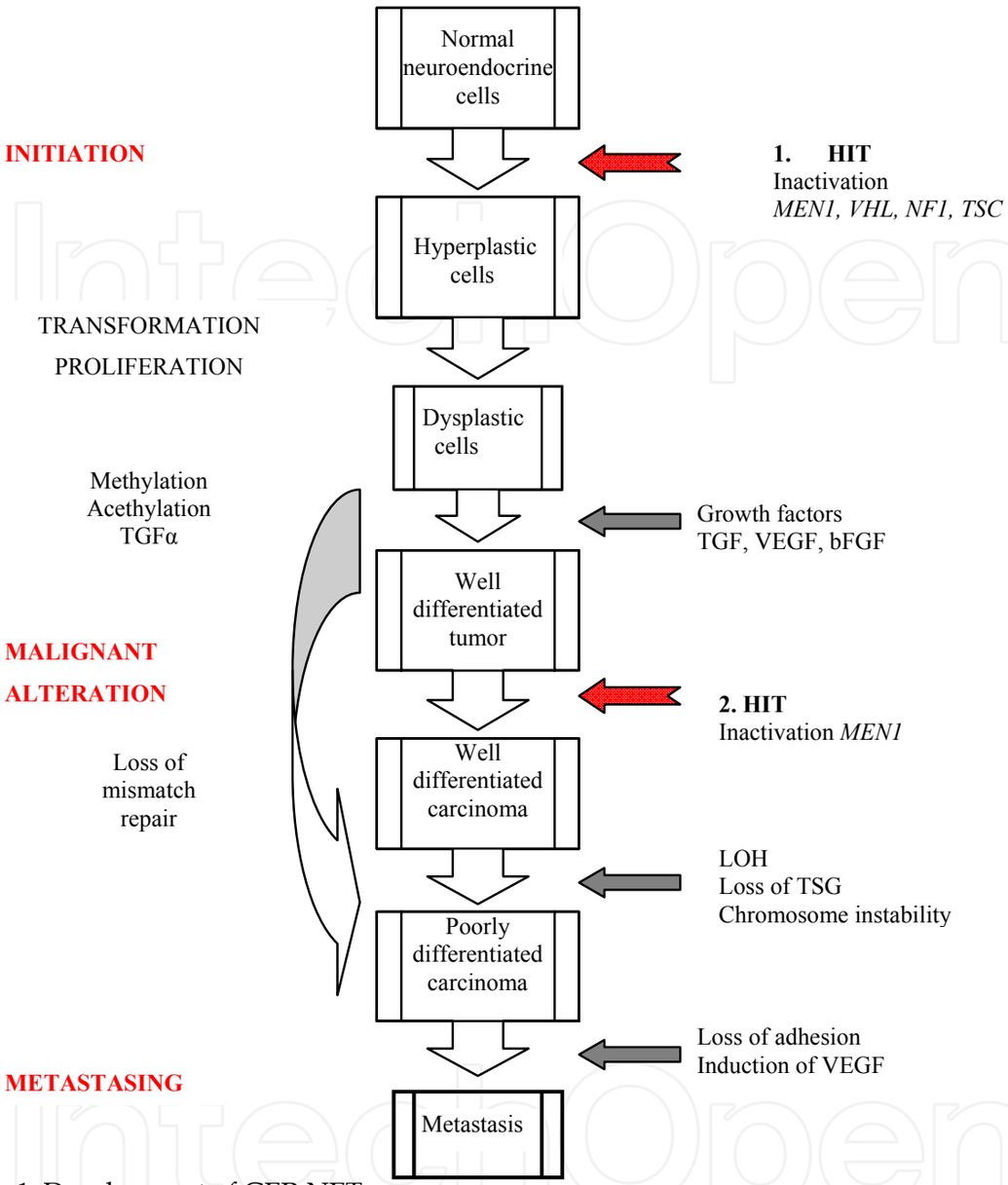


Fig. 1. Development of GEP-NETs.

