Surgery 179 (2025) 108943

Contents lists available at ScienceDirect

Surgery

journal homepage: www.elsevier.com/locate/surg

GLP-1R agonist promotes proliferation of neuroendocrine neoplasm cells expressing GLP-1 receptors

Jonathan S. Shilyansky, BA[a], Casandro J. Chan[a], Sophia Xiao, BA[a], Irena Gribovskaja-Rupp, MD[a], Dawn E. Quelle, PhD[b], James R. Howe, MD[a], Joseph S. Dillon, MB[c], Po Hien Ear, PhD[a,*]

[a] Department of Surgery, University of Iowa Carver College of Medicine, Iowa City, IA
[b] Department of Pharmacology and Neuroscience, University of Iowa Carver College of Medicine, Iowa City, IA
[c] Department of Internal Medicine, University of Iowa Carver College of Medicine, Iowa City, IA

ARTICLE INFO

Article history:
Accepted 25 September 2024
Available online 10 December 2024

Presenting Author: Sophia Xiao

ABSTRACT

Objectives: Semaglutide is a glucagon-like peptide 1 (GLP-1) analog that binds to GLP-1 receptors (GLP-1R) on beta-cells and neuronal cells and is used for treating type 2 diabetes and obesity. Insulin-secreting pancreatic neuroendocrine neoplasms have been reported to express high levels of GLP-1R protein, raising the possibility that GLP-1 receptor agonists could promote tumor growth. Our goal was to quantify GLP-1R expression levels in 6 neuroendocrine neoplasm cellular models and determine their proliferative response to semaglutide treatment.

Methods: Gene expression of *GLP-1R* in neuroendocrine neoplasm cells (BON, GOT1, NT-3, NEC913, NEC1452, and NEC1583) was measured by quantitative polymerase chain reaction. Protein expression was determined by immunofluorescent staining and Western blotting. Neuroendocrine neoplasm cells were incubated with semaglutide, and cell growth was measured using a cell viability assay. Mice harboring GOT1 xenografts were treated with semaglutide, and tumor volumes were measured.

Results: BON, NEC1452, and NEC1583 cells expressed significantly lower levels of *GLP-1R* transcript and protein than GOT1, NT-3, and NEC913 cells. GOT1 and NT-3 showed the highest response to semaglutide treatment, with a 19% and 22% increase in growth. Semaglutide promotes tumor growth in mice with GOT1 xenografts by 72%.

Conclusion: The impact of the GLP-1 receptor agonist semaglutide on neuroendocrine cancer growth is understudied. Our data revealed that 50% of neuroendocrine neoplasm cell lines tested expressed GLP-1R, and semaglutide treatment promoted their growth. These results indicate a potential risk in the use of semaglutide in patients with neuroendocrine neoplasms expressing GLP-1R. Investigations into a larger set of neuroendocrine neoplasms would be important because they are highly heterogeneous.

Introduction

Glucagon-like peptide 1 (GLP-1) is an incretin hormone secreted by intestinal enteroendocrine cells or L cells in response to food intake and is rapidly degraded within local tissue and liver.[1] Although short-lived, GLP-1 has multiple sites of actions where its receptors (GLP-1R) are expressed, including pancreas, kidney, lung, heart, adipose tissue, muscle, and central nervous system.[1] In pancreas, GLP-1 increases glucose-dependent insulin secretion from beta-cells and decreases glucagon release from alpha-cells leading to an improved glycemic control.[1] It also protects beta-cells from glucolipotoxicity and apoptosis.[2,3] Given these desirable effects of GLP-1 on glucose control, various GLP-1R agonists with longer half-lives have been developed for managing type 2 diabetes. Semaglutide is one of the GLP-1R agonists with a half-life of 165 hours when given subcutaneously once weekly and has been approved for diabetes management based on several clinical trials.[4–6]

Neuroendocrine neoplasms (NENs) are a family of rare cancers originating from hormone-secreting cells with neuronal features. NENs are categorized into poorly differentiated neuroendocrine

* Corresponding author: Po Hien Ear, PhD, Department of Surgery, Section of Surgical Oncology and Endocrine Surgery, University of Iowa Carver College of Medicine, 375 Newton Road, MERF 4235, Iowa City, IA 52242.
E-mail address: pohien-ear@uiowa.edu (P.H. Ear);
Twitter: @PoHienEar, @pohienear.bsky.social

https://doi.org/10.1016/j.surg.2024.09.052

carcinomas (NECs) and well-differentiated neuroendocrine tumors (NETs).[7] NETs are generally less aggressive than NECs.[8] NENs are understudied and difficult to culture, resulting in few human NEN models.[9] Gastrointestinal (GI) and pancreatic NET models are especially rare, although a small number of cell lines, such as NT-3,[10] an insulinoma (pancreatic NET) derived from a lymph node metastasis, and GOT1, an ileal carcinoid, have been successfully established.[11] These NET cell lines express common NET markers such as chromogranin A and synaptophysin, and NT-3 cells secrete insulin.[10,11]

