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ORIGINAL ARTICLE

Evaluation of GLP-2 receptor expression in gastrointestinal neuroendocrine tumors

Gastrointestinal nöroendokrin tümörlerde GLP-2 reseptör ekspresyonunun değerlendirilmesi

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Background and Aims: Neuroendocrine tumors arise from cells of the neuroendocrine system. These cells show both nerve and endocrine cell characteristics and can be found in many organs in the body. GLP-1 and GLP-2 are released from intestinal L cells in a 1:1 ratio following food intake. GLP-2 receptor recognizes GLP-2. GLP-2 receptor mRNA transcripts have been detected in the stomach, small and large intestine, brain, and lung. The proliferative effect of GLP-2 has been demonstrated in mice, rats, pigs, and humans by administering exogenous GLP-2. The objective is to evalaute the relation between gastroenteropancreatic neuroendocrine tumors and glukagon like peptid-2 and GLP-2 receptor. **Materials and Methods:** The patients, who were pathologically diagnosed with gastroenteropancreatic neuroendocrine tumor between 2006-2009 were included in the study. There were 47 patients (27 females, 20 males, avarage age: 54 ± 15.5) in the study. There were also 46 control group patients (25 females, 21 males, avarage age: 57.5 ± 14.8). Pathological tissue blocks prepared on poly-L-lysine microscope slides were stained by GLP-2 receptor antibody (1:100 - 1:200, 1 mg/ml) immunohistochemical stain. **Results:** GLP-2 receptor positivity of colon neuroendocrine tumor group was 30% (4/13) and colon control group was %100. GLP-2 receptor positivity of pancreas neuroendocrine tumor group was 25% (3/12) while it was 100% in pancreas control group. The comparison of colon neuroendocrine tumor and control group showed significant difference (p: 0.003). The comparison of pancreas neuroendocrine tumor and control group also showed statistically significant difference ($p < 0.001$). The comparison of gastric neuroendocrine tumor with the control yielded comparable results (p: 0.22). **Conclusions:** We concluded that GLP-2 receptor cannot be as useful as somatostatin receptors in diagnosis and treatment of these tumors. More studies are needed on this subject with different methods.

Key words: GLP-2 receptor, neuroendocrine tumours, GEP-NET

Giriş ve Amaç: Nöroendokrin tümörler, nöroendokrin sistem hücrelerinden kaynaklanır. Bu hücreler hem sinir hem de endokrin hücre özelliği gösterirler ve vücuttaki birçok organda bulunabilirler. GLP-1 ve GLP-2, gıda alımını takiben 1:1 oranında bağırsak L hücrelerinden salınır. GLP-2 reseptörü, GLP-2'yi tanır. GLP-2 reseptör mRNA transkriptleri mide, ince ve kalın bağırsak, beyin ve akciğerde tespit edilmiştir. GLP-2'nin proliferatif etkisi farelerde, sıçanlarda, domuzlarda ve insanlarda eksojen GLP-2 uygulanarak gösterilmiştir. Amaç, gastroenteropankreatik nöroendokrin tümörler ile glukagon benzeri peptid-2 ve GLP-2 reseptör arasındaki ilişkiyi değerlendirmektir. **Gereç ve Yöntem:** 2006-2009 yılları arasında patolojik olarak gastroenteropankreatik nöroendokrin tümör tanısı alan hastalar çalışmaya dahil edildi. Çalışmaya 47 hasta (27 kadın, 20 erkek, ortalama yaş: 54 ± 15.5) alındı. Ayrıca 46 kontrol grubu hastası (25 kadın, 21 erkek, ortalama yaş: 57.5 ± 14.8) vardı. Poli-L-lisin mikroskop lamlarında hazırlanan patolojik doku blokları, GLP-2 reseptör antikoru (1:100 - 1:200, 1 mg/ml) immünohistokimyasal boyama ile boyandı. **Bulgular:** Kolon nöroendokrin tümör grubunun GLP-2 reseptör pozitifliği %30 (4/13), kolon kontrol grubunun GLP-2 reseptör pozitifliği %100 bulundu. Pankreas nöroendokrin tümör grubunun GLP-2 reseptör pozitifliği %25 (3/12) iken, pankreas kontrol grubunda %100 idi. Kolon nöroendokrin tümör ile kontrol grubunun karşılaştırılmasında anlamlı farklılık görüldü (p: 0.003). Pankreas nöroendokrin tümör ile kontrol grubunun karşılaştırılması da istatistiksel olarak anlamlı farklılık gösterdi ($p < 0.001$). Gastrik nöroendokrin tümörlerin kontrol ile karşılaştırılması karşılaştırılabilir sonuçlar verdi (p: 0.22). **Sonuç:** GLP-2 reseptörünün bu tümörlerin tanı ve tedavisinde somatostatin reseptörleri kadar yararlı olamayacağı sonucuna vardık. Bu konuda farklı yöntemlerle daha fazla çalışmaya ihtiyaç vardır.