Comparative studies of pancreatic adenocarcinoma and pancreatic neuroendocrine tumors (pNETs) have helped in giving insight into cellular biology of those specific tumors. Unlike pancreatic adenocarcinomas, pNETs do not exhibit mutations in K-Ras oncogene or p53 tumor suppressor gene, which are often mutated in the former. Also, the pattern of genomic alterations of pNETs differs from that of gastrointestinal NETs, where losses on chromosome 18q are almost a rule (occur in 38-88% of tumors).[13]

It seems that specifics of pNET development are gains and losses of chromosomes, which also appear to influence disease stage. Specifically, genomic gains are common on chromosomes 4pq, 5pq, 7pq, 9q, 12q, 14q, 17pq, 18q and 20q, while losses occur on

chromosomes 1p, 3p, 6q, 10p, 11pq, X and Yq. It is interesting that nonfunctioning pNETs harbor more genetic changes than those functional; in particular they exhibit more losses of 3p and mutations in MEN1 gene. The locus 3p is especially interesting while it harbors several tumor suppressor genes like VHL and retinoic receptor-beta (RAR-β). The later, involved in induction of apoptosis, has been found hypermethylated in 25% of pNETS.[14]

In addition to tumor suppressor genes, some oncogenes have also been found altered in pNETs. Those specifically include over expression of growth factor-related genes such as insulin like growth factor binding protein 3 (IGFBP3), cell adhesion and migration molecules as well as endothelial elements, suggesting an important role of tumor microenvironment.[15]

Dysregulation of DNA methylation patterns is a central feature of colon carcinogenesis, and was also found to be present in development of gastrointestinal neuroendocrine tumors (especially carcinoids). This finding is interesting from the nutrigenomic point of view, and it raises the possibility of tumor prevention with folate and vitamin B12 supplementation.[16,17]

Positive immunohistochemistry staining for different cytokines and growth factors in the GEP-NETs as well as occurrence of GEP-NETs in the setting of inflammatory bowel disease led to the belief that chronic inflammation may play a crucial role in their development and that a number of more prevalent, low penetrance genes contribute to GEP-NET susceptibility in a larger population of patients.[18]

With respect to the role of inflammatory signals in promoting the development of cancer, there is now emerging evidence for an important relationship between macrophage migration inhibitory (MIF) factor expression, oncogenesis and tumor progression. It seems that in different tumors MIF directly promotes tumorigenesis by inhibiting p53 accumulation, promotes cellular proliferation through activation of members of the MAPK family and through induction of COX-2/PGE-2 influences tumor growth and viability. MIF was found to be co-secreted with adrenocorticotrophic hormone (ACTH) by the anterior pituitary, and it has the ability to override its antiinflammatory effects, thus promoting the inflammation and favouring protumor microinvironment.[19]

It seems that immune system through the network of different cytokines and growth factors may also play permissive role in GEP-NET development (Figure 2).[20]

It is now widely acknowledged that chronic inflammatory conditions can both pave the way for and sustain conditions favorable for carcinogenesis and tumor progression. Although the molecular mechanisms of this causal relationship remain to be elucidated, there is strong evidence of association between chronic inflammation and aproximately 1/5 of human cancers confirmed by numerous epidemiologic, gene association and molecular studies.[21]

Overall, it appears that chronic inflammation more often stimulates then inhibits tumor development. The persistence of chronic inflammation plays a critical role in initiating, sustaining and advancing tumor growth, and thus modulating the immune response may still be an alluring goal for therapeutic intervention.[22,23]

Although a pathogenic role for chronic inflammation has been suggested in multiple tumor systems in tumor initiation, progression and metastatic potential, the mechanism of this

important association is still not understood completely. The development of a tumor is associated with the growth and expansion of not only tumor cells but also stroma, vessels and infiltrating inflammatory cells, and it is the interaction between these different cell types that propagates tumor growth. Cytokines found in tumors, acting on paracrine and autocrine loops, are most likely the key players in the mentioned communication[24], and for some of them link has been found between the serum and/or tumor tissue level and cancer survival.[25]