GLP-1R expression is common in GI and pancreatic NETs.[12] Benign insulinomas have high levels of GLP-1R expression, with one study finding that 100% of patient tumors were positive for the receptor.[12] A radiotracer targeting GLP-1R has even been trialed in the preoperative localization of insulinomas.[13] Ninety percent of pancreatic gastrinomas and 30% of ileal carcinoids were positive for GLP-1R using autoradiography.[14] Data on GLP-1R expression in NECs are limited, but immunohistochemical staining of 5 GI and pancreatic NECs showed that only 1 was positive for GLP-1R.[15]

GLP-1R agonists promote proliferation of beta-cells by binding to the GLP-1 receptor, which stimulates pathways including beta-arrestins and PI3K to activate ERK1/2 in rodents.[16–18] In rodents, prolonged exposure to a GLP-1R agonist causes hyperplasia and tumor formation in thyroid C cells,[19] and semaglutide is contraindicated in patients with medullary thyroid carcinoma or multiple endocrine neoplasm syndrome type 2.[20] The effect of GLP-1R agonists on other types of neuroendocrine cancer is unknown. Studying the effects of GLP-1R agonists on a diverse set of cell lines could be important because NENs are heterogeneous and their response to GLP-1R agonists may be unpredictable. Here, we report the response of 6 human NEN cell models to semaglutide. NET and NEC cell lines with high GLP-1R expression showed increased proliferation and activation of ERK1/2 when treated with semaglutide.

Methods

Cell Culture

Six NEN cell lines (BON,[21] GOT1,[11] NT-3,[10] NEC913,[22] NEC1452,[22] and NEC1583[23]) were used in this study. BON cells were cultured in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12) with 10% fetal bovine serum (FBS), 100 μg/mL Pen-Strep, and an additional 2 mmol/L of L-glutamine (Gibco, Waltham, MA). GOT1 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco) with 10% FBS, 100 μg/mL Pen-Strep, 2 mmol/L of L-glutamine, 5 μg/mL insulin, and 5 μg/mL transferrin. NT-3 cells were cultured in RPMI 1640 with 10% FBS, 100 μg/mL Pen-Strep, 2 mmol/L of L-glutamine, 0.1 μg/μL fibroblast growth factor 2 (Peprotech, Cranbury, NJ), and 0.1 μg/μL epidermal growth factor (Peprotech). NEC913, NEC1452, and NEC1583 cells were cultured in DMEM/F12 with 10% FBS, 100 μg/mL Pen-Strep, 2 mmol/L of L-glutamine, 10 μg/mL insulin, and 10 mmol/L nicotinamide. Cells were grown in a 37°C incubator with 5% CO_2.

Cell Viability Assay

Cells were washed with Dulbecco's phosphate-buffered saline (DPBS) and plated in 96-well plates and then incubated with semaglutide (MedchemExpress, Monmouth Junction, NJ). Cell growth was measured using the alamarBlue (BioRad, Hercules, CA) cell viability assay and the results were quantified via fluorescence using an Infinite 200 PRO M Plex plate reader (Tecan, Männedorf, Switzerland) with a 560 nm excitation wavelength and a 590 nm emission wavelength. Wells on the edge of the 96-well plates were used to measure the cell media's background fluorescence.

Quantitative Polymerase Chain Reaction

Gene expression of *GLP-1R* in NEN cells was measured through quantitative polymerase chain reaction. RNA was extracted from NEN cells via TRIzol and chloroform and then transferred to spin columns using an Animal Total RNA Mini Prep Kit (Lamda Biotech, St. Louis, MO) according to the manufacturer's protocol. The extracted RNA was synthesized into complementary DNA (cDNA) using EasyScript Plus cDNA Synthesis MasterMix (Lamda Biotech). *GLP-1R* primers (forward sequence: GGTGCAGAAATGGCGAGAATA; reverse sequence: CCGGTTGCAGAACAAGTCTGT) and GAPDH primers (forward sequence: GGAGCGAGATCCCTCCAAAAT; reverse sequence: GGCTGTTGTCATACTTCTCATGG) were purchased from Integrated DNA Technologies (Coralville, IA) based on recommended sequence from PrimerBank. Quantitative polymerase chain reaction was performed using 2X qPCR Universal Green MasterMix (Lamda Biotech) on a QuantStudio 3 real-time polymerase chain reaction system (Thermo Fisher Scientific, Waltham, MA).

Immunofluorescence

GLP-1R protein levels in NEN cells were detected using immunofluorescent staining. Cells were fixed with 4% paraformaldehyde for 15 minutes and were permeabilized using a buffer containing 3% bovine serum albumin and 0.2% Triton X-100 for 5 minutes. Cells were then incubated for 1 hour with a GLP-1R antibody (SAB4300800, Sigma-Aldrich, St. Louis, MO) at 1:200 dilution, incubated for 1 hour with an anti-rabbit fluorescein isothiocyanate secondary antibody at 1:500 dilution, and counterstained with 4',6-diamidino-2-phenylindole (DAPI) mounting medium (VectaShield). Images were taken under a fluorescent microscope with a 150 ms exposure time.