Anahtar kelimeler: GLP-2 reseptörü, nöroendokrin tümörler, GEP-NET

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INTRODUCTION

Neuroendocrine tumors (NETs) develop from neuroendocrine system cells. These cells have both nerve and endocrine cell properties and can be found in a variety of organs across the body (1). According to hormone secretion, NETs are classed as functional (about 40%) (2) or nonfunctional (3-5).

The proglucagon precursor encodes glucagon-like peptide-2 (GLP-2). The glucagon and GLP-1 sequences are encoded by this precursor. Proglucagon is broken down in tissue-specific ways by prohormone converting enzymes (PC), PC-2 creates glucagon, and the major proglucagon component in the pancreas or PC-1 produces gastrointestinal (GI) glycentin, oxyntomodulin, GLP-1, and GLP-2 in the gastrointestinal system (6-8). Proglucagon-derived peptides (PGDPs) have a wide range of actions. All of these are necessary for controlling nutrition intake and/or maintaining energy balance.

Following meal ingestion, intestinal L cells release GLP-1 and GLP-2 in a 1:1 ratio (9-11). The GLP-2 receptor (GLP-2R) was cloned from rat and human hypothalamus and intestinal cDNA cells (12) and designated as a member of the G protein-coupled receptor family B secretin-like subfamily based on conserved structure (12).

GLP-2R identifies GLP-2 (12,13). Transcripts of GLP-2 receptor mRNA have been found in the stomach, small and large intestines, brain, and lung (14-18). Exogenous GLP-2 administration has been shown to have a proliferative impact in mice, rats, pigs, and humans (19). Given the findings of experimental research demonstrating GLP-2's proliferative and antiapoptotic actions, particularly on the colon, it is hypothesized that it plays a role in the creation of colon polyps and colon cancer.

In this study, we looked at GLP-2R expression in cells using gastroenteropancreatic neuroendocrine tumor (GEP-NET). The discovery of the link between GLP-2R and GEP-NET may help to understand the pathogenic process and may lead to novel treatment techniques that block GLP-2R by locating a secondary target receptor, such as the somatostatin receptor in neuroendocrine tumors.

MATERIAL and METHODS

Group Study

The research comprised patients with pathological diagnosis of GEP-NET between 2006 and 2009. The trial comprised 47 patients in the GEP-NET group and 46 participants in the control group. Patients were investigated in accordance with a procedure authorized by the Human Subjects Institutional Committee of Dokuz Eylül University Medical Faculty (26.01.2011-12). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki and informed consent was taken from the patients.

Pathologic Examine

Five-micron slices were cut from each representative paraffin block and placed on poly-L-lysine coated slides for immunohistochemical staining. For immunostaining using GLP-2 receptor antibody (Genetex, dilution: 1/100-1/200, 1 mg/ml), the standard streptavidin-biotin immunoperoxidase technique was utilized. Tissue slices were deparaffinized in xylene, then rehydrated in a succession of alcohols before being submerged in distilled water. Antigen retrieval was carried out in 0.1 mol/L citrate buffer at 99°C for 20 minutes (pH: 5.5). The Lab Vision Autostainer 360 was then utilized for further staining. Endogenous peroxidase activity was inhibited in 0.3% H2O2 for 15 minutes before the sections were rinsed with Tris. The sections were then incubated for 5 minutes in Large Volume Ultra V Block, followed by 60 minutes in the primary antibody (GLP-2, 1:100-1:200 dilution rate). As a positive control, colon tissue was employed. The staining profile was examined under

a light microscope (Nikon ECLIPSE 80i). For the examination of GLP-2 staining in adenocarcinoma patients, nuclear and cytoplasmic expression in less than 30% of tumor cells was scored (+), while expression in more than 30% of tumor cells was scored (++).