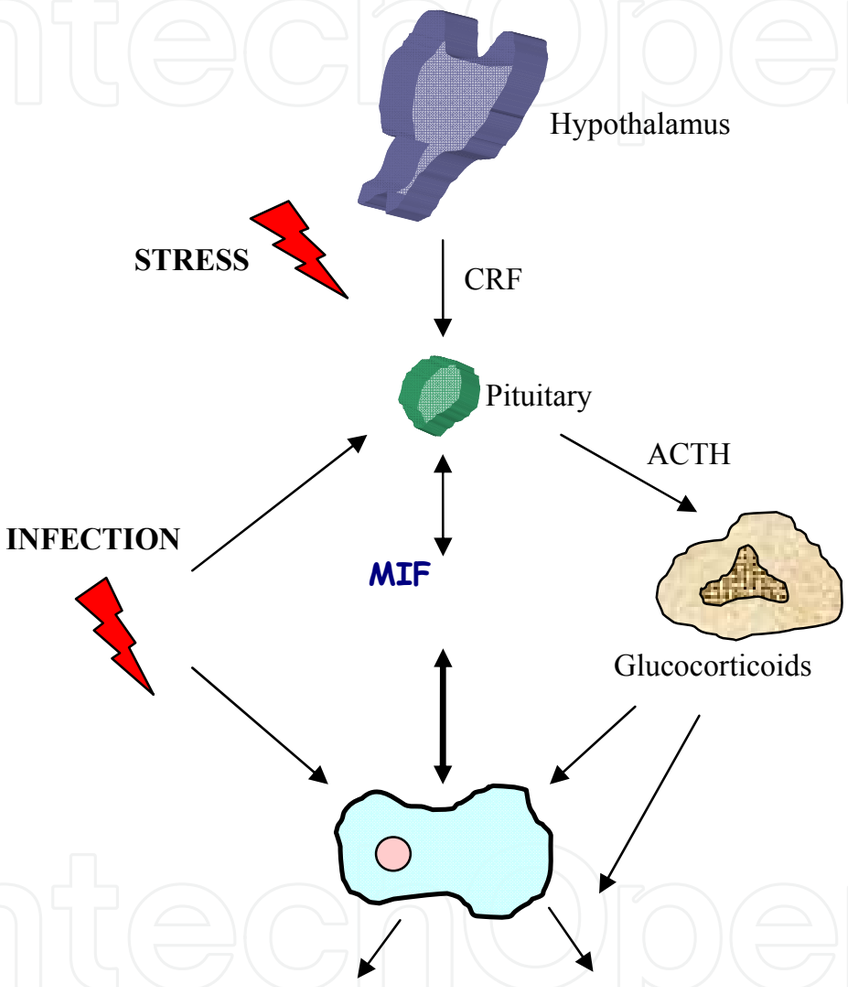


Fig. 2. Connection between the endocrine system and cytokines.

Cytokines and growth factors seem to largely contribute to the development and progression of GEP-NETs[13,17,26,27], but their involvement in the autocrine stimulation of tumor cells, either in genesis and/or in the progression of GEP-NETs has not yet been clearly elucidated.[28]

GEP-NETs represent a tumor entity with an extraordinary high vascularization along with an abundant production and secretion of growth factors, especially vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), insulin like growth factor (IGF), fibroblast growth factor (FGF) and transforming

growth factor-α (TGF-α), which according to both observational and mechanistic data connect chronic inflammation with gastrointestinal carcinogenesis.[20,23]

MEN-1 patients have a higher serum level of fibroblast growth factor (FGF), which correlates with the amount of tumor-associated fibroblastic response. Furthermore, insulin-like growth factor-I (IGF-I) receptors found on GEP-NET cells suggest an autocrine trophic function for the mentioned growth factor in these tumors.[27] Patients with carcinoid syndrome were found to have positive immunohistochemistry for TGF -β on the right sided heart valves, as a consequence of NET progression and metastasis.[29]

For further cancer evolution angiogenesis plays an important role. Proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), IL1 and IL6 once again participate in this process by inducing the production of angiogenic factors, mainly VEGF. The role of vascular endothelial growth factor (VEGF) in the new vessel formation of these highly vascularized tumors is increasingly studied, and it appears to be involved in the metastasing process of the mentioned tumors. Higher levels of cytokines and growth factors detected in GEP-NETs are responsible for neurotrophic effects, smooth muscle cell hypertrophy and proliferation of both intimal and adventitial elastic tissue of the mesenteric blood vessels leading to vascular elastosis sometimes associated with ischemic changes of the near-by tissue (Figure 3).[6,30]