Western Blot

Phosphorylated ERK protein was detected by Western blot. Cells were washed with DPBS, incubated at 37°C with 100 nmol/L of semaglutide for 5 minutes, and lysed with NuPAGE lithium dodecyl sulfate (LDS) sample buffer (Thermo Fisher Scientific). Samples were boiled at 100°C for 10 minutes and run in a polyacrylamide gel. The protein was then transferred to a polyvinylidene difluoride membrane. The membrane was blocked, incubated overnight with phospho-ERK1/2 (Cell Signaling Technology, 4370S) and ERK1/2 (Cell Signaling Technology, 9102S) antibodies at 1:1000 dilution, washed with Tris-buffered saline with Tween-20, and incubated with horseradish peroxidase secondary anti-rabbit (Jackson ImmunoResearch, 111-035-003) antibodies at 1:3000 dilution. Protein was detected via chemiluminescence Clarity Western ECL Substrate (BioRad 1705060).

Xenograft models

Animal experiments were approved by the University of Iowa Institutional Animal Care and Use Committee (IACUC) protocol 2051771. Subcutaneous xenograft tumor models were generated by implanting 5 million GOT1 cells in the flank of 4-month-old NSG male mice (Jackson Laboratory; Stock 005557). Experiment in female mice has not been performed because of the slow growth rate of the GOT1 cells. Two weeks post tumor cell injections, mice were randomized into 2 treatment groups (control and semaglutide), with 9 and 6 mice per group, respectively. Semaglutide or vehicle control solutions were given by subcutaneous injections at 50 μg/kg twice per week for 3 weeks and tumor measurements were performed twice per week using a caliper. GraphPad version 10.2.2 was used to generate graphs and perform statistical analyses. Group

comparisons were made with 2-tailed *t* test and indicated by asterisks when the *P* value was <.05.

Results

The expression of GLP-1R has not been extensively investigated in NEN other than in insulinomas and medullary thyroid carcinoma.[12] Here, we curated our previously published RNA sequencing of 20 small bowel and 30 pancreas NETs to determine the gene expression levels of *GLP-1R* compared with normal adjacent tissue samples.[24,25] These data showed low expression of *GLP-1R* in normal small bowel and higher levels in SBNETs (Figure 1, *A*). Normal pancreas samples showed higher *GLP-1R* levels and a few PNETs showed higher *GLP-1R* levels, but most PNETs have levels