Statistical Evaluation

Using computer software, the immune-histochemical assessment results were statistically evaluated (SPSS 15.0, Chicago, IL, USA). The percentage computation, average, and standard deviation were employed for descriptive findings. Statistical significance was defined as a probability level of 0.05 or less.

RESULTS

The research included 47 individuals with pathologically confirmed GEP-NET (27 females and 20 males) (Table 1). The control group included 25 females and 20 males. The patients' mean age was 54 ± 15.5 (range: 11 - 78), while the control group's mean age was 57.5 ± 14.8. There was no difference in age or gender between GEP-NET patients and the control group.

Table 1 Characteristics of patient and control group.

	Patients	Control Group	p
Gender (male)	20/47 (42%)	20/45 (44.4%)	> 0.05
Age (years)	54 ± 15.5	57.5 ±14.8	> 0.05

Tumors were found in the stomach in 15 patients, the pancreas in 12, the colon in 13, the duodenum in 3, the appendix in 2, and the esophagus in 2. Concerning tumor size, 22 GEP-NETs were smaller than 1 cm, 17 were larger than 2 cm, 5 were between 1 and 2 centimeters, and 3 were of unknown size.

Immunohistochemical Analysis

In all instances, GLP-2R antibody (1:100-1:200, 1 mg/ml) was immunohistochemically examined. Normal mucosa tissues were obtained from the colon, stomach, and pancreas, and enteroendocrine tissues were immunohistochemically stained to reveal GLP-2 receptor expression in enteroendocrine cells (Figure 1). The presence of chromogranin positivity and actin negativity in normal mucosa cells indicates that these GLP-2R-positive cells are enteroendocrine cells.

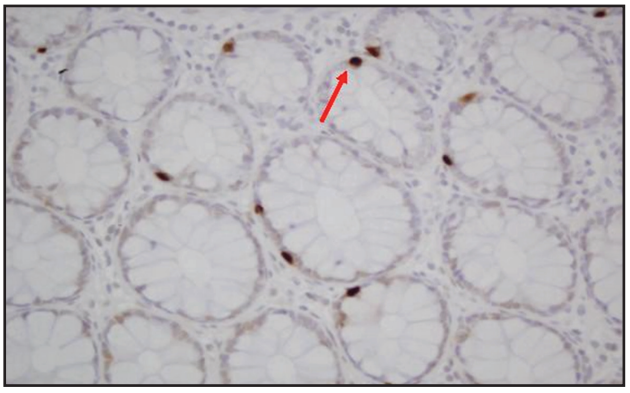

Figure 1 Staining pattern in endocrine cells.

In GEP-NET patients, focal cytoplasmic staining was identified in 6 (20%), no staining was seen in 34 (72%), and focal staining was reported in 7 (15%). GLP-2R expression was significantly higher in pancreatic tissue from the control group than in pancreas neuroendocrine tumor (p: 0.001) (Table 2). Significant staining was detected in the colon tissue control group against the colon neuroendocrine tumor group (p: 0.003) (Table 3). One patient had considerable staining, 1 patient had focal

Table 2 Staining patterns of pancreas.

	Staining (+)	Staining (-)
Patient	3 (25%)	9 (75%)
Control	12 (%100)	

p: 0.001

Table 3 Staining pattern of colon.

	Staining (+)	Staining (-)
Patient	4 (30%)	9 (70%)
Control	13 (%100)	

p: 0.003

staining, and 2 patients had non-significant focal staining in gastric neuroendocrine tumors of the stomach. There was no substantial difference in GLP-2R expression between normal stomach tissue and neuroendocrine tumors.

DISCUSSION

Neuroendocrine tumors are rare heterogeneous category of tumors that can occur in practically any part of the body as a result of malignant transformation of diverse neuroendocrine cells. It is most common in the gastrointestinal system. If GEP-NETs (gastroenteropancreatic neuroendocrine tumors) induce a neuroendocrine syndrome due to excessive production of a peptide hormone, they are called "functional GEP-NETs," and if they don't, they are called "nonfunctional GEP-NETs" (8).

GEP-NETs express a variety of peptide receptors, the most prominent of which being somatostatin. In hormone-secreting symptomatic instances, these tumors can be treated by blocking the somatostatin receptor. Aside from somatostatin receptors, these tumors can also express cholecystokinin 2, bombesin, neuropeptide Y, and vasoactive intestinal peptide receptors, and novel therapies for these receptors are being explored (9).