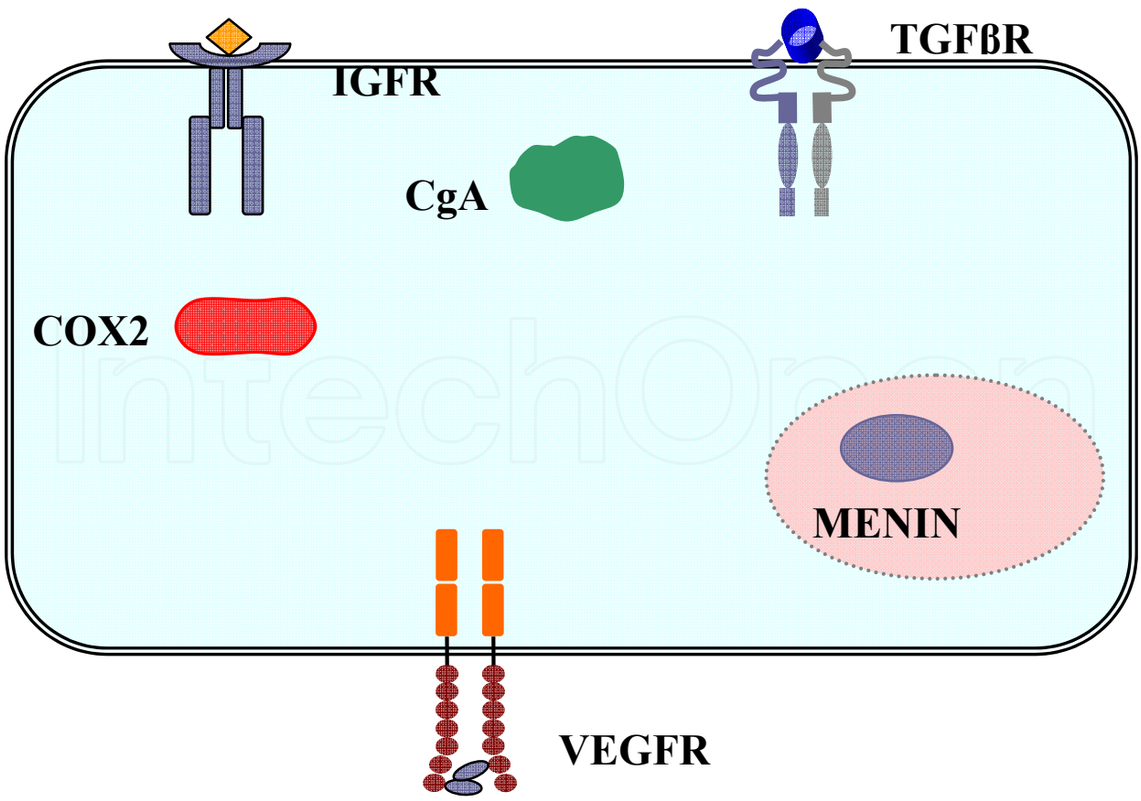


Fig. 3. Tumor cell markers of neuroendocrine cell.

Cytokine genes are highly polymorphic, with polymorphisms frequently located in regions of DNA that regulate transcription, or posttranscriptional events, thus influencing functional activity. Recently published studies connected proinflammatory cytokine genes SNPs with cancer susceptibility and severity, putting them in the spot light as cancer-modifier genes.[31] This is particularly true for cytokine gene polymorphisms and gastrointestinal malignancy, where many authors suggest the role of inflammation-mediated oncogenesis.[16,18,32] It seems likely that they also contribute to GEP-NET development.[33,34]

Genetic polymorphisms directly influence interindividual variation in the cytokine response, and this clearly contributes to an individual's ultimate clinical outcome. Many single nucleotide polymorphisms (SNPs) have been detected within the cytokine gene sequences, particularly within the promoter regions. Several of these SNPs may be associated with differential level of gene transcription, thus influencing levels of cytokines and growth factors in sera and tumor tissue and ultimately altering the disease prognosis by influencing anti-tumor immunologic response or pathways of (neo)angiogenesis.

However, for the ultimate outcome, not only cytokines or growth factors but also (tumor) cell type and stimulus may also be important.[35] In our investigation of the role of IL-6 in GEP-NETs we have found the significantly higher proportion of high expression genotypes (*-174 C/G* and *G/G*) in the nonfunctioning pNETs, discriminating them from functional pNETs and gastrointestinal NETs (mainly of midgut origin). Mentioned patients had also higher concentrations of IL-6 in their sera (it was overall elevated in 36.8% of patients), suggesting the potential role of IL-6 as a novel diagnostic and prognostic marker of nonfunctioning pNETs.[36]