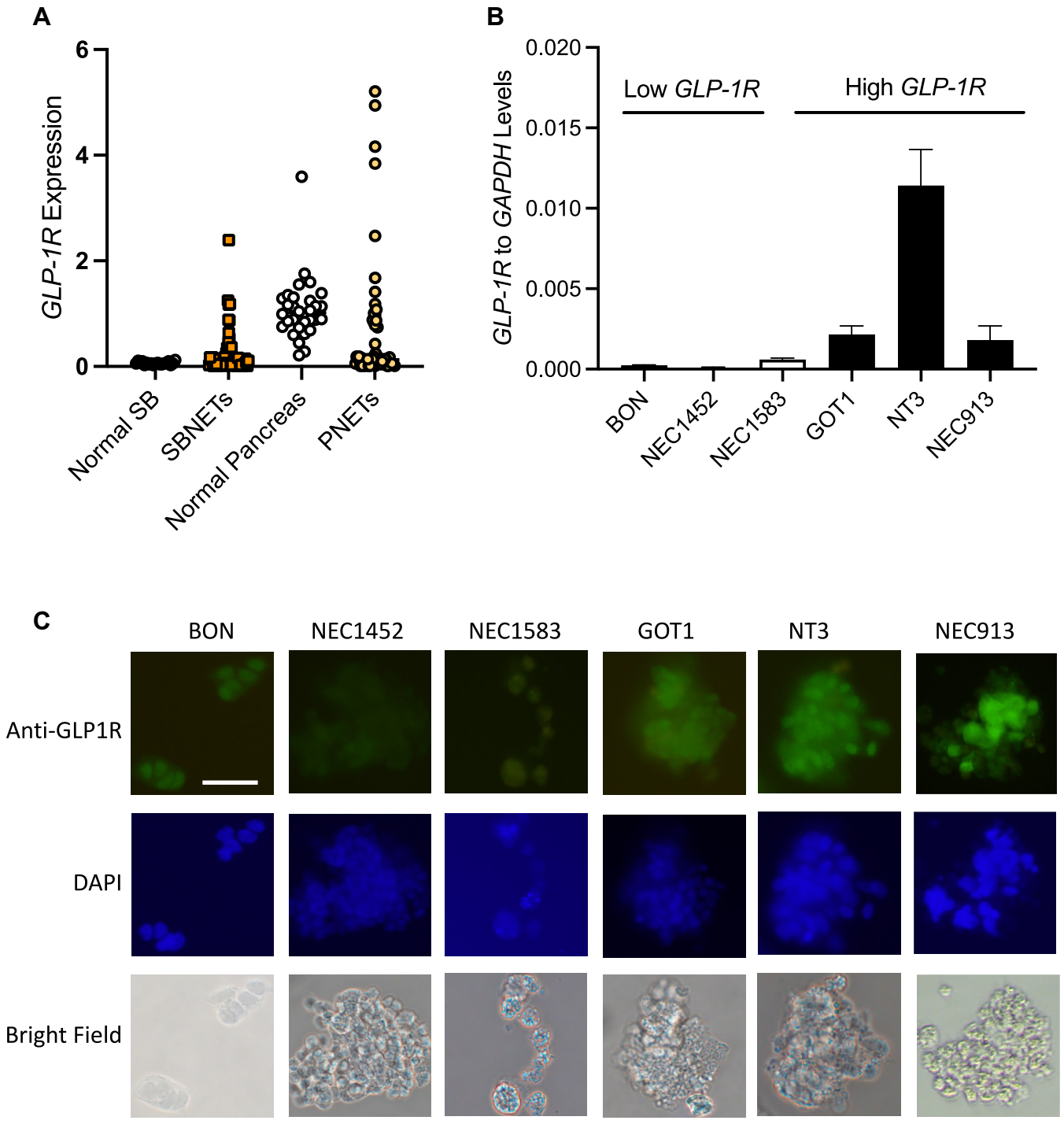


Figure 1. Expression levels of the *GLP-1R* gene and GLP-1R protein in neuroendocrine cancers. (A) Gene expression levels of *GLP-1R* in 20 normal small bowel (SB) samples, 20 small bowel neuroendocrine tumors (SBNETs), 30 normal pancreas samples, and 30 pancreas NETs (PNETs) from RNA sequencing data. (B) Gene expression levels of *GLP-1R* in 6 neuroendocrine cancer cell lines detected using quantitative polymerase chain reaction. Data are expressed as the mean ± SEM from 7 to 14 replicates per group. (C) Protein levels of GLP-1R in 6 neuroendocrine cancer cell lines detected through immunofluorescent microscopy using specific antibody against GLP-1R. *GLP-1R*, glucagon-like peptide 1 receptor; *SEM*, standard error of the mean.

similar to those in normal pancreas tissues because pancreatic islets are known to have high GLP-1R[26] (Figure 1, *A*). To determine the *GLP-1R* expression levels in NEN cell lines, we assembled a collection of our recently established NEC cell lines[22] and available NET cell lines[10,11,21] to investigate the transcript levels of *GLP-1R*. We detected low levels of *GLP-1R* in BON, NEC1452, and NEC1583 cell lines and higher *GLP-1R* levels in GOT1, NT-3, and NEC913 (Figure 1, *B*). We performed immunofluorescent microscopy using antibody specific against GLP-1R to determine the protein levels of GLP-1R in the 6 NEC cell lines. We confirmed that the 3 cell lines with high GLP-1R transcript levels (GOT1, NT-3, and NEC913) also showed higher levels of GLP-1R protein (Figure 1, *C*).

To investigate the growth-promoting activity of semaglutide in NEN cells in vitro, we first incubated the 6 NEN cell lines with and without 100 nmol/L of semaglutide for 7 days and performed the alamarBlue cell viability assay by adding the resazurin metabolic dye to cells and measure its conversion to resorufin, which emits a strong fluorescent signal at 590 nm. Our data showed that semaglutide can enhance the growth of GOT1 and NT-3 cells by 19% and 22%, respectively (Figure 2, *A*). To determine the impact of semaglutide in an in vivo mouse model, we implanted 5 million GOT1 cells in the flank of immunocompromised mice (NSG strain) and measured tumor volume 2 weeks post tumor cell injections. Mice were randomized into the control and semaglutide treatment groups. Semaglutide was given at 50 μg/kg twice per week by subcutaneous injections, and tumor volumes were measured twice per week. Semaglutide promoted GOT1 xenograft growth by 72% in comparison to the control group (Figure 2, *B*). Semaglutide treatment did not cause any body weight change (Figure 2, *C*). Overall, we demonstrated that semaglutide enhances the growth of NEN cells that express GLP-1R in both in vitro and in vivo models.