Increased GLP-2 and other proglucagon-derived peptides were discovered in the blood of experimental animals with intestinal injury during animal investigations (10). These experiments also shown that GLP-2 infusion reversed the intestinal damage caused by non-steroid antiinflammatory drug (NSAID) and chemotherapeutic drug use in experimental mice (11,12). Similarly, in the blood of individuals with inflammatory bowel illness, plasma GLP-2 levels increased, particularly the GLP-2 (1-33) / GLP-2 (3-33) ratio (13). These findings may give evidence that GLP-2 is effective in cell proliferation and may play a role in uncontrolled cell growth.

Although the methods by which GLP-2 reduces apoptosis are unknown, GLP-2 signaling decreases the effects of the proapoptotic protein glycogen synthase kinase-3 (GSK3) both in vitro and in vivo (20).

Exogenous GLP-2 dramatically promotes enterocyte proliferation in vivo, however GLP-2R is not expressed in this cell group. GLP-2 therapy suppresses cell proliferation in cultivated epithelial cells from the small intestine but enhances cell growth in cell lines originating from the large bowel (21), indicating that the impact of GLP-2R activation may be cell-type or tissue-specific. GLP-2's proliferative effects in cell culture systems are frequently reported in the absence of confirmed expression of the known GLP-2R (22), suggesting that GLP-2 may exert some of these actions via as-yet unidentified receptors and/or signaling pathways. Because GLP-2 is a powerful intestinotrophic growth factor, its effect may stimulate the creation or accelerate the growth of intestinal cancers. This is especially relevant in patients taking chemotherapy for pre-existing cancer and in individuals with inflammatory bowel disease (IBD), who are at a higher risk of developing colon cancer (23). Short-term GLP-2 administration had no influence on tumor growth in rats with pre-existing large bowel tumors (24), and GLP-2 administration had no effect on chemotherapy's ability to diminish tumor size in mice (25). However, after the carcinogen 1,2-dimethylhydrazine was administered to mice, one-month treatment of a GLP-2 analog increased tumor burden relative to saline-treated controls (26). As a result, while the present evidence implies that GLP-2 may promote tumor

development in the context of carcinogen delivery, whether GLP-2 alone promotes tumor formation has yet to be established.

GLP-2 receptor expression was found in all 25 normal tissues tested with immunohistochemistry around colon and pancreatic NETs in our investigation. Furthermore, only four (30%) of the NET patients displayed staining indicating GLP-2R expression (Figure 2). Furthermore, only two of these instances exhibited extensive staining, whereas only three of the pancreatic NET cases had GLP-2R showing (Figure 2). Because low levels of antigen expression may not be detected by immunohistochemistry, GLP-2R expression may have been detected in more cancer tissues if a more sensitive RT-PCR approach had been utilized, as Yusta et al. did (16). The second explanation might be that lack of differentiation can lead to loss of GLP-2 receptors. Somatostatin receptors are seen in a similar manner. SSTR2A expression has been found to decrease in high-grade lesions, including gut (27) and lung (28) cancers. Although there is little research on this, we believe that loss of receptors due to differentiation may be the cause.

Another possibility is that GLP receptors are being downregulated. The GLP-2R, like many other G protein-coupled receptors, has considerable signaling down-regulation in response to acute ligand stimulation in vitro. This mechanism, known as receptor desensitization, occurs independently of receptor lipid raft-dependent internalization and leads in a sustained decrease in receptor responsiveness to future agonist activation (29).

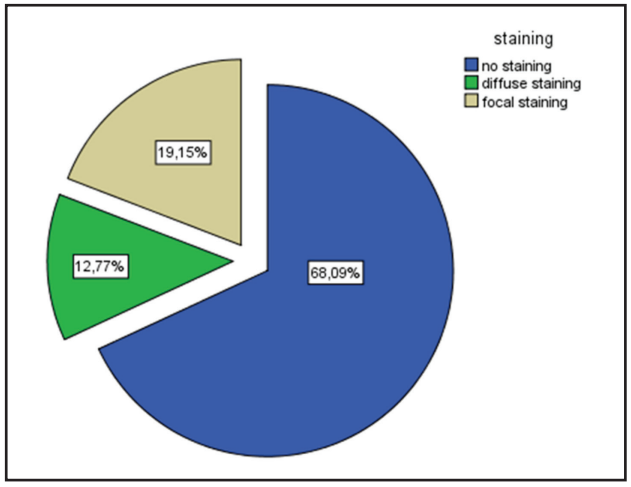

Figure 2 Staining pattern of patient group.