A number of studies have reported associations between TNF-α promoter SNPs with high expression alleles (*-238A, -308A, -1031C*) and susceptibility to cancer.[20,37] Our ongoing studies have strongly confirmed the role of TNF-α *-1031C* (high expression) allele as a potential risk factor for developing GEP-NET. Also, we have found the higher level of the *-308* high expression genotypes (*AG, AA*) as well as high expression *-308A* allele among the patients contracting foregut GEP-NETs than in those with midgut tumors. This finding may provide better insight in the role of cytokines in the development of different GEP-NET types and differentiation, and possibly open new prospective in GEP-NET treatment.[38]

2. References

[1] Plöckinger U, Rindi G, Arnold A, Eriksson B, Krenning EP, DeHerder WW, Goede A, Caplin Guidelines for the Diagnosis and Treatment of Neuroendocrine Gastrointestinal Tumors. Neuroendocrinology 2004;80:394-424.

[2] Cigrovski Berković M, Altabas V, Herman D, Hrabar D, Goldoni V, Vizner B, Zjačić-Rotkvić V. A Single-Centre Experience with Octreotide in the Treatment of Different Hypersecretory Syndromes in Patients with Functional Gastroenteropancreatic Neuroendocrine Tumors. Coll Antropol. 2007;31:531-534.

[3] Rindi G, Bordi C. Highlights of the biology of endocrine tumors of the gut and pancreas. Endocrine-Related Cancer 2003;10:427-436.

[4] Ardill JE. Circulating markers for endocrine tumors of the gastroenteropancreatic tract. Ann Clin Biochem 2008;45:539-559.
[5] Cigrovski Berkovic M, Jokic M, Zjacic-Rotkvic V, Kapitanovic S. The role of cytokines and their polymorphisms in the gastroenteropancreatic neuroendocrine tumors (GEP-NETs): mini review. Periodicum Biologorum. 2007;109:111-114.
[6] Delle Fave G, Corleto VD. Oncogenes, growth factors, receptor expression and proliferation markers in digestive neuroendocrine tumors. A critical reappraisal. Ann of Oncol 2001;12 (suppl 2):S13-S17.
[7] Zikusoka MN, Kidd M, Eick G, Latich I, Modlin IM. The molecular genetics of gastroenteropancreatic neuroendocrine tumors. Cancer 2005;04:2292-2309.
[8] Perren A, Komminoth P, Heitz PU. Molecular genetics of gastroenteropancreatic endocrine tumors. Ann NY Acad Sci 2004;1014:199-208.
[9] Chan AO, Kim SG, Bedeir A, Issa JP, Hamilton SR, Rashid A 2003 CpG island methylation in carcinoid and pancreatic endocrine tumors. Oncogene 22:924-934.
[10] Pannett AA, Thakker RV 2001 Somatic mutations in MEN type 1 tumors, consistent with the Knudson "two-hit" hypothesis. J Clin Endocrinol Metab 86:4371-4374.
[11] Duerr E-M, Chung DC. Molecular Genetics of pancreatic neuroendocrine tumors. In: A century of advances in neuroendocrine tumor biology and treatment. (Ed. Modlin IM, Oberg K.), Felsenstein C.C.C.P. 2007.
[12] Leotlela PD, Jauch A, Holtgrave-Grez H, Thakker RV. Genetics of neuroendocrine tumors and carcinoid tumors. Endocrine Related Cancer 2003;10:437-450.
[13] Öberg K. Carcinoid tumors-current considerations. In: A century of advances in neuroendocrine tumor biology and treatment. (Ed. Modlin IM, Oberg K.), Felsenstein C.C.C.P. 2007.
[14] Speel EJ et al. 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(13):5186-92.
[15] Östman A. Tumor stroma-a perspective of therapeutic and prognostic opportunities. In: A century of advances in neuroendocrine tumor biology and treatment. (Ed. Modlin IM, Oberg K.), Felsenstein C.C.C.P. 2007.
[16] House MG et al. Aberrant hypermethylation of tumor suppresor genes in pancreatic endocrine neoplasms. Ann Surg 2003;238(3):423-31.
[17] Shimizu T et al. Growth characteristics of rectal carcinoid tumors. Oncology 2000;59:229-237.
[18] Terris B et al. Expression of vascular endothelial growth factor in digestive neuroendocrine tumors. Histopathology 1998; 32:133-138.
[19] Conroy H, Mawhinney L, S. C. Donnelly SC. Inflammation and cancer: macrophage migration inhibitory factor (MIF)—the potential missing link. Q J Med 2010; 103:831–836.
[20] Wulbrand U, Wied M, Zofel P, Goke B, Arnold R, Fehmann HC. Growth factor receptor expression in human gastroenteropancreatic neuroendocrine tumors. European J of Clin Invest 1998;28:1038-1049.
[21] De Marzo AM et al. Inflammation in prostate carcinogenesis. Nat Rev Cancer 2007;7:256–269.