We sought to determine how semaglutide promotes tumor cell growth in GLP-1R–expressing NEN cells. It has been reported that GLP-1R agonists activate the GLP-1R and the β-arrestins signaling pathway to promote the phosphorylation of 1/2 (p-ERK1/2)[16] (Figure 3, *A*). In a rodent insulinoma cell line, GLP-1 activated p-ERK1/2 and a GLP-1R agonist increased cell proliferation by inducing Wnt signaling in an ERK-dependent manner.[27] We found an increase in p-ERK1/2 activation when cells were treated with 100 nmol/L of semaglutide for 5 minutes in the NT-3, GOT1, and NEC913 cell lines (Figure 3), which express higher levels of GLP-1R (Figure 1, *B* and *C*). There was no increase in p-ERK1/2 levels in BON, NEC1452, and NEC1583 cells (Figure 3). The Western blots were reprobed using antibodies against total ERK1/2 to serve as loading controls.

Discussion

NENs are a family of rare cancers originating from neuroendocrine cells throughout the body. Previous studies have shown that the GLP-1R is expressed at variable levels in many NENs. Although insulinomas universally express high levels of GLP-1R,[12,28] other NENs have not been investigated extensively for receptor expression. We evaluated the expression levels of the *GLP-1R* transcript in our previously published cohort of 20 SBNETs[24] and 30 PNETs[25] and found that approximately 30% of SBNETs express high levels of *GLP1-R* in comparison to the normal adjacent tissues (Figure 1, *A*). Normal pancreas tissues already express high levels of *GLP1-R* because islet cells highly express *GLP-1R*.[26] Hence, PNETs have similar expression of *GLP1-R* transcripts to normal pancreas tissues. NET and NEC cell lines are extremely rare and difficult to culture. Only a few cell lines are available to the research community.[9] Among the 6 NEN cell lines we curated, the 3 cell lines expressing low levels of *GLP-1R* transcripts and GLP-1R protein