More research is needed to determine if functional GLP-2R desensitization occurs in vivo in response to either short-term activation by endogenous GLP-2 or persistent receptor signaling caused by exogenous injection of long-acting GLP-2 analogs. GLP-2's proliferative effects in cell culture systems are frequently reported in the absence of confirmed expression of the known GLP-2R (22), suggesting that GLP-2 may exert some of these actions via as-yet unidentified receptors and/or signaling pathways. As a result, GLP-2 may still have a function in the development of NET without affecting GLP-2R. There were no other studies in the literature that showed GLP-2R positivity in GRP-NETs. However, because carcinoid tumors are derived from intestinal endocrine cells, Yutsa et al. examined GLP-2R expression in four patients based on the idea that these tumors should be GLP-2 positive. GLP-2R expression was not unique in these instances, however three individuals showed positive in small localized regions. Despite the fact that it is a small group of patients, these findings are consistent with the findings of our study (16).

We found no GLP-2R (+) cells in either stomach NETs or normal stomach tissue in our investigation. The explanation for this might be that GLP-2R expression in the stomach is lower than in the colon and small intestine. Yusta et al. discovered substantially more GLP-2R positivity in the small intestine and colon than in the stomach in their investigation. They demonstrated that expression rises as it approaches the distal gastrointestinal tract.

Finally, because GLP-2R expression is derived from endocrine cells in GEP-NETs, it is expected that it will be employed for diagnostic and therapeutic reasons. However, our analysis found no evidence of GLP-2R expression in these malignancies. As a result, it does not appear conceivable to benefit from GLP-2-like medicines such as somatostatin analogs in GEP-NET therapy at this time. It also does not appear acceptable to employ GLP-2R as a diagnostic technique in the same way as somatostatin receptors are. More research is needed to back up our results of GLP-2R expression in GEP-NETs.

Ethics: *This study protocol was approved by Ethics Committee of Dokuz Eylül University (Date: 13.01.2011, and number 17-GOA). The study was complied with The World Medical Association Declaration of Helsinki.*

Conflict of Interest: *The authors declare that they have no conflict of interest.*

Authorship: *All the author has contributed substantially to the research, preparation and production of the paper.*