[22] Jackson L, Evers BM. Chronic inflammation and pathogenesis of GI and pancreatic cancers. Cancer Treat Res 2006;130:39-65.

[23] Höpfner M, Schuppan D, Scherübl H. Treatment of gastrointestinal neuroendocrine tumors with inhibitors of growth factor receptors and their signaling pathways: Recent advances and future perspectives. World J Gastroenterol 2008;14(16):2461-2473.

[24] Gonda TA, Tu S, Wang TC. Chronic inflammation, the tumor microenvironment and carcinogenesis. Cell cycle 2009;8 (13):2005-13.

[25] Westbrook AM, Szakmary A, Schiestl RH. Mechanisms of intestinal inflammation and development of associated cancers: Lessons learned from mouse models. Mutat Res 2010;705(1):40-59.

[26] Wiedenmann B, Pape UF 2004 From basic to clinical research in gastroenteropancreatic neuroendocrine tumor disease-the clinician-scientist perspective. Neuroendocrinology 80(suppl 1):94-98.

[27] Wild A et al. Frequent methylation-associated silencing of the tissue inhibitor of metalloproteinase-3 gene in pancreatic endocrine tumors. J Clin Endocrinol Metab 2003; 88:1367-1373.

[28] Barakat MT, Meeren K, Bloom SR. Neuroendocrine tumors. Endocrine-Related Cancer 2004;11:1-18.

[29] Lester WM, Gotlieb AI 1991 The cardiovascular system. In *Functional Endocrine Pathology*, vol. 2, pp 724-747. Eds k Kovacs and SL Asa, Boston: Blackwell

[30] Ardill JES, Erikkson B. The importance of the measurment of circulating markers in patients with neuroendocrine tumors of the pancreas and gut. Endocrine-related Cancer 2003;10:459-462.

[31] Seike M et al. Use of a cytokine gene expression signature in lung adenocarcinoma and the surrounding tissue as a prognostic classifier. J Natl Cancer Inst 2007;99:1257-1269.

[32] Bidwell J et al. Cytokine gene polymorphism in human disease: on-line databases. Genes Immun 1999;1:3-19.

[33] Wilkening S et al. Interleukin promoter polymorphisms and prognosis in colorectal cancer. Carcinogenesis 2008;29(6):1202-1206.

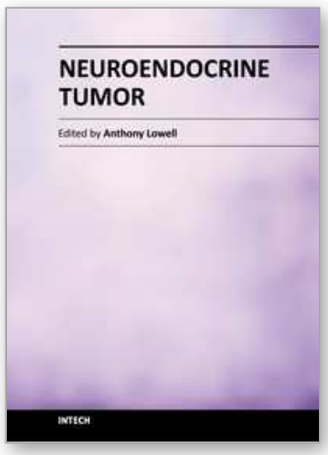
[34] Cigrovski Berkovic M. The role of cytokines and growth factors in development and progression of gastroenteropancreatic neuroendocrine tumors (GEP-NETs). Doctoral thesis. University of Zagreb, 2009.

[35] MacArthur M, Hold GL, El-Omar EM. Inflammation and Cancer II. Role of chronic inflammation and cytokine gene polymorphisms in the pathogenesis of gastrointestinal malignancy. Am J Physiol Gastrointest Liver Physiol 2004;286:G515-G520.

[36] Cigrovski Berković M, Jokić M, Marout J, Radošević S, Zjačić-Rotkvić V, Kapitanović, S. IL-6-174 C/G polymorphism in the gastroenteropancreatic neuroendocrine tumors (GEP-NETs). Experimental and Molecular Pathology 2007;83:474-479.

[37] Anderson GM, Nakada MT, Dewitte M. Tumor necrosis factor-α in the pathogenesis and treatment of cancer. Curr Opin Pharmacol 2004; 4314-4320.

[38] Berkovic M, Cacev T, Zjacic-Rotkvic V, Kapitanovic S. TNF-α promoter SNPs in gastroenteropancreatic neuroendocrine tumors (GEP-NET). Neuroendocrinology 2006;84:346-352.



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