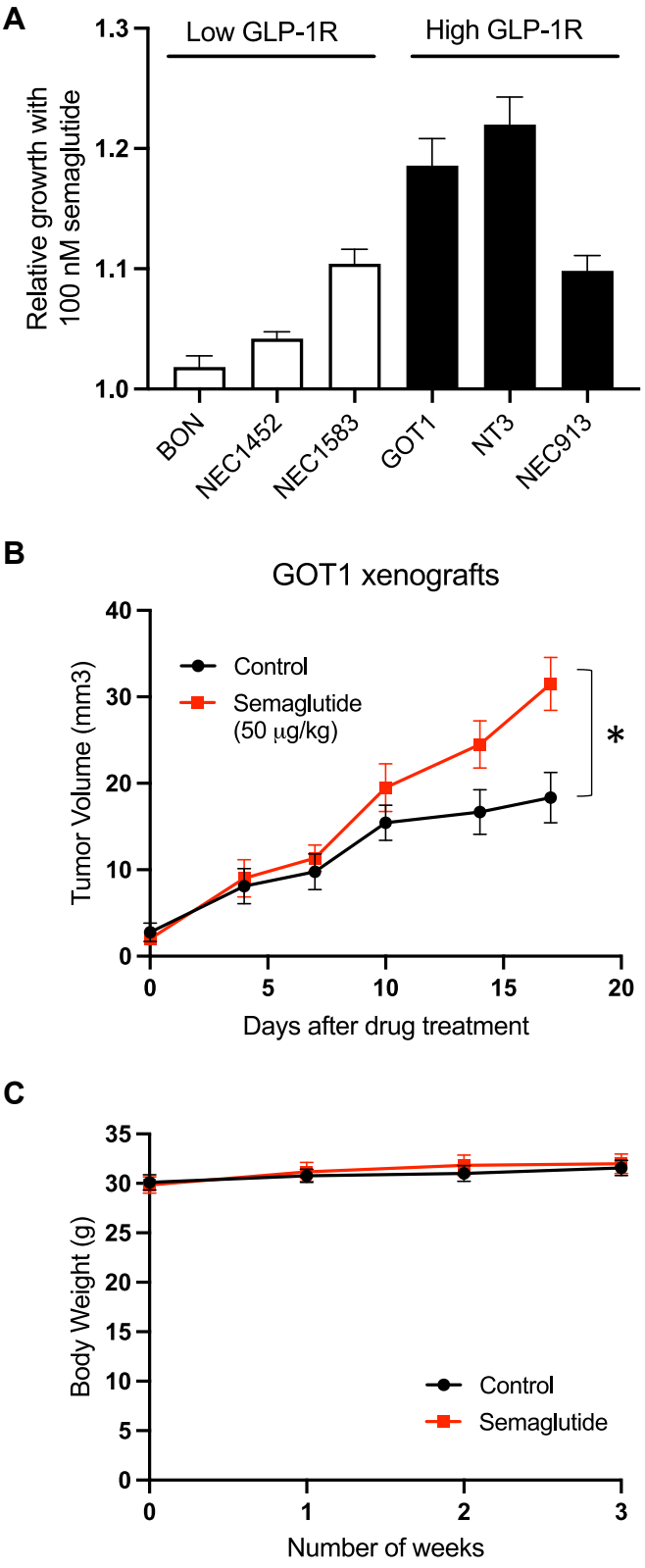
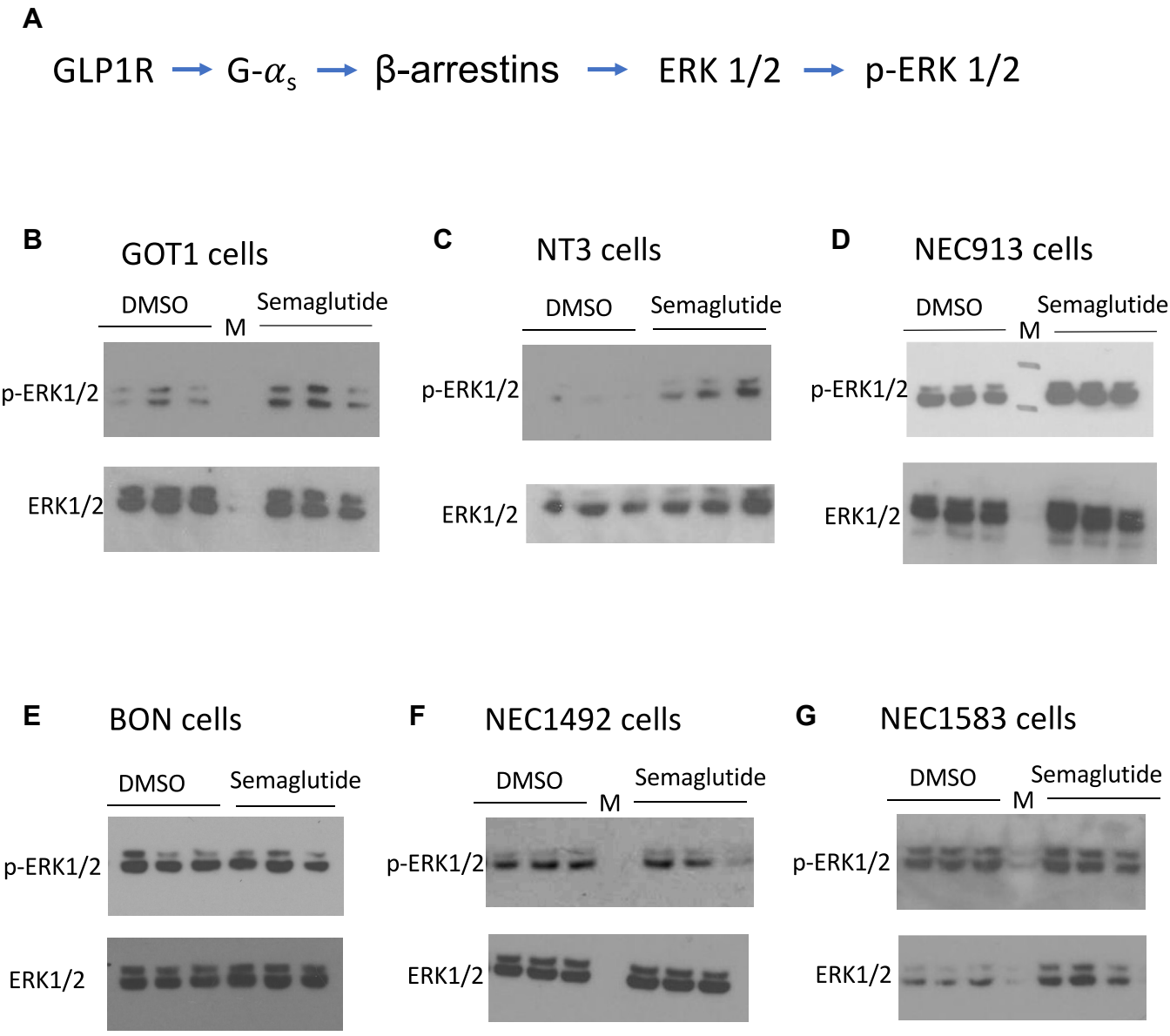


Figure 2. The GLP-1R agonist semaglutide promotes tumor cell growth in GLP-1R–expressing neuroendocrine cancers. (A) Neuroendocrine cancer cell growth in the presence of 100 nmol/L of semaglutide normalized to the vehicle control group. Data shown as mean ± SEM (n = 30 per group). (B) Tumor volume measurement of an SBNET xenograft model with and without semaglutide treatment twice per week at 50 μg/kg. Data are depicted as mean ± SEM (n = 6 and 9 mice per group). (C) Body weight measurement of mice from the semaglutide treatment experiment reported in B. *$P < .05$. *GLP-1R*, glucagon-like peptide 1 receptor; *SBNET*, small bowel neuroendocrine tumor; *SEM*, standard error of the mean.