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REFERENCES

1. Modlin IM, Champaneria MC, Bornschein J, Kidd M. Evolution of the diffuse neuroendocrine system–clear cells and cloudy origins. Neuroendocrinology 2006;84:69-82.
2. Ahmed M. Gastrointestinal neuroendocrine tumors in 2020. World J Gastrointest Oncol 2020;12:791-807.
3. Cives M, Strosberg J. An update on gastroenteropancreatic neuroendocrine tumors. Oncology (Williston Park) 2014;28:749-756, 758.
4. Grande E, Capdevila J, Barriuso J, Antón-Aparicio L, Castellano D. Gastroenteropancreatic neuroendocrine tumor cancer stem cells: do they exist? Cancer Metastasis Rev 2012;31:47-53.
5. Klöppel G, Anlauf M, Perren A. Endocrine precursor lesions of gastroenteropancreatic neuroendocrine tumors. Endocr Pathol 2007;18:150-5.
6. Dhanvantari S, Seidah NG, Brubaker PL. Role of prohormone convertases in the tissue-specific processing of proglucagon. Mol Endocrinol 1996;10:342-55.
7. Furuta M, Yano H, Zhou A, et al. Defective prohormone processing and altered pancreatic islet morphology in mice lacking ac active SPC2. Proc Natl Acad Sci USA 1997;94:6646-51.
8. Larsen PJ, Tang-Christensen M, Holst JJ, Orskov C. Distribution of glucagon like peptide-1 and other preproglucagonderived peptides in the rat hypothalamus and brainstem. Neuroscience 1997;77:257-70.
9. Drucker DJ. Biological actions and therapeutic potential of the glucagon-like peptides. Gastroenterology 2002;122:531-44.
10. Ghatei MA, Uttenthal LO, Christofides ND, Bryant MG, Bloom SR. Molecular forms of human enteroglucagon in tissue and plasma: plasma responses to nutrient stimuli in health and in disorders of the upper gastrointestinal tract. J Clin Endocrinol Metab 1983;57:488-95.
11. Roberge JN, Brubaker PL. Secretion of proglucagon-derived peptides in response to intestinal luminal nutrients. Endocrinology 1991;128:3169-74.
12. Munroe DG, Gupta AK, Kooshesh F, et al. Prototypic G protein-coupled receptor for the intestinotrophic factor glucagon-like peptide 2. Proc Natl Acad Sci USA 1999;96:1569-73.
13. DaCambra MP, Yusta B, Sumner-Smith M, et al. Structural determinants for activity of glucagon-like peptide-2. Biochemistry 2000;39:8888-94.
14. Lovshin JA, Huang Q, Seaberg R, Brubaker PL, Drucker DJ. Extrahypothalamic expression of the glucagonlike peptide-2 receptor is coupled to reduction of glutamate-induced cell death in cultured hippocampal cells. Endocrinology 2004;145:3495-506.
15. Tang-Christensen M, Larsen PJ, Thulesen J, Romer J, Vrang N. The proglucagon-derived peptide, glucagonlike peptide-2, is a neurotransmitter involved in the regulation of food intake. Nat Med 2000;6:802-7.
16. Yusta B, Huang L, Munroe D, et al. Enteroendocrine localization of GLP-2 receptor expression in humans and rodents. Gastroenterology 2000;119:744-55.
17. Bjerknes M, Cheng H. Modulation of specific intestinal epithelial progenitors by enteric neurons. Proc Natl Acad Sci USA 2001;98:12497-502.
18. Orskov C, Hartmann B, Poulsen SS, et al. GLP-2 stimulates colonic growth via KGF, released by subepithelial myofibroblasts with GLP-2 receptors. Regul Pept 2005;124:105-12.
19. Benjamin MA, McKay DM, Yang PC, Cameron H, Perdue MH. Glucagonlike peptide-2 enhances intestinal epithelial barrier function of both transcellular and paracellular pathways in the mouse. Gut 2000;47:112-9.

20. Jasleen J, Ashley SW, Shimoda N, Zinner MJ, Whang EE. Glucagon-like peptide 2 stimulates intestinal epithelial proliferation in vitro. Dig Dis. Sci 200;47:1135-40.

21. Bulut K, Meier JJ, Ansorge N, et al. Glucagonlike peptide 2 improves intestinal wound healing through induction of epithelial cell migration in vitro evidence for a TGF-beta-mediated effect. Regul Pept 2004;121:137-43.

22. Rocha FG, Shen KR, Jasleen J, et al. Glucagon-like peptide-2: divergent signaling pathways. J Surg Res 2004;121:5-12.

23. Martins NB, Peppercorn MA. Inflammatory bowel disease. Am J Manag Care 2004;10:544-52.

24. Chance WT, Sheriff S, Foley-Nelson T, Thomas I, Balasubramaniam A. Maintaining gut integrity during parenteral nutrition of tumor-bearing rats: effects of glucagon-like peptide 2. Nutr Cancer 2000;37:215-22.

25. Boushey RP, Yusta B, Drucker DJ. Glucagon-like peptide (GLP)-2 reduces chemotherapy-associated mortality and enhances cell survival in cells expressing a transfected GLP-2 receptor. Cancer Res 2001;61:687-93.

26. Thulesen J, Hartmann B, Kissow H, et al. Intestinal growth adaptation and glucagonlike peptide 2 in rats with ileal–jejunal transposition or small bowel resection. Dig Dis Sci 2001;46:379-88.

27. Wada H, Matsuda K, Akazawa Y, et al. Expression of somatostatin receptor Type 2A and PTEN in neuroendocrine neoplasms is associated with tumor grade but not with site of origin. Endocr Pathol 2016;27:179-87.

28. Righi L, Volante M, Tavaglione V, et al. Somatostatin receptor tissue distribution in lung neuroendocrine tumours: A clinicopathologic and immunohistochemical study of 218 'clinically aggressive' cases. Ann Oncol 2010;21:548-55.

29. Estall JL, Yusta B, Drucker DJ. Lipid raft-dependent glucagon-likepeptide-2 receptor trafficking occurs independently of agonist-induced desensitization. Mol Biol Cell 2004;15:3673-87.