Semaglutide activates GLP-1R pathway to promote phosphorylation of ERK1/2. (A) Schematic of the GLP-1R signaling pathway activating β-arrestins to promote phosphorylation of ERK1/2. (B-G) Detection of phosphorylated-ERK1/2 (p-ERK1/2) in neuroendocrine cancer cell lines with and without 100 nmol/L of semaglutide treatment for 5 minutes, detected by Western blot. Lanes with protein markers are labeled as "M." Representative data from n = 3 samples of 3 independent experiments. Blots were striped and reprobed for total ERK1/2 to serve as loading controls. *GLP-1R*, glucagon-like peptide 1 receptor.

(BON, NEC1452, and NEC1583) are poorly differentiated cells. The 3 NEN cell lines expressing high levels GLP-1R (GOT1, NT-3, and NEC913) have well-differentiated characteristics that are defined by lower levels of Ki67 and higher levels of neuroendocrine markers such as SSTR2. Recently, it has been demonstrated that NETs can be cultured as patient-derived tumor spheroids or organoids.[29–33] It will be important to investigate the expression levels of GLP-1R in these novel 3-dimensional culture models and compare them to the original patient tumors. However, the study limitations are due to the scarcity of these NEN models.

Semaglutide is a long-lasting GLP-1R agonist that binds to GLP-1R and promotes beta-cell and neuronal cell growth[17] and is used as a medication in type 2 diabetes and, more recently, in obesity. Many PNETs are derived from beta-cells and other NEN cells that do express GLP-1R. This raises the concern that semaglutide could have a significant impact on tumor progression in GLP-1R–expressing NENs,[15,34,35] although this has not been investigated to date. In this study, we identified the growth-promoting effect of semaglutide in GLP-1R–expressing NEN cells (Figure 2). In addition, we demonstrated that semaglutide promotes tumor cell growth by activating the mitogen-activated protein kinase (MAPK) pathway through phosphorylation of ERK1/2 in NEN cells expressing the GLP-1R protein (Figure 3). The MAPK pathway plays a key role in promoting cell proliferation in many cancers. Once phosphorylated, ERK1/2 are translocated to the nucleus, where they regulate the activity of pro-proliferative and antiapoptotic transcription factors.[36] The expression of the GLP-1R protein can be found in the nucleus, in cytoplasm, and on the membrane of NEN cells (Figure 1, *C*). Although the NT-3 cells were determined to have the highest *GLP-1R* transcript levels, the cell surface GLP-1R protein levels are similar to those of the GOT1 cells, and the growth-promoting effects of semaglutide on these cells are similar (Figure 2, *A*). This suggests that the cell surface expression levels of GLP-1R on cancer cells is a better predictor of response to semaglutide than the gene expression levels.

A few recently published articles reported no effect of semaglutide on pancreas and colon cancer risks.[37,38] Other studies reported beneficial effect of semaglutide on reducing tumor growth in diabetic or obese mouse models.[39,40] Although this might seem contradictory to our findings, it should be noted the cancer models used in those studies were based on cancers that do not express GLP-1R.[12,40] Moreover, the growth inhibitory effects of semaglutide on breast and colon cancer models is not a direct effect of semaglutide on the tumor cells because those cancer cells had low or no GLP-1R expression.[39,40] In those scenarios, semaglutide is promoting weight loss in the obese and diabetic mice, by decreasing tumor inflammation similarly to putting the mice on a calorie restriction diet.[40] In conclusion, our data support the notion that semaglutide promotes cancer growth in GLP-1R–expressing NEN in preclinical models. These results raise potential concerns about the use of GLP-1R agonists for patients with NEN and for those with multiple endocrine neoplasm syndrome type 1 (MEN1) because these agonists may promote NEN growth or development.[41] Expanding the investigation into more NEN models such as the MEN1 mouse models will be valuable because of the heterogeneous nature of these cancers.

Funding/Support

We are grateful for support from the University of Iowa Holden Comprehensive Cancer Center. We thank Drs Mark Evers, Jorg Shrader, Ola Nilsson, and Yvonne Arvidsson for the BON, NT-3, and GOT1 cells. In addition, we thank Dr Shujie Yang for generously donating NSG mice for this study.

Conflict of Interest/Disclosure

The authors declare no conflict of interest.

CRediT authorship contribution statement

Jonathan S. Shilyansky: Writing – original draft, Investigation, Data curation. **Casandro J. Chan:** Methodology, Investigation, Data curation. **Sophia Xiao:** Investigation. **Irena Gribovskaja-Rupp:** Writing – review & editing, Investigation. **Dawn E. Quelle:** Resources, Methodology. **James R. Howe:** Writing – review & editing, Resources, Investigation. **Joseph S. Dillon:** Writing – review & editing, Resources, Investigation, Conceptualization. **Po Hien Ear:** Writing – original draft, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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Discussion

Dr Anai Kothari: The discussion of this paper will be opened by Dr Chris McHenry.

Dr Christopher McHenry: Chris McHenry from Cleveland, Ohio. Congratulations to you and to Dr Po on some excellent research. Your presentation was well done, and the manuscript is well written. As you indicated, semaglutide is a class of medication called secretin mimetics, and it is a GLP-1 [glucagon-like peptide 1] receptor agonist with a half-life of 165 hours when given subcutaneously once weekly and it is approved for the treatment of type 2 diabetes and is now being used for the management of obesity and it mimics the GLP-1 hormone that is released into the GI tract in response to eating to stimulate increase in insulin secretion and suppression of glucagon, which reduces blood sugar. I think, most importantly, in rodents, prolonged exposure to this GLP-1 receptor agonist causes thyroid C-cell hyperplasia and medullary thyroid cancer. In humans, approximately 20–30% of medullary thyroid cancer expresses the GLP-1 receptor and, as a result, semaglutide is contraindicated in patients with a history of medullary thyroid cancer or MEN2 [multiple endocrine neoplasm syndrome type 2]. The results of your study raised concerns for the potential risk of using semaglutide with other neuroendocrine neoplasms expressing GLP-1 receptor, so I have 4 questions for you, and if you can't remember them, I will repeat. So, first, based on your in vitro and in vivo results, semaglutide should also be contraindicated in patients with MEN1 [multiple endocrine neoplasm syndrome type 1] because of the occurrence of pancreatic neuroendocrine tumors in 30–80% of these patients. Secondly, what are the implications of semaglutide activation of the MAPK [mitogen-activated protein kinase] pathway through phosphorylation of ERK1 and ERK2. Can you comment on the use of radiolabeled GLP-1R agonist for localizing insulinoma and then, finally, in your in vitro experiments, do you have any explanation for why semaglutide only stimulates 20% cell growth? So, I enjoyed your presentation, and I thank you and the Central Surgical for asking me to be the discussant.

Sophia Xiao: Thank you so much for those questions. So, regarding your first question about MEN1 our data did support this hypothesis that we think that semaglutide should not be recommended for MEN1 patients. We actually do have an ongoing experiment with 11 other collaborators with MEN1 knockout mice showing that one GLP-1 receptor is highly expressed. Secondly, I think the blood sugar and insulin levels were increased in these mice, and we predict that tumor growth will be increased as well. Regarding your second question, MAPK phosphorylation, phosphorylation of ERK1/2, so MAPK is the pathway that is important for cell growth, ERK1/2 is a key factor in that pathway, phosphorylation of ERK1/2 allows for transport into the nucleus where it can affect cell growth, cell survival, and cell migration as well, which has implications for a tumor progression. Your third question regarding insulin, will you repeat your third question, please?

Dr Christopher McHenry: The *use* of radiolabeled GLP-1R agonists to localize insulinoma sometimes can be very challenging, especially in patients with MEN.

Dr Sophia Xiao: Yes. Insulinoma do express a lot of GLP-1 receptors, and so often, what is done is we can use it, GLP-1R radiolabeled agonists, to target those, and I think they do line up very well from what we have seen and then, fourthly, your fourth question was regarding the growth, the 20% of the growth that we saw, it is related to the design, to the, I guess, the methods of the experiment. Usually these kinds of tumors are slow-growing; they take 1-2 weeks to divide, and we only had it running for a couple of weeks. We have continued this experiment, so it is still ongoing. We hope to determine if tumor growth will increase with time.

Dr E. Christopher Ellison: Chris Ellison, Columbus, Ohio. I enjoyed the paper very much, and I must admit I learned about GLP-1R agonists during the cocktail reception last night. Dr Henry gave me a beautiful dissertation on the entire physiology. However, I would like to make a historical comment. As you know, before the development of PPI [protein pump] inhibitors, total gastrectomy was recommended for patients with gastrinoma, which is the most common neuroendocrine tumor of the pancreas of patients with MEN1. If you look at the data in that group of patients that had a total gastrectomy with MEN1, there is no evidence that disease progression was worse in that population. Frequently, when the tumors were left behind. In addition, more modern series look at pancreatic duodenectomy or total pancreatectomy in that setting, which also increases GLP-1. So GLP-1 is increased in total gastrectomy and in pancreatic duodenectomy. There is no evidence that there is a more rapid progression of tumors in those patients, so your evidence is suggestive. We have natural history occurrences that people can look back on in this specific population. I enjoyed your paper very much, thank you.

Miranda Addie: Hello, thank you very much for your presentation. I have 1 quick question. You said you used NSG mice, and those mice are severely immunocompromised. Do you think that this has an impact on your results, and are you thinking perhaps in the future of using humanized models or more immunocompetent mice or other forms?

Sophia Xiao: That is a great question, and we will consider it for future experiments. The thought process behind using NSG mice was just to ensure that these tumors were really implemented in these mice. Even with the GOT1 model, I think only 80% of the mice were able to take up the tumor or show a tumor.

Miranda Addie: Thank you very much. I use NSG mice as well, so I am not complaining. I was just asking for clarification.